Demonstrated ways to use rapid cycling *Brassica rapa* in ecology instruction and research

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FOCUS OF INVESTIGATIONS

The National Science Foundation has a long supported the use of "Wisconsin Fast Plants" (rapid cycling *B. rapa*) in the teaching of Biology (K-12). I believe that the opportunity is at hand for biologists to significantly extend past efforts made by our colleagues at the k-12 level to higher education. Biology faculty can realize the many practical benefits of RCBr as a model organism with known genotype and phenotype for student research in the lab and in the field. One area of investigative student experience that is significantly lacking, is the development of ecological investigations using Fast Plants. Here, I demonstrate how I incorporated Fast Plants into three college courses to provide students with: 1) An authentic and enriching research experience. 2) A link between “classroom and professional practice”. 3) A connection between what students read and reality. 4) Experience with Fast Plants as a model system for their research and teaching.

OVERVIEW

One species that botanists have broadly employed in all areas of research is *Brassica rapa* (syn. *campestris*). This species is endemic throughout Europe eastward to Siberia. This plant is also widely cultivated in cool, temperate climates as Pak Choi, Turnip Rape, Choy Sum, Chinese Cabbage, Tendergreen, Turnip, Sarson, and Broccoli Raab (Williams and Hill, 1986). In addition, *B. rapa* has established weedy and naturalized populations in Asia, Australia, and the Americas. Of special importance to these investigations is rapid cycling *B. rapa* (RCBr, AKA Wisconsin Fast Plants). RCBr was derived using classical methods of artificial selection and breeding (Williams and Hill, 1986).

ABOUT RAPID CYCLING *BRASSICA rapa*

RCBr was derived from a global collection of *B. rapa* (L.) varieties (Williams and Hill, 1986). Plants were selected for the following six qualities (Tomkins and Williams, 1990):

1. Reduced size at maturity
2. Minimum time from germination to flowering
3. Uniformity of age at first flowering
4. High flower production
5. Rapid maturation of seeds
6. Lack of seed dormancy

Individuals that flowered fastest were used as the base population. These individuals were out-crossed to generate seeds. In the next generation, the 10% of the offspring population that flowered first were selected as parents. These 288, or more, plants were mass pollinated to produce the next generation of seeds; artificial selection continued until the response to selection was stabilized (Williams and Hill, 1986).

Under optimal laboratory conditions, RCBr flowers within 16 days of seed germination and has a life cycle of 35-40 days, from parental seed sown to offspring seed harvest (Williams and Hill, 1986). Compared to normal *B. rapa*, which can produce two generations in a year, RCBr can produce upto ten generations in a year. The potential applications of RCBr to experimental plant biology are diverse (Kelly, 2004; Musgrave, 2000).

It is important to recognize that RCBr retains considerable genetic variation (Williams and Hill, 1986). When inbreeding is forced, fitness is significantly reduced (Evans, 1991). Both outcomes suggest that *B. rapa* did not passed through a genetic bottleneck in order to establish the rapid cycling lines.

RCBr are available in a wide variety of known genotypes with distinct phenotypes. Seeds may be purchased from Carolina Biological Supply Company (USA). RCBr seed stocks for the same self-incompatible genotypes may also be purchased from Blades Biological Ltd. (UK).

After Williams and Hill (1986) summarized the results of their selection for rapid cycling *Brassica* species, plant ecologists were among the early adopters. Ecologists have employed both naturalized and rapid cycling varieties of *B. rapa* in a wide variety of studies (references that follow cited in Kelly, 2004). Though Miller and Schemske (1990) were the first to publish ecological research using RCBr, almost all of our knowledge about the growth and development of RCBr is based on plants raised in controlled environments. Yet, in a direct comparison of *B. rapa* grown in growth chambers and the field, Torresen and Lotz (2000) concluded that it was not possible to use the phenotypic outcomes of plants grown in growth chambers as predictors for plants grown in the field. Kelly and Terrana (2004) were the first to employ RCBr in a field experiment.

APPROACH TAKEN & EVIDENCE GATHERED

Here, I demonstrate how I incorporated Fast Plants into three college courses to provide students with:

- An authentic and enriching research experience
- A link between "classroom and professional practice"
- A connection between what students read and reality
- Experience with Fast Plants as a model system for their research and teaching

Independent Study (BIO 495), Summer 2002
Student: Sebastian Terrana

A Method to Teach Age-Specific Demography with Field Grown Rapid Cycling *Brassica rapa* (Wisconsin Fast Plants)

Published in J. Nat. Resour. Life Sci. Educ. 33:40–46 (2004)
http://www.JNRLSE.org/

In spite of the academic and personal benefits fieldwork can provide, the use of fieldwork in biology education is declining (TIEE, 2003). Factors that decrease student participation in fieldwork include class size, that the academic calendar doesn't conveniently permit time for fieldwork, cost and difficulty associated with transporting students to the field site, and the availability of suitable field sites. In the United States, 90% of undergraduate courses in ecology only employ lecture, 34% of students never go outside to learn ecology, and open ended laboratory exercises are rarely used (TIEE, 2003). Without fieldwork, fewer students will have the opportunity to personally experience the range of complex and connected factors that shape the natural world.

In this paper, we demonstrated that rapid cycling *Brassica rapa* (Wisconsin Fast Plants) could be used for inquiry-based, student ecological fieldwork. We were the first to describe age-specific survival for field grown Fast Plants and identified life history traits associated with individual survival. This project can be adapted by educators to teach age-specific demography or ecology with Fast Plants.

Four genotypes of Fast Plants (dwarf = dwf1/dwf1, elongated internode = ein/ein, standard = Rbr/Rbr, and rosette = ros/ros) were used. Fast Plants were grown in a "common garden" experiment. The site was surveyed every day to monitor emergence, and every two days to record mortality.

Day	**Activity**
-3	Friday, prepare plot by clipping grass and weeds to soil level.
0	Monday, sow seeds 1 through 256 (number can be reduced) according to plan. Lightly cover seeds with soil mix and water (~15 liters) to moisten soil.
1-2	Survey for seedling emergence. No seedlings observed.
3	Survey: first 4 seedlings emerged.
4-19	Survey for new seedlings and juvenile deaths. First seedling death recorded on Day 10. Survey all positions without germination to confirm lack of seedling. Clip grass and weeds in the plot if obstructing view of seedlings. Can also measure length and width of cotyledons.
20-30	Survey for mortality and harvest dead plants. If a known emergent is missing, score as dead. Can also measure leaf number and size (length and width), and plant height at known ages or developmental stages. First flowering plants observed Day 20. Plants in flower until Day 30. Insects seen on flowers Days 21, 22, and 23.
31-53	No plants in flower. Can monitor plants for fruit development. Survey for mortality and harvest dead plants. If known emergent missing, score as dead. Last plant harvested on Day 53. Harvested plants can be dried and weighed, and the number of seeds per fruit measured for reproductive data.

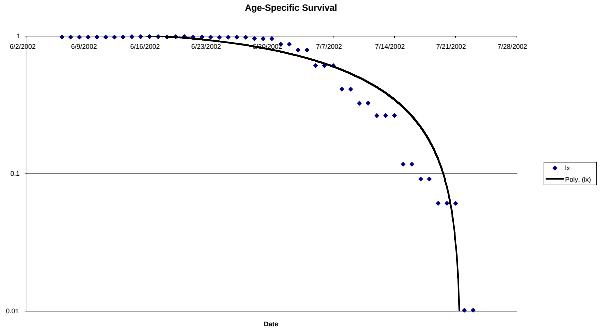
Seven life-history traits were measured: days to emergence, emergence date, death date, life span, flowering date, juvenile duration (days), and adult duration (days). Most seedlings emerged 5-6 days after sowing and plants lived an average of 30 days. Flowering began about 17 days after seedling emergence.

*	**dwf1/dwf1**	**ein/ein**	**Rbr/Rbr**	**ros/ros**
Days to Emergence	5.4±0.2 (52)	5.8±0.2 (48)	5.7±0.3 (47)	5.6±0.2 (50)
Emergence date	June 8±0.2 (52)	June 8±0.2 (48)	June 8±0.3 (47)	June 8±0.2 (50)
Deaath date	July 7±1.1 (53)	July 9±0.9 (48)	July 8±1.0 (48)	July 9±0.8 (50)
Life span	29.3±1.2 (52)	30.9±1.0 (48)	30.4±1.0 (47)	30.4±1.0 (50)
Flowering date	June 24±0.4 (17)	June 25±0.3 (12)	June 26±0.6 (12)	June 24±0.4 (9)
Juvenile days	17.2±0.4 (17)	16.9±0.3 (12)	18.0±0.5 (12)	17.0±0.4 (9)
Adult days	15.3±1.6 (17)	16.2±1.8 (12)	14.5±2.2 (12)	12.6±1.1 (9)

*Average ± standard error of the mean and the number of plants measured (sample size)

Analysis of Variance showed that these four genotypes did not differ for any of the life-history traits measured. Correlation analysis revealed that life span was negatively related to emergence date and flowering date was positively related with emergence date.

Life table analysis showed that rapid cycling *B. rapa* has Type I age-specific survivorship.



We suggested that ecologists extend the use of Fast Plants to include student fieldwork. Inquiry based work, can permit students to construct a personal understanding about the process, practice, and outcomes of science. Students can also gain understanding about the collection of numerical data, its analysis, and limits to their interpretation.

Plant Ecology (BIO 617), Fall 2002

Students: Pat Creamer, Rolfe Freidenberg Jr., Dan Godwin, Tonniele Naeher, David Rosa, and Harry Shoemaker

Characterizing Genotype Specific Differences in Growth and Fitness for Field Grown, Rapid Cycling *Brassica rapa*

Article in press. Environmental and Experimental Botany (2004)
Available online through www.sciencedirect.com

This study was the first to describe the genotype specific variation in traits describing survival, growth, and reproduction for field grown rapid cycling *Brassica rapa* (RCBr). Six students in a Plant Ecology course collected data. This semester long course met one evening per week (Fall 2002). From data collected through five independent projects, I identified RCBr traits associated with fitness.

Five genotypes of RCBr were used: standard, anthocyaninless, yellow-green, anthocyaninless & hairless, and anthocyaninless & yellow-green.

Genotype	**Phenotype**
Rbr/Rbr	"standard", the basic Fast Plant type
anl/anl	"anthocyaninless", lacks purple pigment
ygr/ygr	"yellow-green", deficient in chlorophyll
anl/anl & hir(0-1)	"anthocyaninless & hairless", lacks both purple pigment & epidermal hairs
anl/anl & ygr/ygr	"anthocyaninless & yellow-green", lacks purple pigment & chlorophyll deficient

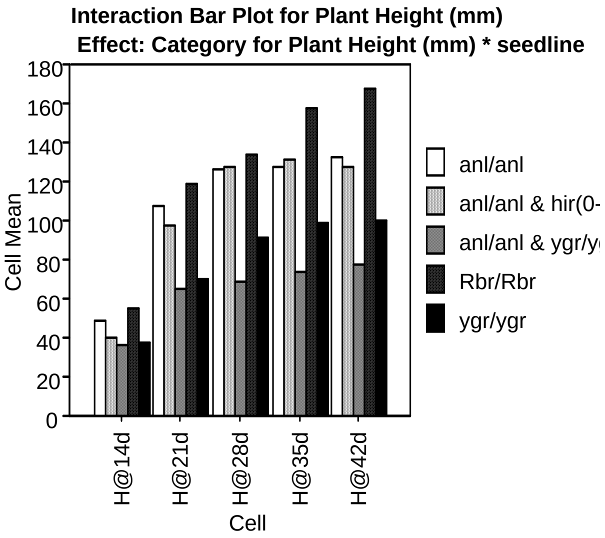
Plants were grown outside in a "common garden".

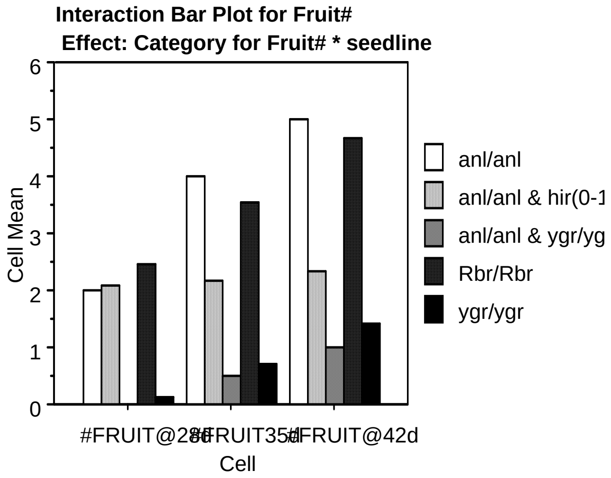
The site was surveyed every week to monitor plant presence (September 5 - October 17). Each seed's place was marked with an individually numbered plastic stake.

Death was recorded when a plant could not be located at the base of its marker, or the plant had browned and turned brittle. Individual plants were identified every census; the plant's marker was not removed until the end of the experiment (October 17th). The experiment was terminated and all plants harvested three weeks after the last fruits were initiated.

Date	Class Activity
AUG 29	• Sow "Fast Plant" seeds for plant observation & measurement • Record Data in Excel & SAVE
SEP 5	• Census plot for seedling emergence & mortality • Record Data in Excel
SEP 12	• Census plot for later seedling emergence & plant mortality • Measure juvenile plant size for SEP 5 plants • Record Data in Excel
SEP 19	• Census plot for plant mortality • Measure juvenile plant size for SEP 12 plants • Count # flowers per plant • Record Data in Excel
SEP 26	• Census plot for plant mortality • Measure juvenile plant size for SEP 19 plants • Count # flowers per plant • Count # fruits per plant • Record Data in Excel
OCT 3	• Census plot for plant mortality • Count # flowers per plant • Count # fruits per plant • Record Data in Excel
OCT 10	• Census plot for plant mortality • Count # flowers per plant • Count # fruits per plant • Record Data in Excel
OCT 17	Tentative harvest date (3 weeks after last fruits initiated)

Eight plant traits were measured: life span, height, growth rate, leaf size, number of flowers and fruits, fruit set, and fitness.





All traits, except life span, differed significantly among the five plant genotypes. Correlation analysis revealed that fitness increased as height, growth rate, leaf size, number of flowers and fruits, and fruit set, increased. This study demonstrated that RCBr could serve as a model organism in ecological field studies. That genetic and genotypic variation in RCBr produced significant and informative variation at the phenotypic level in the field.

General Botany (BIO 115, Fall 2003)

Intraspecific Competition between Standard and Yellow-Green rapid cycling *Brassica rapa*

In 4-labs, students performed 3-experiments on the effects of genotype (Standard and Yellow-Green Fast Plants) and density on plant growth. This experiment started in the 1st week of November and was completed in the 1st week of December.

The term competition as understood and used by ecologists is different from the popular meaning of the same word. *Ecological competition occurs when the use of a LIMITED resource by one individual reduces the availability of that same resource to another individual.* For plants, limiting resources can include sunlight, water, mineral nutrients, pollinators, and seed dispersers. For competition to occur, the resource must be required by both individuals and promote survival or reproduction. A second necessary condition for competition is that the resource is finite and scarce, a "limiting" resource.

Competition can occur between individuals of the same species or different species. If competing individuals belong to the same species, it is termed "INTRA-specific competition". If competing individuals belong to different species, then it is called "INTER-specific competition".

In this laboratory our experiment was modest. Students conducted a series of replicated experiments to determine if rapid cycling *Brassica rapa* seedlings, Standard and Yellow-Green, compete for the limited resources present in a small pot of soil (eg. space, water, or mineral nutrients). It is likely that rapidly growing plants should compete for one or more of these limited resources. Only INTRA-specific competition will be studied. The procedure is simple: your lab will sow seeds at different densities and in different mixtures, allow them to germinate and grow plants in the laboratory over their lifespan.

General Experimental Procedure

You will examine the effect of population density on seed germination rate and seedling growth. You will be randomly assigned to sow specific numbers seeds from Standard and Yellow-Green rapid cycling *Brassica rapa* in a minipot (2.25 inch diameter) at specific densities. Your lab will sow the species pairwise combinations. Over all of the lab, *the density of added seeds per pot will double from 0 to 1 to 2 to 4 seeds per pot* in a fixed volume of soil. Germination is expected to be 95% or more.

EXPERIMENT SET UP
TARGET PLANT IS ___________________________
SURROUNDING PLANTS ARE ________________
TYPE OF SURROUNDING PLANT IS ___________

1) Get your specific experimental set from your lab instructor
2) Get a 2 1/4 inch standard pot (4 drain holes)
3) Label you pot with tape. Include on your label: your lab section, your name, and your experimental set
4) Put 2 blue diamond™ wicks half-way through 2 of the 4 drain holes
5) Measure & add 20ml of jiffy-pot #901 soil mix to the bottom of your pot
6) Have your lab instructor add 1ml of slow release N-P-K (14-14-14) onto this soil
7) Measure & add another 20ml of jiffy-pot #901 soil mix to your pot
8) Count out the exact number of seeds needed for your experiment with the target plant seed in the center
9) Measure & add another 20ml of jiffy-pot #901 soil mix to your pot
10) Place in the tray for your lab section where they will be watered and grown

Results

- No competitive effects were detected
- Standard and Yellow-Green Fast Plants differed in cotyledon size at 7 & 14 days ($P=0.051$ and $P=0.027$)
- Standard and Yellow-Green Fast Plants differed in leaf production ($P=0.077$)
- Standard and Yellow-Green Fast Plants differed in reproductive effort, as measured by the sum of flowers and fruits ($P=0.0016$)

OVERALL RESULTS FROM THREE COLLEGE COURSE PROJECTS

Under optimal growth conditions, RCBr is characterized by its reduced size at maturity, minimal time from germination to flowering, increased uniformity at age of first flowering, high flower production, rapid maturation of seeds, and lack of seed dormancy.

Musgrave (2000) made the general case for using RCBr in areas of plant research conducted in the laboratory. I make the case that RCBr can valuably serve as a model organism for ecological studies conducted in the field. There are at least six benefits that can be realized by plant ecologists using RCBr in the field:

1) Because of its origin through artificial selection from a global collection of *B. rapa*, RCBr has substantial allelic variation.
2) Genetic and genotypic variation in RCBr produces significant and informative variation at the phenotypic level in the field.
3) For RCBr grown in the field, all of the phenotypic traits measured, except life span, differed significantly between genotypic lines. It may be that past selection for rapid cycling, has compressed life span to the point where slight differences are statistically difficult to detect in small field grown populations.
4) RCBr is commercially available in a wide variety of known genotypes with distinct, contrasting phenotypes.
5) The close taxonomic relation of *Brassica* species to *A. thaliana* makes the molecular genetic tools derived for *A. thaliana* transferable to RCBr.
6) The larger flowers, fruits, and seeds in RCBr are easier to handle, count, and measure than flowers, fruits, and seeds in *A. thaliana*.

The present liability in the application of RCBr to plant field ecology is the lack of comparable studies. I welcome the efforts of other plant ecologists in this validation by performing parallel ecological trials in the field with RCBr and naturalized *B. rapa*. With this type of comparison, it is possible to know what tradeoffs in RCBr plant performance represent life history tradeoffs also evident in naturalized *B. rapa*.

IMPLICATIONS FOR TEACHING AND LEARNING

In October 2000, I participated in *Research Link 2000*, an NSF-funded project of the Council on Undergraduate Research. A goal of *Research Link 2000* was to promote research activities by students and faculty on all levels of the undergraduate curriculum. There I was introduced to the Wisconsin Fast Plants as a model organism. I believe that the opportunity is at hand for biologists to significantly extend past efforts made by our colleagues at the k-12 level to higher education. Biology faculty can realize the many practical benefits of RCBr as a model organism with known genotype and phenotype for student research in the lab and in the field. Using Wisconsin Fast Plants it becomes more possible to provide students with:

- An authentic and enriching research experience
- A link between "classroom and professional practice"

- A connection between what students read and reality
- Experience with Fast Plants as a model system for their future research and teaching

HELPFUL PUBLICATIONS

Kelly, M.G. 2004. Characterizing Genotype Specific Differences in Growth and Fitness for Field Grown, Rapid Cycling *Brassica rapa*. Article in press. Environmental and Experimental Botany.

Kelly, M.G., Terrana, S. 2004. Age-Specific Survivorship of Field Grown rapid cycling *Brassica rapa* (Wisconsin Fast Plants™). J. Nat. Resour. Life Sci. Educ. 33: 40-46.

Musgrave, M.E. 2000. Realizing the potential of rapid-cycling *Brassica* as a model system for use in plant biology research. J. Plant Growth Regul. 19: 314-325.

Tomkins S.P. and Williams P.H. 1990. Fast plants for finer science - an introduction to the biology of rapid cycling Brassica campestris (rapa) L. Journal of Biological Education 24: 239-250.

Williams, P.H., Hill, C.B. 1986. Rapid-cycling populations of *Brassica*. Science, 232: 1385-1389.

HELPFUL RESOURCES

Experiments to Teach Ecology, Volume 2, Copyright 2003 - Ecological Society of America. Valerie A. Barko, Beth A. Burke, David J. Gibson, and Beth A. Middleton

Research Link 2000. Sponsored by the Council on Undergraduate Research
Funded by the National Science Foundation. http://www.cur.org/reslink2000.html

TIEE. 2003. Teaching Issues and Experiments in Ecology: Overview. Education and Human Resources Committee, the Ecological Society of America. http://www.ecoed.net/tiee/misc/about.shtml

The Carolina Biological Supply Co. homepage (www.carolina.com). Exclusive license to sell Wisconsin Fast Plant seed stocks in the United States.

The Crucifer Genetics Cooperative at Madison, WI. A source of unique rapid cycling *Brassica* genotypes not sold by Carolina Biological.

The Wisconsin Fast Plants homepage (www.fastplants.org) provides the following four college-level activities:

- *Evolution By Artificial Selection*. A 9-Week Classroom Investigation using Rapid-cycling *Brassica rapa*: Developed by Bruce Fall, University of Minnesota
- *Teaching Recurrent Selection in the Classroom with Wisconsin Fast Plants*.

Developed by Irwin L. Goldman, University of Wisconsin - Madison

- *Inbreeding Depression and the Evolutionary Advantage of Outbreeding in Brassica rapa.* Developed by Chris Eckert, Queen's University Kingston
- *The Ecological Significance of Intra-specific Competition in Plant Populations*. Developed by Mike Moser, University of California - Berkeley