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A PLANT BIOCHEMISTRY UNIT DESIGNED FOR A HIGH ACHIEVING SECONDARY CLASSROOM

By

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ABSTRACT

A PLANT BIOCHEMISTRY UNIT DESIGNED FOR A HIGH ACHIEVING SECONDARY CLASSROOM

By

Cheryl L. Hach

A unit on plant biochemistry was formulated for high achieving students enrolled in an elective organic chemistry class. The focus of the instruction was to improve the attitudes of students toward plant science and activities were chosen to provide high interest and hands-on experience. The main areas of study included some mechanisms that plants have evolved to assist in defense against predators and competitors, pollination strategies and energy conversions using photosynthesis and enzymes. Alternative assessment techniques were used for evaluation. These techniques included observation, group work and journal writing. Students responded positively to the content of the unit and reported, both verbally and in writing, that they gained insight into plants that they did not possess previously.

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A final kudo is due to Bell's Greenhouse for allowing a group of students to visit during a very busy time of year for them. The assistance of their staff was most helpful to both students and their teacher.

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INTRODUCTION

It is commonly held that students have less interest in plants than they do in animals. Surveys of student attitudes have shown that among many topics in life sciences, botany and plant science are clearly not the choices of most interest (Wandersee, 1986). Furthermore, gender differences and also cultural differences exist which further differentiate interest in plants (Wandersee, 1986). Even teaching practitioners find plants less exciting than animals (Flannery, 1991).

I chose to include a new unit on plant biochemistry to an existing eleventh grade elective biochemistry course at an area high school program for gifted and talented students. The school curriculum is designed to present courses that offer "enriched" rather than "accelerated" opportunities. In other words, the content is made broader by varied activities and encouragement to expand the scope of instruction. Many science classes provide up to two-thirds of the contact time with students actively involved in hands-on and laboratory activities. Because students are bright, motivated to attend and have made the personal commitment to succeed, classroom management is easy to maintain and students are eager for instruction.

The school population is approximately three hundred students evenly distributed throughout the ninth through twelfth grades. Students are selected from eleven different school systems throughout the local intermediate school district; they attend on a half day basis. Invitation to be part of the program is based on several criteria including standardized test scores, teacher recommendation and student interest. Students attend by their choice. Ninth and tenth graders attend in the morning when they participate in three different classes. Ninth graders are required to take biology, a mathematics course on a

level with their experience and ability and a course entitled interdisciplinary studies which encompasses research skills, keyboarding and writing skills. There is heavy emphasis in this course on time management and study skills. Tenth grade students must enroll in chemistry, computer science and continue in a math course.

Eleventh and twelfth graders attend in the afternoon and have the opportunity to elect from a variety of course offerings. All students need to complete a math and a science course each year, with physics being the required science in eleventh grade. This allows the opportunity to choose an additional science or an additional math or a course such as advanced computer science, mentorship or introduction to engineering.

I developed this course as part of a two-semester elective course providing an additional credit in chemistry. It is elected primarily by juniors. The first semester of the course involves the study of basic organic chemistry including various functional groups and the chemistry associated with these entities. The second semester of the course takes up the study of major biochemical classes. It reinforces the skills and techniques taught in the first semester. Additionally students learn some basic molecular biology activities.

Structuring of the school day is unusual for secondary schools in the local area. Block scheduling has been in place for ten years. The small teaching staff is very open to rearranging time to allow for the special needs of classes to take field trips, attend lectures, and invite guest speakers. Sometimes an entire afternoon is spent in one class. Typically, however, students are in class for forty-eight minutes on Mondays and Fridays. Classes meet twice during the intervening three days for one hour and twelve minutes. The class participating in this study met on Tuesdays and Wednesdays. There was no class period on Thursdays.

An additional opportunity for all students to contact teachers and laboratory time is Wednesday night "open lab". Instructors voluntarily make themselves available to students on Wednesdays from 7:00 until 8:30 P.M. This allows students to make up missing work, work additionally on a current lab, consult a teacher for extra help or just meet friends or

talk to instructors on a more casual basis.

The demographics of the classroom involved with this unit plan includes twenty junior students and one senior. The students represent eleven different home high schools. All except two have attended the school since their ninth grade year. Two are new to the school this year. Three students are of Oriental descent; one is an Orthodox Jew. No students are African-American or American Indian. Except the two new students to the school, all of the students had the same ninth grade biology class that I taught. Most, but not all, were at least above average students academically in the ninth grade.

The goal for the unit is to enable high achieving students with a year of general chemistry and a semester of basic organic chemistry to experience some aspects of plant chemical capabilities that are not obvious on the cursory examination common in a first year biology course. It is an unusual experience for students to have access to such a course; the instructor is suited to teach such a specialty, possessing a double major in chemistry and biology. Further consideration included access to many local growers and native plant specialists. Our school is located in the "bedding plant capital of the world", and city in bloom in May and June may be relied upon to spark interest in the beauty and variety of plants.

The development of this unit is an important opportunity both for students and myself since, like many colleagues teaching basic biology in a ninth grade curriculum, I give plants short shrift both in time and interesting activities (Flannery, 1991). If "deliberation begins with a concrete practical situation that disturbs us, and is the means by which we develop and construct curriculum" and "the purpose of deliberation is to probe into the nature of the problem in order to orchestrate change" (Tamir, 1974), this researcher undertook the examination of a troubling aspect of her curriculum with the goal of making a change. As the teacher of the previously mentioned first-year biology course, I feel a certain amount of guilt yearly as the calendar winds to a close. Plant biology becomes a filler during the final days of the school year, when students' interests are

waning and survival until final examination time is the primary motivation for continued scholarship. Although the textbook (Miller and Levine, 1991) is generally adequate, the level of interest in the chapters involving plants is quite low. This text stresses low interest topics such as plant anatomy, physiology and systematic classification (Hershey, 1992). My experiences have shown that this material is perceived as dull and irrelevant. Activities involving basic plant anatomy and physiology do not engage students with the same excitement that activities from other areas generate. Each autumn I make the commitment to rearrange topics to allow more time for interesting plant activities that demonstrate how plants are the basis for life and, as such, are critical to many aspects of the world. Somehow, however, the year seems to get away and days dwindle to few with no exciting plant activities in sight. This is not to imply that plants are totally neglected in the curriculum. Students study photosynthesis as part of the unit on cell biology and ecological aspects of plants in the ecosystem are stressed early in the first semester of biology. Topics such as cell division mention plants in passing, but basically, like many courses, the focus of most of the material is on animals, specifically mammals and the anatomy and physiology of man (Honey, 1987).

The sorry state of plant biology teaching on a secondary level is not new. It has been said that "plants are the most important, least understood, and the most taken-for-granted of all living things " (Wilkins, 1988). Some researchers have referred to high school teachers as "animal chauvinists" (Darley, 1990) who are not well educated in plant science and therefore teach the content in this area with little enthusiasm and considerable vagueness that may be the result of many factors. Studies have shown that the use of vague terms are perceived by students as indicative of an instructor being "unconfident, disorganized, and unprepared, despite the substantive content the teacher presents" (Smith and Bramblett, 1981). Opportunities to relate the relevance of plants to the world, which should be abundantly clear to students, are frequently missed in favor of "canned" book activities that are of little practical interest to students. Since "learners who have little need

to know and understand, quite naturally, expend relatively little learning effort" (Ausubel, 1968), teachers must be educated to relate plants to everyday events in the lives of their students (Wandersee, 1986). Most novice teachers have not been prepared to teach plant science. Although many have taken one or more botany courses, the course material has been on an advanced academic level. The content was not intended to expose new educators to activities and teaching techniques they might use in the classroom to spark interest in this life science area (Hershey, 1992). The honest ignorance of teachers to the various adaptations that have resulted from plant evolution has reduced their use in many classrooms to the level of decoration. In response to a survey, currently practicing biology educators listed the need to make science meaningful to students as their number one inservice need, with the desire to help students develop an understanding of interrelationships between science and society as a strong second (Rubba, 1981). The result of this lack of experience and the associated lack of confidence in their abilities in plant sciences encourages teachers who are otherwise quite competent to disregard plants. These same instructors, with proper training, could be connecting plants to such diverse societal concerns as economics, geography and historical events. Matt Edwards presented a look at phrases and discussions of biology topics collected from school children in an article in Bioscience (Edwards, 1991). These reflections of school children are humorous and also perceptive. I have used the article to amuse students for several years. However, there is not one reference to plants (Stern, 1991).

Although plant scientists decry the state of botany teaching at the secondary level, plant science journals do not accept articles on education and plant science has lagged behind in publishing a journal on education in that field. These issues have led to call for the establishment of such a publication (Hershey, 1989).

It was noted by Bloom that interest is mediated by a sense of adequacy about a topic (Baird, Lazarowitz, and Allman. 1984). Students and teachers alike are uncomfortable with ignorance on a topic and would prefer to be engaged in areas on which they feel some

level of understanding.

Learners themselves bring little to the classroom that might fan a spark of interest in developing more engaging plant science teaching materials. It has been documented in student preference studies that both boys and girls would prefer to study topics relating to animals over topics that relate to plants. Plants are perceived as boring because they do not appear to share the attributes of animals; they do not move, breathe or respond rapidly. "As animals draw attention to themselves, plants need to have attention drawn to them..." (Honey, 1987). It is worth noting as an aside that when students refer to animals, they generally are thinking almost exclusively of mammals and their responses reflect this bias (Wandersee, 1986). Although students of both sexes report the preference to study animals over plants, girls show slightly higher interest in plant topics than do boys of the same age (Wandersee, 1986).

There is a wealth of historical precedent for female interest in botany and I believe that this history may play a part in the relative disinterest of boys in plant sciences. "Although women were seldom professional botanists in the United States during the Nineteenth Century, they did constitute a significant part of the botanical community during that century, particularly in its latter part, and were most important in the furtherance of botanical activity" (Rudolph, 1982). In Europe the popular scientific study of botany was considered de rigueur for young ladies of the higher social classes. The encouragement of young women to study plants was viewed as a means to discipline the mind while allowing the "fair sex" to appreciate the beauty of native plants. Beatrix Potter's popular stories contain many references to plant life. The prevalence of botany as a fashionable pastime for females allowed them to be occupied by science although few could break into professional levels of scientific study. An acceptable way to increase the rigor with which plants were studied was as a teacher. Of the few females who were identified with any profession in the Nineteenth Century, most were teachers, and it was clearly part of their professional charge to carry on instruction in the feminine arts, which included the amateur

study of botany.

Wealthy young men were also, of course, educated in the study of the natural sciences. Perhaps some relative reticence of adolescent males to identify with interest in plant sciences stems from the perception that interest in the study of plants is somehow unmanly. The study of plants as horticulture, an applied science, would not have any negative connotation, as it is part of a commercial venture. However, among early adolescent males, the macho image is not in keeping with the gentle study of plants.

It is common for students, particularly in the early secondary years, to anthropomorphize and perceive animals as relating to their own experiences. Students show great difficulty in imagining what it would be like to be a plant. They, understandably, have no conception of what it would be like to undergo photosynthesis or live under the constraints of being a plant, unable to move freely, see, hear or communicate (Wandersee, 1986).

It was noted by Fox in a 1971 address delivered to the Convention of the American Educational Research Association on "A Practical Image of the Practical" that "botany is simply under a severe handicap that cannot be beaten for at least two reasons. First, children at a very early age are attracted to things that move and very few plants do, and second, it is much easier to identify with animals than with plants. If I compare our folklore to Indian folklore I realize the difficulties we must encounter in trying to 'sell' botany. There are practically no children's stories about plant life, while we have many delightful stories about animals." "...Our Western conception of science which views matter as a continuum from inanimate things to human beings tends to minimize the significance of plant life as it is rather far away from man in the continuum. If we were to teach other conceptions of science besides our Western conception, plant life would assume a much more significant role and children might respond differently" (Tamir, 1974).

Students, despite gender, typically bring experiences to the science classroom. Participation in out of school organizations such as Boy or Girl Scouts, 4-H and YW- or

YMCA offers students the opportunity to spend time outside and exposes them to plant and animal life. Many students have been to a zoo, planetarium or museum, but few have visited an arboretum or conservatory (Kahle, 1982). Although, or perhaps because, economic hardship is the primary determiner of the absence of so-called extras in a student's background, minority students of both genders come to school with a significant lack of experiences with plants. Many report that they have not helped tend a garden and, as compared to other students, 16 percent fewer black 17 year olds have even watched a seed sprout (Kahle, 1982).

Although students have considerable differences in their interests in plants versus animals, these differences are mediated by the inclusion of outdoor activities into the curriculum. Students prefer any activity outside, either concerning plants or animals, over activities conducted inside (Jungwirth, 1975). Educators should be encouraged to take advantage of outdoor opportunities to increase the interests of students in real world, just-outside-the-back-door, plant science.

It seems paradoxical that, in spite of low student interest, Gallop polls suggest that gardening has long been the nation's most popular hobby. Popular literature on that topic probably lacks the scientific point of view and rigor necessary for an academic course on the secondary level. It does indicate, however, that many people grow to have a general appreciation of plants. Perhaps it might be in the interest of education to use this as a stepping off place for a practical plant biology curriculum that stresses the common availability and appreciation of plants (Hershey, 1992). The traditional approach of using charts, herbarium specimens and microscope slides should be reduced in favor of a hands-on, minds-on approach. Students should grow, collect, prepare and extract plant materials themselves and use a group-think approach to their explorations in plant science (Lumpe and Oliver, 1991).

I chose to focus on this topic after hearing a lecture given by a faculty member who invited the audience to consider that plants live on a different timeline as compared to

animals. Many activities we consider as essential to organisms (when we think of organisms as animals) are also engaged in by plants. Reproductive success, movement, the need for energy, the need to mount an effective defense against an invader, are all needs that are common to animals. Students will accept this readily and eagerly offer more aspects of animal existence. They have not considered, however, the need for plants to engage in the same strategies to be suited for success in their environment.

The goal of this unit was to allow students to learn something about the varied and unusual ways that plants have adapted to meet some of their needs. Since the students in this study have had some organic chemistry, they are familiar with chemical characteristics of aromatic compounds and the chemistry of acids and bases. They have the capacity to examine the structure of basic pigment compounds and make predictions about the activity of these classes of molecules. Previous experience using both paper and thin layer chromatography can be reinforced by different activities related to plant biology. Since the curriculum has previously included a study of unsaturated hydrocarbons and esters, it is possible for students to assess plant scents on a molecular structural basis. Extraction of essential oils is yet another reinforcement of earlier laboratory techniques. Inclusion of activities that prove photosynthetic generation of ATP and the hormonal induction of hydrolytic enzymes allows students to ponder the ways in which plants convert light energy into chemical energy and then convert that chemical energy into readily accessible monosaccharides. Finally, students are asked to consider some ways in which plants may mount a defense against interspecies competitors as well as fungal pathogens.

The focus of this series of activities was affective. I hoped to awaken interest in plants by having students examine them in new and different ways. It was not a goal to require students to memorize structures of anthocyanin pigments or to ask them to recite the means by which plants synthesize phytoalexins or allelopathic compounds. Taking a new look at plants with a view toward the "crafty" chemical ways they have had to adapt to be successful in varied environments is quite enough for my purposes.

Asking students to change or develop new attitudes toward a topic in which they express little interest is a challenging undertaking. Nearly all of the material in the published curriculum for this organic chemistry and biochemistry course is cognitive, with emphasis on structure, nomenclature and characteristic reactions. Assessment of cognitive objectives is a relatively simple matter: teach then, assign practice problems and then test then and move on. The decision to look at attitudinal information represents a departure from anything I have done before. This change in pedagogy has required my trying different schemes to get at the information in question. Pre- and posttests, writing in journals and more extensive use of larger laboratory groups were new activities, both for students and the teacher. Very little time was spent in organized lecture, instead students were given reading assignments and background information as laboratory textual material was integral to the laboratory protocol.

Students and their parents were, of course, aware that they were participating in the master's thesis requirement of their instructor to study these notions. Consent forms were designed and distributed prior to instruction and the unit was discussed at parent-teacher conferences (APPENDIX 1, Figure C). The researcher offered students and parents the choice to participate in the activities. Learners were offered alternative instruction if they desired and were assured that whether or not they chose to participate, their success in the class was not in question. The mere fact that students were allowed to choose to participate or not in instruction has been shown in several studies to be a powerful motivator that correlates with interest and achievement (Lazarowitz and Lazarowitz, 1979). It is reasonable to expect that these highly motivated pupils would experience a high level of engagement.

Educational research indicates that there is a positive correlation between student engagement and student achievement. Science teachers typically try to present materials that will be interesting enough to promote engagement. Educators also are encouraged to exhibit behaviors that will keep students on-task such as moving among learners, frowning

at misbehavior, and other pertinent nonverbal behavior. A classroom that is well managed and provides an atmosphere conducive to learning makes paying attention easier for the student (McGarity and Butts, 1984). This unit was designed with an eye toward making the material relevant, interesting and inviting to further inquiry. Accepting the stereotype that students find plants boring and somehow "less" than animals, I invited the students to question that belief. Acknowledging that plants appear to do nothing and have no particular skills to adapt to their environment, learners were challenged to examine some aspects of plant life that we take for granted in animals. Focusing on the need for energy, the need to reproduce and the need to defend itself against invaders, students were asked to examine some strategies employed by plants to service these needs in ways that perhaps students had not thought about before.

To aid in accomplishing this task, I made use of living materials that were housed in the chemistry classroom. In addition to providing living material available for laboratory study, "the mere presence of living materials, in and of itself, have been shown to influence attitudes toward science, motivation, student interest, curiosity and learning (Saunders and Young, 1985). Additionally, pupils would use these materials as sources for laboratory explorations.

Since the aim of this unit was to invite students to change their conceptual framework about plants, it would be reasonable to expect that the activities and assessments accompanying the unit be aimed at guiding students to be reflective and deliberate in their consideration of the topics presented for examination. It was made clear to students that their work would be evaluated differently than in the past, where pencil and paper tests and chemical yields were a basis for grading. The students would not be to asked to memorize and exhibit proficiency in the chemical structures involved in plant biochemistry. The decision was made to try using techniques described in current educational research as authentic assessment. These activities are a means to "assess the rich and varied experiences that constitute doing science by devising ways for the actions and their

products to become part of the assessment. If the assessment of science is limited to passive responses, we will never fully understand what our students know" (Hein and Price, 1994). The use of these new techniques of assessment required me to become very deliberate in the way the classroom activities would be presented. I had to carefully consider what sorts of interactions with students would be most likely to present information that would prove useful in examining student attitudes.

Hein and Price (1994) stated, "if we consider assessment as using our senses to find out what someone else is thinking, we more readily comprehend the diverse forms available to us. ...It is considered reasonable to try to 'read' a person's facial expressions, to question friends and neighbors, to review a sample of the person's work. In short, we draw on all the resources we can find to make an assessment ". To this end, observation, conversation, photography and journal entries were used as the major assessment pieces of the unit, in addition to a set of pre- and posttest activities (APPENDIX 2, Figures A and B).

These, and other, means of assessment have been endorsed as part of the National Science Education Standards for all grade levels. Given the responsibility of teaching students that science is a process, it is incumbent on practitioners to model that paradigm shift by taking science out of the lab and classroom and encouraging students to experience science in all their activities with all their cognitive and affective abilities. The standards charge science educators to engage learners in active science by shifting emphasis away from teachers presenting information and covering science topics that will then be tested (National Science Standards, 1996).

In presenting the standards, the National Research Council lists several assumptions about the nature of science teaching. These relate that students are greatly influenced by how they are taught. Teachers are charged with the responsibility of transmitting knowledge, but even more importantly, attitudes toward science. We are asked to allow students to construct knowledge from activities that stimulate both individual

and social processes. Finally, teachers are requested to build strong relationships with students based on trust, integrity and mutual respect (National Science Standards, 1996). I tried to keep these charges in mind as the unit and its activities were developed.

The aim of the pretest was to ascertain the level of student interest regarding various biology topics and get students to think about the needs of plants and animals. There was no basis for using a pretest to assess the level of expertise on the topic of plant biochemistry since it is a topic on which they have had no experience. Since the content of the unit was selected to allow students to change their schema regarding botany, it was necessary to design tools that would allow learners to reflect and address their attitudes.

Journal writing as a classroom assessment tool was a way of enabling students to reflect on their feelings regarding the activities in the classroom. The entries were focused on one or two specific questions, presented several times a week, with directions that the focus questions were to be a springboard that could be enlarged or expanded to include other observations of the material at hand (APPENDIX 2, Figure C). The introduction of journal writing into a science classroom situation was a new undertaking for students at this school. Although many have written in journals as a requirement of language arts instruction, this type of reflection in science has never been tried before at our school.

Student activities would be undertaken in groups of two, or for some activities, four. Many times students work more effectively in groups and they probably benefit from the stimulus and informal tutoring of others in the group. Each individual is allowed to make a contribution to the end product and the result is frequently better due to the convergent thinking of the group members. Additionally, group centered activities "place greater emphasis on student activity; on pupil participation, initiative and responsibility" (Ausubel, 1968).

A primary focus of the unit was the structures of terpenes and isoprene polymers common in essential oils. Using college organic chemistry textbooks as resources, the structures were used to show students the similarities between the essences of many

common scents as well as to demonstrate how minor variation on these chemical structures change the aroma in significant ways.

The chemistry of anthocyanin pigments also demonstrated this concept. Within the same class of plant products there is commonality of structure. Minor modifications of this structure may produce marked changes in the appearance and chemical properties of its derivatives. Many resource materials were used for background on pigment biochemistry.

Plant defense mechanisms were a second focus area of the unit with reprinted materials from plant pathology journals and books as resources.

By far the most useful day-to-day resource for this unit was Introduction to Ecological Biochemistry, Third ed. by J. B. Harborne. This book could have been used as a textbook for the unit. Chapters included material dealing with mechanisms by which plants interact within their biotic and abiotic communities.

A popular science source was useful in the study of scent and color. A Natural History of the Senses, by Diane Ackerman, is uniquely suited to the study of the sensual quality of plants and their use of the chemical senses in the enticement of pollinators. Written on a level that is interesting to the general non-professional, it contains worthwhile information and the language conjures up the intangible quality of those hard to describe sensations.

IMPLEMENTATION OF UNIT

In organizing the unit, a three pronged approach to the central question, "How Do Plants Meet Their Needs?" was chosen. It was important to set the stage for students by asking them to think about what kinds of needs are common to all organisms. This preinstruction brainstorming activity was intended to get students thinking about organisms in general. Student responses led to a more organized overview of the unit. The first aspect studied was an examination of the evolution of strategies that aid plants in reproduction: scent, pigments and flower morphology. Little lecture accompanied this area of the unit. Instead, lab activities revolved around these themes and some reading sufficed to provide chemical basis for the activities. A second thrust of investigation included an examination of ways in which plants supply their energy needs. Instead of lecturing on photosynthesis, a brief review was sufficient and laboratory exercises reinforced the major concepts to students. A final study was an examination of defense mechanisms. Outside reading by students allowed contact time to be spent in hands-on, "minds-on" activities in the laboratory.

Students were aware that this was a new series of activities. The initial brainstorming resulted in a concept map outlining the areas of concentration. (APPENDIX 1, Figure A). This practice allowed students to have an idea of where we were going and helped them develop a context in which to anticipate instruction. It is my common practice to be very clear with students about "where we are going and how we will get there". The focus is on the path of learning, not the ultimate grade. Students were assured that their teacher was interested in sharing some aspects of plant life that perhaps they had not considered.

The brainstorming led to some basic aspects of plant life: They can't run and move only very slowly. They are required to produce their own food and must have ways of utilizing stored food when needed and a means of controlling the rate at which this stored

food is metabolized. Plants require a "yenta" of a different species to make the "introduction" and to transfer gametes. The same selective pressures that students associate with the success of animals in their habitats are present for plants as well. The anticipatory activity of the unit allowed students to begin to examine their beliefs and understanding about plant life.

Students were also introduced to the practice of journal writing in the science classroom. Entries required answering one or more focus questions, but learners were invited to comment on other aspects of the instruction as well.

The unit utilized eleven activities and demonstrations for students to complete over a fourteen day, five week period. A set of daily lesson plans is included in APPENDIX 1, Figure B. This is a large number of activities and students were encouraged to organize their studies so that they could maximize their time in lab. In some instances, students worked in groups of two. In others, groups of four were necessary due to equipment constraints or the complex nature of the lab. For some activities, the product or activity itself was sufficient enough that further evaluation was not necessary. In some cases, a product was turned in and in others a short lab report was required. Students were granted the opportunity to submit lab reports as a group. In several instances, focus questions relating to the lab activity or reading assignment were the evaluative tools.

Science courses at this school are characterized by the amount of hands-on, inquiry based activities that are incorporated into the curriculum. Daily scheduling is based on the premise that lab work must take a high priority and that students must become adept at the skills of a laboratory scientist. In this unit, however, more laboratory activities than usual were scheduled and students sometimes were working at several activities at once. Several of the scheduled activities did not require an entire lab period; in this case another activity was started to be continued at another class meeting. Students and teacher alike were required to be organized in order to make the best use of time. I included a helpful introduction to each activity and prelab discussion to put it in context with the goals of the

unit.

This almost-total laboratory approach to learning was new for all of us. It has been customary to include more formal lecture as a way of leading students to the conceptual material and using that time in lecture to set up each activity. The new approach, which truly required learners to be responsible for their own time and to work as a group to achieve the desired outcomes.

As stated before, journal writing in science is a new skill for the students in the class. Observation and reflection is a skill that is constantly stressed in science courses and recounting what is experienced in class on a more or less daily basis required a shift in thinking. The assignment also required me to think very carefully about what the students were to take from each activity. Constructing a cleanly worded question that invited learners to reflect and construct knowledge was a difficult undertaking.

STUDENT ACTIVITIES

The following section summarizes each hands-on activity or laboratory exercise incorporated into the unit and a rationale for its inclusion.

1. Extraction of Essential Oils (APPENDIX 3, Figure A)

Distillation and solvent extraction were used to isolate and crudely purify a natural product, essential plant oils. I would have preferred to allow students to extract essential floral oils, but the extraction of citrus and clove oils was within budgetary means and allowed students to experience the process. Working in groups of two, students either extracted limonene from oranges and lemons by enfleurage, whereby the essence was dissolved in a lipid then extracted by vapor distillation, or used steam distillation of cloves to yield eugenol, its essential oil. Students were evaluated on their yield of essential oil. They were also asked to reflect on their lab experience in a journal entry.

2. Demonstration of Essential Oils

Students were shown various essential oils and extracts of plant materials courtesy of a local laboratory, Kalsec. Kalsec is a major supplier of scent and flavoring chemicals, extracted from natural sources, to industry. Their spice chemist supplied the class with some extracts which are marketed by the company as well as some interesting chemicals used as artificial fragrances. Students were able to sample the fragrance of very well refined limonene, essential oil of red and black peppers, dill and others. In this way they were able to demonstrate to themselves the chemical nature of scent.

3. Perfume Making (APPENDIX 3, Figure B)

This activity fell on the last class day before Mother's Day. It was suggested to the students that they might use this opportunity to make something truly unique for their mothers. Perfume oils were purchased from a local craft and hobby store. The manufacturer supplied some recipes, but students were allowed to choose a known formulation or concoct their own fragrance. This activity allowed students to experiment with scent and the mixing of various essences to create a product of their own design.

4. Identification of Plant Pigments (APPENDIX 3, Figure C)

This activity, derived from a workshop activity offered at Michigan State University, utilized microscale techniques to identify chemical characteristics of anthocyanins and betacyanins based on their differing chemical properties. Many of the characteristic reactions, particularly those dealing with acid-base chemistry, were studied earlier in the year as part of organic chemistry. Knowledge of structure and basic chemical characteristics of various functional groups could be transferred from reaction types involving long, complex, chemical-named ingredients to something common, like raspberry pigment. The chemistry is the same. Students extracted pigments from fruit and

vegetable materials and, using well plates, exposed their extracts to acids, bases, oxidizing agents and heat to detect differences in response to these agents. They were then given two unknown extracts and directed to identify them as either anthocyanins or betacyanins. Students were evaluated on a written statement of their rationale for their identification.

5. Anthocyanin Pigments of Flowers and Vegetables (APPENDIX 3, Figure D)

This laboratory exercise was developed at Michigan State University under the direction of Dr. Ken Nadler, with input from Dr. Ray Hammerschmidt. Students extracted and isolated pigments from fruits, flowers and vegetables using reverse phase (C-18) cartridges. Understanding a solid phase column to isolate a component of a mixture required students to recall previous knowledge of polarity, molecular size and charge characteristics and basic tenets of chromatography involving stationary and mobile phase considerations. Once isolated, anthocyanins were hydrolyzed to form anthocyanidins and monosaccharides. Both pigment products were chromatographed on paper using various solvent mixtures and R_f values were calculated and compared to known pigments. Students were asked to identify the pigment present in their plant sample by comparing the experimental R_f value with known values obtained from reference materials.

6. Hill Reaction (APPENDIX 3, Figure E)

In this classic laboratory activity involving electron transport during the light reactions of photosynthesis, students isolate chloroplasts from spinach and use an oxidation-reduction indicator, 2,6-dichlorophenol-indophenol (DCPIP) to show that electron transfer has occurred. In this way learners can prove to themselves that electrons are indeed transferred during the light reactions. The reaction is followed spectrophotometrically as blue DCPIP is reduced and loses its color. We examined whether the reaction proceeds under fluorescent light, in darkness, or using boiled, presumably non-functioning chloroplasts. Students were asked to hypothesize, gather and

plot their data and draw conclusions as a group.

7. Bioluminescence -"The Firefly-Butt Experiment" (APPENDIX 3, Figure F)

This activity is really a demonstration of bioluminescence using firefly lanterns and ATP produced in the light reactions of photosynthesis. An adjunct to the Hill Reaction, this exercise allows students to actually harness ATP made in photosynthesis and use it to provide energy for the firefly reaction using dead insect material. A popular feature of the activity involves students gathering in a completely darkened room.

8. Gibberellin Induction of Amylase: The Halo Halfseed Reaction (APPENDIX 3, Figure G)

This activity utilizes serial dilutions of gibberellic acid to activate the expression of hydrolytic enzymes in barley endosperm. When exposed to gibberellic acid, amylase production is induced in the aleurone and starch in the endosperm is converted to monosaccharides. There is a relationship between the amount of gibberellic acid and the amount of amylase produced. The seeds are incubated on starch agar to which varying amounts of gibberellic acid has been added. When bathed in Lugol's Iodine solution, a test for the presence of starch, those areas on the agar which have undergone hydrolysis to monsaccharides should not turn the characteristic blue-black color that indicates the presence of polysaccharides. The diameter of the resultant "halo" is logarithmically proportional to the concentration of hormone. Enzyme regulation has been studied earlier in the semester. In this activity, students are able to apply what they have learned previously to hormone regulation of enzyme activity.

9. Induction of Camalexin in *Arabidopsis thaliana* (APPENDIX 3, Figure H)

The idea of plants having defense systems is a new concept to students. That plants may respond to infection by production of a non-specific defense represents a level of

complexity that students should address. Fresh leaves of *Arabidopsis thaliana* are infected with a fungus and allowed to produce a phytoalexin compound as a defense mechanism. The antimicrobial camalexin is collected from the leaves and extracted with ethyl acetate. The residue is then subjected to thin layer chromatography using a chloroform:methanol solvent system. The recovered camalexin should fluoresce. Its presence may be presumed when compared with residue collected from non-infected leaves. The isolation of the phytoalexin was demonstrated by student groups.

10. Demonstrating a Chemical Defense Mechanism: Allelopathy (APPENDIX 3, Figure I)

Extract of black walnut plant material was used to demonstrate a defense mechanism used by some species of plants. The walnut's active allelopathic ingredient, juglone, was extracted and used to treat filter paper. Seeds of different vegetables were counted out and laid on the extract-laden paper as well as a distilled water-soaked control and kept in the dark for several days to germinate. The germination rate was compared with the control and data were obtained for several different types of common garden vegetables indicating that some seeds, notably tomatoes and lettuce seem to be more severely inhibited than others. Data were shown on a bar graph and conclusions were drawn by students. Once more, this was an opportunity to impress students with some mechanisms evolved by plants to increase the chances of their success in their environment.

11. Flower Pollination Strategies: Use of a Polyclave Key (APPENDIX 3, Figure J)

The class utilized a large local greenhouse for this activity. Students were instructed in the techniques required to use a polyclave key. A blooming peony was used to demonstrate this exercise. Various flower characteristics, including color, flower size, presence of nectar guides and fragrance allowed students to eliminate certain pollinator categories until only one or two remained. By process of elimination, students could learn

to recognize what are the characteristics of plants serviced by bees, birds, flies and a variety of other possible pollinators. This activity also allowed students to walk freely among many different types of horticultural plants and examine some common flower characteristics in a more detailed way than just wandering through a garden or examining photographic material. I prepared a laminated, flower-shaped guide for students to use during their visit. Each "petal" of the guide used a different flower characteristic.

EVALUATION

Students participated in this unit by their own choice. Parents as well as pupils were appraised that their participation was voluntary and that evaluation would be part of the unit and that various means of alternative assessment tools might be used. The learning process in this series of activities would be as much for the teacher as the students. This type of cooperation characterizes the classroom environment of this researcher. It was made clear that any difficulties would be dealt with cooperatively and that students were free to give feedback regarding both the content and the process.

The primary evaluation tools of the unit utilized anonymous pre- and posttest instruments as well as journal entries and anecdotal discussions during the progress of the unit. Of the interest areas surveyed on the pretest, plant biology carried the lowest level of interest or tied for the lowest level of interest on fourteen of the twenty-two responses. Since the thrust of the unit was to challenge student beliefs that plants were boring and of little interest, it was imperative that I utilize some alternative assessment tools. This task was one in which I had no background and sought the counsel of professionals in the field with experience in these types of evaluation, in addition to some very current written material. The use of journal writing in science is a skill being mandated for students in preparation for the current Michigan Education Assessment Program (MEAP) science evaluation tool. It is, however, a new activity for students and science teachers and one which will take time and patience to utilize effectively.

The data obtained from the pre and post test instruments provided information on basic attitudes and levels of previous knowledge in several areas of biology. Students were asked to anonymously assign a discrete numerical value to their interest in science, biology /biochemistry, plant science and animal science. I chose to focus on levels of interest expressed pre- and post-instruction in that area (Table 1). In another portion of the tests, they ranked their interest in areas of biology, one of which was botany (Table 2).

Table 1
Unmatched Pretest and Posttest Data Reflecting Student Self-reported Interest in Plant Biology Ranging from 1 (Low) to 10 (High)

Pretest	Posttest
5	6
3	7
7	8
9.5	7
8	3
1	7
7	5
4	7
5	7
4	6
6	9
4	4
5	5
7	4
6	5
5	7
3	8
8	10
7	9
6	6
7	
8	
$x = 5.7045$	$x = 6.5000$
$s_x = 2.0275$	$s_x = 1.8209$
First Quartile = 4.00	First Quartile = 5.00
Fourth Quartile = 7.00	Fourth Quartile = 7.50
$IQR/s_x = 1.48$	$IQR/s_x = 1.37$

Table 2
Unmatched Pretest and Posttest Data Ranking Interest in Botany Among Selected Biology Topics Ranging from 1 (Low) to 10 (High)

Pretest	Posttest
6	4
3	7
1	8
3	7
7	3
3	6
5	7
5	7
9	9
3	1
1	5
3	6
7	4
10	7
7	9
	2
	5
$\bar{x}$ = 5.1364	$\bar{x}$ = 6.0000
s_x = 2.5874	s_x = 2.2361
First Quartile = 3.00	First Quartile = 4.00
Fourth Quartile = 7.00	Fourth Quartile = 7.50
IQR/s_x = 1.54	IQR/s_x = 1.33

It is apparent from the demographics of the students at a math and science center that interest in science in general would be expected to be very high. This was borne out with average scores of 8.66 and 8.78 on the pretests and posttests respectively, with 10 being the highest level of interest. I chose not to evaluate the rise in interest level as a result of instruction because it was determined that this data would not indicate normalcy as a population (Sincich, 1992). In other words, there would not be normal distribution round the mean value of 8.66 or 8.78. Clearly this population is skewed far to the right with respect to interest in science in general.

The researcher did, however, treat the remaining data as reflecting normalcy, or normal distribution around a mean. The mean value of the data is located far above the center of the range, as to be expected from this non-random group of students, but standard descriptive statistics indicate that there is sufficient variation around that mean that the data can be inferred to be approximately normally distributed, although skewed to the right. A calculation useful for determining whether normalcy can be inferred is to determine the interquartile range (the difference between the seventy-fifth and twenty-fifth percentiles). The interquartile range is then divided by the standard deviation of the sample. These statistics are readily available using standard statistics programs on hand-held scientific calculators. If the ratio is approximately 1.35, then normalcy can be assumed and a variety of descriptive statistics may be employed to assess significance (Sincich, 1992). For the data considered, the values are reflected in an unmatched arrangement in Tables 1 and 2.

Once the normalcy of the datum was established, I chose to use a small sample ($n<30$) t-statistic to test the null hypothesis. The null hypothesis would suggest that there was no significant difference in interest levels in botany, as expressed by students, between the pretest and the posttest activities. T-values were obtained from standard tables and considered at the 0.05 confidence level. For the data reflecting the rise in interest in plant biology before and after instruction, $t = 2.0$, with any value above 1.7 being the rejection value. For the data ranking interest in botany among biology topics, $t = 1.5$, with any

value above 1.8 being the rejection value. The null hypothesis can be rejected for the data in Table 1 reflecting increased interest in plant biology but must be accepted for the data in Table 2 reflecting increased interest in plant biology as compared with other areas of biology. In other words, student attitudes regarding plant science became more positive with the instruction although the preference of other biology topics over botany was maintained in spite of the instruction.

It should be noted that the number of data points is not equal in the pre and posttest instruments. This is due to the absence of two sets of data which were not submitted for inclusion. One student graduated and did not complete the unit. The second student declined to complete the journal and posttest instruments due to personal and academic problems.

It is also noted that the number of responses to the interdisciplinary interest survey reported in Table 2 do not equal the number of students completing the pretest and posttest instruments. This discrepancy is due to a design flaw in the document. I gave instructions to students to "rate" their interests when what was desired was a ranking. Some students, therefore, rated several areas with the same number and did not place the choices in a hierarchical order. Those responses were disregarded, due to the lack of clear preferences between the different areas of study.

In addition to statistical analysis of the pre- and posttest data, a rubric was designed for use in evaluating the journal entries submitted by students (APPENDIX 2, Figure D). Hein and Price (1994) provide guidelines for evaluating student work which include reminders to evaluate the quality of response as well as expressions of attitudes and feelings. Evaluators are asked to notice evidence of acquired information as well as changes in conceptual knowledge. Since students were asked to focus on specific questions in their entries, some entries were primarily statements reflecting new knowledge or skills. Other days, students were asked to reflect on what had taken place in lab or in a reading assignment. These entries were looking for attitude statements. The rubric provided a tool

to analyze the insights of students and allowed the researcher to categorize the statements contained within the entries. I chose to categorize statements as reflecting attitudes, either positive or negative, knowledge, again, either positive or negative, skills or other statements. Using different colored highlighting markers, I read the journals and color-coded the statements and entered the data for each journal entry on the student's rubric. Learners were encouraged to express their opinions freely in their entries. There would be no penalty for candor, even if it was not complimentary to the teacher or the unit material. Students apparently felt free to reflect, since they offered constructive criticism that was relevant to the unit makeup and the method of instruction as well as suggestions for future years.

The journal entries offered some insight into student thinking that was not obvious despite the fact that I feel I know the students quite well in both an academic and personal way. Some very bright students had great difficulty expressing in writing the feelings they experienced. Writing about science, without numbers or chemical structures seemed very hard for young learners. I felt that their reluctance to discuss science as a passionate subject would involve betrayal of an intimacy. Clearly, for some pupils, feelings are not easy to express. Other students, however, were quite happy to express interest and curiosity using rich, animated language. I read these entries as if looking more at a personal diary than a science journal.

The researcher was able to assess a definite interest in the instructional material. One initial journal entry informed the reader that the student, "basicly (sic) just likes to learn". Another echoed a similar sentiment, "I just want to learn something". Despite the low interest level of students indicated on the pretest, students reported that they were willing to change this attitude. Students confessed hope that instruction would diminish their "fear of biology" and lack of knowledge of plants. The students felt that they were not well educated in plant biology and were motivated and eager to change that situation. No student openly declared a dislike of plants and most expressed a neutral feeling. Many

were able to say that they "don't know much about them" (referring to plants) and thought that the unit would be fun.

Student feedback was also collected informally in daily discussions occurring as I "touched base" around the lab. Each laboratory aspect of the unit elicited useful pieces of information, either as part of the learning process of the student, teacher or both.

EVALUATION OF STUDENT ACTIVITIES

The following are observations about each activity and reflections both from the student and teacher viewpoints:

1. Extraction of Essential Oils

Students commented on the wonderful scents elicited in this extraction lab. One student commented that she could now recognize a scent that she associated with visiting her grandmother. She learned the scent was of cloves. Several students wrote about the small amount of essential oils that were extracted from a large amount of plant material. This gave them some understanding of the expense of essential oils used in perfumes and students could grasp the cost involved in extracting essences from floral materials. The average yield of recovered essence from each lab was higher than expected. I expect that this was due to the inclusion of solvents with the oils. With the exception of one group, however, students were able to successfully extract a somewhat crude product.

2. Demonstration of Essential Oils

The goal of this adjunct demonstration was to allow students to make the connection between chemicals and scents. Some of the scents were derived from natural materials. Some were artificial in nature. Students commented about the crispness of some of the scents as well as the ethereal nature of others and were interested in smelling the

"real" limonene and comparing it to an artificial citrus fragrance. Students were amazed to smell the difference between limonene samples that had been distilled and redistilled many times to create very pure extracts. A favorite sensation was the scent supplied when manufacturers call for the smell of "green", *cis* -3-hexen-1-ol. The scent is similar to newly mowed grass. Students had never identified a smell with a color before but this surely was an apt description for a unique olfactory sensation. Student journal responses related the extreme difficulty in expressing scent sensations as words and noted that scents frequently evoked memories.

3. Perfume Making

Many comments were received both in journal entries and in informal discussion concerning the popularity of this exercise. Students from other classes came into the lab after school to ask to make a perfume for their mothers. Other teachers remarked at how pleasant their afternoon classrooms smelled and asked to make their own. This activity was definitely high in interest level and students throughout the school were engaged.

4. Identification of Plant Pigments and

5. Anthocyanin Pigments of Flowers and Vegetables

These activities seemed to take students longer to complete than I believe it should have. Although students worked in groups of four, they seemed to have a difficult time organizing their time and the researcher noticed that considerable time was spent in unproductive ways.

An example that comes to mind concerns a student that extracted the anthocyanins for the chromatography lab. The procedure calls for a portion of the anthocyanin pigment extract to be boiled in aqueous hydrochloric acid to break the glycoside bonds, releasing the anthocyanidin. The student put the sample into a water bath and set it on the hotplate. He neglected, however, to turn on the hotplate and neither he nor his lab partners noticed for

over thirty minutes. He then came in after school to try again. This time, he turned on the hotplate and allowed the water in the boiling water bath to boil dry. This resulted in a burned sample. Several other groups seemed to experience different, but just as frustrating difficulties and seemed to fall behind. One group failed to complete the identification lab. The others completed the labs and did fairly well at the activity, although the level of interest was not as high as the previous set of labs. Students experienced difficulty identifying their pigments using the known R_f values for reference.

The students, although bright and certainly well acquainted with lab activities, have difficulties doing several things at once, particularly new things. This makes them human but also contributes to inefficiency. Students also were beginning to experience the first few days of spring. Although all the students in class were juniors with one exception, the waning days of the senior class was beginning to wear on the junior class and the desire for a "junior skip-day" was high.

6. Hill Reaction

This activity was hindered by a bad reagent bottle of 2,6-dichlorophenol-indophenol on the first day. Much confusion occurred when the student's samples exhibited no change in color or random change as time progressed. A newer bottle of reagent was opened and the lab was repeated the following day, with much better results. Although the school is well-equipped, only six spectrophotometers were available. This resulted in groups of four performing the lab. I made the chloroplast solution before class and boiled a portion to save time. Automatic pipettors were also used to hasten the addition of sugar solution and phosphate buffer. These shortcuts allowed students to perform the lab in an efficient manner and they got very good results. Graphical datum was reasonable and comments in the student journals indicated that students had grasped the concepts that were presented and that their data confirmed earlier hypotheses.

7. Bioluminescence -"The Firefly-Butt Experiment"

This activity was performed in conjunction with the second, successful day concerning the Hill Reaction. Students working in groups of four to accommodate the number of spectrophotometers were able to complete this activity while waiting to gather data for the other lab. Many students commented on the activity. They were intrigued to learn that it is possible to buy firefly lanterns and several humorous conversations revolved around this as a possible future career. Students like to go into a dark room, and this afforded them the possibility to engage in that pastime as well as experience the dim glow of a firefly. The chemistry involved in the reaction, as well as the entire concept of oxidation and reduction is a difficult one for students and while students enjoyed the experience, it is unclear if they understood the underlying concepts behind the activity, that ATP is the "currency" of biological activity.

8. The Halo Halfseed Reaction

My classes have performed this activity in earlier years, coupling the activity with instruction on preparation of serial dilutions. For this unit the starch agar was prepared for students in the interest of saving time. Students found the preparation of the barley halfseeds to be a tedious undertaking. Working in groups of four, the imbibition and extraction of the embryo was rather carelessly done by several groups. As a result, the data obtained were not in keeping with the expected values. At this point, it was impossible to try again, so a "postmortem" on the lab was performed and students were guided in a discussion of what might have happened and what should have been reflected in their data.

9. Induction of Camalexin in *Arabidopsis thaliana*

Induction was begun on the Friday before the Memorial Day weekend. Students, working in groups of four, spotted ten leaves with fungus suspension and ten leaves with distilled water. The leaves were then allowed to sit, closed, over the long weekend.

Extraction was completed on the following Tuesday. Three groups were successful in demonstrating a scant amount of fluorescent extract on thin layer chromatography plates. Two groups were unsuccessful.

Students commented on the concept of this lab as being a particularly surprising adaptation of a plant. The reading assignment that accompanied the exercise allowed students to consider the selective success that might be achieved by plants that are able to accomplish this type of response to an invader. Several students, however, compared this to the presence of antibodies in animals. The analogy is incorrect. Antibodies are specific immunochemicals, whereas phytoalexins are non-specific antibiotics. The induction of an antimicrobial is a clever response; its nonspecificity allows the plant to be successful against a variety of microbes.

10. A Demonstration of a Chemical Defense Mechanism: Allelopathy

This activity was very successful. Students demonstrated different levels of germination inhibition with differing types of seeds. Done in conjunction with the induction of camalexin, the walnut extract was applied to the plates and allowed to incubate with seeds in a closed cabinet for the long weekend. Some students did not apply enough liquid extract to keep the seeds moist; they re-wet and waited another day or so before tabulating their results. Tomato and lettuce seemed to be fairly severely inhibited by the walnut extract. Carrots were less affected. Students were able to demonstrate that some vegetable species were inhibited by the walnut extract.

11. Flower Pollination Strategies: Use of a Polyclave Key

This was a perfect closing to the unit. Students enjoyed the opportunity to complete their exam early so that they could have a field trip. Although the only feedback received was anecdotal through informal discussion both at the greenhouse and afterward, during an ice cream outing, students were fascinated at the various types of plants and the variety of

flower colors that exist. In addition to learning how to use a different type of identification tool, students got to spend time examining some morphological characteristics of flowering plants. Students examined flowers under UV light and were able to see nectar guides on a white clematis. They predicted that peonies would be insect pollinated, then found ants on open and unopened buds. Much time was spent enthusiastically showing friends (and the teacher) the "neat flower I found". Students were able to apply much of what they had learned about scent and color. Flower morphology, scent and pigment were tied together in a beautiful and fun-to-study way. The instructor allowed each student was allowed to find an inexpensive plant that they liked and purchased flowers for the group. A special plant was found at the greenhouse. Several flats of mimosas were available for students to examine. A number of students selected mimosa plants at the greenhouse to take home. Others selected more common plants such as coleus, petunias and primroses.

Students were asked to ponder the effects of man on the pollination strategies of plants as a final journal entry. Responses indicated that the idea of man as an agent of selective pressure did not enter the minds of many students. One student did report that her mother receives catalogs detailing "new" flower colors. It was suggested that the actions of humans have decreased competition between species as humans favor some plants over others in their habitats. This student suggested that perhaps man, by genetically manipulating horticultural species, might be changing the balance between pollinators and plant species. Other students suggested that man's actions have severely limited the diversity of species in the environment. One suggested that acid rain might play a part in color changes and that this change may not be advantageous to the plant with respect to its pollinators. Several responses concluded that the use of pesticides might reduce the populations of some pollination vectors, allowing for increased competition between remaining plant species. These responses suggested that students had begun to think about the role of plants in the environment and the ways in which they must interact in order to be successful in very competitive circumstances.

Affective responses to the unit as a whole were elicited as an open-ended question on the posttest. Students were asked to respond to the content and activities of the unit. They were asked what was interesting and what suggestions they would make for the future. The responses indicated a generally positive attitude toward the material and activities and suggestions were made to allow much more time on the unit in the future. One response indicated that "I thought the choice of labs was excellent; the basic needs of the plants were clearly demonstrated through the variety of experiments....I would have liked to see more time to regroup as a class and take 'time outs' just to see where everyone was and what each person planned to acomplish (sic) that day". Some students indicated that more labs should be included. "I realize now what an intensive lab course in college will be like." Some indicated that fewer labs would help. "...I didn't get to enjoy them all." Some students enjoyed working in lab every day and were satisfied with omitting lecture in favor of reading assignments. One student stated, "It was nice to be in the lab almost every day and have reading at night because I felt very accomplished at the end". Others indicated the need for more lecture and less outside reading. "I wish we could have had time to hear lecture and then do each lab." "There was a lot of reading outside of class when all lab-time was chosen, but I believe the extra work was worth the extra lab time." Most felt that the groups were too large and able to operate too independently from within. One student reported that "things were quite hectic and I had trouble remembering which experiments I was completing and what the purpose of each was".

DISCUSSION AND CONCLUSIONS

Many of the activities, as well as the conceptual material conveyed in this unit were new to both students and me. The unit was prepared to be entirely different than any other plant unit being taught at a high school level. Although a few high schools offer courses in organic chemistry to upper level students, it is unusual to find an offering that included biochemistry at the high school level. Given the uniqueness both of the material and the characteristics of both instructor and learners there are several areas worthy of discussion.

A number of the activities were certainly high energy, high interest events for students as well as me. Perfume making, the bioluminescence lab and the use of the polyclave key were events that elicited many positive responses to students. If the 1984 research by McGarity and Butts is correct and student engagement is highly correlated to student achievement, motivation to understand the concepts involved was high.

Although student responses were mostly positive as expressed in writing and by physical energy in the lab, there are changes that would improve the unit in future years. The criticisms offered by students were noted and will be responded to when the unit is next taught.

Students began to feel an increased level of stress during the second week of the unit. Although students responded positively to the idea of having many lab activities and little passive time in the classroom, performing labs every day began to get too confusing for most of them. Upon reflection, too many labs were done over too short a period of time. Students were struggling to keep up and began to feel that they were losing track of the concepts involved. They freely expressed their distress to me. I, however, had not allowed enough time to allow the pace to slow down. I dropped two planned activities. The viewing of the video, "Sexual Encounters of the Floral Kind" was omitted, as was an activity using garlic and onion extracts as a plant defense deterrent to insects. For this reason, the students submitted group lab reports and worked even more cooperatively to

get the work done. This, however, was to prove distressing in another way. Students divided up the tasks and completed them independently so that within the group there were members who had no idea of what was being done by others.

A troublesome aspect of group learning from my experience deals with the sense of ownership among group members. This does not seem to be a problem for groups of two, but groups of four are more fragmentary than cohesive. Students naturally pair up and a single task becomes two tasks performed independently. Ownership of the task, and written work associated with it becomes diffuse. When it was time to turn in the lab report, the party who had it "is sick" or "is at the state track meet" or even, "Wasn't so-and-so supposed to do that?". This was frustrating to all of us.

Teaching the unit in May was also problematic. Although the season is right for the study of flowers, the timing for students is wrong. May, for high school teachers and students, is the month of athletic events, graduations, Advanced Placement Exams, honors award assemblies and a host of other activities that take students from school. There was not a day of instruction, with the exception of the days that the final exam was given, that there were not at least two students absent.

For students as well as teachers, the stress level is high. The end of the year approaches and students begin to feel the push to finish. Other class instructors have a set amount of material they are trying to cover and all teachers seem to be pushing their students to finish up. Whereas I normally "back off" during this time of the year, the fact that this unit needed to be completed added to the end-of-year burnout.

I could have eliminated these difficulties by allowing more time to complete the activities of the unit. Despite having done the activities and providing the best estimate of the time involved for each lab, students take much longer to complete tasks. These problems could have been eliminated if the unit had not been placed at the end of the year.

One of the results of the rushed time frame of the unit was a lack of closure on some of the activities. When time becomes short, it is usual to complete the activity but not

allow for a post-lab period. This is a mistake because students miss the opportunity to discuss their findings and receive feedback from the group about the significance of the activity. The teacher also misses the opportunity to hear about the difficulties students faced and allow them to make suggestions. Many misconceptions could have been addressed during this period.

Future pretest and posttest instruments would include inquiry about the gender of the respondent. That was a piece of information that the researcher was very aware of, but failed to include. Since many attitudinal interests involve gender differences, that knowledge would have been most helpful in assessing the success of the project.

Additionally, as stated earlier, the item on the pre- and posttests would ask students to "rank" their interests in various biology topics, not "rate" them from one to ten. Students were confused by that portion of the assessment. I believe it can be made more clear to students in the future.

The practice of using a journal will be used for the entire year in the future. Students seemed more willing to express frustration or confusion in a written format compared with a face to face discussion with me. Journal writing will serve as a means of "taking the temperature" of the class in general. It will allow me to continue to refine the skills involved with effective questioning that have begun to be practiced. I also would like to consider post-unit questions like, "The piece that was hardest for me was....." or "My best piece of work was....". Hein and Price (1994) suggest that students assess themselves and share that assessment. "A teacher we know asks her students what they would do differently tomorrow: 'It gives me food for thought... I also ask them what they would want me to do differently tomorrow.'" This researcher will remember these types of questions, not only because they elicit worthwhile feedback, but they allow students to feel a sense of ownership about the process occurring in the classroom.

I have given consideration to teaching this unit differently in the future. It might be just as effective to use the theme of plant biochemistry as a focus area throughout the year

and, instead of taking three or four or six weeks to teach the unit as a stand-alone entity, take the pieces and intersperse them throughout the curriculum at appropriate areas of study. For example, during the first semester study of alkenes, the aspects of smell could be taught. Since the study of terpenes and isoprene subunits could fit there, it might be appropriate to look at them from the standpoint of their chemical functional group. Likewise, color is many times associated with aromatic groups. This would place the study of pigments within the portion of the class dealing with benzene rings and their derivatives. In this way, the instructor could reinforce the unit concepts over the entire year as opposed to trying to compress the material into a single focus area.

In spite of the difficulties encountered, I believe that the goal of the unit, to increase student interest in the study of plants by providing students with insight about plant adaptations that allow them to be successful, was successful. Attitudes, as expressed by the participants, remained mostly positive. In the posttest unit evaluation, respondents strongly urged me to teach the unit again. I believe that students will look differently at plants than they did before. While it is unclear whether or not any prospective botany majors have come from the instruction given in this unit, students have had the opportunity to explore some relevant, everyday areas related to plant life that were unknown to them before.

APPENDIX 1.

APPENDIX 1, Figure A.

HOW DO PLANTS MEET THEIR NEEDS?

Defense

1.) Induction of Immunochemicals: (Induction of Camalexin in *Arabidopsis thaliana*)

2.) Allelopathy: A Demonstration of a Chemical Defense Mechanism: Allelopathy

3.) Mimosa plant demonstration

Pollination

Scent

1.) Essential Oil Extraction: Steam Distillation of Citrus and Cloves

2.) Custom-Blended Perfume

Pigment

1.) Anthocyanins and Betacyanins: Identification of Plant Pigments

2.) Extraction and Isolation of Anthocyanins: Anthocyanin Pigments of Flowers and Vegetables

Flower Morphology

1.) Pollination Strategies: Use of a Polyclave Key

2.) Video: Sexual Encounters of the Floral Kind

Energy

Photosynthesis

1.) Electron Transfer: The Hill Reaction Bioluminescence: "The Firefly-Butt Experiment"

Enzyme Control

1.) Hormone Induction of Amylase: The Halo Halfseed Reaction

APPENDIX 1, Figure B.

LESSON PLANS FOR PLANT BIOCHEMISTRY UNIT

As designed, the unit was to be completed over a 14 class-day, four week period of time. This unit would be that last instruction of the school year. The instructor made arrangements to include a field trip during the final exam period to take students to a local nursery for the portion of the unit to include flower morphology. The required final examination was split into portions to be given on two class days prior to the exam period. The instructional sequence of events is shown below.

WEEK 1

Day 1 Tuesday, 5/7/96 (72 min)
* Begin unit - Pretest
* Brainstorming activity

Essences
* Explain Journaling
* Begin Discussion of Scent
* Prelab - Extraction of Essential Oils

Day 2 Wednesday, 5/8/96 (72 min)
* Begin Extraction Lab
* Scent Demo: Kalsec

Day 3 Friday, 5/10/96 (48 min)
* Make Custom Perfume

WEEK 2

Day 4 Monday, 5/13/96 (48 min)
* Prelab: Identification of Plant Pigments Anthocyanin Pigments of Flowers and Vegetables (to run concurrently)
* Continue Essential Oil Extraction

Day 5 Tuesday, 5/14/96 (72 min)
* Work in lab on activities

Day 6 Wednesday, 5/15/96 (72 min)
* Continue to work in lab
* Assigned Reading: Harborne - Ch.2 -Biochemistry of Plant Pollination Ackerman - "Scent"
* Assignment: 1 page reflection paper on Ackerman chapter.

Day 7 Friday, 5/17/96 (48 min)
* Finish lab activities
* Turn in Essential Oils and % yield
* Identification of Anthocyanin or Betacyanin due Friday, 5/24
* Lab Report on Anthocyanin Isolation and Chromatogram due Friday, 5/24

WEEK 3

Day 8 Monday, 5/20/96 (48 min)
* Review where we've been
* Talk about Energy -review photosynthesis - hydrolysis of starch
* Prelab: Hill Reaction
  Halo Halfseed Reaction

Day 9 Tuesday, 5/21/96 (72 min)
* Hill Reaction
* Prepare and imbibe barley for Halo Halfseed Reaction

Day 10 Wednesday, 5/22/96 (72 min)
* Hill Reaction (re-do with new DCPIP)
* Plate Halfseeds
* Bioluminescence Activity
* Assignment: Harborne -
  Ch. 9 - p.277-282 Allelopathy
  Ch. 10 - p.302-327 Phytoalexins

Day 11 Friday, 5/24/96 (48 min)
* Read and measure halos
* Discuss defense strategies
* Prepare and spot *Arabidopsis* for Camalexin Induction
* Plate seeds in walnut extract

WEEK 4

Day 12 Tuesday, 5/28/96 (72 min)
* Rewet walnut plates, if necessary
* Extract Camalexin

Day 13 Wednesday, 5/29/96 (72 min)
* Count sprouts on walnut plates
* Run TLC of camalexin
* Assignment: Final focus questions in journal
* Posttest

WEEK 5

Day 14 Thursday, 6/6/96 (120 min)
* Field trip to Bell's Greenhouse
* Use of Polyclave Key to Identify Plant Pollination Strategies
* Examine Mimosa
* Ice Cream and Thanks!

APPENDIX 1, Figure C.

April 15, 1996

Dear ,

This marking period, I will be teaching a unit that I have constructed concerning plant biochemistry. Students in my class will be examining some strategies used by plants to meet their needs for energy, defense against invaders and reproduction. This unit has been devised to complete my masters' studies at Michigan State University and I will be assessing the learning that takes place as this material is presented.

I estimate that the unit will require 3 -5 weeks of class time. Much of the time will be spent in laboratory activities. The laboratory techniques that we will use are the same or similar to many of the activities students have already learned earlier in the class, such as distillation, chromatography and other means of separating mixtures. We will be using some ordinary plant materials, including garlic, oranges and some small fast cycling plants. All of the exercises will be pre-labbed with students before we begin and, as always, I will point out any potential hazards, such as use of solvents and heat sources.

Students will be tested conventionally using paper and pencil, interviewed and possibly videotaped for viewing later. I will be assessing student attitudes and knowledge, since they will be graded during this time, but their identities will be confidential within the bounds of my thesis or its defense. Upon completion, I would be delighted for students to read my thesis and examine the unit and my assessment of their learning as I have submitted it to my master's committee.

Your student must agree to freely consent to be part of this project. Their participation is voluntary and they may choose not to participate at all or only for some of the exercises. They may also choose to discontinue their participation at any time. I will arrange for alternative instruction if this is their desire and they will not be penalized for their withdrawal.

Please do not hesitate to contact me concerning any questions that you may have regarding the content and activities of this unit, or your student's participation. I may be reached by telephone at school (337-0019) or at home (329-1526).

If you consent to allow your student to participate in the described activities, please complete the form below and ask your son or daughter to return it to me as soon as possible.

Thank you for your support.

Cheryl Hach
Biochemistry Instructor

_____ has my consent to participate in Mrs. Hach's teaching unit on plant biochemistry. I have had the opportunity to speak with Mrs. Hach concerning these activities and will notify her of any concerns that may arise during the period of instruction.

Parent or Guardian's Signature _____
Phone Number _____

APPENDIX 2.

APPENDIX 2, Figure A.

PLANT BIOCHEMISTRY PRETEST

Teacher's Name _____

1. On a scale of 1 - 10, with 10 being the highest score, how would you describe your level of interest in science?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in biology and/or biochemistry?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in plant biology?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in animal biology?

0 1 2 3 4 5 6 7 8 9 10

Comments...

2. Please rate your interest in the following biology topics from 1 to 10, with 10 being the highest score.

_____ ecology
_____ cell biology
_____ biochemistry
_____ zoology
_____ botany
_____ microbiology
_____ molecular biology
_____ genetics
_____ embryology
_____ anatomy and physiology

3. What are some common needs of all living organisms? Please try to list three.

4. For each of the needs listed, please describe ways that these needs are met in plants and animals.

Need:	Plant Means of Meeting This Need	Animal Means of Meeting This Need

5. How familiar are you with the processes, mechanisms and/or behaviors employed by plants and animals for meeting their needs?

APPENDIX 2, Figure B.

PLANT BIOCHEMISTRY POSTTEST

Teacher's Name _____

1. On a scale of 1 - 10, with 10 being the highest score, how would you describe your level of interest in science?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in biology and/or biochemistry?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in plant biology?

0 1 2 3 4 5 6 7 8 9 10

Comments...

in animal biology?

0 1 2 3 4 5 6 7 8 9 10

Comments...

2. Please rate your interest in the following biology topics from 1 to 10, with 10 being the highest score.

_____ ecology
_____ cell biology
_____ biochemistry
_____ zoology
_____ botany
_____ microbiology
_____ molecular biology
_____ genetics
_____ embryology
_____ anatomy and physiology

3. What are some common needs of all living organisms? Please try to list three.

4. For each of the needs listed, please describe ways that these needs are met in plants and animals.

Need: Plant Means of Meeting This Need Animal Means of Meeting This Need

5. How familiar are you with the processes, mechanisms and/or behaviors employed by plants and animals for meeting their needs?

6. Please review the activities of the unit we just completed and make any suggestions for the future. What worked for you? What was interesting? What suggestions would you make for the next time around?

APPENDIX 2, Figure C.

JOURNAL QUESTIONS

5/7/96
1. What would you like to learn or experience in this unit?
2. What kind(s) of characteristics have plants evolved over time to aid them in reproductive success? Do they work?

5/8/96
1. What most amazed you about today's activities? (essential oil extraction)
2. How could this activity be expanded to include other extractions? What other oils could we try to extract?

5/10/96
1. Characterize your "custom" perfume. How easy it to put this sensation into words?
2. Name your perfume.

5/14/96
1. Today you extracted anthocyanins from fruits, vegetables and flowers. Based on color, of the following, which do you think may contain the same pigments:

geranium	blueberries	grapes
tuberous begonia	raspberries	cherries
petunia	strawberries	apples
savoy cabbage	red cabbage	red pepper
radish		

5/16/96 Based on your reading assignment, the chapter on Scent from the Natural History of the Senses by Diane Ackerman, write a one page opinion and reflection piece.

5/20/96 Based on your reading assignment, write a synopsis of the use of color and scent as a pollination strategy. I.e., what are the key things to remember?

5/22/96 Daily reflection: Firefly butts, Hill reaction

5/24/96 Daily reflection: Begin allelopathy, phytoalexin, finish halo-halfseed

6/4/96 Final Journal Assignment

1. For each of the biochemical defense methods studied, describe how these adaptations contribute to the ability of plants to compete on an interspecies level.
2. Relate scent and color to the ability of a plant to compete successfully in its environment. Have the actions of humans changed these competitive strategies? If so, how?

APPENDIX 2, Figure D.

JOURNAL RUBRIC

Name _____

DATE	TOPIC	ATTITUDES (+)	ATTITUDES (-)	SKILLS	KNOWLEDGE (+)	KNOWLEDGE (-)	OTHER	COMMENTS
7-May	Anticipation, Brainstorm							
8-May	Oil Extraction							
10-May	Perfumes							
14-May	Pigment Ext.							
16-May	Scent Reflection							
20-May	Pollin.-Key Points							
22-May	Hill Rxn, Fireflies							
24-May	Allelopathy, camalexin							
4-Jun	Defenses, Compet. success							

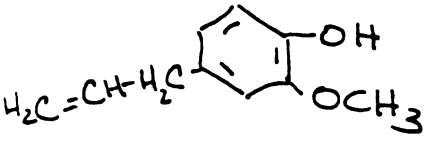
APPENDIX 3.

APPENDIX 3, Figure A.

ISOLATION OF ESSENTIAL OILS: Eugenol from Cloves Limonene from Citrus Rind

Introduction

This exercise will allow you to investigate several ways of isolating essential oils from their natural sources. Eugenol,



is the principal oil present in cloves. It is highly aromatic with phenolic and ether groups and an unsaturated side chain. In the past, oil of cloves served as a dental antiseptic and analgesic. We will extract this oil using a technique called steam distillation.

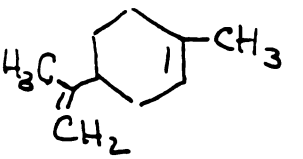
Steam distillation is an effective way to isolate high boiling compounds that are not soluble in water. As an immiscible mixture is heated, the vapor pressure of each substance rises until the total vapor pressure is equal to atmospheric pressure. Since that vapor pressure is the sum of the partial pressures of each component, the mixture would be expected to distill at a temperature below the boiling point of either pure substance. In effect, eugenol, which has a boiling point of 255° C, will co-distill with water vapor at a temperature below 100° C. We will then extract the oil from the watery mixture using solvent extraction and re-distill the extract to isolate the organic residue.

Extraction of Eugenol from Cloves

Procedure

1. Crush 10 g of whole cloves in a mortar and pestle and add them to 150 ml of distilled water in a 500 ml round bottomed flask. Connect the flask to a distillation apparatus and heat, using a mantle to boiling.
2. Continue, collecting distillate until about 100 ml has been collected. The distillate will be turbid, owing to the colloidal nature of the codistillation product. Note the odor of the distillate.
3. Cool the distillate to room temperature and transfer it to a 125 ml separatory funnel. Extract the distillate three times with 10 ml aliquots of methylene chloride. (NOTE: Which layer will you save? THINK about it!)
4. Transfer the combined methylene chloride extracts to a dry 100 ml round bottomed flask and redistill until only about 5-10 ml of the extracts remain in the flask.
5. Cool the residue, transfer it to a 50 ml beaker, rinse the flask with 2-3 ml of methylene chloride and add the rinse to the contents of the beaker.
6. Use a steam bath to evaporate the remaining methylene chloride from the beaker. You should recover approximately 1/2 gram of the pale yellow oil known as "oil of cloves", or eugenol. Note the odor of the oil.

Another way of extracting essential oils involves the use of "enfleurage", the transfer of those fragrant oils to lard and the subsequent extraction from the lipid matrix. This method is based on the old adage, "Like dissolves like". To speed up the process, which would be very time consuming if performed under cold conditions, we will heat the fruit material with lard. Heat makes essential oils dissolve into the fat more quickly, but it also allows some of the volatile oils to evaporate, with some loss of quality and yield. We will then distill the fat using an alcoholic solvent which will codistill the oil along with the ethanol. You may choose to perform the extraction either on lemons or oranges; the major essential oil constituent is limonene in either case, with differences in composition due mainly to other oils present in extremely small quantities. Limonene,



with a boiling point of 176° C, is insoluble in water but quite soluble in oils and somewhat soluble in alcohol.

Extraction of Limonene from Orange and Lemon Rind

Procedure

1. Peel two lemons or one large orange. Use a paring knife to remove only the outside colored portion, chopping the rind very fine or grate the peel to expose surface area.
2. Place 75 g of lard in a 500 ml round bottom flask and heat in a beaker of water on a hot plate over low heat until it is melted. Add the grated peel to the fat and cover loosely with foil.
3. Heat the lard-peel mixture, swirling the contents frequently until the water reaches a full boil. Remove the mixture from the heat and allow it to sit for at least an hour, or overnight.
4. Reheat the fat mixture on a water bath and add 50 ml of ethanol to the fat mixture. Swirl to mix the alcohol into the fat, but do not stopper the flask as heat will send the stopper flying!
5. Connect the flask to a distillation apparatus and heat the mixture, collecting the distillate that is recovered from the condenser. Note the odor of the distillate.
6. Extract the distillate with 10 ml aliquots of methylene chloride twice, collecting the organic layer. (NOTE: Which layer do you want? THINK!)
7. Redistill the methylene chloride extracts in a 50 ml round bottomed flask until 3-4 ml of residue remains in the flask.
8. Transfer the residue into a 50 ml beaker and rinse the flask with 2-3 ml of clean methylene chloride. Transfer the rinse into the beaker and place the beaker over a steam bath to evaporate any remaining solvent. The crude lemon oil should remain in the beaker. Note its odor.

References:

Ault, Addison. Techniques and Experiments for Organic Chemistry, 2nd Edition. Holbrook Press, Boston, MA . 1976.

Hart, Harold. Laboratory Manual for Organic Chemistry: A Short Course, 7th Edition. Houghton Mifflin Co., Boston, MA 1987.

Morris, Edwin, T. Fragrance: The Story of Perfume from Cleopatra to Chanel. E. T. Morris and Co., Greenwich, CT. 1984.

Cobb, Vicki. The Secret Life of Cosmetics. J. B. Lippincott, New York, N.Y. 1985.

APPENDIX 3, Figure B.

PERFUME MAKING

Introduction

Extracting and blending essential floral oils into perfumes is an ancient art. The burning of incense was a way of contacting the spirits, who were perceived to enjoy the fragrant smoke. Later, incense was burned in sickrooms to drive off evil spirits and, probably more importantly, to mask the odors of illness and death. Even the word, perfume, means "through smoke".

The art of perfumery was probably first practiced by ancient Egyptians, who used flowers to scent the bath oils of which the nobility was fond. Perfume, itself, a fragrant oil dissolved in alcohol probably originated about that time. Cologne, named for the German industrial city, is an inexpensive variation, consisting of a far more watery blend of oils and alcohols.

Modern perfumes are available to most everyone, but for many centuries, only the aristocracy were able to afford the costly oils from which they were made. Commoners were fond of a scented atmosphere, but they made do with scented wax necklaces called pomanders which were designed to hang around their necks to disguise body odors and the smell of rarely washed clothing. Rich or poor, it was common to carry a handkerchief filled with fragrant spices which could be brought quickly to the nose when passing an open sewer. The practice of covering a casket with flowers owes its origin to the fact that, as well as being a tribute to the dead loved one, it also served to help mask the smell of a rotting corpse.

Today, fragrance is a powerful selling point in the marketplace. We scent almost everything we come in contact with: detergents for both the laundry and kitchen, soaps, fabric softeners and deodorants. It's no wonder that many times we are such a jumble of cosmetic scents, that one can clash with another.

The smells that come from fine perfumes are a blend of as many as 200 to 500 ingredients, many of them only in trace amounts. A perfumer, one who blends perfumes, is trained to tell the differences between hundreds of essential oils and derivatives based on a finely developed sense of smell.

I have researched some easy to prepare perfume recipes and encourage you to try one or two and share them with a friend. These are courtesy of the manufacturer of the essential oils that I purchased, but you may mix or match the same approximate volumes to act as your own "perfumer".

Recipe 1
- 6 drops Ylang Ylang
- 2 drops Peppermint
- 2 drops Almond
- 2 drops Sandalwood
- 22.5 ml ethanol

Recipe 5
- 5 drops Mango
- 3 drops Coconut
- 2 drops Almond
- 1 drop Peppermint
- 19.5 ml ethanol

Recipe 2
6 drops Lilac
5 drops Ylang Ylang
3 drops Rose
2 drops Neroli Orange
28 ml ethanol

Recipe 3
5 drops Rose
3 drops Sandalwood
3 drops Ylang Ylang
2 drops Neroli Orange
23.5 ml ethanol

Recipe 4
5 drops Sandalwood
4 drops Avocado
1 drop Patchouli
18.5 ml ethanol

Recipe 6
5 drops Freesia
2 drops Peppermint
1 drop Neroli Orange
15 ml ethanol

Recipe 7
5 drops Freesia
4 drops Avocado
2 drops Peppermint
19.5 ml ethanol

Recipe 8
5 drops Jasmine
4 drops Botanical Blend
16.5 ml ethanol

References:

Cobb, Vickie. The Secret Life of Cosmetics. J.B. Lippincott, New York, N.Y. 1985.

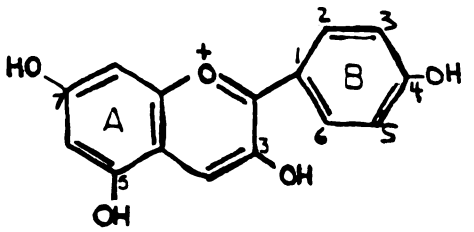
Gottschalk, S., Powell, A. and Grinnell, C. "Romantic Scentsations", Botanical Pleasures. Harold Mangelsen and Sons, Inc., Omaha, NE. 1995.

APPENDIX 3, Figure C.

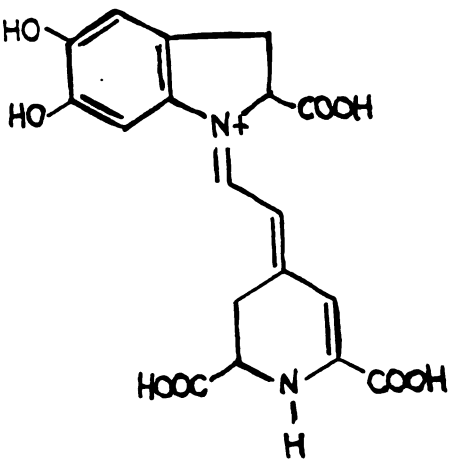
IDENTIFICATION OF PLANT PIGMENTS

Introduction

Plants contain a number of water-soluble red or blue hued pigments. Some of the pigments are known as anthocyanins and others are known as betacyanins. The pigments have the general chemical structures illustrated below.



General Structure of an Anthocyanin



General Structure of a Betacyanin

The red pigments in most plants are anthocyanins. These pigments are present only in angiosperms. Beets and lamb's quarter, however, have betacyanins as their red pigment. In this exercise you will extract the red pigments from the root of table beets and the fruits of strawberries or raspberries. You will then use these extracts to determine if an unknown set of red pigments are anthocyanins or betacyanins.

Methods and Materials

raspberries or strawberries, fresh
beet roots
unknown plant pigments
13 x 100 test tubes
single-edged razor blades
1% acidified methanol (1% HCl in methanol) (10 ml/group)
plastic well plate
1.0 M HCl (3 ml/group)
0.5 M NaOH (3 ml/ group)
2.5 M HCl (6 ml/group)
boiling water bath
0.5% $KMnO_4$ solution (1 ml group)
1M NH_3 solution (3 ml/group)
Bromine water

Procedure

1. Using the data template provided, you will perform several chemical tests and compare the data you obtain with standard solutions of anthocyanins and betacyanins. To prepare these standard solutions, obtain a small piece (2-3 cm^3 cube) of each fruit tissue and chop it very finely with a razor blade. Place the pieces of each fruit and any juice that was released in chopping, in a clean, labeled test tube and add 5 ml of acidified methanol. Swirl the solvent and gently crush it against the sides of the tube with a stirring rod. Allow the pigment solution to sit for 5 minutes.

2. Your teacher will provide you with beral pipettes containing unknown pigment solutions. They will be either anthocyanins or betacyanins.

3. Obtain 6 clean, dry test tubes. Label them for identification and add 10 drops of the appropriate pigment to each. Add 20 drops of 2.5 M HCl to each tube and boil for 5 to 10 minutes. Note any changes in the solutions.

4. You will be provided with a clean well plate. Orient it on a piece of white paper in such a way that it has six wells across and four rows down. Using your data template as a guide, in the first row place 10 drops of each known and unknown solution in their appropriate wells. Record the color and any other distinguishing characteristics on your template. This row will serve as a control for future comparison.

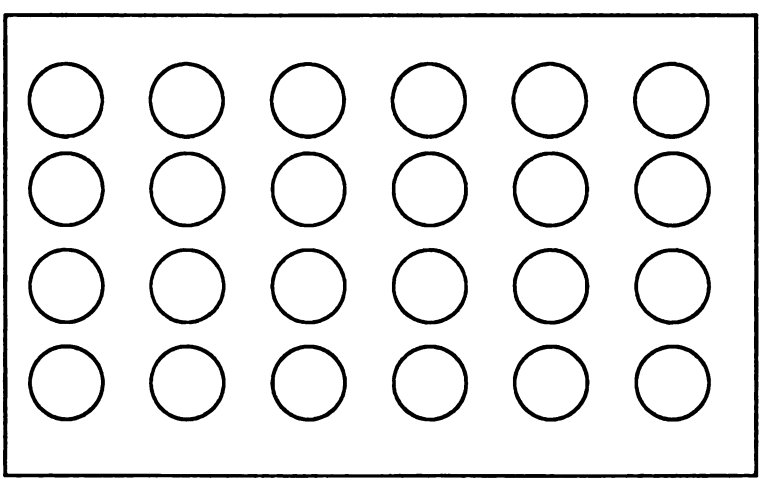
5. In the second row, place 10 drops of pigment in each corresponding well. Add 10 drops of 0.5 M NaOH to each well across. Record any color changes. After 10 minutes, add 10 drops of 1.0 M HCl to each well and record any further changes.

6. In the third row of wells, add 10 drops of each pigment as directed above and add 3 drops of 0.5% $KMnO_4$ to each well. Note any color changes.

7. In the fourth row of wells, add 10 drops of pigment to each well and then add 10 drops of 1 M NH_3 solution. Note any color changes.

8. Obtain 6 more clean, dry test tubes and label them appropriately. Add 10 drops of pigment to each appropriate tube. Add 10 drops of bromine water. Shake and note decolorization, if it occurs. What does this tell you about chemical structure.

Results and Conclusions



1. Complete the data template and identify your unknowns as either anthocyanins or betacyanins. You will not be able to identify the source of the pigment, but you should be able to classify it.

2. Explain how your reached the conclusions you formed. What was your logic? How did the chemistry of each class of pigment differ from the other?

References:

Moore, Randy and Vodopich, Darrell (1987). "The Influence of pH on the Color of Anthocyanins and Betalains", American Biology Teacher, 49:2, pg.111-12.

APPENDIX 3, Figure D.

Especially for Teachers Section

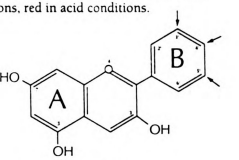
Anthocyanin Pigments of Flowers and Vegetables

Water-soluble flavonoid pigments (i.e. anthocyanins, flavonols and flavones) are widely distributed in plants. Anthocyanins are responsible for violet and blue flower color, and are largely responsible for bright red flowers and the spectacular colors of autumn leaves. This is in contrast to the colors of dandelion flowers and tomato fruits in which yellow and orange carotenoids predominate. Anthocyanins tint autumn leaves when their presence is revealed, as chlorophylls and carotenoids are destroyed. In this exercise, one can identify the major anthocyanins from common plant products.

The structures of several common anthocyanidins are shown below in the figure and table. Pigment color is clearly dependent on the number and orientation of hydroxyl and methoxyl groups on the B-ring of the molecule. In nature, these pigments occur as the 3-, or 3,5-glycosides, called anthocyanins; one or more of the A-ring hydroxyl groups are linked to one or more sugars (structures tabulated below do not indicate these sugar derivatives). Glycosylation increases the water-solubility of the insoluble anthocyanidin. Anthocyanins are virtually characteristic of higher plants.

Flowers with different colors often contain the same anthocyanin; compare the blue cornflower and the red rose. Obviously, other factors are involved in pigmentation. Of these, chelation with metals (iron or aluminum), changes of pH, and "co-pigmentation" (the "blueing" of anthocyanins due to weak chemical interaction with different flavones) are most common.

The systematic study of anthocyanins dates from 1664, when Robert Boyle (of the famous Gas Laws) showed that the purple pigment of *Viola tricolor* is a natural pH indicator, becoming green in alkaline, red in acid solution. Similarly, anthocyanins in *Hydrangea* flowers indicate the pH of the plant cell vacuole: blue in alkaline conditions, red in acid conditions.



anthocyanidin pigment	Substitution on 3'	4'	5'	color
pelargonidin	H	OH	H	scarlet
cyanidin	OH	OH	H	crimson
peonidin	OCH_3	OH	H	rose red
petunidin	OCH_3	OH	OH	purple
delphinidin	OH	OH	OH	blue-violet
malvidin	OCH_3	OH	OCH_3	mauve

In the exercises described here, you will purify, separate, and identify the anthocyanin pigments in flowers, vegetables, and if available, autumn leaves.

Materials:

Red cabbage; flowers of petunia, geranium, mallow; dark grape juice; frozen grape and cranberry juices, strawberries; skin of eggplant fruit, radish tuber; autumn leaves; etc.

boiling water baths: ringstands, burners, 100 ml flasks for each team

5 N HCl; ca. 100 ml per team

12.5 cm circles of Whatman #1 filter paper for funnels

small (50 ml) funnels

distilled water

95% ethanol, in squeeze bottles (100 ml, or more)

C_{18} reverse phase column ("SepPak" from Waters, Inc.); 3 ml syringes to fit

20 µliter capillary pipets; to be used as chromatography applicators

sheets of Whatman #3 MM paper, to be cut to proper size

Mason jars as chromatography tanks (1 per team of 3-4 students)

Chromatography solvents: 100 ml of each for each team of students

- Solvent A: 1% HCl (3 ml conc. HCl + 97 ml water), or 5% acetic acid
- Solvent B: BAW = n-butanol/acetic acid/water (4/1/5 by vol)
- Solvent C: "Forestal" = acetic acid/ conc.HCl/water (30/3/10 by vol)

Methods and Procedures: To identify the anthocyanins in several different plant samples.

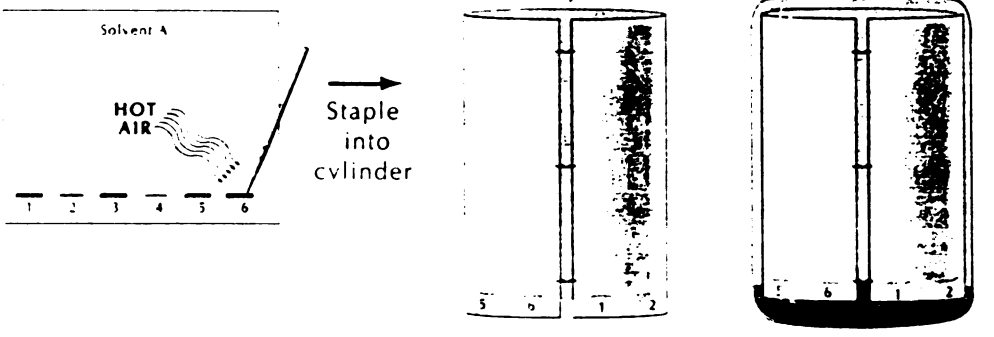
1. Select several samples of flower petals, fruits (or fruit juices), and/or leaves of several plants. Cut up or tear the pigmented tissues into small pieces and quickly immerse the tissue in boiling water (4 gms. tissue in 30 ml water works well for most flowers). Let the tissue steep for 5-10 minutes.

2. Filter through one layer of cheesecloth and/ or Whatman #1 paper as required; wash the filter with an additional 5 ml distilled water. These filtrates contain the anthocyanins.

3. Adjust each filtrate to 2 N HCl (add 20 ml 5 N HCl to 30 ml filtrate); the pigments are more stable in aqueous acid solution.

4. Set aside a 10 ml aliquot of the acidified filtrate as "crude anthocyanin."

5. Place the remaining 40 ml acid filtrate in a boiling water bath for 40 mins. to hydrolyze the sugars from the pigment, releasing the anthocyanidin. Label this hydrolyzate "crude anthodyanidin."



In fume hood; run about 60 min.

6. While the anthocyanins are being hydrolyzed, equilibrate a C18-reversed phase cartridge ("SepPak") with 2 ml 95% ethanol, followed by 2 X 2 ml of distilled water.

7. Concentrate your pigments on the reverse phase cartridge as follows: run 1-2 ml of "crude anthocyanin" or "crude anthocyanidin" through the equilibrated SepPak. The pigment should form a sharp band at the top of the SepPak; if the band is faint, run more pigment through.

Wash the SepPak with 2 X 2 ml distilled water.

Elute the pigment with 1 ml 95% ethanol; catch just the highly concentrated first fractions in a test tube or Eppendorf tube. These concentrated ethanolic pigment samples will be used for chromatographic identification of the pigment.

Regenerate the SepPak cartridge by washing the cartridge with 2 X 2 ml distilled water; use it again for the next pigment sample.

8. Prepare 3 Whatman 3MM paper chromatograms (A, B and C), as follows: Draw a pencil line 2 cm. above the bottom of each of the papers. This will serve as the origin for your pigments.

Streak both your anthocyanin and your anthocyanidin concentrates from step 7. on the A and B chromatograms; label the pigment spots. Streak only the anthocyanidins on chromatogram C. Streak enough pigment concentrate on each chromatogram so that a dark line, about 1 cm is deposited; two 20 µliter applications usually are sufficient. Blow hot air over the samples as you apply them, driving off the solvent. The chromatogram develops best if it is thoroughly dry, so place it in a hot oven for about a minute (or heat in a microwave oven for 20 secs.) Be sure to label your chromatograms and your streaked samples.

Roll each chromatogram into a cylinder and staple the ends together, taking care that the sides **do not touch each other**; see figure on page 12. Place each paper cylinder in the appropriate solvent in chromatography jars in a fume hood. Adequate development requires ca. 60 minutes. Mark the solvent front with a pencil.

Allow the paper chromatograms to dry in a fume hood for several minutes before recording your data and calculating each Rf.

Results and conclusions:

Tabulate the chromatographic behavior and colors of your pigment samples on the tables provided on the following pages.

How many major pigment bands are resolved in each sample?

Do the anthocyanins run differently than the anthocyanidins? Explain the chromatographic differences between the anthocyanins and the anthocyanidins based on their comparative structures.

Identify the major anthocyanin (including the extent of glycosylation) from each sample, based on data provided in Tables 2.10 and 2.11 (from Harborne, 1984). If you wish, you may confirm your identification by referring to Harborne (1965).

Table 2.10 (Harborne, 1984)

Pigment*	Forestal	R_f (x100) in formic	BAW	Visible color	Visible max.(nm) in MeOH-HCl	Color shift with $AlCl_3$ **
Pelargonidin	68	33	80	red	520	-
Cyanidin	49	22	68	magenta	535	+
Peonidin	63	30	71	magenta	532	-
Delphinidin	32	13	42	purple	546	+
Petunidin	46	20	52	purple	543	+
Malvidin	60	27	58	purple	542	-

* There are about six other known anthocyanins, but these are all rare in occurence.

** This bathochromic shift to blue colors with $AlCl_3$ may be observed in the visible spectrum by measuring the spectrum in methanol-0.01% HCl and then adding 5% $AlCl_3$; it may also be detected by spraying or dipping chromatograms which have been thoroughly dried to remove acid vapor in 5% alc. $AlCl_3$.

Table 2.11 (Harborne, 1984)

Anthocyanin	BAW	R_f (x100) in But-HCl	1% HCl	Petal Source
Monoglycosides				
Pelargonidin 3-glucoside	44	38	14	Chinese Aster
Cyanidin 3-glucoside	38	25	07	Chrysanthemum
Malvidin 3-glucoside	38	15	06	*Primula polyanthus*
Diglycosides				
Pelargonidin 3,5-diglucoside	31	14	23	*Pelargonium*
Cyanidin 3-rhamnosylglucoside	37	25	19	Snapdragon
Peonidin 3,5-diglucoside	31	10	17	Peony
Delphinidin 3,5-diglucoside	15	03	08	*Verbena*
Triglycosides				
Cyanidin 3-rhamnosylglucoside-5-glucoside	25	08	36	Cape Primrose
Cyanidin 3-(2"-glucosylrhamnosylglucoside)	26	11	61	*Begonia coccinea*
Acylated Diglucoside				
Pelargonidin 3-(p-coumaryl-glucoside)-5-glucoside	40	46	19	*Viola x williamsii*

NOTES:

Most pink, scarlet, and orange-red flowers have pelargonins and/or its methoxylated derivative peonin; similarly, crimson and magenta flowers have cyanins; purple, blue flowers have delphinins or the methoxylated derivatives petunins and malvidins.

For anthocyanidin "standards," you may use grape juice as a source of delphinidin; cyanidin from blackberry, cranberry, appleskin, and roses; petunia petals contain petunidin; geranium flowers pelargonidin; strawberry fruit has a mixture of pelargonidin and cyanidin. You **must** deglycosylate these anthocyanins (by hydrolysis at 100°C in 2 N HCl) before using them as standards! Alternatively, you may hydrolyze the glycosidic bonds with the β–glucosidase activity present at high levels in almond seeds. It's a relatively simple demonstration of enzyme activity.

Adapted by Dr. Ken Nadler with input from Ms. Cheryl Hach (Kalamazoo Math. and Science Center) and Dr. Ray Hammerschmidt from:

1. Harborne, J. B. 1984. Flavonoid Pigments in *Phytochemical Methods*, 2nd ed. Chapman and Hall publishing, London. 288 pp.

2. Harborne, J. B. 1965. Flavonoids: Distribution and Contribution to Plant Colour. In *Chemistry and Biochemistry of Plant Pigments*. (ed. T. W. Goodwin), Academic Press, London and New York. 583 pp.

3. Swain, T. 1965. Nature and Properties of Flavonoids. In *Chemistry and Biochemistry of Plant Pigments*, (ed. T. W. Goodwin), Academic Press, London and New York. p.211.

4. Swain, T. 1965. Analytical Methods For Flavonoids. In *Chemistry and Biochemistry of Plant Pigments*, (ed. T. W. Goodwin), Academic Press, London and New York. p.533.

TABLE I. Chromatographic behavior of anthocyanins and derivatives on Whatman #MM paper in solvent _____

SAMPLE	DISTANCE MIGRATED	R_f	COLOR
Solvent front		1.0	

APPENDIX 3, Figure E.

THE HILL REACTION

Introduction

During the light phase of photosynthesis, two distinct but interrelated processes occur. Both processes occur on the membranes of the thylakoid and are in close proximity to each other. In one process, located in Photosystem II (PS II), electrons are passed through a transport chain and the resultant energy is conserved in the phosphate bonds of ATP. In the other process, located in Photosystem I (PSI), the electrons that flow from PS II are used to reduce a coenzyme called Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$) in the reaction:

$$NADP^+ + H^+ + 2e^- \rightarrow NADPH$$

The reduction process can be demonstrated in a cell-free extract of chloroplasts by using a dye, 2,6-dichlorophenol-indophenol (DCPIP) as the electron acceptor in place of $NADP^+$. DCPIP changes from blue to colorless when it is reduced. If DCPIP, in the presence of active chloroplasts, is subjected to light, an observable color change should occur. The degree of color change can be measured on the spectrophotometer as a function of time.

In this exercise we will demonstrate that during the light cycle of photosynthesis, an oxidation-reduction reaction occurs, as electrons are transferred to different receptor molecules.

Methods and Materials

Fresh spinach leaves
Spectrophotometer, with red filter
Ice buckets
2-500 ml beakers
Boiling water bath
2-250 ml beakers
Hotplate
Phosphate buffer, pH 7.2, chilled
0.25 M sucrose
Parafilm
Food blender
Cheesecloth
Crushed ice
13 x 100 mm test tubes (4/group)
Test tube rack
Aluminum foil
Fluorescent light source
0.02% 2,6-dichlorophenol-indophenol (DCPIP)

To prepare phosphate buffer: Dissolve 19.162 g of $Na_2HPO_4 \cdot 7H_2O$) and 4.816 g of KH_2PO_4 in sufficient distilled water to make 1 liter of solution. Adjust the pH if necessary with 6 M HCl or NaOH.

To prepare 0.25 M sucrose: Dissolve 85.5 g of sucrose in distilled water sufficient to make 1 liter.

Procedure

NOTE: Prepare the chloroplast suspension for the entire class. WORK UNDER SUBDUED LIGHT CONDITIONS.

1. Place 8 - 10 fresh spinach leaves in a food blender and add 25 ml of cold phosphate buffer and 25 ml of cold sucrose solution.
2. Blend for 10 seconds at low speed.

3. Add equal volumes of buffer and sucrose solution to cover the slurry and blend again at low speed for 15 seconds, then high speed for an additional 10 seconds.
4. Filter the slurry through four layers of cheesecloth into a 500 ml beaker that is held in ice. Discard the filtered material and save the filtrate. Alternately, you may centrifuge the slurry for 5 minutes at low speed. Immediately cool the chloroplast suspension on ice.
5. Place about 10 ml of the filtered chloroplast solution in a test tube and heat it for 5 minutes in a boiling water bath.
6. Prepare four spectrophotometer cells according to the table below:

Contents	Tube #1	Tube #2	Tube #3	Tube #4
Buffer	1 ml	1 ml	1 ml	1 ml
DCPIP	-----	15 drops	15 drops	15 drops
Sucrose	4 ml	3 ml	3 ml	3 ml
Boiled Chloroplasts	-----	-----	-----	5 drops
Fresh Chloroplasts	5 drops	5 drops	5 drops	-----

7. Cover the tubes with Parafilm.
8. Cover Tube #3 with aluminum foil to block out all light
9. Use Tube #1 as a blank to adjust the spectrophotometer to 100% transmittance at 660 nm.
10. Place the four tubes in a test tube rack in front of a fluorescent light source.
11. At time intervals of 0, 1, 2, 4, 6, 8, 12, 16 and 20 minutes, place each tube in the spectrophotometer and read the % transmittance. Record each reading. You may want to extend the experiment for another half hour, if time permits. Take subsequent readings every five minutes.
12. Take readings as quickly as possible and return Tube #3 to its aluminum sleeve immediately afterwards.
13. At the conclusion of the lab period, plot the readings for Tubes #2, #3 and #4 on the same graph using %T on the y-axis and Time on the x-axis.

References:

Stryer, Lubert. (1981). Biochemistry, Second Edition, W.H. Freeman and Co., New York, N.Y.

College Entrance Examination Board. (1981). Laboratory Exercises I - VI. Princeton, N.J.

APPENDIX 3, Figure F.

BIOLUMINESCENCE - "THE FIREFLY-BUTT EXPERIMENT"

Introduction

The reaction responsible for bioluminescence is complicated but can be briefly summarized. The active molecule, luciferin, requires ATP to provide the energy responsible for the attachment of an oxygen molecule. In the presence of an enzyme, luciferase, the luciferin-O_2 complex is broken down into CO_2, light and several breakdown products.

In this experiment you will synthesize ATP from chloroplasts, add it to firefly extract and see the light produced. In addition, we will supply extra ADP and an artificial electron acceptor, phenazine methosulfate which will enhance the production of ATP.

Procedure

IMPORTANT: ALL TUBES, FLASKS AND PIPETTES MUST BE KEPT ON ICE THROUGHOUT THIS PROCEDURE!!

1. Use the chloroplast suspension prepared in the Hill Reaction procedure.
2. Put exactly 12 drops of phosphate buffer into a small test tube. Obtain four firefly lanterns and add them to the buffer. Homogenize them with a glass stirring rod. Place on ice for 30 minutes.
3. In the meantime, prepare the following solution in a clean, chilled 50 ml flask. Measure out:
   - 9 drops of phosphate buffer
   - 6 drops of 0.25% ADP
   - 6 drops of 0.25% phenazine methosulfate
   - 6 drops of chloroplast solution

   Shake to stir and place in an ice bath that is illuminated under fluorescent light for at least 10 minutes.
4. When each group has illuminated its flask for at least 10 minutes, suck up the contents of the chloroplast mixture into a pasteur pipette.
5. The next step MUST be done in total darkness. Gather in a darkened room and once your eyes have adjusted to the darkness, and on the instructor's count, squirt the contents of the chloroplast mixture into the chilled firefly extract. Observe.

References:

Stryer, Lubert. (1981). Biochemistry, Second Edition, W.H. Freeman and Co., New York, N.Y.

College Entrance Examination Board. (1981). Laboratory Exercises I - VI. Princeton, N.J.

APPENDIX 3, Figure G.

GIBBERELLIN INDUCTION OF AMYLASE: The Halo Halfseed Reaction

Introduction

The synthesis of hydrolytic enzymes in specialized protein granules within the endosperm of grain seeds is controlled by gibberellins which are secreted by the embryo at the time of germination. The synthesis of the enzyme ∂-amylase in barley seeds can be induced in vitro using seeds that have had the embryo removed. The effect of varying concentrations of gibberellins on amylase production will be measured semi-quantitatively using starch agar and Lugol's Iodine indicator.

Methods and Materials

Gibberellic acid
Barley seeds - 10/team soaked in cold water for 12-24 hours
Sterile Petri Dishes
Starch agar
1% Bleach solution (1:10 dilution of commercial bleach)
Lugol's Iodine Reagent
Sterile forceps
Sterile paper towels
Sterile water
Sterile 500 ml Erlenmeyer flasks

Procedure

1. Prepare starch agar by mixing 2 g of soluble starch with 30 grams of agar and adding distilled water to make 1000 ml of solution.
2. Autoclave the agar for 20 minutes and keep warm.
3. Meanwhile, prepare the gibberellic acid serial dilutions:

   a.) Carefully weigh 1 mg (0.001 g) of gibberellic acid into 100 ml of methanol. (0.001 mg/ml)
   b.) Remove 1.00 ml of the above solution and dilute to 100 ml with methanol in a volumetric flask. (0.00001 mg/ml - 10 ug/ml) Label this solution #4.
   c.) Remove 0.2 ml of solution #4 and add to 1.8 ml of methanol. (1.0 ug/ml) Label this solution #3.
   d.) Remove 0.2 ml of solution #3 and add to 1.8 ml of methanol. (0.1 ug/ml) Label this solution #2.
   e.) Remove 0.2 ml of solution #2 and add to 1.8 ml of methanol. (0.01 ug/ml) Label this solution #1.

4. Add 0.1 ml of each of solutions #1 through #4 to a sterile petri dish. Label an additional dish as a control. Add 25 ml of sterile agar to each of the five dishes, mix them to disperse the hormone and allow them to solidify at room temperature.

5. Store the dishes in the refrigerator until ready to use.

6. Imbibe barley seeds in water in the refrigerator at least overnight.

7. Hull the seeds and, using a sterile scalpel blade or razor blade, separate the halves of the seeds. Remove the embryo from the endosperm.

8. Soak the barley halfseeds in 1% sodium hypochlorite solution for 20 minutes and then rinse several times with sterile water.

9. Place 10 halfseeds in each of the petri dishes and incubate under refrigeration until the next class period.

10. Remove the dishes and discard the half seeds from the agar.

11. Bathe each dish with Lugol's Iodine solution to allow the zone of hydrolysis to be visualized.

12. Measure each zone and obtain an average diameter for each gibberellic acid concentration.

13. Graph the log [GA] versus zone diameter.

References:

Jones, R.L. and Varner, J.E. (1967), Planta, 72, 155-61.

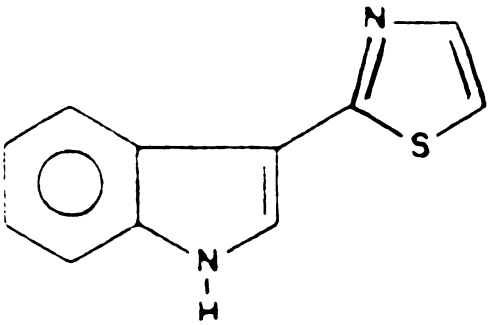
APPENDIX 3, Figure H.

INDUCTION OF CAMALEXIN IN *Arabidopsis thaliana*

Introduction

One way plants defend themselves from invaders is by the production of low molecular weight, non-specific antifungal compounds called phytoalexins. These compounds have been found to be produced only in plant tissues that have come in contact with a fungus; they are not a persistent component of the tissues. It appears that the infected cell synthesizes the compound and it is active only to infected tissues and those cells in the immediate vicinity. The ability of a plant to, in effect, produce its own antimicrobial that works only in infected tissue can be thought of as an evolutionary advantage; those plants which are able to produce phytoalexins may be presumed to be more successful in their habitats by being better able to defend themselves.

This activity will attempt to induce the synthesis of camalexin, an antimicrobial phytoalexin, in a small Brassica, *Arabidopsis thaliana*. This small crucifer is currently being used extensively in research and educational situations for many of the same reasons that Wisconsin Fast Plants are used. *Arabidopsis* is small,easy to grow and has a very short life-cycle. We will infect leaves of young plants with a spore suspension of a fungus, *Cochliobolus carbonum*. After several days, we will extract the camalexin and demonstrate its presence using TLC. We will also inoculate yeast plates with our camalexin and observe its antifungal properties.



Camalexin
Camelina sativa
Arabidopsis thaliana

Methods and Materials

Cochliobolus carbonum culture
Young *Arabidopsis thaliana* plants
Petri dishes
Filter paper to fit petri dishes
Chloroform
UV light source
Cheesecloth
Pasteur pipettes
Ethyl acetate
Methanol
Thin Layer Chromatography plates

Procedure

1. Flood a culture plate of *Cochliobolus carbonum* and release its spores by scraping. Filter the suspension through cheesecloth into a culture tube.
2. Detach 20 leaves per group from full size Arabidopsis plants. Place ten leaves on moistened filter paper in two petri dishes with their underside facing up, forming a well.
3. Using a Pasteur pipette, inoculate the underside of the leaves in one dish using spore suspension. Using another clean pipette, inoculate the underside of the leaves in the other dish with distilled water. These leaves will serve as a control.
4. Place covers on the dishes and allow them to incubate at room temperature for 2 to 3 days.
5. Collect the droplets from the infected leaves in a Pasteur pipette and place them in a small labeled vial. Extract the droplets three times with equal volumes of ethyl acetate, collecting and combining the ethyl acetate.
6. Repeat the extraction above with the control leaves and place the extracts in an appropriately labeled vial.
7. Evaporate the ethyl acetate extracts to dryness and dissolve the residue from each vial in 100 µl of methanol. Spot half of each sample (50µl) onto TLC plates and label the lanes as infected and control samples.
8. Develop the plates in chloroform:methanol (9:1, v:v).
9. After development, view under UV light for the presence of camalexin, which will fluoresce.
10. Submit your observations and conclusions on a separate piece of paper along with your chromatography plate.

References:

Davis, Keith R. and Raymond Hammerschmidt. (1993) *Arabidopsis thaliana* as a Model for Plant-Pathogen Interactions. APS Press, St. Paul, MN

APPENDIX 3, Figure I.

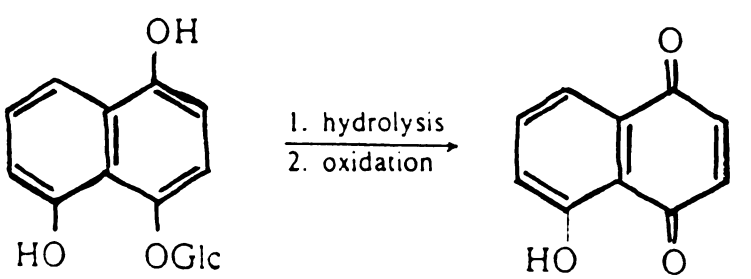
DEMONSTRATING A CHEMICAL DEFENSE MECHANISM: Allelopathy

Introduction

Darwin's theories propose that organisms struggle for survival, constantly in competition for resources. Plants must compete with each other for adequate moisture, light and soil nutrients. In this pursuit, plants have adopted various strategies to deal with encroaching neighbors. One way some plants have discouraged interspecies territorial invaders is by the evolution of chemicals that interfere with their growth. This chemical "warfare" is known as allelopathy.

Allelopathic compounds are usually low molecular weight chemicals with simple structures derived from terpenes or phenols. Although the evolution of allelopathic compounds was first noted in desert species where it would be reasonable to expect the competition for the sparse resources to be fierce, we are fortunate to have a plant species noted for its allelopathic properties virtually right in our own backyards.

As long ago as the times of the Greek philosophers, it was noted that other species would not grow in close proximity to a black walnut tree. The toxin in question, juglone, was isolated in the 1920's. Studies indicate that juglone is produced in a bound state in the leaves, stems and branches of the tree and rain leaches the compound from these structures and deposits the chemical into the soil. The bound state consists of a 4-glycoside of the toxin which is converted by hydrolysis and oxidation into the active allelopath which is released into the soil underneath the leaf canopy of the tree.



bound form of toxin

juglone
(5-hydroxynaphthoquinone)

Repeated years of deposition, renewed by each rainfall, can produce quite a toxic substrate under the tree which will discourage the growth of other plants, be they grasses, shrubs or other tree species.

This activity will attempt to simulate the effect of the toxin on the germination rate of several types of vegetable seeds.

Methods and Materials

Walnut extract (sun-brewed leaves, twigs, bark of a black walnut tree)
Petri dishes
Filter Paper for Petri dishes
Garden seeds, several varieties

Procedure

1. Collect plant material from a black walnut tree. Place the material in a glass jar, cover and allow it to sit in the sun for several days to "brew". Filter, saving the liquid and allow some of the liquid to evaporate off to create a thick extract. This pigment may also be used as a natural dyestuff.

2. For each type of seed to be tested, obtain two petri dishes and label one "control" and the other "walnut".
3. Place 2 or 3 circles of filter paper in each dish.
4. Wet the circles in the control plates with distilled water. They should be thoroughly moistened but water should not be standing in them.
5. Wet the circles in the walnut-treated plates with a solution of 10% walnut extract.
6. Place ten seeds of each species on each plate. Cover them and allow them to sit undisturbed in a darkened spot (inside a drawer) for 3 or 4 days.
7. At the end of the incubation period, count the number of seeds of each treatment that have germinated. We may want to combine the data for the class.
8. Illustrate your data in a bar graph and submit it, along with your observations and conclusions.

References:

Harborne, J.B. (1988) Introduction to Ecological Biochemistry. 3rd Ed. Academic Press, London, England.

APPENDIX 3, Figure J.

FLOWER POLLINATION STRATEGIES: USE OF A POLYCLAVE KEY

Introduction

Pollen is transported between flowers by pollen vectors - wind, water and animals such as birds, bats and insects. Many flowers appear to be specialized by their color, shape, odor and nectar and pollen rewards, increasing the chances that pollination will occur by a specific vector to. These flower specializations are collectively known as pollination syndromes or floral syndromes.

A pollination system refers to the interaction between the plant's floral syndrome and its corresponding pollen vector. For example, red, tubular, nectar-rich flowers (a floral syndrome) have been thought to attract hummingbirds (the pollen vector). This combination results in bird pollination.

Many identification keys for animals and plants are dichotomous keys. This lab will use a key of a different sort, a polyclave key. A polyclave key is an approach using the process of elimination to reach a tentative identification. They key presents characteristics that can be selected in any order.

Plants differ in the degree to which their flowers show these pollination-related specializations. More than one pollen vector may be involved in the pollination of a particular flower species. Some insects may visit flowers without actually transferring pollen. A limitation of this key is that it cannot determine with certainty which pollination system is involved with a given flower. By matching the observed flower characteristics with the possible pollination systems, however, a student can decide which one or ones are more likely to be involved.

Methods and Materials

flowers
polyclave key
ultraviolet light

Procedure

Abbreviation Codes for Pollination Systems

WI	Wind pollination
BT	Beetle pollination
F - M	Fly pollination - syrphid or bee flies
F - S	Fly pollination - carrion and dung flies
BE	Bee pollination
BU	Butterfly pollination
MO	Moth pollination
BI	Bird pollination
BA	Bat pollination

1. Choose a plant in flower.
2. Select any one of the flower characteristics given in the key that is observable for your flower (such as flower size). You can choose the flower characteristic in any order, but near the end you may have to examine a particular character in order to distinguish between two possible pollination systems.
3. Choose the description of the character that matches the flower you are observing (such as large).
4. Following this description is a list of the codes of the pollination systems NOT involved. Those listed in parentheses are unlikely, but possible candidates. Cross the rejected pollination systems off the list provided on the worksheet. (For example, if the flower size is large, you would cross "WI" off your worksheet, and put "F -M" in parentheses.
5. Select any other characteristic and continue to eliminate pollination systems from your list. It may not be possible to narrow the choices down to just one because of the characters available for your examination on the flower that you have selected.
6. The pollination system or systems that are NOT crossed off the list are the ones involved with your flower.

PRACTICE WITH A RED HIBISCUS

1. Select a flower characteristic such as flower size.
2. In the case of the hibiscus, the flower is 10 cm across. Choose "showy, large, conspicuous flower" as the description.
3. Following this description, the key lists "NOT: WI,(F-M)". On the row of letters on your worksheet , cross off "WI" and put parentheses around" F-M". This indicates that wind pollination has been eliminated as a possible choice for the hibiscus. Syrphid and bee flies are now unlikely candidates for its pollination.
4. Choose another characteristic such as flower symmetry. Hibiscus has a flower exhibiting radial symmetry. Following this description the key reads "- - -", indicating that no pollination choice can be eliminated on this basis alone.
5. Repeat the above steps for nectar guides: The hibiscus does not appear to have any, so eliminate "BE" and "BU" from the worksheet.

NOTE: * Nectar guides are lines of contrasting color on the petals of a flower, directing the pollinator to the food source. If the lines are of UV wavelength, the lines will only be visible under a black light source.

6. Selecting flower color as red allows you to cross off "WI,BT,F-M,F-S and BA" and put parentheses around "(BE) and (MO)". Since "WI and BE" were already eliminated in the previous rounds, the new eliminations are "BT,F-M, and BA". "F-S" is no longer a possibility. The worksheet now looks like the following:

HIBISCUS ~~WI~~ ~~BT~~ ~~(F-M)~~ ~~F-S~~ ~~BE~~ ~~BU~~ (MO) BI ~~BA~~

7. All possibilities have been eliminated except one. The most likely pollination system for the red hibiscus is "BI". Checking the codes, "BI" translates as a bird pollinated flower.

DATA

POLLINATION SYSTEMS WORKSHEET

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

WI BT F-M F-S BE BU MO BI BA

Flower Name Likely Pollinator(s)

FLOWER CHARACTERISTIC: Flower Symmetry
Description:
- radial — NOT: - - -
- bilateral — NOT: BT,F-M,F-S,(BU),MO

FLOWER CHARACTERISTIC: Flower Shape
Description:
- tubular — NOT: WI,BT,F-M
- not tubular — NOT: MO,(BU),(BI),BA

FLOWER CHARACTERISTIC: Flower Size
Description:
- small, inconspicuous — NOT: BT,BE,MO,BU,BI,BA
- showy, large or small flowers in a conspicuous group — NOT: WI,(F-M)

FLOWER CHARACTERISTIC: Flower Color
Description:
- white — NOT: WI,F-S,(BE),(BI)
- yellow — NOT: WI,BT,F-M,F-S,(MO),BA
- blue — NOT: WI,F-M,F-S,(MO),(BI),BA
- red — NOT: WI,BT,F-M,F-S,(BE),(MO),BA
- dull or dark — NOT: BE,BU,(BI)

FLOWER CHARACTERISTIC: Flower Display
Description:
- night only — NOT: (BE),(BU),BI
- day only — NOT: (MO),BA
- day and night — NOT: - - -

FLOWER CHARACTERISTIC: Odor
Description:
- no odor — NOT: F-S,MO,BA
- putrid — NOT: WI,MO,BI,BA
- fragrant — NOT: WI,F-S,BI

FLOWER CHARACTERISTIC: Pollen
Description:
- few grains — NOT: WI,BA
- abundant — NOT: - - -

FLOWER CHARACTERISTIC: Nectar
Description:
- none — NOT: BE,BU,MO,BI,BA
- abundant, unconcealed — NOT: BT,F-S,BU,MO,BI
- abundant, concealed — NOT: BT,F-S,BA

FLOWER CHARACTERISTIC: Nectar Guides*
Description:
- present — NOT: WI,BT,F-M,F-S,MO,BI,BA
- absent — NOT: BE,BU

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