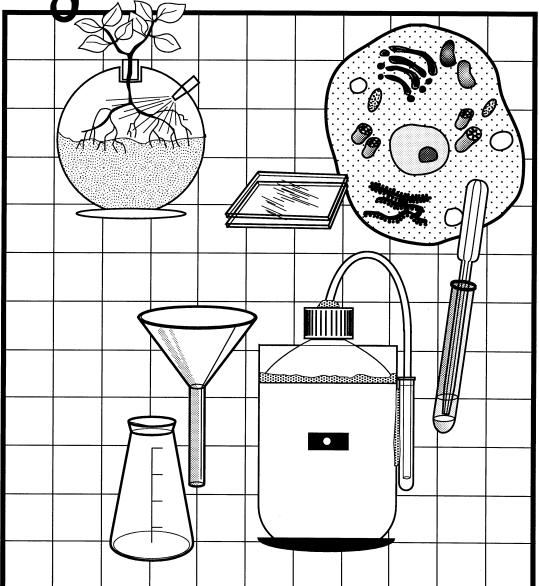


Agricultural Science



# LAB ACTIVITIES

INSTRUCTIONAL MATERIALS LABORATORY • UNIVERSITY OF MISSOURI-COLUMBIA

In cooperation with Agricultural Education
Department of Practical Arts and
Vocational-Technical Education
College of Education and College of
Agriculture, Food and Natural Resources
University of Missouri-Columbia



In cooperation with
Agricultural Education Section
Division of Vocational and Adult Education
Department of
Elementary and Secondary Education
Jefferson City, Missouri

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Produced by, and available from: Instructional Materials Laboratory College of Education University of Missouri - Columbia 1400 Rock Quarry Center Columbia, MO 65211-3280 1-800-669-2465 www.iml.coe.missouri.edu

The activity that is the subject of this report was supported in whole or in part by funds from the Department of Elementary and Secondary Education, Division of Vocational and Adult Education. However, the opinions expressed herein do not necessarily reflect the position or policies of the Missouri Department of Elementary and Secondary Education or the Division of Vocational and Adult Education, and no official endorsement should be inferred.

# AGRICULTURAL SCIENCE LAB ACTIVITIES

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Volume 27 Number 2

Catalog Number 10-0005-I February 1995

### **ACKNOWLEDGMENTS**

Recognition is given to advisory committee members for providing their valuable time and suggestions in developing these activities for Agricultural Science I and II courses. The committee consisted of Bob Birkenholz, Patty Bratton, Lyndon Brush, Kenny Graham, Larry Henneke, Delbert Lund, Mike McCrory, Marsha Kelly Nelson, David Twente, Vance Vanderwerken, Jim Welker, and Lyle Whitaker.

These activities were field tested by agricultural instructors, science instructors, and industry representatives. They included Bob Birkenholz, Harold Bossaller, Patty Bratton, Lyndon Brush, Larry Crutsinger, Kenny Graham, Larry Henneke, Steve King, Mike McCrory, Chuck Miller, Andy Robinson, Sherie Rodekohr, David Twente, Vance Vanderwerken, and David Wells. In addition, Steve Pallardy of the School of Natural Resources, University of Missouri-Columbia, provided a technical review of part of the material.

Appreciation is given to the following staff members of the Instructional Materials Laboratory for their efforts in producing this material: Harley Schlichting, Director; Phyllis Miller and Dan Stapleton, Assistant Directors; Ann Statler and Leslie Forbes, Graphic Designers; Veronica Feilner, Editor; Janice Trimble, Word Processor; and Felicia Lathon and Zuaelie Ahmed, Student Assistants.

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### **FOREWORD**

Development of *Agricultural Science Lab Activities* is the result of MVATA Teaching Aids Committee suggestions. The activities were developed to enhance Agricultural Science I and Agricultural Science II courses for grades 9 and 10.

Much has been written about the value of experiential learning and the problem-solving instructional approach. These activities are designed to enrich existing curriculum by providing additional lab-based activities for Agricultural Science I and II classes. Missouri Core Competencies and Key Skills have been identified in most activities to encourage attainment of those educational outcomes. These 20 activities are cross-referenced to related agricultural science competencies. This cross-reference is identified in each activity (Student Outcomes and Scientific Principles sections), as well as in the Organizational Chart found in these introductory pages.

For tips on managing lab-based classes, see the Instructor Notes section of these introductory pages.

This instructor guide and the corresponding student reference contain activities in the areas of: Animal Nutrition, Animal Breeding, Poultry, Plant Science, and Crop Science.

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Animal Nutrition  • Absorption of Nutrients  • Bacteria and Disease  • Enzyme Digestion  • Mycotoxins
Animal Breeding  • Artificial Insemination  • DNA Extraction  • Genetics  • Sperm Motility
Poultry • Embryology
Plant and Crop Science

# **Plant Science**

- Growth Regulators
- Herbicides
- Photosynthesis
- Tropism
- Tissue Culturing

# **Crop Science**

- Fermentation
- Hydroponics

# AGRICULTURAL SCIENCE LAB ACTIVITIES ORGANIZATIONAL CHART

VIII VIII VIII VIII VIII VIII VIII VII		Science competency (from Missouri Core	Preparation and	
Microscope Use			Preparation time: 20 min. Class time: 50 min.	Compound microscope, cloth or paper towel, dropper, lens paper, scissors, dish soap, glass slide, coverslip, magazine, probe, water, absorbent cotton
		Animal Nutrition		
Absorption of	Animal Nutrition 1: Compare the	100-5: Identify the variables and controls	Activity 1 Length:	Hot water hath 750 ml heaker 1000 ml heaker four
Nutrients	digestive systems of ruminants and	in a laboratory experiment involving	55 minutes	test tubes, test tube rack with test tubes, string or
	nonruminants and determine how	osmosis and reach a conclusion from the		thread, distilled water for rinsing, 20 cm dialysis
	the differences between them affect	given data.		tubing, 5 ml Benedict's solution, two drops iodine
	feeding practices.			solution, 5 grams cornstarch, one amylase enzyme
	Animal Nutrition 2: List the basic nutrient requirements and six elements of a ration.	10F-3: Predict the direction and rate of water flow in two different concentrations of a solute separated by a semipermeable membrane.		tablet, one wax pencil
			Activity 2 Length: 10 minutes for four days	(Per group) one egg, 200 ml vinegar, 100 ml corn syrup, 250 ml beaker, scales for weighing
Bacteria and     Disease	Dairy 3: Plan a dairy herd health	8C-1: Hypothesize several ways	Preparation time:	One sterile petri dish with nutrient agar per student or
00000	Piogram:	environmental changes.		group of 2-4 students, one bearer of glass for alcorror, grease marking pencil or felt-tip pen, three sterile
	Plant Science 3: Describe factors	,	Class time: 45 minutes	cotton swabs, alcohol (ethanol or isopropyl), parafilm
	that affect plant growth and development	10L-1: Design/identify an experiment which tests the effects of systematically		or plastic wrap, pipettes or droppers (disposable or reusable), distilled water
	: : : : :	altering variables.		
	Small Grains 5: Describe the factors involved in controlling diseases of small grains.			
• Enzyme	Animal Nutrition 1: Compare the	Þ	Preparation time:	Hot plate, two 150 ml beakers, four test tubes, test
Digestion	digestive systems of ruminants and	products of a chemical reaction.	25 minutes	tube rack, dropper or 1 ml pipette, 150 ml of water, 2 ml iodine solution 5 ml Benedict's solution (indicates
	the differences between them affect	8H-9: Design and conduct an experiment	Class time: 50 minutes	color), 1 gram cornstarch
	feeding practices.	which demonstrates physical and/or chemical changes.		
<ul> <li>Mycotoxins</li> </ul>	Animal Nutrition 2: List the basic nutrient requirements and six	8H-9: Design and construct an experiment which demonstrates physical	Sample extraction: 15 minutes	Grain samples, Aflatoxin Field Kit (available from Neogen Corporation)
	elements of a ration.		:	
	Animal Nutrition 4: List common rules of thumb for feeding livestock.	jni/	Running the field test: 30 minutes	
		things would adapt in order to survive.		

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
		Animal Breeding		
Artificial Insemination	Animal Breeding 2: Identify and describe the function of the parts of the female reproductive system in cattle and swine.	10B-4: Describe the structure and function of human reproductive organs.	Preparation time: 30 minutes Class time: 45 minutes	Dissecting microscope, artificial insemination equipment (semen - frozen or fresh; catheter - plastic, glass, or metal; polybulb - syringe; ice water - a can or plastic container for holding frozen semen; paper towels; female reproductive tracts), scissors, scalpel, dissection tray, dissection pins, magnifying glass, tweezers
DNA Extraction	Animal Breeding 3: Describe the heritability of traits in animals. Animal Breeding 4: Describe procedures for making genetic improvements in livestock.	Science 10B-3: Describe the structure and function of DNA. Science 10D-5: Devise an experiment that will test a stated hypothesis.	Activity 1 Length: Preparation time: 10-20 minutes Class time: 30-45 minutes	1 ml 3M sodium acetate solution, one test tube (1 ml) of DNA (salmon testes), 2 ml of ethanol or isopropyl alcohol, one glass stirring rod , stirring rods, pipettes, 2 ml pipette
		Science 10D-9: Analyze the risks and benefits of genetic engineering to society.	Activity 2 Length: Preparation time: 10-20 minutes Class time: 30-45 minutes	250 ml beaker, 3.5 g yeast, 95 percent ethanol, 5 ml liquid detergent (Palmolive Green preferred), 3 g meat tenderizer, sodium bicarbonate (baking soda), warm (50-60°C) water, thermometer, glass stirring rod, 600 ml beaker, 100 ml graduated cylinder, pH meter, safety glasses for each student
• Genetics	Animal Breeding 3: Describe the heritability traits in animals. Animal Breeding 4: Describe procedures for making genetic improvements in livestock.	10C-1: Predict the phenotypic and genotypic ratios of the offspring of a dihybrid cross using a Punnett square. 10C-4: Associate the roles of genetic variation and natural selection with changes in organisms over time.	Preparation time: 60 minutes Class time: two periods	One cup, watch, 10 red beans, 10 speckled beans, a bag of mixed beans (50 percent red and 50 percent speckled), 10 toothpicks (dyed green), 10 toothpicks (natural color or dyed brown), 10 toothpicks (dyed red)
• Sperm Motility	Animal Breeding 1: Identify and describe the function of the parts of the male reproductive system in cattle and swine.  Animal Breeding 2: Identify and describe the function of the parts of the female reproductive system in cattle and swine.	10B-4: Describe the structure and function of human reproductive organs.	Preparation time: 30-60 minutes (Microscope Use activity should be completed before this activity.) Class time: up to three periods	Microscope with 10X, 40X, and 100X (oil immersion) objectives; laboratory thermometer; wide-mouth thermos; hot plate; pan; prepared slide of sperm; prepared slide of an ovary; prepared sperm slide of another animal; straw of bull semen; paper towel; two slides per team; one cover slip per team; eye dropper; scissors

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
		Poultry		
• Embryology	Poultry 2: Select and evaluate poultry.	Science 10C-7: Sequence the developmental stages of the human fetus.	Preparation time: 20 minutes	Incubator; sharp, pointed, dissecting scissors; magnifying glass or dissecting microscope; petri dish; metric ruler; forceps; dropper; one fertile egg
	Poultry 6: Describe various marketing channels for poultry and		Activity time: 45 minutes on first day, 5-10	
	eggs.		minutes for each observation day, and 20-30 minutes when eags are opened	one unfertilized egg; paper towels
		Plant and Crop Science	nce	
Germination	Plant Science 5: Identify the steps	10A-5: Classify selected plants into	Preparation time:	Per student:
	in germination of monocot and dicot	taxonomic groups (e.g., moss, fern,	60 minutes	10 turnip seeds or Wisconsin Fast Plants seeds; petri
	seeds.	gymnosperm, monocous, arcous).	TZ test: 45 minutes	disn; piece or niter paper (9 cm, No. 2); torceps; plastic grid sheet; water reservoir (plastic, 2-liter
	Crop Science 5: Explain the			bottle); graph paper
	requirements for establishing a crop		Rag doll test: 20	For TZ Test (per student):
	stand.	pnotosyntnesis.	minutes	10-20 sweet corn seeds; 1 g of 2,3,5 TZ; small con-
			Germination test observation: four partial	tainer to soak seeds; single-edged safety razor or utility knife: paper towels: magnifying lens
			periods	For entire class:
				Hand lens or steriomicroscope; fluorescent light bank
				For Rag Doll Test: Danor townle, mithor hande, condo (inctructor)
				raper towers, rubber barrus, seeds (instructor s choice), plastic bags
Plant Taxonomy	Crop Science 3: Identify common plants and weeds in Missouri.	10A-5: Classify selected plants into taxonomic groups (e.g., moss, fern.	Preparation time:	Different tree leaves and tree parts to use for identification (at instructor's discretion)
	Plant Science 2: List and describe		Class time: One class	
	methods to classify plants.		period or more, depend-	
	: :		ing on the depth the	
	Forestry 2: Classify Missouri trees		instructor would like to	
	by use and grade.		have students involved	

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
Water Quality	Plant Science 4: Describe how pests affect plant growth. Crop Science 9: Describe methods of plant pest control. Small Grains 3: Describe the practices necessary to establish a satisfactory stand of small grains.	8C-1: Hypothesize several ways organisms would respond to drastic environmental changes.  8D-4: Predict the effects of pollution (e.g., acid rain, smog, oil spills, excess pesticides, and fertilizer runoff) on the environment.  9D-4: Predict possible local environmental consequences based on factors such as increased population, amount of available fresh water, and amount and types of pollution.  9K-5: Observe the effects of man-made	Preparation time: 90 minutes to construct bottles and for laboratory setup Class time: 10 minutes during four days to collect data	Six teams of 2-5 students each, six 2-liter plastic soda bottles with caps, distilled water, 100 ml of 5 percent detergent solution, 100 ml graduated cylinder, 10 ml graduated pipette, 48 two-frond colonies of duckweed ( <i>Lemna sp.</i> ), hand lens for counting fronds, graph paper, liquid fertilizer (mixed to standard strength), scalpel, felt-tip marker
		Plant Science		
• Growth Regulators	Plant Science 7: Describe how plants reproduce sexually and asexually. Plant Science 8: Describe how genetics influences plant growth.	1	Preparation time: 60- 90 minutes Observation time: 20 days (none during days 2-7, 5-10 minutes during days 8-16, and 50 minutes on day 20)	Hormone Kit (Carolina Biological, 15-8728 or 15-8730), rulers
• Herbicides	Horticulture 3: Identify characteristics and production practices for warm season solanaceous crops.  Plant Science 6: Describe good crop production practices.  Plant Science 8: Describe how genetics influences plant growth.	9K-5: Observe the effects of man-made pollutants on the atmosphere.  10A-5: Classify selected plants into taxonomic groups (e.g., moss, fern, gymnosperm, monocots, dicots).  10C-2: Hypothesize how genetic resistance develops from continued exposure to pesticides or antibiotics.	Preparation time: 45 minutes Observation time: two weeks	Monocotyledon and dicotyledon seeds (Corn and soybeans or oats and radishes work well.); planting flats or small pots; potted broadleaf plants; selective herbicide (2,4-D, 2,4,5-T, MCPP, Weed-B-Gon <sup>TM</sup> and Grass-B-Gon <sup>TM</sup> are readily available at garden stores.); non-selective herbicide (Roundup <sup>TM</sup> , Kleenup <sup>TM</sup> )

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Preparation and Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
• Photosynthesis	Small Grains 1: Describe the soil and climatic adaptations and the uses of small grains.  Plant Science 3: Describe factors that affect plant growth and development.  Plant Science 6: Describe basic plant processes.	9D-1: Predict the rate of photosynthesis under various environmental conditions. 9D-2: Predict the rate of respiration under various environmental conditions. 9G-1: Infer how energy can be transformed from one form to another. 10A-7: Describe the significance of the light and dark phases of photosynthesis. 10C-6: Design an experiment in which the growth of plants is affected by photosynthetic condition. 10D-8: Describe general ways in which human activities affect environmental quality.	- Rate of Photosyn- thesis (30 min.) - Sugar Production and Photosynthesis (20 min.) - Light and Photosynthesis (10 min.) - Carbon Dioxide and Photosynthesis (30 min.) - Chlorophyll and Photosynthesis (20 min.) - Chlorophyll and Photosynthesis (20 min.) - Water and Photosyn- thesis (20 min.)	Rate of Photosynthesis:  Anacharis (Elodea) from aquarium supply, sodium bicarbonate (baking soda), four test tubes, test tube rack, bright light source, four glass stirring rods or cut pieces of glass tubing and Photosynthesis:  Two coleus plants, clinitest tablets, two test tubes, mortar and pestle, beaker of water, graduated cylinder, filter paper, funnel, labels or wax marking pencil Sugar Production and Photosynthesis:  Cornstarch, test tube with about 25 mm of distilled water, pinch of sugar, ethyl alcohol, hot plate or Bunsen burner, pan, petri dish  Light and Photosynthesis:  Black cardboard, paper clips, potted plant  Carbon Dioxide and Photosynthesis:  Petroleum jelly; see Photosynthesis and Starch activity  Water and Photosynthesis and Starch activity  Water and Photosynthesis and Starch activity
• Tropism	Plant Science 3: Describe factors that affect plant growth and development. Plant Science 6: Describe basic plant processes.	9C-1: Observe tropisms in plants due to stimuli.	Preparation time: Three days (Seedlings from Germination activity could be used to shorten this time.) Class time: One class period for set-up; three partial periods for observations	Lightproof, 35 mm plastic film canisters; plastic film sheets (red, blue, and green); 1/4" hand-held paper punch; clear plastic tape and black electrical tape; 2 cm square pleces of blotting paper or absorbent paper towels; waterproof pen; turnip seeds or RCBr seeds (available from Wisconsin Fast Plants, Carolina Biological Supply); 3- to 5-day-old seedlings; com seeds; small pieces of cardboard, 13 cm x 13 cm; chlorine bleach, 5 percent solution; straight pins; plastic bags

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
• Tissue Culturing	Plant Science 7: Describe how plants reproduce sexually and asexually. Plant Science 8: Describe how genetics influences plant growth.	10C-4: Associate the roles of genetic variation and natural selection with changes in organisms over time.  10D-9: Analyze the risks and benefits of genetic engineering to society.	Creating a transfer chamber Preparation time: 15-30 minutes Class time: 45 minutes Tissue culturing a weeping fig Preparation time: 15-30 minutes Class time: 50 minutes Plansferring ficus plantlets to rooting media Preparation time: 15-30 minutes Class time: 50 minutes Class time: 50 minutes	Creating a transfer chamber: Cardboard box (approximate size 30"L x 18"W x 18"H), 70 percent ethanol or isopropanol, plastic wrap, aluminum foil, spray bottle, paper towels, surgical gloves, empty beaker, sterile petri dish, scalpel  Tissue culturing a weeping fig: Ficus benjamina plant (weeping fig): 95 percent ethanol or isopropanol, 100 ml; 70 percent ethanol or isopropanol in a spray bottle; two test tubes of shoot multiplication medium  4 x 4" squares of cheesecloth; forceps; transfer chamber; marking pen; scissors; 100 ml distilled water; 10% bleach, 75 ml; dishwashing detergent; sterile petri dish; parafilm; scalpel  Transferring ficus plantlets to rooting media: 95 percent ethanol/isopropanol, 100 ml; 70 percent ethanol in a spray bottle; 100 ml distilled water; two test tubes of ficus rooting medium; ficus explants with shoots (previous activity); transfer chamber; grow shelf space; marking pen; forceps; sterile petri dish; parafilm
		Crop Science		
• Fermentation	Crop Science 8: Describe factors related to harvesting and storing quality forages.  Small Grains 6: Determine the most profitable practices and methods of	8C-1: Hypothesize several ways organisms would respond to drastic environmental changes.  8E-8: Identify methods of determining whether a solution is basic, neutral, or acidic.  8H-7: Describe the reactant and products of a chemical reaction.  8H-9: Design and conduct an experiment which demonstrates physical and/or chemical changes.  9D-6: Identify the control, the dependent, and the independent variables in an experiment.	Two hours to collect materials and prepare solutions  Collect plant material 12-24 hours prior to the experiment.  One class period to demonstrate and for students to construct bottle chambers (Allow 24 hours for silicone in the chambers to dry.)  One class period to chop materials, seal the bottle, and add the indicator solution	Per group:  Two clear, 2-liter plastic bottles with plastic tops; two small test tubes; two 45 cm long pieces of plastic tubing (Tygon 5/16" O.D., 3/16" I.D.); five ears of com (with husks) or one grocery bag of alfalfa, grass clippings, or other green material; tube of silicone sealant; safety razor; sturdy, large kitchen knife; cutting board (or a flat piece of wood or tray); hole punch; sharp needle; heat source to heat a hole poker (needle or paper clip); balance and/or scale; tapered reamer or drill; mortar and pestle; scissors; beaker, 250 ml or larger; thermometer; black electrical tape; pH paper (to measure from a pH of 3-7 in increments of 0.5 units or less); microwave; lime water (calcium hydroxide) (optional); cabbage juice pH indicator solution (optional)

Activity title	Agri. Science I/II competency	Science competency (from Missouri Core Preparation and Competencies/Key Skills)	Preparation and class time	Materials and equipment needed
• Fermentation (cont.)		9E-6: Identify the four basic types of chemical reactions: composition, decomposition, single replacement, and double replacement.	If possible, start materials fermenting on a Monday so students can observe the chambers for the remainder of the week. Take 2-3 observations the next week and one the following week.  An additional period might be needed for discussion of the product and the read-	
• Hydroponics	Horticulture 1: Describe preparations necessary for fruit and vegetable production.  Horticulture 3: Identify characteristics and production practices for warm season solanaceous crops.  Plant Science 3: Describe factors that affect plant growth and development.	9C-2: Infer from examples of environmental conditions the ways in which living things would adapt in order to survive.  10E-1: Compare and contrast organic and inorganic compounds.	Class time: 50 minutes	Electrical conductivity (EC) meter, two-liter soda bottles (2), felt-tip marker, sharp knife, scissors, aluminum foil, plastic aquarium tubing, plastic soda straw, aquarium pump with three-way valve, plants, plastic bag, water (either tap or distilled), hydroponic nutrient solution (can be ordered from science education suppliers or hydroponic suppliers)

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4. Kits

a. Bacteria: Bacteria Student Materials Set (15-4600). Carolina Biological, 2700 York

Road, Burlington, NC 27215-3398. 800/334-5551 (orders) and 800/227-1150

(technical support).

b. DNA Extraction: Bacteria DNA Extraction Activity, Demonstration Kit (88 W 8106) or a 24-

student, class-sized kit (88 W 8112). Ward's, PO Box 92912, Rochester, NY

14692-9012. 800/962-2660.

Bacteria DNA Extraction Kit, Teacher Demonstration Kit (15-4702) or a

30-student, class-sized kit (15-4701). Carolina Biological.

DNA Spooling Kit (D-8666). Sigma Chemical, PO Box 14508, St. Louis, MO

63178-9916. 800/325-3010 or FAX 800/325=5052.

c. Growth Regulators: Hormone Kit (15-8728 or 15-8730). Carolina Blological.

d. Mycotoxins: Aflatoxin Field Kit. Neogen Corporation, 620 Lesher Place, Lansing, MI

48912. 800/234-5333.

5. Other

 Mathematics and Science Education Center, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, MO 63121. 314/553-5650. b. *Interactive Multimedia Courseware.* Technical Writing and Education, 82 Park Ave., Rutherford, NJ 07070. 201/507-0030, FAX 201/507=0192.

# **INSTRUCTOR NOTES**

These suggestions may help the teacher managing lab-based activities. Recommended procedures that should be employed when teaching in this manner are:

- 1. Describe the purpose of an experiment.
- 2. Outline why experiments are used in teaching.
- 3. Identify available resource materials.
- 4. Develop the ability to organize an experiment.
- 5. Develop the ability to prepare students for an experiment.
- 6. Identify the usefulness of an experiment.
- 7. Develop a method of evaluating the material learned.

Students should have a knowledge of experimental science and the laboratory equipment to be used. Never take for granted that students know the proper names of laboratory equipment. (See the following three pages of illustrations.)

Preparation is the key to success with any experiment. Before presenting an experiment, always do a dry run. If the experiment involves a perishable product and you only have enough material for one experiment, go through a fake experiment (without students). Have all equipment in place before starting the experiment. If you or a student have to go to the science room to borrow equipment, the experiment may fail before it begins.

Recording data from the experiment is essential. Be sure students record results, dates, conditions, what they did, etc. Never leave anything to memory. Keep a copy for yourself.

Check the experiment's running time. If it is an experiment that will take longer than the class period, find a good stopping point or a method to continue it after the bell rings.

Take care in scheduling the day of the week you perform the experiment. You don't want to perform an experiment that takes six days if the final day's procedure is on a weekend or holiday.

The students should be in the proper mind-set to perform experiments. They should act and feel like scientists to follow proper procedure, make proper measurements, perform proper recordings, and use proper sanitary techniques. Students must know *why* they are performing the experiment--what they trying to discover. The experiment should closely follow the lesson or unit that you are teaching.

When the experiment is finished, the students should write a report or summarize what they found. (If they know this in advance, they will pay closer attention.)

For a "sponge activity" (filler for awkward times), the students can record results of the experiment each day.

Pair up lab partners; don't let students pick their own partners. Strategically do this so you pair struggling students with ones who find it easy. (Do not make this strategy obvious.) Students will get the opportunity to work with people from different backgrounds, which helps build your Agricultural Education family.

Record results on wall charts, bulletin boards, etc., so other classes can see what is happening in class. This helps with recruiting and gives younger students a chance to see what is going on in the advanced classes. Exhibiting the results of experiments to the school through the FFA trophy case will be a great public relations activity for your program.

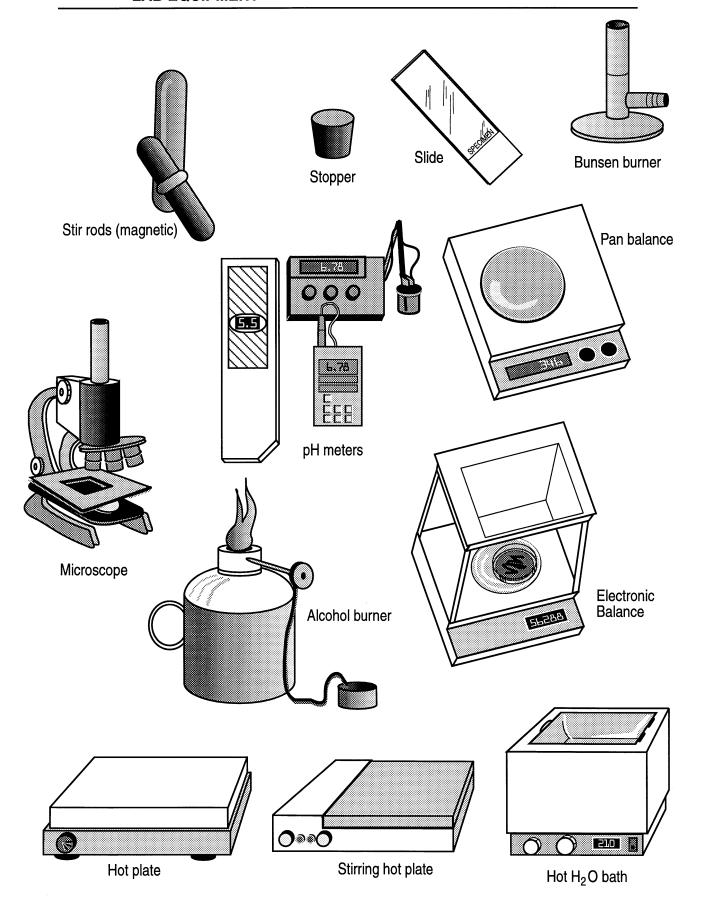
Work closely with the science teacher. Science students who see you up in the science room will realize that agriculture is more than just cows and plows. It can be a great recruiting instrument.

If you are performing the experiment, be sure all students can see what is happening, are involved in the process, and/or understand the process.

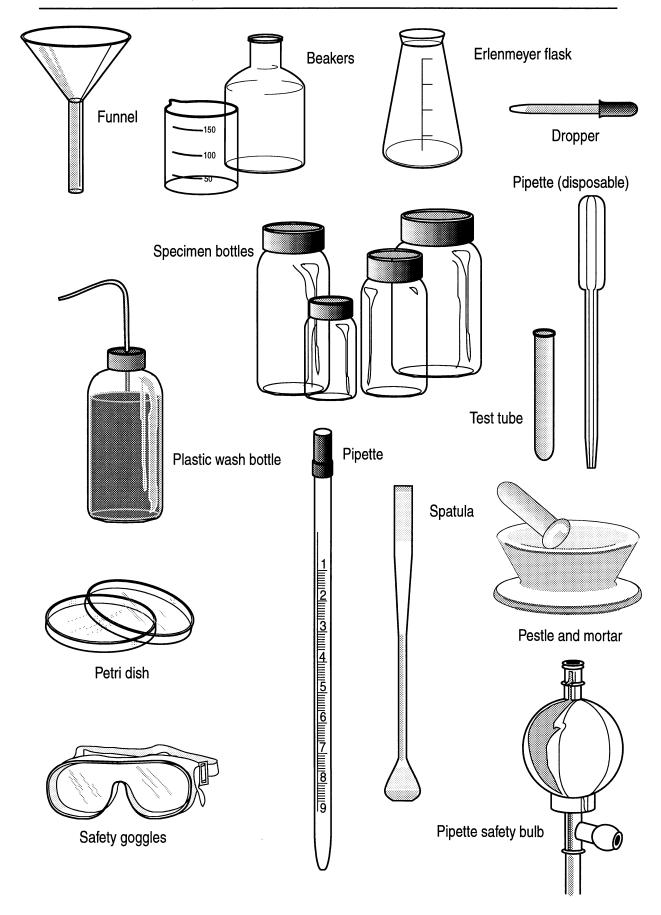
# Common questions about teaching with experiments

- Q: What is the purpose of an experiment?
  - A: A test, trial, or process used to discover something not yet known or to demonstrate something already known.
- Q: What is the value of using experiments in teaching?
  - Experiments create a high degree of interest because of active involvement.
  - Students learn better and more because they are actively involved in the learning process and use their own minds to draw conclusions.
  - Students create their own information by setting up the experiment, making observations, collecting data, and developing conclusions.
  - This method visually illustrates important concepts while applying theories and concepts.
  - Experiments teach students to think systematically, clearly, and thoroughly.
  - Students learn to keep careful records and carry out procedures in an orderly, step-by-step fashion.
  - Students learn to examine the facts and draw conclusions.
- Q: Where are resource materials found?
  - Scientific catalogs
    - Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. 800/334-5551 (orders) and 800/227-1150 (technical support).
    - ii. Daigger, 199 Carpenter Avenue, Wheeling, IL 60090. 800/621-7193.
    - iii. Edmund Scientific, 101 E. Gloucester Pike, Barrington, NJ 08007-1380. 609/573-6250.
    - iv. Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219-9919. 800/388-8355.
    - v. NASCO, 901 Janesville Ave., Fort Atkinson, WI 53538-0901. 800/558-9595.
    - vi. Neogen Corporation, 620 Lesher Place, Lansing, MI 48912. 800/234-5333.
    - vii. Sargent-Welch, 911 Commerce Court, Buffalo Grove, IL 60089-2362. 800/727-4368.
    - viii. Science Kit and Boreal Laboratories, 777 East Park Drive, Tonawanda, NY 14150-6784. 800/828-7777.
    - ix. Sigma Chemical, PO Box 14508, St. Louis, MO 63178-9916. 800/325-3010.
    - x. United States Biochemical Corporation, PO Box 22400, Cleveland, OH 44122-9619. 800/321-9322.
    - xi. Wards, PO Box 92912, Rochester, NY 14692-9012. 800/962-2660.
  - Resource books, especially laboratory manuals
  - Curriculum guides

- Agricultural and science magazines
  - 1. Science of Food and Agriculture
  - 2. Agricultural Research
  - 3. Monsanto
  - 4. Many different news magazines
- Colleagues are a great resource. Always leave an agricultural education department or science classroom with something new or an idea that works.
- Q: How will you organize the experiment?
  - Compile a list of equipment and materials needed for the experiment. Do you need equipment/materials for each group, or can the groups share?
  - Practice (dry run) the experiment.
  - Estimate the time needed to complete the experiment.
  - Decide the grouping, number of lab partners, and how you plan to split the group in advance.
  - Establish a lab leader for each group.
  - Develop a written plan for the student and/or teacher to follow in carrying out the procedure.
  - Develop a system for gathering and reporting the data and conclusions.
- Q: How is the experiment carried out?
  - Create student interest in the experiment.
  - Develop a laboratory research attitude among the students.
  - Distribute written directions; go over the directions orally with the class.
  - When it is time for the students to begin the experiment, supervise students closely.
  - Once the data is collected, help in analyzing the results and developing sound conclusions.
  - Stimulate class discussion and/or have each group report results to the class.
  - · Summarize and check for understanding.



# **LAB EQUIPMENT**



# **LAB EQUIPMENT**



• Microscope Use

# Microscope Use

### Situation/Problem:

Many times, scientists must enlarge an organism to identify the cause of disease in plants and animals. When the organism is identified, treatment or prevention programs can be utilized. What instrument can be used to help identify very small organisms that cause many plant and animal diseases?

# **Activity Length:**

Preparation time: 20 min. Class time: 50 min.

One of the basic tools used by agricultural research scientists is the compound microscope. This activity will help you develop skills in using the microscope.

# **Activity Objectives:**

- 1. Identify parts of the microscope.
- 2. Adjust the microscope.
- 3. Prepare slides to study different organisms and materials that affect agriculture.

# **Vocabulary Terms:**

Arm - supports the body tube.

Base - supports the microscope.

Body tube - maintains a set distance between the eyepiece and objective lens.

Coarse focus - moves the body tube up and down.

Coverslip - thin piece of glass or plastic that is placed over the specimen on the slide.

*Diaphragm* - regulates light from the mirror or light source.

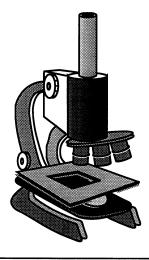
Eyepiece - contains lenses for magnification.

Fine focus - focuses and sharpens the image.

Mirror or light - provides light to illuminate specimen.

Nosepiece - contains high- and low-power objective lenses.

Objective (lens) - the microscope lens nearest to the object observed; focuses light to form the image of the object.



# Microscope Use

Resolution - capability of a microscope to provide fine detail.

Slide - small glass or plastic plate used to mount objects to be examined under a microscope.

Stage - supports the slide being viewed.

Stage clips - holds the slide in place on the stage.

Turret - rotating wheel on which objective lenses are mounted.

Working distance - space between the lens and the top of the coverslip.

# **Equipment and Materials:**

Compound microscope Cloth or paper towel

Dropper Lens paper Scissors Dish soap Glass slide Coverslip Magazine Probe

Water Absorbent cotton

# **Procedures:**

Microscope parts and handling

- 1. To move the microscope, place one hand beneath the base and firmly grasp the arm of the microscope with the other hand.
- 2. Place the microscope on the table with the arm toward you and the stage away from you. The base should be about 8-10 cm from the table's edge.
- 3. Identify the parts of the microscope.

Preparing wet-mount slide

NOTE: Specimens observed though a microscope are placed on a glass slide. A coverslip is placed over the specimen on the slide. Water is usually placed between the slide and the coverslip.

# Microscope Use

- 1. Rinse a slide in soapy water. Gently wipe both sides of the slide with a clean paper towel.
- 2. To prevent smudges, always handle slides by the edge rather than the flat surface.
- 3. From newsprint, cut out a small square with a lower case "e."
- 4. Place the paper square with the letter "e" in the center of the slide. Use a medicine dropper to place a drop of water in the center of the slide.
- 5. Place the coverslip at a 45-degree angle over the drop of water and the paper. With the probe, gently lower the coverslip into position on the slide.
- 6. If air bubbles are present, gently tap the coverslip directly over the air bubbles with the eraser of a pencil.
- 7. Clip the slide to the stage with the stage clips.

# Focusing the microscope

- 1. Using the coarse focus, raise the body tube of the microscope until the objective lens is 2-3 cm above the opening in the stage.
- 2. Revolve the nosepiece so the low-power objective (10X) "clicks" and locks directly in line with the body tube.
- 3. Adjust the diaphragm so the greatest amount of light is coming through the opening in the stage.
- 4. Looking from the side, use the coarse focus to lower the body tube as far as it will go without hitting the slide. **Do not force the body tube.**
- 5. Look through the eyepiece. Using the coarse adjustment, focus until the "e" comes into view.

# Microscope Use

- 6. Use the fine focus knob to bring the letter "e" into proper focus.
- 7. Sketch the letter as you see it under low power.
- 8. Move the slide toward your right.
- 9. Move the slide away from the microscope arm. Recenter the slide.
- 10. Rotate the turret to the high-power lens and focus with the fine-focus knob.
- 11. Compare your observations of the letter under high and low power.
- 12. Sketch the letter under high power.
- 13. Compute the total magnification image by multiplying the eyepiece magnification by the magnification of the objective lens. The magnification rate of the lenses of your microscope should be imprinted on the barrel of the lens.

# Resolving power and depth of focus

- 1. Prepare a wet mount of a very small piece of absorbent cotton. Pull or tear the cotton to create air spaces between the fibers.
- 2. Observe the cotton fibers with low power and then with higher power.
- 3. Sketch the cotton as observed in the field of view with both powers.

# **Answers to Key Questions:**

 It seemed to move to the left. It seemed to move in the opposite direction.

# **Key Questions:**

•	What happened to slide to the right?	the letter when you moved the
		_ Away from the microscope arm

Ą	gricultural Research:	Mi	croscope Use
2.	It is actually moving from the bottom to the top.	2.	If you see a living organism moving from the top to the bottom of the field of view, what do you know about the organism's direction of travel?
3.	It may move out of the field of view because of its original position in the field of view.	3.	How did the letter "e" change position upon switching to high power?
4.	The magnification rate reduces the light.	4.	Why did the field of view get dimmer as you switched to high power?
5.	Low power is usually 100x magnification.	5.	What is the total magnification of the image with the microscope on low power?
6.	High power magnifies the image anywhere from 400X to 440X, depending on the microscope being used.	6.	What is the total magnification of the image with the microscope on high power?
7.	As you change the focus of the lens, one strand will go out of focus, and another one then comes into sharp focus.	7.	How can you decide which strand of cotton overlays another?
8.	The greater the depth of field, the more of the specimen that will be in focus. As the resolution of the lens changes, the depth of focus also change.	8.	In microscope observations, why is it important to resolve power and depth of focus problems?
9.	As the objective is switched to high power, the focusing ability is greatly narrowed. The depth of field is reduced, and the focusing range is very narrow and hard to focus.	9.	How does the focusing ability change when the objective is switched from low to high power?

# Microscope Use

# Supplemental References:

Agriscience Activity Manual. Pierre: South Dakota Curriculum Center, 1994.

Lee, J. S., and D. L. Turner. *Introduction to World AgriScience and Technology (Activity Manual)*. Danville, IL: Interstate Publishers, Inc., 1994.

# **Applications:**

The microscope is not a new invention. It was developed in the 17th century by the Dutch scientist Anton van Leeuwenhoek. Refinement of Leeuwenhoek's crude microscope has led to the development of a very powerful tool. The microscope has enabled scientists to discover new worlds of knowledge in medicine, industry, and agriculture. Magnification is accomplished by a series of ground and adjusted lenses. With a microscope, even small pieces that cannot be seen with the naked eye becomes visible.

# **Extensions:**

- 1. Using a microscope, compare the differences between a plant cell and an animal cell.
- 2. Examine pond water with a microscope.

# **Animal Nutrition**

- Absorption of Nutrients
- Bacteria and Disease
- Enzyme Digestion
- Mycotoxins

# **Animal Nutrition:**

# **Absorption of Nutrients**

### **Student Outcomes:**

Animal Nutrition 1: Compare the digestive systems of ruminants and nonruminants and determine how the differences between them affect feeding practices.

Animal Nutrition 2: List the basic nutrient requirements and six elements of a ration.

### Situation/Problem:

How does a feed substance such as grain become digested and absorbed into the animal's bloodstream?

What happens to feed that is not digestible?

Where do most of the digestion and absorption occur in a ruminant?

Where do most of the digestion and absorption occur in a nonruminant?

Why is the digestibility of a feed important?

# **Scientific Principles:**

10C-5: Identify the variables and controls in a laboratory experiment involving osmosis and reach a conclusion from the given data.

10F-3: Predict the direction and rate of water flow in two different concentrations of a solute separated by a semipermeable membrane.

Activity 1 Length: 55 minutes

Activity 2 Length: 10 minutes for

four days

This activity will show how feed substances are broken down and absorbed into the bloodstream. What factors affect the digestibility of a feedstuff?

# **Activity Objectives:**

- 1. Describe the role of the plasma membrane in animal cells.
- 2. Explain how a selectively permeable membrane works.
- 3. Relate the role of enzymes to the process of absorption.

# **Vocabulary Terms:**

Absorption - the passage of digested food from the alimentary tract to the circulatory system.

Alimentary tract - from mouth to anus, the passage through which food passes.

Digestion - changes that food undergoes within the digestive tract to prepare it for absorption and use in the body.

Nutrients - components of a feed that aid in the support of life.

Osmosis - flow of a fluid through a semipermeable membrane separating two solutions, which permits the passage of the solvent but not the dissolved substance. The liquid will flow from a weaker to a stronger solution, thus tending to equalize concentrations.

# **Animal Nutrition:**

# **Absorption of Nutrients**

# Activity 1

# **Equipment and Materials:**

Hot water bath
750 ml beaker
1000 ml beaker
Four test tubes
Test tube rack with test tubes
String or thread
Distilled water for rinsing
20 cm dialysis tubing
5 ml Benedict's solution
Two drops iodine solution
5 grams cornstarch
One amylase enzyme tablet
One wax pencil

### **Instructor Procedure:**

Prepare a starch suspension ahead of time by mixing 5 grams of cornstarch in 500 ml of hot water. Bring the mixture to a boil until it thickens and forms a clear suspension. Set aside to cool.

# **Procedure:**

- Cut a 20 cm piece of dialysis tubing and place in water for a few minutes. Rub the dialysis tubing between your thumb and forefinger until it separates and the tubing opens completely. Twist one end of the tubing, fold backward, and secure with string to close the end.
- 2. Pour cornstarch suspension into the tubing until it is two-thirds full.
- 3. Add an enzyme tablet to the solution and tie the top of the dialysis "bag" closed, leaving a small airspace in the top. Make sure the bag doesn't leak.
- 4. Rinse the bag's outside thoroughly in distilled water.
- 5. Place the bag in a beaker of distilled water and allow to stand 30 minutes.
- 6. Fill a test tube one-third full of starch suspension.
  Add two drops of iodine solution and observe color.

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# **Absorption of Nutrients**

- 7. Fill a separate test tube one-third full of starch and enzyme suspension and add 5 ml of Benedict's solution. Heat in a water bath for five minutes and observe color change.
- 8. Label four test tubes 1, 2, 3, and 4. After the dialysis bag has set for 30 minutes, remove 5 ml of water from the beaker and place in test tube 1. Repeat for test tube 2.
- 9. Open the bag. Remove 10 ml of starch suspension and place equal amounts in test tubes 3 and 4.
- 10. Add two drops of iodine solution to test tubes 1 and3. Add 5 ml of Benedict's solution to test tubes 2and 4. Place the test tubes containing Benedict's solution in a hot water bath for five minutes.

11.	Record c	olor cha	nges in e	each of the	test tubes.	

### **Answers to Key Questions:**

- The airspace gets slightly smaller because water moved into the bag from the beaker. This happened because the concentration of water inside the bag was less than in the beaker.
- The amylase enzyme tablet converted some of the starch suspension to sugar, which moved through the membrane of the dialysis tubing into the beaker.
- 3. There was no color change because there was no presence of sugar or starch.

# Key Questions:1. How would you explain the change in the size of the

Aı	nimal Nutrition:	Ab	sorption of Nutrients
4.	It allows small molecules to pass through, while preventing movement of larger molecules.	4.	What does a selectively permeable membrane do?
5.	Through absorption	5.	How do nutrients and waste products move in and out of a cell?
6.	When the concentration of nutrients outside a cell is greater than the concentration of nutrients within the cell, osmosis occurs.	6.	When does osmosis occur in living cells?
7.	Proteins, carbohydrates, and fats	7.	What nutrients can be absorbed by cells?
8.	After nutrients are absorbed, they are transferred through the body by the blood.	8.	How are nutrients transferred through the body?
		Act	tivity 2
		1 eg 200 100 250	uipment and Materials (per group): gg ml vinegar ml corn syrup ml beaker ales for weighing
		<b>Pro</b> 1.	cedure: Soak the egg in 200 ml of vinegar for approximately 48 hours.
		2.	Carefully remove the egg shell. Be careful not to break the inner egg membrane.
		3.	Weigh and record.
		4.	Place the egg in enough corn syrup to cover the entire egg for 24 hours.
		<u> </u>	

Animal Nutrition:	Absorption of Nutrients		
	<ul> <li>5. Observe the egg after 24 hours. Rinse, weigh, and record.</li> <li>6. Place the egg in enough water to cover it; allow it to stand for 24 hours. Rinse, weigh, and record.</li> </ul>		
Answers to Key Questions:  1. It absorbed the corn syrup. Osmosis causes the egg to absorb syrup until the inner concentration is equal to the outer concentration.	Key Questions:  1. What happened after the egg was placed in corn syrup for 24 hours?  Why?		
It swelled up after absorbing more water.	2. What happened when the egg was placed in water?  Why?		

# **Absorption of Nutrients**

#### **Supplemental References:**

Flanders, F., and R. Herren. Lab Manual to Accompany the Science of Animal Agriculture. Albany, NY: Delmar Publishers, Inc., 1994.

Osborne, E., J. Moss, and A. Barger. Biological Science Applications in Agriculture: Animal Science (Teacher's Guide). Urbana-Champaign, III.: University of Illinois, 1993.

## **Applications:**

Proteins, carbohydrates, and fats must be broken down to move from the digestive canal into cells that line the digestive tract and then into the blood. This movement of nutrients is called absorption. Vitamins, minerals, and water are also absorbed from the digestive tract and transported by the blood throughout the body.

Nutrients are absorbed from the digestive tract, mostly the small intestine, at specific locations. Minerals, such as calcium, magnesium, and iron, are absorbed in the uppermost region of the small intestine. Water-soluble vitamins and simple sugars are absorbed from the alkaline environment of the small intestine. Fats and proteins are absorbed in the lower small intestine.

### **Extension:**

Create opposite conditions by putting distilled water in the dialysis bag and immersing it in a starch suspension.

## **Bacteria and Disease**

#### **Student Outcomes:**

Dairy 3: Plan a dairy herd health program.

Plant Science 3: Describe factors that affect plant growth and development

Small Grains 5: Describe the factors involved in controlling diseases of small grains.

#### Situation/Problem:

What are some causes of plant and animal diseases?

What causes meat, milk, and other products to spoil?

What do bacteria look like?

Are all bacteria harmful?

What are some agricultural uses of bacteria?

### **Scientific Principles:**

8C-1: Hypothesize several ways organisms would respond to drastic environmental changes.

10L-1: Design/identify an experiment which tests the effects of systematically altering variables.

#### **Activity Length:**

Preparation time: 30 minutes Class time: 45 minutes

#### **Equipment and Materials:**

There are four different options, depending on budget, time, and/or ability.

 Kit--Carolina Biological student materials set: Bacteria (15-4600, 10 students, \$11.95).
 Contains 10 (15 x 100 ml) petri dishes, two (125 ml) bottles of nutrient agar, 10 alcohol pads, Bacteria are microscopic, one-celled organisms. While many bacteria are beneficial, some bacteria spoil food, and others cause diseases of plants and animals. Bacteria are constantly present in the environment--most of the time without your awareness of them.

This experiment will help you understand the sources of bacteria. Even though the bacteria you culture are not necessarily those that cause animal diseases, these organisms are very common in the environment. You will become aware of the frequent exposure of animals to disease-causing organisms. In this experiment you will culture bacteria so you can see the growth of a bacteria colony.

## **Activity Objectives:**

- Demonstrate the presence, concentration, and bacteria types by inoculating cultures with common items found in the environment.
- 2. Demonstrate the effectiveness of cleanliness and sterility when around plant and animal working areas.
- 3. Observe colonies of bacteria growing on plates of agar.

### **Vocabulary Terms:**

Agar - sugary gel derived from certain algae; used as a medium for cultures.

Aseptic - being free of infectious microorganisms.

Bacteria - single-celled microorganisms. Some cause human, animal, or plant diseases, while others are beneficial.

Control - standard sample used for comparative purposes in experiments; also called check or check plot.

## **Bacteria and Disease**

10 sterile applicator swabs, and an autoclavable disposal bag.

- Petri dishes already poured--NASCO Science (Z13903M, \$11/10 plates). The instructor secures alcohol pads and sterile cotton swabs.
- Ready-to-pour agar--Carolina Biological (77-6360, \$3.60/125 ml, enough for five plates). The instructor secures plastic 15 x 100 ml petri dishes, alcohol pads, and sterile cotton swabs.
- Instructor-prepared agar
   To make 1 liter of agar, add 10 g bacto-typtone, 5 g bactoyeast extract, 10 g NaCl, 950 ml distilled water, adjust pH to 7.4 with NaOH, add 15 g agar, add distilled water to make 1 liter, and autoclave to sterilize.
  - Pour into petri plates on a level surface. Use approximately 25 ml for 90 or 100 mm plates. The final depth of medium in petri plate will be approximately 0.3 cm.
  - · Let agar cool.

#### **Instructor Procedures:**

- Heat bottles of nutrient agar in boiling water for 30 minutes. Let cool.
- When bottle is cool enough to handle barehanded, pour approximately 0.2-0.3 cm of agar in each plate.
- Cover plates and let stand approximately 30 minutes. (You can mix up the agar overnight for the next day, also.)

Disease - any deviation from a normal state of health in plants, animals, or people that temporarily impairs vital functions. It can be caused by viruses, pathogens, parasites, poor nutrition, congenital or inherent deficiencies, unfavorable environment, or any combination of these.

Pathogen - living, microscopic, disease-producing agent, such as a bacterium or virus.

Sterile - free from contamination by living bacterial, fungal, or viral organisms.

Toxin - poisonous protein produced by some higher plants, certain animals, and pathogenic bacteria.

## **Equipment and Materials:**

One sterile petri dish with nutrient agar per student or group of 2-4 students
One beaker or glass for alcohol

Grease marking pencil or felt-tip pen Three sterile cotton swabs

Alcohol (ethanol or isopropyl)
Parafilm or plastic wrap

Pipettes or droppers (disposable or reusable)

Distilled water

#### **Procedures:**

- Obtain sterile petri dish with nutrient agar. Keep the dishes closed until directed to open them.
- 2. Turn the dish over on the table and divide the bottom into fourths by drawing on the bottom with a grease pencil. Number each section. Put your initials on one section of the dish for easy identification.
- 3. Collect three sources of bacteria from the environment. The fourth quadrant of the dish will not be inoculated and will serve as a control. Select

## **Bacteria and Disease**

Caution: Pour plates in a sterile working environment. Air currents or drafts can contaminate plates with bacteria. Proper pouring procedure involves holding the lid of the petri dish slightly opened and covering immediately after pouring. Petri dishes can set for 2-3 days to check for contamination. If fungi or bacteria are growing on plates after 2-3 days, they were contaminated during pouring or during the waiting period.

NOTE: For variety, innoculate an area of a dish with disinfectants (e.g., dilution of isopropyl alcohol or ethanol) to show how they prevent growth.

one source that appears clean, such as the lunchroom tabletop. Other sources may be areas such as your hand, a doorknob, a window sill, the rim of a fish tank, or a piece of your hair.

- 4. Record your sources in the Results section (at the end of this activity) *before* you collect the samples. See attached.
- 5. Obtain a clean cotton swab from a newly opened package. Keep one end of the swab sterile. (Do not touch the sterile end or allow it to contact anything except your source of bacteria.)
- 6. Put a drop of distilled water on one of the chosen sources of bacteria. Gently rub the sterile end of the swab through the drop several times.
- 7. Lift the lid of the petri dish only far enough to insert the cotton swab. Gently touch the swab to the agar surface in the center of the first quadrant. Dispose of the contaminated cotton swab by dipping it into a container of alcohol.
- 8. Repeat steps 5, 6, and 7 to inoculate the other two sections of the dish. Do not inoculate section 4 (control).
- Seal the lid onto the petri dish with parafilm or plastic wrap. The dish should not be opened again, especially after the incubation period.
- 10. At the end of the incubation period (approximately 1-2 weeks), examine the dish for evidence of bacterial growth and record in the Results section. The colony is likely to show a lot of growth if it was inoculated well; if not, hold the dish toward a light source to see the bacterial growth.
- 11. Never open the petri dish. Dispose of the bacterial colony and petri dish as directed by your instructor.

INSTRUCTOR NOTE: Petri dishes should be autoclaved (or cooked in a

## **Bacteria and Disease**

pressure cooker) before disposal to kill living organisms.

### **Answers to Key Questions:**

- To prevent bacteria from escaping the petri dish and contaminating the environment and to prevent outside bacteria from entering the dish
- 2. To prevent airborne, microscopic bacteria from entering the petri dish and contaminating the agar
- 3. No. If yes, the bacteria were airborne from the observer breathing on the agar or from microscopic bacteria falling from the observer or the inoculating cotton swab. Later, bacteria from the other quadrants will infect the control area.
- 4. There are many forms and colonies of bacteria. Bacteria were selected from different areas where different types of bacteria live.
- 5. Technician: to sterilize the environment, preventing bacteria from contaminating an experiment. Veterinarian: to prevent the spread of disease, especially during operations. Producer: to understand the importance of sanitary health procedures, such as when needles and operating equipment.
- 6. Airborne organisms may contaminate the sterile work

## **Key Questions:**

Why is it necessary to tape the dishes shut and keep them shut once the culture has been started? \_ 2. During inoculation of the petri dish with bacteria, why was it important to open the dish only part Were there bacteria in the control area? If so, 3. where might these bacteria have come from? \_\_\_\_\_ 4. Why are the bacteria growths in each quadrant different? Through your observation of bacterial growth, why 5. do you think this is important to learn as a laboratory technician? A veterinarian? A livestock producer?

6. Why are aseptic techniques important when working in sterile environments?

## **Bacteria and Disease**

area and destroy the experiment.

- 7. Inoculation, good nutrition, and a sound herd health program.
- 8. Bacterial infection can alter the plant's growth or kill the plant.
- Yeasts, brewing, vaccines for diseases, hormones for animal metabolism.

7.	What are some methods to prevent bacterial-borne diseases in animals?
8.	How can bacterial infection affect plant growth?
9.	What are some useful benefits of bacteria?

## **Credit:**

Lab Manual/The Science of Animal Agriculture. By Frank Flanders with Ray Herren. Delmar Publishers, Albany, NY, copyright 1994.

## **Bacteria and Disease**

#### **Supplemental References:**

"Educating the Educators in Science and Agriculture," *Biotechnology Protocols.* Princeton, NJ: American Cyanamid Company, Agriculture Research Division, n.d.

York, Walter. *Agriscience*Fundamentals & Applications
Laboratory Manual. Albany, NY:
Delmar Publishers Inc., 1991.

Carolina Biological Supply, 2700 York Road, Burlington, NC. 800/334-5551.

Ward's Biology Supply Catalog, PO Box 92912; Rochester, NY 14692-9012.

## **Applications:**

Bacteria, as well as other harmful and beneficial organisms, are present in the environment. The bacteria provide evidence of the many concentrations and types of bacteria present in the environment. Sterile (aseptic) work areas are important when designing experiments. It is also important to maintain a sterile veterinary and health program when working around animals. The elimination of harmful bacteria when giving animal injections will help reduce the possibility of harmful pathogens entering the animal's immune system.

Parasitic bacteria can harm the animal by feeding off the body cells or by secreting material known as toxins. When large numbers of bacteria invade an animal's body, the animal becomes ill. The type and form of illness depends on the type of bacteria that invades the animal.

Fortunately, animals have lines of defense in fighting disease-causing bacteria. Animals can also be injected with antigens that help produce immunity to some bacteria-borne diseases. Many bacteria are beneficial and live on and in the bodies of animals.

#### **Extensions:**

- Culture, stain, and identify different nonpathogenic bacteria.
- 2. Use different, identified bacteria for experiments in fermentation, antibiotic inhibition of bacterial growth, and many other industrial activities.
- 3. Swab a sterilized surface and compare to an unsterilized surface, or swab a washed surface and compare it to a swab from an unwashed surface.

# **Bacteria and Disease**

Results, Quadrant 1	Results, Quadrant 2
Description:	Description:
·	
Results, Quadrant 3	Results, Quadrant 4 (control)
Description:	Description:

# **Enzyme Digestion**

#### **Student Outcomes:**

Animal Nutrition 1: Compare the digestive systems of ruminants and nonruminants and determine how the differences between them affect feeding practices.

#### Situation/Problem:

How are plant foods such as carbohydrates broken down and used in the digestion process?

How are starches chemically changed in the digestion process?

How are carbohydrates digested by animals?

How are starches and carbohydrates related?

What role do carbohydrates play in animal nutrition?

What foodstuffs are sources of carbohydrates? Cane molasses, ground corn, wheat middlings, and dehydrated alfalfa meal, to name a few

#### **Scientific Principles:**

8H-7: Describe the reactants and products of a chemical reaction.

8H-9: Design and conduct an experiment which demonstrates physical and/or chemical changes.

#### **Activity Length:**

Preparation time: 25 minutes Class time: 50 minutes

## **Activity Objective:**

Investigate how a digestive enzyme breaks down starch (a carbohydrate).

## **Background:**

Animals need a balanced diet to grow and remain healthy. This balanced diet consists of six important nutrients: carbohydrates, lipids, protein, minerals, vitamins, and water. Energy is provided by carbohydrates, lipids and protein.

Plants normally provide the nutrients for animal growth. These nutrients are present in a complex form that prevents easy utilization by animals. The digestive system breaks feed down into simple substances that can be absorbed by the body.

One important nutrient which provides energy for animal growth and development is carbohydrates. Carbohydrates are common in starches, a major component of livestock rations.

## **Vocabulary Terms:**

Amylase - enzyme secreted by the pancreas and delivered to the small intestine to aid in starch digestion.

Carbohydrates - the body's primary source of energy; made from compounds of carbon, hydrogen, and oxygen.

*Digestion* - changes that food undergoes within the digestive tract to prepare it for absorption and use in the body.

Enzymes - complex protein molecules produced by the body that speed up chemical reactions, such as starch breakdown.

Maltase - enzyme found in the mouth and intestine that acts on maltose to produce glucose.

Maltose - compound sugar.

## **Enzyme Digestion**

Monosaccharide - simplest form of sugar; a single sugar unit.

Polysaccharide - large, molecular weight carbohydrate made up of many sugar units, e.g., starches, cellulose, and glycogen.

Starch - carbohydrate manufactured by plants and stored in the seeds, roots, leaves, stems, and fruit in high concentrations; the major component of livestock feeds. Forages contain lower concentrations of starch.

Substrate - the material upon which an enzyme or fermenting agent acts.

### **Equipment and Materials:**

Hot plate
Two 150 ml beakers
Four test tubes
Test tube rack
Dropper or 1 ml pipette
150 ml of water
2 ml iodine solution
5 ml Benedict's solution (indicates color)
1 gram cornstarch

#### Instructor Procedures:

Preparing the first two steps before the class period will save 10-15 minutes.

#### **Procedures:**

- Prepare a starch solution by adding 1 gram cornstarch to 100 ml boiling water. Let starch dissolve and set aside to cool.
- 2. Prepare a hot water bath by filling a 150 ml beaker half full of water and bringing to a boil.
- 3. Add 5 ml of the prepared starch solution to each of the four test tubes and label them 1-4.

4.	Observe and record color.

Ai	nimal Nutrition:	En	<b>Enzyme Digestion</b>		
		5.	Add 5 ml of Benedict's solution to test tube 2 and place in hot water bath for five minutes. Observe and record color.		
		6.	Add 2 ml of your saliva to test tubes 3 and 4. Mix and allow to stand for five minutes.		
		7.	Put 2 drops of iodine in test tube 3. Observe and record color change.		
		8.	Put 5 ml of Benedict's solution in test tube 4 and place in hot water bath for five minutes. Observe color change.		
<b>Ans</b> 1.	wers to Key Questions: Amylase, which is an enzyme secreted by the pancreas. The alkalinity or high pH level helps break down the starch.	Key 1.	/ Questions: What is present in saliva that caused the breakdown of starch?		
2.	Starch is a complex carbohydrate found in most cereal grains. Carbohydrates are found in starches, a major component of livestock feed. Starch is used in this activity to demonstrate how it is broken down by enzymes and converted to sugars, which are more easily digested.	2.	What is the function of starch in this activity?		
3.	In most animals, saliva contains enzymes that help speed up digestive action. Saliva is slightly alkaline, which is needed to continue enzyme action. The saliva in this experiment demonstrates the use of the enzyme amylase.	3.	What is the function of saliva in this experiment?		
4.	Animals cannot absorb starches directly into the bloodstream. Starches must be changed into	4.	Why must starches be chemically changed in digestion?		

Ar	nimal Nutrition:	Enzyme Digestion
<b>5</b> .	maltose by pancreatic amylase. Pancreatic amylase does most of the starch digestion.  Enzymes speed up the digestive action but remain unchanged in the process. Enzymes cause amylase to change starch to maltose (malt sugar), and maltase changes maltose to glucose.  A high pH or alkalinity. This is	5. What role do enzymes play in digestion?  ———————————————————————————————————
	the reason that very few starches are broken down in the stomach.	What conditions are necessary for starch digestion

# **Enzyme Digestion**

#### Supplemental References:

Flanders, F., and R. Herren. *The Science of Animal Agriculture*. Albany, NY: Delmar Publishers, Inc., 1994.

Gillespie, J. R. *Animal Nutrition and Feeding*. Albany, NY: Delmar Publishers, Inc., 1987.

Osborne, E., J. Moss, and A. Barger. Biological Science Applications in Agriculture: Animal Science (Teacher's Guide). Urbana-Champaign, III.: University of Illinois, 1993.

#### **Conclusions:**

Digestion of food is the breaking down of complex molecules into smaller molecules. In starch digestion, the enzyme amylase converts starch molecules to glucose. Glucose is a simple sugar that can be absorbed and supplies energy to cells.

Saliva contains the enzyme amylase, which breaks down starch. Since food remains in the mouth for only a short time, amylase is secreted by the pancreas into the small intestine for further breakdown of starch molecules. Most starch is converted to sugar in the small intestine.

The pH also has an effect on the function of amylase. Amylase ceases to function in the stomach, where acidic pH levels may vary from 2.0-3.0.

Enzymes are special proteins that exist in all living things and speed up chemical changes. Enzymes act as a catalyst by speeding up the chemical reaction without being used up in the process.

#### **Extensions:**

To expand this activity, add a pair of test tubes containing a piece of hard-boiled egg white (protein) and a pair containing lard (lipid). When saliva is added, it does not affect protein or fat digestion.

## **Mycotoxins**

#### **Student Outcomes:**

Animal Nutrition 2: List the basic nutrient requirements and six elements of a ration.

Animal Nutrition 4: List common rules of thumb for feeding livestock.

#### Situation/Problem:

The U.S. Food and Drug Administration has set allowable levels of total aflatoxins at 20 parts per billion (ppb). Commodities used for human and animal consumption must be tested to ensure that aflatoxin levels are below this number. How can you be sure that you're not affected by aflatoxin? Who should be concerned about aflatoxin?

#### **Scientific Principles:**

8H-9: Design and construct an experiment which demonstrates physical and/or chemical changes.

9C-2: Infer from examples of environmental conditions the ways in which living things would adapt in order to survive.

#### **Activity Length:**

Sample extraction: 15 minutes Running the field test: 30 minutes Toxins (poisons) such as aflatoxins are dangerous to humans and animals. How can we find out if the grain sold or fed to livestock contains dangerous amounts of aflatoxin?

## **Activity Objectives:**

- Identify environmental conditions that promote grain molds.
- 2. Detect aflatoxin in food, feed, and environment.
- 3. Identify a mycotoxin that is harmful to human and animal health.

### **Vocabulary Terms:**

Aflatoxin - a mycotoxin produced by some strains of the fungus Aspergillus flavus, the most potent natural carcinogen discovered, which contaminates corn and other grains.

Carcinogen - chemical, physical, or biological agent that increases the incidence of cancer.

Conjugate - a toxin that has been chemically marked with an enzyme.

ELISA (Enzyme-Linked Immunoassay) - test to detect the presence of a substance due to a reaction with substance-specific antibodies.

Mycotoxins - chemical substances produced by fungi that can result in illness and death of animals and humans when foods containing it are eaten.

Pathogen - living, microscopic, disease-producing agent, such as a bacterium or virus.

Reagent - any substance found in a chemical reaction.

Substrate - the material upon which an enzyme or fermenting agent acts.

## **Mycotoxins**

Toxin - poisonous protein produced by some higher plants, certain animals, and pathogenic bacteria.

Wash - using a wash bottle or gently running tap water to fill wells with water. Shake out. Repeat 10 times. Turn wells upside-down and tap on a paper towel to remove water droplets.

### **Equipment and Materials:**

Neogen Corporation's Aflatoxin Field Kit contains an extraction kit and 12 tests for \$94. (Refrigeration is needed.)

## **Equipment and Materials:**

Grain samples
Aflatoxin Field Kit (available from Neogen Corporation)

#### **Procedures:**

### Sample Extraction

- Obtain a representative sample of corn.
- 2. Grind the sample to the size of fine instant coffee (20 mesh). Thoroughly mix sample prior to subsampling for extraction.
- 3. Weigh out a 5-gram sample of the ground corn.
- 4. Using the paper funnel, pour the sample into a prefilled extraction bottle. Tightly cap and shake vigorously for one minute.
- 5. Ensure that glass wool is tightly packed in the base of the syringe assembly. Remove the plunger from the syringe assembly and hold the syringe tip over a collection dropper bottle. Pour the liquid from the extraction bottle into the syringe until the syringe is one-half full.
- 6. Replace plunger in filter syringe and apply gentle pressure until collection dropper bottle is approximately one-half full. Secure the dropper top in the bottle. The sample is ready for testing.

## **Mycotoxins**

Running the Field Test (ELISA Test)

- 1. Break off two antibody-coated wells (in foil pack) and place in white paddle. Label control well #1 with a felt-tip marker. Return unused wells to foil envelope, insert in a sealable bag, and seal.
- 2. Add three drops of control from the bottle labeled "yellow" to the control antibody well (well #1).
- 3. Add three drops from sample collection bottle to the sample antibody well (labeled #2).
- 4. Add two drops of conjugate from the bottle labeled "blue" to each antibody well. Tap wells gently to mix. Wait five minutes.
- 5. Initial reaction is now complete. Turn upside-down and shake the contents out of the antibody-coated wells.
- 6. "Wash" both wells 10 times. Remove all water droplets by tapping wells on paper towels.
- 7. Add two drops of TMB (Tetramethyl Benzidine) from bottle labeled "green" to each well.
- 8. Add one drop of H<sub>2</sub>O<sub>2</sub> from the bottle labeled "green" to each well. Tap wells gently to mix. **Wait five minutes.**
- 9. Add two drops of red stop from the bottle labeled "red" to each well. Tap wells gently to mix them.
- Place the wells on a white background and read, looking down through the solution, to observe color differences.

11.	Record results.	 		

Anim	nal N	Vutri	ition:
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## **Mycotoxins**

<b>Answers</b>	to	Key	Qu	estio	ns
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- 1-2. (Answers will vary and are dependent on the samples used.)
- Mycotoxins are a potent, naturally occurring carcinogen and have been linked with a variety of health problems.
- 4. When they produce toxins such as aflatoxin
- 5. Warm temperatures and high humidity
- 6. Blend with enough untainted grain to be below USDA allowable levels (20 ppb).
- A toxin is extracted from the 7. sample and mixed in a solution with a conjugate. The mixed solution is transferred to the antibody coated wells, where the free toxin and the conjugate bind to the antibody sites. Excess conjugate and other reagents are then washed away. A substrate is added that changes color in the presence of the enzyme-marked conjugate. A red stopping reagent is added. The amount of red color in the well is proportional to the amount of toxin present (i.e., the more blue color, the less toxin that is present).

## **Key Questions:**

- 1. How many samples turned more pink than the 20 ppb?
- 2. How many samples were as blue or more blue than the control?
- 3. What are the effects of mycotoxins on animal and human health?
- 4. When can molds be harmful to humans and animals?
- 5. What conditions promote the growth of molds? \_\_\_\_\_
- 6. What should be done with grains that contain mycotoxins?
- 7. How does the ELISA test work? \_\_\_\_\_

**Credit:** Aflatoxin Field Kit. Lansing, MI: Neogen Corporation.

## **Mycotoxins**

#### **Supplemental References:**

Neogen Corporation; 620 Lesher Place; Lansing, MI 48912. 800/234-5333.

### **Applications:**

Aflatoxins are a potent, naturally-occurring carcinogen. They have been linked to many health problems in both humans and animals. An aflatoxin is a byproduct of mold growth that affects many commodities. These soil-borne fungi are found everywhere in the world and grow on the rich nutrients of seeds. Mold and aflatoxin growth is favored by warm temperatures and high humidity. Corn is especially vulnerable to contamination during extended periods of drought. Closed, poorly aerated conditions help create an ideal environment for mold growth.

The U.S. Food and Drug Administration has set a maximum allowable level of total aflatoxins at 20 parts per billion (ppb). There are several methods of detecting aflatoxins. Some are highly sensitive and accurate, but very costly. Others are inexpensive but less accurate, such as using a black light.

#### **Extension:**

A black light set-up can be used to detect aflatoxins in grain. Simply pass the corn under a black light and the kernels with aflatoxins present will glow. However, it is not a reliable method of detecting aflatoxin since the compound that produces the bright, greenish-yellow fluorescence is kojic acid, not aflatoxin. The black light method can be used as a presumptive screening method but not as an analytical method since fluorescence may occur without aflatoxins.

- Artificial Insemination
- DNA Extraction
- Genetics
- Sperm Motility

## **Artificial Insemination**

#### **Student Outcome:**

Animal Breeding 2: Identify and describe the function of the parts of the female reproductive system in cattle and swine.

#### Situation/Problem:

A.I. is a quick method of increasing the quality of a herd. Ask an A.I. technician to speak to the class. Show a video on artificial insemination. Ask students if they have ever seen an artificial insemination. Pass around magazines and catalogs from an A.I. company. What are the advantages and disadvantages of artificial insemination?

If I had two champion heifers that I wanted to use to start a herd of cattle, what method would be best to breed the heifers? How would a person choose the bull to use when breeding those heifers?

#### **Scientific Principle:**

10B-4: Describe the structure and function of human reproductive organs.

#### **Activity Length:**

Preparation time: 30 minutes Class time: 45 minutes

In this activity, students have the opportunity to identify, study, and practice artificial insemination techniques using the reproductive tract of a cow. The tract can then be frozen to be used in years to come. Also, students can dissect a rat to inspect its reproductive tract, which is similar to reproductive tracts in many other mammals.

## **Activity Objectives:**

- 1. Define artificial insemination.
- 2. Identify the parts of the female reproductive tract.
- 3. Identify the procedures and equipment used in artificial insemination.

## **Vocabulary Terms:**

Ampule - unit of packaging for frozen semen (usually glass).

Artificial insemination (AI) - the placement of semen into the female reproductive tract by artificial (rather than natural) means.

Catheter - slender; tubelike; metal, rubber, plastic, or glass instrument inserted into body passages to deposit or remove fluids.

Cervix - part of the female reproductive tract that forms a seal between the uterus and the vagina.

Dorsal - refers to the back or toward the back of an animal.

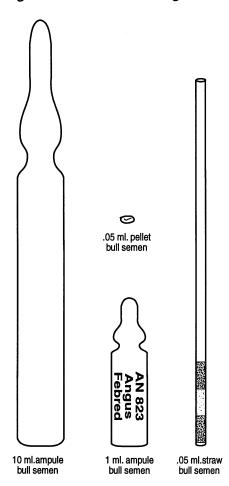
Estrous - period of sexual excitement (heat) when the female will accept sexual intercourse with the male.

Estrous cycle - reproductive cycle measured from the beginning of one estrous to the beginning of the next.

Gestation period - length of time from conception to birth of young in a particular species.

## **Artificial Insemination**

Figure 1 - Semen storage



INSTRUCTOR NOTE: Reproductive tracts can be found at a local slaughter house. The reproductive tracts can be frozen to reuse in other classes.

INSTRUCTOR NOTE: Two A.I. methods are described, depending on whether ampules or straws of semen are available.

Ovary - organ in female animals that produces the egg.

Oviduct - tube that leads from the ovary to the uterus; also called the Fallopian tube.

Ovum - female sex cell (egg) produced in the ovary; carries half of the genes contributed by the female from which it was produced.

Semen - sperm-containing fluid that is ejaculated by the male.

Sperm - male sex cell, which is produced in the testicles.

Straw - unit of packaged, frozen semen that is more commonly used than the ampule because of storage ease.

*Urethra* - canal or tube that carries urine away from the bladder.

*Uterus* - womb; in female mammals, an organ in which the young develops before birth.

Vagina - the canal in female mammals extending from the uterus to the vulva.

Vulva - external genital (reproductive) organ of the female; the opening to the vagina.

## **Equipment and Materials:**

Dissecting microscope

Artificial insemination equipment:

Semen - frozen or fresh

Catheter - plastic, glass, or metal

Polybulb - syringe

lce water - a can or plastic container for holding frozen semen

Paper towels

Female reproductive tracts

## **Artificial Insemination**

Scissors
Scalpel
Dissection tray
Dissection pins
Disposable gloves
Magnifying glass
Tweezers

### **Procedures:**

## Dissection

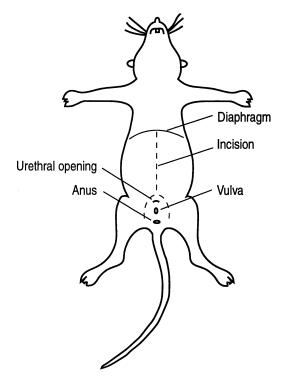
- Obtain a euthanized ("put to sleep") female rat. Put on disposable gloves, then use the scalpel to make a longitudinal incision in the abdominal cavity. Make an incision in the abdominal cavity. (See Figure 2.) Do not cut into the intestines.
- 2. Gently move the intestines so that the reproductive tract can be seen. Locate the structures labeled in Figure 3. Using the small, pointed scissors, cut the vagina open and expose the cervices. Insert a probe into each cervix.
- 3. Carefully remove the reproductive tract (cervix, uterus, oviducts, and ovaries). Observe the oviducts and ovaries using a dissecting microscope.
- 4. Compare the reproductive anatomy to that of a cow's.

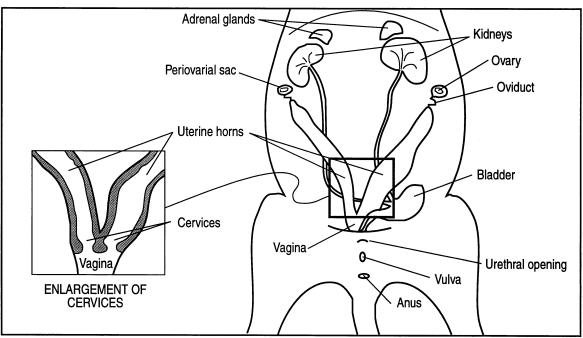
## A.I. ampule preparation

- 1. Fill a metal or plastic container with ice and water. (A coffee can is convenient.)
- 2. Remove an ampule from the N<sup>2</sup> (nitrogen) tank and place it quickly in the ice water. Let the ampule thaw naturally. Do not remove the ice shell that forms around it. (This will take about 10 minutes.)
- 3. Keeping catheter sterile, prepare it by placing a polybulb on one end.

# **Artificial Insemination**

Figures 2 and 3





## **Artificial Insemination**

- 4. Break the ampule using the following steps.
  - a. Remove the ampule from the water and dry it.
  - b. Prescored ampules will have a dot or mark on the scored side. Put a towel over the ampule to prevent finger cuts. Break the top away from this mark.
- 5. Draw the semen into the catheter by doing the following.
  - a. Roll the polybulb over your thumb to expel all the air.
  - b. Insert the catheter into the ampule and slowly release pressure on the polybulb to siphon semen into the catheter. Practice until you can remove all the semen from the ampule.

## A.I. straw preparation

- 1. Carefully remove a single straw with tweezers.
- 2. Wrap the straw in the fold of a paper towel and place in someone's shirt pocket to thaw. Straws can also be thawed in ice water or warm water.
- 3. Prepare the inseminating tube and plunger. Draw back plunger, if necessary.
- 4. Using a scissors, cut off the sealed end of the straw with a square cut.
- 5. Insert straw into inseminating tube; the plunger should be behind the straw.

#### Insemination

- 1. Check vulva to make sure it is clean and clear of obstructions.
- 2. Insert catheter into the vulva.

Animal Bree	edina:
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## **Artificial Insemination**

- 3. Pass the catheter through the vagina by doing the following.
  - a. Grasp the cervix and stretch or straighten the vagina.
  - b. Keep the catheter tip against the vagina's dorsal wall for 4-6" to avoid penetrating the cow's urethra.
- 4. Because of the concentric rings or folds in the cervix, passage through it may be difficult. Manipulate the cervix to align the catheter with the cervix; thread the catheter into the cervix. The catheter should not be pushed or punched in the process because of potential damage to vaginal or cervical walls. The catheter tip should pass to the anterior end of the cervix.
- 5. Deposit the semen slowly over about five seconds.
- 6. With the polybulb depressed, remove the catheter. This will prevent siphoning of the semen back into the catheter prior to removal.

### **Answers to Key Questions:**

- The placement of sperm into the female reproductive tract by artificial means.
- Estrous is another term for heat.
   It is the time during which the female will accept the male for breeding.
- 3. It depends upon the animal being inseminated. The optimum time for cattle is 12-20 hours after the start of estrous.
- Estrous length ranges are: cattle, 6-35 hours; swine, 1-5 days; sheep, 1-3 days; goats, 2-3 days; and horses, 1-37 days.

## **Key Questions:**

What is estrous?  When is the best time to inseminate?  What are the estrous differences between species

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## **Artificial Insemination**

5.

7.

- 5. The ovaries produce the eggs. The oviducts carry the eggs to the uterus. In the uterus, the fertilized egg grows into a fetus. The cervix is the neck of the uterus that separates the uterus from the vagina. The bladder collects the body's waste liquid, urine. The external opening of the reproductive and urinary systems is the vulva. The clitoris is a sensory organ of the female.
- 6. The membrane wall between the rectum and the reproductive tract is thin, allowing the technician to feel the reproductive tract. The technician feels the cervix and helps guide the straw through the cervix, allowing the semen to be deposited at the proper place in the reproductive tract. Without this guidance, semen could get caught in the folds of the cervix and be deposited in the wrong location.
- After the egg is fertilized, it moves to the uterus, where it grows into a fetus.

During artificial insemination of a cow, why doe technician have to put an arm into the cow's
technician have to put an arm into the cow's
technician have to put an arm into the cow's
technician have to put an arm into the cow's
technician have to put an arm into the cow's
technician have to put an arm into the cow's
technician have to put an arm into the cow's
rectum?

What are the functions of the different parts of the

**Credit:** Sorenson, A. M. *Repro Lab: A Laboratory Manual for Animal Reproduction*. 4th ed. Boston, MA: American Press, 1979.

Where does the fetus develop?

## **Artificial Insemination**

#### **Supplemental References:**

Sorenson, A. M. Repro Lab: A Laboratory Manual for Animal Reproduction. 4th ed. Boston, MA: American Press, 1979.

Gillespie, J. R. *Modern Livestock and Poultry Production*. 4th ed. Albany, NY: Delmar Publishers, Inc., 1992.

Herren, R. V. *Science of Animal Agriculture*. Albany, NY: Delmar Publishers, Inc., 1994.

Herren, R. V., and R. L. Donahue. *The Agriculture Dictionary*. Albany, NY: Delmar Publishers, Inc., 1991.

### **Applications:**

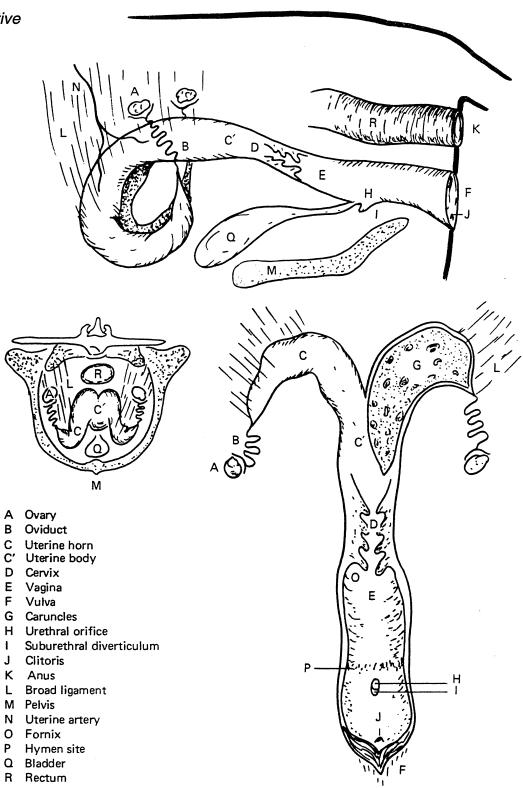
Probably the fastest growing area of technology in agriscience is genetics. Artificial insemination has done much to improve the quality of livestock. Artificial insemination has allowed for more milk production improvement in the last 20 years than had occurred in the previous 200 years.

There is a high demand for qualified A.I. technicians. A qualified technician usually performs the actual insemination. Many breeding services have schools for individuals interested in becoming A.I. technicians.

There are many advantages to using artificial insemination. Producers can use sires of higher quality than they could otherwise afford. Producers select the type of sire needed for a particular group of females. Data from the progeny of A.I. sires is available to help producers determine sire quality. Producers do not have to keep the more aggressive, often dangerous, male animals. Artificial insemination also lessens the likelihood of disease. Sires from all over the world can be used, and sires can be replaced easily.

# **Artificial Insemination**

Figure 4 - Reproductive system of a cow



## **DNA Extraction**

#### **Student Outcomes:**

Animal Breeding 3: Describe the heritability of traits in animals.

Animal Breeding 4: Describe procedures for making genetic improvements in livestock.

#### Situation/Problem:

Animal characteristics are passed on to an offspring by its parents. Genes are the genetic material responsible for all animal characteristics. Genes occur at specific locations on chromosomes. Information about an animal's characteristics are passed on through chromosomes contributed by each parent. Chromosomes are composed of long strands of molecules called deoxyribonucleic acid (DNA).

#### **Scientific Principles:**

Science 10B-3: Describe the structure and function of DNA.

Science 10D-5: Devise an experiment that will test a stated hypothesis.

Science 10D-9: Analyze the risks and benefits of genetic engineering to society.

#### Activity Length (for each activity):

Preparation time: 10-20 minutes Class time: 30-45 minutes

# Equipment and Materials (per class of 25):

One DNA Spooling Kit (from Sigma Chemical, D-8666, \$18.90).
Each kit contains enough solution for 25 samples.

50 ml of 95 percent ethanol or isopropyl alcohol
25 small (10-15 ml) test tubes
25 petri dishes
Stirring rods and pipettes
Microscope

DNA is a very complex substance that serves as the coding mechanism for heredity. This activity will isolate a DNA strand so it can be studied more closely.

## **Activity Objectives:**

- 1. Describe the physical appearance of DNA.
- 2. Explain how DNA is spooled from a solution of salmon sperm using ethanol.

### **Vocabulary Terms:**

Chromosome - in a cell nucleus, a filamentous structure that is composed of DNA.

Density - mass per unit of volume.

DNA (deoxyribonucleic acid) - protein-like nucleic acid component of plant and animal genes that controls inheritance. Each DNA molecule consists of two strands in the shape of a double helix (i.e., ladder).

Ethanol - the alcohol product of fermentation.

Gene - the smallest unit of inheritance. Genes are DNA sequences that code for a single protein.

Soluble - capable of changing form or dissolving into a solution.

Spooling - to wind material around something.

#### Activity 1

### **Equipment and Materials:**

1 ml 3M sodium acetate solution
One test tube (1 ml) of DNA (salmon testes)
2 ml of ethanol or isopropyl alcohol
One glass stirring rod
Stirring rods
Pipettes
2 ml pipette

# **DNA Extraction**

**Procedures:** 

NOTE: The two activities will show DNA. Activity 1 will exhibit long, easily seen strands. Activity 2 is an inexpensive method of extracting DNA. However, the fragile DNA strands may shear and be hard to spool. The strands will be floating in the solution and can be observed.

#### **Instructor Procedures:**

- Pipette 1 ml DNA solution into each of 25 test tubes and label "Salmon testes DNA."
- Pour isopropyl into beakers from which students can pipette.
- Distribute one test tube, pipette, stirring rod, and petri dish to each student.

# Answers to Key Questions:

NOTE: Answers will vary slightly.

1. Colorless but cloudy, fairly thick

- 2. Clear and thin

# 1. To slowly add 1 ml of 3M sodium acetate into the salmon testes in DNA solution, tilt the test tube and let the acetate

run down the side of the tube. Mix by gently swirling the test tube.

- 2. Slowly layer in 2 ml of alcohol and observe the layering.
- 3. Stir gently with a glass or plastic rod or pipette to mix the layers. Gently spool the DNA around the glass rod. The DNA will separate into long fibers. Most of the sperm extract will stick to the glass stirring rod. Be sure to stir the mixture until all

sperm extract that will stick has done so.

4. Carefully remove the DNA and place on a petri dish to dry. Remove the purified extract by pulling the glass stirring rod out of the test tube. Try to pull out long, slender threads. (It may take 20-24 hours for the DNA to properly dry.) The isolated DNA is ready for storage or examination under a microscope. It can also be used for other laboratory projects. Let the DNA dry on a petri dish or try to blot it dry. It can be stored in a petri dish.

# **Key Questions:**

1.	Record the color, appearance, and any other descriptive properties of the salmon sperm extract

2.	Record the color, appearance, and any other
	descriptive properties of the isopropyl.

Animal Breeding:		DN	A Extraction
3.	There were distinct layers; the bottom one was cloudy, and the top was clear.	3.	What did the two layers of isopropyl and salmon sperm extract look like before the spooling process?
4.	The extract was stringy. There may also be large clumps.	4.	Describe the appearance of the salmon sperm extract that stuck to the glass stirring rod during spooling.
5.	Because of the differences in density	5.	Why did the mixture layer?
6.	Under low magnifications, the DNA looks like threads.	6.	After the DNA dries, examine it under a microscope.  Describe the appearance.
7.	DNA is composed of long, thin strands of genetic material.	7.	What do your observations indicate about the nature of DNA?
		<u>Acti</u>	ivity 2
		Equ 250 3.5 ( 95 p 5 ml 3 g i Sodi Wari Thei Glas 600 100 pH r	ipment and Materials: ml beaker g yeast percent ethanol liquid detergent (Palmolive Green preferred) meat tenderizer ium bicarbonate (baking soda) m (50-60°C) water rmometer es stirring rod ml beaker ml graduated cylinder meter ety glasses for each student

# **DNA Extraction**

### **Procedures:**

- 1. Heat 100 ml water in a 250 ml beaker by placing the beaker in a hot water bath. When the water reaches 50-60°C, add 3.5 g of yeast. Stir until the yeast is thoroughly dissolved.
- 2. Add 5 ml of liquid detergent (if possible, use Palmolive Green). Maintain temperature and stir occasionally for five minutes. **Caution:** Do not exceed 60°C as this might shear the DNA. (The purpose of this step is to dissolve the cell's lipid component and nuclear membranes.)
- 3. Dissolve 3 g of meat tenderizer in the yeast solution. Add 10 ml sodium bicarbonate (baking soda) solution and measure the pH. If needed, add more sodium bicarbonate to bring the pH level to 8. Maintain the temperature at 50-60°C for 20 minutes. (The purpose of this step is to clean off the proteins from the DNA strand.)
- 4. Slowly pipette 10 ml of ice cold ethanol down the side of the beaker so that the ethanol forms a separate layer on top of the yeast mixture.
- 5. Gently stir the glass rod through the two mixtures, twirling the rod as you stir. You should see cloudy white strands begin to collect on the rod. *This is the DNA!*

#### **Answers to Key Questions:**

- Meat tenderizer contains an enzyme, which breaks down the yeast cell walls.
- 2. The papain enzyme is the important ingredient.
- Yeast. They are more advanced than bacteria cells.

# **Key Questions:**

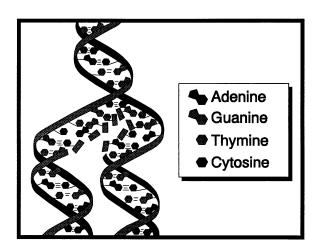
•	Why was meat tenderizer used in the solution?
•	Read the label of the meat tenderizer. What is the active component in the meat tenderizer that might do this?
•	Are the cells more like bacteria cells or yeast cells?  Explain your answer.

A	nimal Breeding:	DN	A Extraction
4.	It will dissolve the lipid structure of the yeast cell membrane. Liquid detergents are used because they break down fats and grease.	4.	Why was liquid detergent used in the solution? Why are liquid detergents used for cleaning purposes?
5.	DNA is a long, double-stranded helix that is tied together by base pairs. It appears flexible only because it is long in comparison to its thickness. Actually, DNA is stiff and brittle and can be bent only so far without breaking.	5.	What is the basic structure of DNA?
6.	Coded instructions are translated into proteins, which form all other chemical constituents of protoplasm through enzyme catalyzed reactions. Information carried on DNA in an animal cell is equal to 1,000 books, each 1,000 pages long, with 3,000 letters per page (three billion base pairs).	6.	How is information stored within the DNA structure?
7.	DNA is soluble in water and cannot be seen. However, DNA is insoluble in alcohol and will move upward when alcohol is added to the mixture. The floating DNA can then be wound (spooled) around a glass rod and isolated.	7.	How can DNA be isolated?
8.	DNA cannot be manipulated unless it is isolated from the plant or animal tissue. Scientists isolate DNA to extract it from the tissue.	8.	Why is DNA isolated?
		1	

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# **DNA Extraction**

- 9. When DNA is isolated and separated from the tissue, scientists can manipulate the genes. DNA can be cut, and other pieces of DNA can be inserted into the gene. Isolated DNA can be run on gels to be used in electrophoresis.
- DNA is the molecule that controls all cellular activity.
   DNA contains the code of life.
   It directs the synthesis of all proteins and enzymes.
- 9. What can be done after DNA is isolated? \_\_\_\_\_
- 10. What is the basic function of DNA?



# **DNA Extraction**

### **Supplemental References:**

Mathematics and Science Education Center, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, MO 63121. 314/553-5650.

Agriscience Activity Manual. Pierre: South Dakota Curriculum Center, 1994.

#### Kits:

Bacteria DNA Extraction Activity, Ward's Demonstration Kit (88 W 8106, \$26.95) or a 24-student, class-sized kit (88 W 8112, \$79.95)

Bacterial DNA Extraction Kit, Carolina Biological Teacher Demonstration Kit (15-4702, \$5.95) or a 30-student, class-sized kit (15-4701, \$21.95)

### **Applications:**

DNA is present in all cells and is very important. With an electron micrograph, the DNA molecule looks like a tangled mass of thread. It is a chemical and is soluble in a water solution that is slightly basic (pH 8.0). DNA is *not* soluble in ethanol. When the two liquids were mixed with a swirling motion, the DNA came out of solution. DNA has a fibrous nature, so it can be spun out. Chemically, DNA is a giant molecule, often containing billions of atoms linked together.

The invisible has become visible and the abstract concrete. DNA's physical nature is seen, and a chemical property (solubility) is used to extract it. A simplified two-dimensional model of DNA has been created.

Biotechnology is being used to improve animal reproduction and health, create useful products from animal waste, and increase lean meat and milk production. With biotechnology, animal scientists can save, store, and split valuable embryos, to reproduce genetically identical offspring. Both the egg and the sperm contain DNA that dictates what the young animal will look like and how that animal will develop. DNA is the code of life.

The use of biotechnology in animal agriculture is growing at a rapid pace. New techniques and products are being continuously developed that will greatly affect producers and consumers.

#### **Extensions:**

- 1. Make a DNA model using endstrips of computer paper and colored markers.
- 2. Diagram structural components of DNA molecules.
- 3. Diagram the chemical structure of DNA based on the analogy of a ladder.
- 4. Analyze the complexity of DNA molecules using the four base units in a genetic code.

# **Genetics**

#### **Student Outcomes:**

Animal Breeding 3: Describe the heritability traits in animals.

Animal Breeding 4: Describe procedures for making genetic improvements in livestock.

#### Situation/Problem:

Why did animals change?

How did humans change animals?

What was the first step to change the breeding of animals?

How have breeding programs changed over the years?

#### **Scientific Principles:**

10C-1: Predict the phenotypic and genotypic ratios of the offspring of a dihybrid cross using a Punnett square.

10C-4: Associate the roles of genetic variation and natural selection with changes in organisms over time.

#### **Activity Length:**

Preparation time: 60 minutes Class time: two periods

Understanding the principles of animal genetics helps producers improve their herds. How do animals today differ from those of prehistoric times?

### **Activity Objectives:**

- 1. Describe how animal characteristics are passed from generation to generation through genes.
- 2. Explain how traits are distributed.
- 3. Determine the probability of a genetic phenotype and genotype of an animal.
- 4. Contrast dominant and recessive traits.

### **Vocabulary Terms:**

Dominant - one of any pair of genes that dominates (or masks) the other when both are present in the germ plasm.

Recessive - a gene or trait that is masked by a dominant gene when both are present in the germ plasm.

Homozygous - a cell that contains two identical genes for a single characteristic (both dominant *or* both recessive).

Heterozygous - a cell that carries both the dominant and recessive genes for a single characteristic (impure).

Genotype - genetic makeup/expression of an organism. Individuals of the same genotype breed alike.

Phenotype - observed character of an individual without reference to its genetic makeup. Individuals of the same phenotype look alike but may not breed alike.

Probability - ratio of specified events to total events.

# **Genetics**

NOTE: For a classroom activity, use colored construction paper circles.

**Equipment and Materials: (per student or group)** 

One cup

Watch

Ten red beans

Ten speckled beans

A bag of mixed beans (50 percent red and 50 percent speckled)

Ten toothpicks, dyed green

Ten toothpicks, natural color or dyed brown

Ten toothpicks, dyed red

### **Procedures:**

Activity 1 will simulate natural selection. The results can help explain why animals with certain traits have survived, while others became extinct or lost certain characteristics. Nature helps control the gene pool of a population.

- 1. Mix the 30 toothpicks together.
- 2. In a grassy area designated by the teacher, mark out a 10' square area in which to work.
- 3. Throw the toothpicks high into the air above the grassy area for even distribution.
- 4. Try to find as many toothpicks as possible in one minute.
- 5. Count the number of each color found. Record your answers below.

Red:	_
Green:	
Brown:	
Total found:	

Activity 2 will simulate random mating, where animals are bred without regard for their particular characteristics.

 Without looking at the beans, place your hand in the mixed bean sample and draw out 20 beans.

# **Genetics**

- 2. Count the number of beans of each color.
- 3. Put the beans in the cup. These 20 beans represent your animal and its genotype for this exercise.
- 4. One at a time, "breed" your animal to the animals of students sitting in front of you, behind you, to your left, and to your right. Do this by pouring your beans into their cups. Shake the beans to get a good mix.
- 5. Pour the beans into your hand. Without looking at them, count out 20 beans into your classmate's cup.
- 6. Record the new genotype in Table 1.

Table 1. Random Breeding Record

	Red beans	Speckled beans
Initial genotype		
Genotype after breeding #1		
After breeding #2		
After breeding #3		
After breeding #4		

**Activity 3** involves developing an animal with a solid red hair coat through selective breeding. A solid red coat will be represented by 20 red beans.

- Obtain 10 red beans and 10 speckled beans. Put them in a cup to hold them. This is the gene pool of your animal.
- 2. Pour the beans into your neighbor's cup. Shake the beans to get a good mix.

Ar	nimal Breeding:	Ge	netics
		3. 4. 5.	Pour the beans into your hand. Without looking, count out 20 beans into your classmate's cup.  Record the new genotype on Table 2. This will simulate record keeping by the producer.  Continue mixing your beans with others in the class. (You will want to mix beans with someone who has more red beans than yours.) Consider asking the classmate for his/her records to examine the animal before committing to mixing (breeding). The more breedings with animals genetically stronger than yours, the better the chance that animal will have a pure red coat.
		6.	After a member of the class has reached the objective or time is called by the teacher, return the beans to the source and complete Table 2.
Answers to Key Questions:  1. Natural selection. The results explain why animals with certain traits have survived while others became extinct or, as a group (breed), lost certain characteristics. Nature helps control the gene pool of a population.		<b>Key</b> 1.	Questions: What did Activity 1 simulate? Why?
2.	Random mating. Animals are bred without regard for their particular characteristics.	2.	What did Activity 2 simulate?Why?
3.	Selective breeding. To get the desired results, animals are developed by breeding a particular animal to another particular animal.	3.	What did Activity 3 simulate?Why?

# **Genetics**

Table 2. Selective Breeding Record

Table 2. Selective breeding necold			
Red beans	Speckled beans		

4. Some animals are living because of survival of the fittest. They can adapt to certain environmental conditions. Since changes in genes occur naturally in animals, only animals with changes that make them better adapted to their environment will survive. Table 1 implies that animals with protective coloration will survive better than those without protective coloration.

Animal Breeding:		Genetics		
5.	If the color of the environment (grass) would have been a different color.	5.	Under what conditions would you have found more of the other toothpick colors?	
6.	It will be almost impossible to get a purebred.	6.	What results did you expect with the random mating of animals as simulated in Activity 2?	
7.	Yes. The probability of getting a purebred (all beans of the same color) is very small.	7.	Were the results of the random breeding exercise what you expected? Explain	
8.	(Each answer will be different.)	8.	If you were not able to develop a pure animal in Table 2, how many more breedings do you think it would take to develop a pure animal?	
9.	It takes approximately nine months for a cow to have a calf, and a cow should be two years of age before she can be bred. The first calf will take three years. Multiply by 18 breedings for a total of 54 years.	9.	Suppose the beans in this exercise represent cattle and it takes you 18 breedings to develop a pure red animal. How many years of development would this represent?	
10.	Identify the characteristic wanted (color, height, etc.). Keep breeding back for that same characteristic; keep offspring with that characteristic.	10.	How would you go about the process if you wanted to start a new breed? How long do you think it would take?	
11.	To know the ancestry and breeding line for purebred purposes or to know what is contributing to a certain characteristic	11,	Why is it important to keep records on animals?	

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An	imal Breeding:	Ge	netics
12.	By controlled breedings and through genetic engineering	12.	How do scientists use genetics to eliminate genetic disorders?
13.	By examining the animal's ancestors (pedigree) and by technologically examining the DNA	13.	What are some ways to determine genetic makeup of an animal?
14.	The phenotype is what the producer is able to observe and is used in the selection process, whereas the genotype is the actual genetic makeup of the animal. Phenotypic factors such as nutrition, stress, climatic conditions, exposure to parasites and diseases can all be somewhat controlled.	14.	Why is it more difficult to determine the genotype of an animal as compared to its phenotype?

# **Genetics**

### **Supplemental References:**

Acker, D., and M. C. Cunningham. Animal Science and Industry. 4th ed. Englewood Cliffs, NJ: Prentice-Hall, Inc., 1991.

Flanders, F., and R. Herren. *The Science of Animal Agriculture*. Albany, NY: Delmar Publishers, Inc., 1994.

Gillespie, J. R. *Modern Livestock and Poultry Production*. 4th ed. Albany, NY: Delmar Publishers, Inc., 1992.

Herren, R. V., and R. L. Donahue. *The Agriculture Dictionary*. Albany, NY: Delmar Publishers, Inc., 1991.

### **Applications:**

Animal producers use genetics to improve their herds/flocks by natural or planned selection. "Survival of the fittest" occurs in natural selection. As changes in genes occur naturally in animals, only the animals with changes that make them better adapted to their environment will survive. In planned or artificial selection, producers decide which traits they want and use animals with desirable traits in their breeding programs.

Understanding the principles of animal genetics helps producers improve their herds. Producers can formulate breeding programs to get the best traits of the animals. Crossbreeding is another advantage of genetics. Producers can take advantage of the positive traits of different breeds by mating two different breeds of a species.

### **Extensions:**

- Give an example of how producers can formulate a breeding program to get the best traits of their animals.
- 2. Give an example of improving a herd by crossbreeding.
- 3. Use three different bean colors to illustrate the effect of multiple gene action on the expression of traits.

# **Sperm Motility**

#### **Student Outcomes:**

Animal Breeding 1: Identify and describe the function of the parts of the male reproductive system in cattle and swine.

Animal Breeding 2: Identify and describe the function of the parts of the female reproductive system in cattle and swine.

#### Situation/Problem:

As a livestock producer, you have been having problems with the calving crop in a herd. In the fall, you pregnancy check the females and find many of them open. In the spring, you notice that calves do not have the characteristics of your favorite herd bull. What could be the problem? How could you check to find out if that bull is sterile?

You are an artificial inseminator and have been having many complaints about the percentage of cows that are open after you artificially inseminate them. Only two producers have been complaining, and they are both using the same bull. How could you find out if it is bad semen or if your A.I. technique is causing the problem?

### **Science Principles:**

10B-4: Describe the structure and function of human reproductive organs.

### **Activity Length:**

Preparation time: 30-60 minutes (Microscope Use activity should be completed before this activity.) Class time: up to three periods Placing a sterile, infertile male into a herd greatly decreases the profitability of animal production. This activity will show differences in sperm motility, which is one measure of fertility.

### **Activity Objectives:**

- 1. Compare the size and shape of sperm and egg cells.
- 2. Demonstrate the use of a compound microscope in observing sperm cells and egg cells.
- 3. Compare sperm cells of different animals.
- 4. Determine the motility of a semen sample.

### **Vocabulary Terms:**

Artificial insemination (AI) - the placement of semen into the female reproductive tract by artificial (rather than natural) means.

*Ejaculate* - the discharge of semen from the male reproductive tract.

Embryo - any organism in its earliest stages of development.

Embryo transfer (transplant) (ET) - the removal of developing embryos from one female and transfer to the uterus of another; usually involves the superovulation of superior females and the transfer of their embryos to increase the number of superior offspring.

Estrous - period of sexual excitement (heat) when the female will accept sexual intercourse with the male.

Fertility - ability of an animal to produce offspring.

Follicle - small, blister-like development on the ovary's surface; contains the developing ovum.

# **Sperm Motility**

Morphology - branch of biological science that deals with forms or shapes.

Motility - active movement of the sperm in a male's semen.

Objective (lens) - the microscope lens nearest to the object observed; focuses light to form the image of the object.

Ovary - organ in female animals that produces the egg.

Ovum - female sex cell (egg) produced in the ovary; carries half of the genes contributed by the female from which it was produced.

Semen - sperm-containing fluid that is ejaculated by the male.

Sperm - male sex cell, which is produced in the testicles.

Spermatozoa - mature male gametes.

Superovulation - stimulation of more than the usual number of ovulations during a single estrous cycle due to the injection of certain hormones.

### Background:

Animal reproduction has always been important economically to the production of agriculture animals. Artificial insemination, in-vitro fertilization, and embryo transplants have made it more important than ever for technicians, as well as producers, to understand the process of animal fertility.

Semen collected from male animals can be used fresh or stored frozen in liquid nitrogen (-320°F) for later use. One ejaculate can be diluted and used to breed several females. Because many types of semen can be frozen and stored for long periods of time, the mating of an outstanding male can be greatly extended.

# **Sperm Motility**

Once semen is collected, it should be examined under a microscope for quality. Quality is determined by the number of sperm present in a milliliter of semen, the sperm's motility, and the sperm's morphology. Very active sperm are desirable because of the distance they must travel to reach the oviduct of the female. A large number of sperm with an unusual shape is not desirable. Examining semen under a microscope enables trained technicians to determine the motility, shape, and quantity of sperm.

This laboratory procedure is a sample of the test used by artificial insemination companies to evaluate semen quality. You will also have the opportunity to compare semen samples from different animals and to examine the female's ovary and follicles.

#### **Equipment and Materials:**

Prepared slide of sperm (Available from Carolina, H4247. Bull: \$2.40 ea.)

Prepared slide of an ovary (Available from Carolina, H3785. Graafian follicles, cat, or rabbit: \$3.90 ea.)

Prepared sperm slide of another animal (Available from Carolina, H4253. Pig: \$2.40 ea.; others available, also.)

Straw of bull semen (Order from local artificial inseminator.)

### **Equipment and Materials:**

Microscope with 10X, 40X, and 100X (oil immersion) objectives

Laboratory thermometer Wide-mouth thermos

Hot plate

Pan

Prepared slide of sperm Prepared slide of an ovary

Prepared sperm slide of another animal

Straw of bull semen

Paper towel

Two slides per team

One cover slip per team

Eye dropper

Scissors

#### **Instructor Procedures:**

Divide the class into teams with 2-3 students per team. (The number of microscopes available will determine the number of students per group.) Students will be examining sperm cells from animals to illustrate the differences; examining the motility and morphology of sperm cells; and

### **Procedures:**

**Caution:** Microscope lenses and slides are very fragile. Never lower the objective while looking through the eyepiece.

examining the ovary and follicles of the female reproductive tract. Since students will be sharing slides, set up the activity so one group is examining the ovary while another is examining the sperm from one animal. Another group can be viewing sperm from the second animal. This will help the activity go more smoothly and give all students the opportunity to view all segments of it.

Students will view slides of active sperm and inactive sperm. The instructor will treat one semen sample by slowly cooling the sample to slow down the sperm.

# **Sperm Motility**

- 1. Place the prepared slide of sperm on the microscope stage. Hold the slide in position by placing the slide under the stage clips.
- 2. Use the coarse adjustment on the microscope to lower the objective as close as possible to the stage without touching it.
- 3. Look through the eyepiece. Turn the coarse adjustment slowly to move the objective away from the stage until the slide comes in focus.
- 4. Locate sperm cells by adjusting the slide and using the fine adjustment to bring the sperm into sharp focus.
- 5. If your microscope has a 100X (oil immersion) objective, ask your teacher for further instructions on the use of immersion oil before continuing.
- 6. Switch to high power by turning the eyepiece until it clicks into position. Focus with the fine adjustment.

7.	Sketch a sperm cell.	

- 8. Switch back to low power. Replace the sperm slide with a prepared slide of another animal.
- 9. Repeat steps 4 and 5 above to focus on the sperm using low power. Use the fine adjustment as necessary.

Animal Breeding:	Spe	erm Motility	
	10.	Sketch the sperm cell.	
	11.	Switch back to low power. with a prepared slide of the	•
	12.	Repeat steps 4 and 5 to for the fine adjustment as neo	<u> </u>
	13.	Notice the many round, maswollen with fluid. Note the eggs within the follicles.	
	14.	Locate a mature follicle an Use the fine adjustment to mature follicle.	<b>.</b>
	15.	Obtain a straw of semen fi	rom the teacher.
	16.	Place the straw in the water minute but no longer than	
	17.	Place two slides and one of warm water (approximately	
	18.	Remove the thawed straw mix it by inverting it two or	
	19.	Follow directions from you straw. Place one drop of slide. Place the coverslip	semen on the warm, clean
	20.	Place the sperm slide on the Hold the slide in position be the stage clips.	

# **Sperm Motility**

- While looking at the low-power objective from the 21. side, use the coarse adjustment to lower the objective as close as possible to the slide without touching it.
- 22. Look through the eyepiece; turn the coarse adjustment slowly to move the objective away from the stage. Continue to turn until the slide comes in focus.
- Switch to high power (40X) by turning the eyepiece 23. until it clicks into position. Focus with the fine adjustment.
- 24. Observe individual sperm cells and their movements. Strong, healthy sperm will move in a straight line across the viewing field. Rate the sample with the following scale.

Sperm motility rating scale (estimated)

Excellent:

80 percent or more of sperm moving

vigorously

Very good:

70-80 percent of sperm moving

vigorously

Good Poor

50-70 percent motility

30-50 percent motility

Less than 30 percent motility Very poor

### Sperm motility rating

	Percent motile sperm cells	Rating
Sample 1	·	
Sample 2		

25. Obtain a second sample. Follow steps 15-24 to score the second sample on motility.

Animal Breeding:
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# **Sperm Motility**

### **Answers to Key Questions:**

- The sperm is shaped like a tadpole with a tail. It uses the tail to propel itself toward the oviduct.
- Embryo transplanting is an costly process. Using sperm of poor quality would be an expensive mistake.
- 3. To be sure the stored sperm is still of good quality
- 4. The bull may become sterile, making that animal unproductive and costing the producer a calf crop.
- 5. Temperature, age, poor nutrition, exposure to direct sunlight, contamination (by blood, water, urine, etc.)
- Very active sperm are desirable because of the distance they must travel to reach the female's oviduct.
- 7. They have a greater chance of making it to the oviduct to fertilize the egg.
- 8. Semen can go bad over time.
- 9. Five of the following: old age, poor nutrition, temperature, exposure time, exposure to direct sunlight, contamination (by blood, water, etc.)

# **Key Questions:**

- 2. Why would a technician who specializes in embryo transplanting want to check sperm motility?
- 3. Why would a company that specializes in artificial insemination check sperm motility regularly?
- 4. Why does the semen from a particular bull need to be checked over a period of time?
- 5. What factors could have accounted for the difference in motility rating of semen samples? \_\_\_\_\_
- 6. Why are vigorous, quick-moving sperm desirable? \_\_\_
- 7. Why are more motile sperm considered more fertile?
- 8. Why might a test of sperm motility at the time of collection have limited value?
- List five reasons for poor semen motility or morphology.

# **Sperm Motility**

 So you don't get them mixed up and waste money by trying to A.I. a different animal

10. Why would the difference between sperm cells of the two different animals be important to know? \_\_\_\_\_

### **Supplemental References:**

Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. 800/334-5551 (orders) and 800/227-1150 (technical support).

Acker, D., and M. C. Cunningham. Animal Science and Industry. 4th ed. Englewood Cliffs, NJ: Prentice-Hall, Inc., 1991.

Flanders, F., and R. Herren. *The Science of Animal Agriculture*. Albany, NY: Delmar Publishers, Inc., 1994.

Gillespie, J. R. *Modern Livestock and Poultry Production*. 4th ed. Albany, NY: Delmar Publishers, Inc., 1992.

Herren, R. V., and R. L. Donahue. *The Agriculture Dictionary*. Albany, NY: Delmar Publishers, Inc., 1991.

### **Applications:**

Motility is one measure of the fertility of the sperm. Active sperm are important because of the distance they must travel to reach the female's oviduct. The greater the number of motile spermatoza, the greater the chances for conception. Checking the semen quality before breeding season can determine a male's fertility.

#### **Extension:**

Compare sperm counts from boars of various ages and breeds. Can any conclusions be made?

• Embryology

# **Embryology**

#### **Student Outcomes:**

Poultry 2: Select and evaluate poultry.

Poultry 6: Describe various marketing channels for poultry and eggs.

#### Situation/Problem:

Where does Missouri rank in egg production in the United States? (fourteenth)

Approximately how many eggs are produced annually in Missouri? (1,575 million; approximately 2.2 percent of the total egg production in the U.S.)

How many eggs does a chicken lay per year? (approximately 251. In 1968, the average was 220 per year.)

What factors determine the grades of eggs? (shell, air cell, white, and yolk) As an activity, grade a fertilized egg.

How is the interior quality of an egg determined? (Eggs are candled by using a high-intensity light to determine the interior quality and to see if the egg has a developing embryo.)

Display fertilized and unfertilized eggs and ask students about ways the eggs could be identified. Use an egg candler to view the embryonic development in the fertilized egg.

For an observation activity, place fertilized and unfertilized eggs in vinegar 2-3 days prior to the activity. The acetic acid will dissolve the egg shell and expose the membrane. Students can observe the environment of a developing chick.

NOTE: Inform students of the fact that developing embryos will not survive most of these procedures.

Embryo development of most mammals is difficult to study because of the embryo's location in the mother's body. Chick embryos closely resemble those of mammal embryos and are easy to access. This activity will explore embryo development in chickens.

### **Activity Objectives:**

- Identify characteristics of 1-day, 7-day, and 14-dayold embryos.
- 2. Identify body structures of developing embryos.
- 3. Candle fertilized and unfertilized eggs.

### **Vocabulary Terms:**

Albumen - the white of an egg.

Blastoderm - fertilized egg.

Blastodisc - a true egg that has not been fertilized.

Chalaza - (pronounced ka-lay-za) two white cords attached to the yolk, which position it in the center of the albumen. The chalaza serve as a rotating axis to keep the germ cell on the top side of the yolk.

Embryo - any organism in its earliest stages of development.

Fertile - egg that is fertilized and has the capability of developing into an embryo.

Incubation period - the time between egg laying and hatching.

Incubator - an apparatus or chamber that provides favorable environmental conditions for embryo development or egg hatching.

Infertile - unfertilized egg that will not hatch.

# **Embryology**

### **Scientific Principles:**

Science 10C-7: Sequence the developmental stages of the human fetus.

### **Activity Length:**

Preparation time: 20 minutes Activity time: 45 minutes on first day, 5-10 minutes for each observation day, and 20-30 minutes when eggs are opened

NOTE: Students can be divided into groups of 2-4, or the activity may be demonstrated. An incubator can be used to get the proper incubation period. Show how fast the embryo develops by incubating eggs every 24-48 hours and examining their progress.

#### **Instructor Procedure:**

This activity can be divided into two separate mini-activities with student groups moving to different stations during the class period to observe and record results.

This activity is broken down into: 1) candling and examining unfertilized eggs and 2) examining (observing) a 24-hour fertilized egg, a 7-day-old egg, and a 14-day-old egg. The instructor can change the age of the fertilized eggs to fit classroom needs.

Shell membranes - two thin membranes that surround the yolk and the albumen. The membranes provide protection against bacterial invasion.

Somite - segment of tissue in the vertebrate embryo that becomes muscle, bone, etc.

*Yolk* - the round, yellow mass that provides nutrients for the developing embryo.

### **Equipment and Materials Required:**

Incubator

Sharp, pointed, dissecting scissors

Magnifying glass or dissecting microscope

Petri dish

Metric ruler

Forceps

Dropper

One fertile egg incubated for 24 hours

One fertile egg incubated for 14 days

One fertile egg incubated for seven days

One unfertilized egg

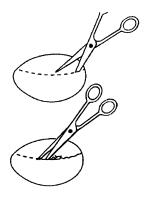
Paper towels

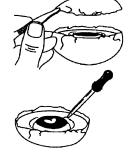
#### **Procedures:**

#### Activity 1

- 1. Candle a fertilized and unfertilized egg by holding a light up to the egg to examine the contents.
- 2. Break the unfertilized egg into a petri dish.
- 3. Examine the shell and notice the shell membrane.
  - a. Examine the yolk and look for a small, white spot called the blastodisc.
  - b. Observe the clear fluid surrounding the yolk (egg white or albumen).
  - c. Locate the air space on the large end of the egg.

# **Embryology**





Art credit: Lee and Turner

### Activity 2

- 1. Before selecting the egg from the incubator, mark the egg on the top with a marker. Be careful to keep the egg in the same position as it was in the incubator as you move it to your work station.
- 2. Nest the egg in a paper towel in the petri dish so it will not roll. Let it remain undisturbed for two minutes before you continue.
- 3. Insert the point of scissors just through the shell at a point high on the shell. Cut away a circular shape in the top one-third of the egg. (See diagram at left.)

NOTE: Cut just below the shell surface so the embryo is not disturbed.

- 4. Carefully lift off the top of the shell with the forceps.
- 5. Use a dropper to remove as much albumen as possible to make observation of the embryo easier.
- 6. Locate the embryo on top of the egg. If the embryo is not there, gently rotate the yolk with the dropper to locate the embryo.
- 7. Use a magnifying glass or dissecting microscope to view the embryo. Locate the head, eyes, ears, nose, legs, wings, somites, nervous system, spinal cord, circulatory system, heart (Is it beating?), and digestive tract.

NOTE: Not all parts will be developed in the earlier stages of growth.

Measure and record the embryo's size.	
	Measure and record the embryo's size.

Po	oultry:	Em	bryology
		9.	Locate the two white, cord-like structures called the chalaza. Use forceps to pull on one cord; observe what happens.
		10.	Measure the small, white, round dot on the surface of the yolk (germ disk).
		11.	Locate the air space between the shell membrane and the shell.
		12.	Rotate to the next work station to observe, following steps 6-11. Record results.
Ansv 1.	wers to Key Questions:  (Will vary according to age of egg)	<b>Key</b> 1.	Questions: How many pairs of somites can be observed?
2.	The blood vessels lead to the heart and serve as the circulatory system.	2.	Where do the blood vessels lead? What function do you think they perform?
3.	The chalaza serve as a rotating axis to keep the germ cell on the top side of the yolk.	3.	How is the germinal disk held on top of the egg?
4.	The shell protects the egg from injury, provides gas exchange, and provides calcium for the developing embryo.	4.	What is the shell's function for the developing embryo?
5.	The egg white or albumen cushions the embryo and provides water and protein for development.	5.	What does the egg white provide for the developing embryo?
6.	Nutrients for the embryo are provided by the yolk.	6.	What does the yolk provide for the developing embryo?
7.	The development of unfertilized eggs	7.	What is parthenogenesis?
<del></del>			

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Poultry:		Embryology			
8.	Chick embryo and human embryo development are very similar in the early stages.	8.	Compare a chick's development to human embryo development.		
9.	If the egg is not turned, the germ spot may attach to the shell membrane.	9.	Why must an egg be turned during the incubation process?		
10.	The porous shell provides air exchange for the embryo.	10.	How does the embryo get air during the incubation process?		
11. Proper temperature and humidity allow the embryo to develop and prevent it from drying out.  11.		11.	Why is temperature and humidity so important during incubation?		
		Flar the Pub Lee	dits: Inders, F., and R. Herren. Lab Manual to Accompany Science of Animal Agriculture. Albany, NY: Delmar Ilishers, Inc., 1994.  J. S., and D. L. Turner. Introduction to World iScience and Technology (Activity Manual). Danville, Interstate Publishers, Inc., 1994.		

# **Embryology**

#### **Supplemental References:**

Flanders, F., and R. Herren. Lab Manual to Accompany the Science of Animal Agriculture. Albany, NY: Delmar Publishers, Inc., 1994.

Lee, J. S., and D. L. Turner.

Introduction to World AgriScience and
Technology (Activity Manual).

Danville, IL: Interstate Publishers,
Inc., 1994.

Osborne, E., J. Moss, and A. Barger. Biological Science Applications in Agriculture: Animal Science (Teacher's Guide). Urbana-Champaign: University of Illinois, 1993.

### **Applications:**

It is difficult to examine embryo development of most animals because they develop in the placenta within the mother's uterus. Chick embryos develop outside the mother's body, providing an easy way to study embryo development.

A fertile chicken egg requires an incubation period of 21 days at the proper temperature and humidity. Chick embryos closely resemble those of mammal embryos. During the early stages of development, it is difficult to distinguish a chick embryo from a human embryo.

### **Extension:**

Acquire 21 fertilized eggs, incubate, and crack one egg each day for 21 days to see the development.

# **Plant and Crop Science**

- Germination
- Plant Taxonomy
- Water Quality

# Plant and Crop Science:

# Germination

#### **Student Outcomes:**

Plant Science 5: Identify the steps in germination of monocot and dicot seeds.

Crop Science 5: Explain the requirements for establishing a crop stand.

### Situation/Problem:

Show students several samples of seed left over from the previous planting season. Include field crop and vegetable seeds in the samples. What would be the results if the seeds were planted this year? Will any seeds germinate? Will germination be less than normal? Why?

### **Scientific Principles:**

10A-5: Classify selected plants into taxonomic groups (e.g., moss, fern, gymnosperm, monocots, dicots).

10C-6: Design an experiment in which the growth of plants is affected by photosynthesis.

### **Activity Length:**

Preparation time: 60 minutes TZ test: 45 minutes Rag doll test: 20 minutes Germination test observation: four partial periods Many factors affect seed germination, including germination potential of the seed itself. How can the viability of seeds be tested?

### **Activity Objectives:**

- 1. Observe and measure germinating seeds.
- 2. Explain how moisture, temperature, air, and light affect seed germination processes.
- 3. Explain how planting depth and soil conditions affect germination processes.
- 4. Describe the stages of seed germination, including the function of major seed parts.

### **Vocabulary Terms:**

Cotyledon - first leaf to be developed by the embryo; also called the seed leaf.

Dormancy - a condition of interrupted in which the plant does not begin to grow without special environmental cues.

Embryo - any organism in its earliest stages of development.

Endosperm - nutritive portion in the seed, which contains stored food for the growing plant during germination.

Germ - embryo of the seed.

Germination - sprouting of the seed and the beginning of plant growth.

Hypocotyl - the short stem of an embryo seed plant; portion of the embryo seedling's axis between the attachment of the cotyledons and the radicle.

Ovule - egg-containing unit of the ovary that develops into a seed after fertilization.

# Plant and Crop Science:

# Germination

Radicle - part of the seed embryo that develops into the root.

Seed - fertilized ovule containing an embryo, which forms a new plant upon germination.

Viability - the capacity of seeds to germinate.

### **Equipment and Materials:**

Wisconsin Fast Plants seeds, #15-8810 (pack of 50) and #15-8811 (pack of 200), available from Carolina Biological Supply Co., 2700 York Road, Burlington, NC 27215 (800/227-1150)

### **Equipment and Materials:**

Per student:

10 turnip seeds or Wisconsin Fast Plants seeds (Rapid Cycling <u>Brassica</u> rapa, available from Carolina Biological Supply Co.)

Petri dish

Piece of filter paper (9 cm, No. 2)

Forceps

Plastic grid sheet

Water reservoir (plastic, 2-liter bottle)

Graph paper

For TZ Test (per student):

10-20 sweet corn seeds

1 g of 2,3,5 triphenyl tretrazolium chloride (TZ) (available from science supply companies or

**Crop Improvement Association)** 

Small container to soak seeds

Single-edged safety razor or utility knife

Paper towels

Magnifying lens

For entire class:

Hand lens or steriomicroscope

Fluorescent light bank

For Rag Doll Test:

Paper towels

Rubber bands

Seeds (instructor's choice)

Plastic bags

# Plant and Crop Science:

# Germination

#### **Instructor Procedures:**

Photocopy the master grid sheet (at the end of this activity) onto overhead transparency material. Cut out and place on petri dish. The plastic grid is a simple, indirect method for measuring root length based on the background grid. The line intersection formula to be used is:  $R = N \times L$ , where R = root length in mm; N = number of intersections the roots make with the grid; L = length conversion factor. The length conversion factors are:

<u>MM</u>	L
0.5	0.3928
1.0	0.7857

The grid has 1 mm squares. Thus, R (in mm) = N x 0.7857. For experiments involving the measurement of root length, approximately five seeds with radicles emerging (Day 1) should be selected from the initial 10 seeds. Orient them along the top of the grid with the radicles pointing downward. Roots can then be measured at successive days after planting. Mean root lengths can be calculated and graphed versus time.

For step 6, the water reservoir is made from a one-pound margarine tub or the base of a plastic, 2-liter soft drink bottle.

If fungal or bacterial contamination becomes a problem after step 8, seeds can be disinfected in a 10 percent bleach solution (10 ml of chlorine bleach + 90 ml of water for about 10 minutes). Rinse with sterile or distilled water before seeding.

Students will need to add water every day or two. Be sure they add water at the sides, not the top, to keep from washing off the seeds into the pool of water.

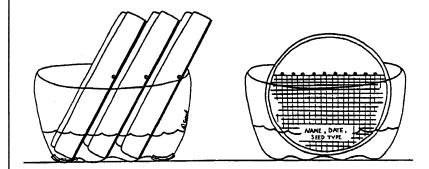
### **Procedures:**

### Germination observation, day 1

- Cut out the plastic grid and place in the top of a petri dish. (A small amount of water between the plate and the plastic will help keep the grid flat.) Smooth out any wrinkles or air pockets.
- 2. Label the edge of the filter paper with seed type, date, time of sowing, and initials or name.
- 3. Place the filter paper over the grid in the top of a petri dish. Wet the paper thoroughly.
- 4. With forceps or fingers, place 10 seeds along the top line of the grid. (Even seed placement will make measurement easier later.)
- 5. Cover with the bottom half of the petri dish.
- 6. Place the petri dish with seeds at the top at a slight angle in the water reservoir.
- 7. Add water to a depth of 2 cm in the reservoir.
- 8. Place under fluorescent lights.

# Day 2

Observe germination. Record data. Look for any changes in seed size; shedding of the seed coat; or emergence of primary root, root hairs, cotyledons, and



## Germination

young shoots. Examine with a hand lens or steriomicroscope. Measure the length of root and record.

### Day 3-5

Observe seedlings. Record observations of the root and hypocotyl on Table 1 and 2.

For the TZ test, soak the corn seeds in warm water at a temperature of 85-100°F (29-38°C) for about two hours. To demonstrate differences, soak seeds for varying lengths of time.

Table 1 - Germination

No. of seeds placed on paper:

	No. of seed coats split	No. of radicles emerged	Changes in hypocotl	Changes in root
Day 2				
Day 3				
Day 4				

Table 2 - Root length (millimeters)

	Seed number									
	1	2	3	4	5	6	7	8	9	10
Day 2										
Day 3										
Day 4										

#### The TZ Test

- After soaking, cut the seeds to be tested lengthwise through the center to expose the full length of the germ.
- 2. Immediately place one-half of each sectioned seed in a 1% solution of TZ. Discard the other half. In a warm water bath, warm the solution to 85-100°F and let the seeds soak for 30-45 minutes.

## Germination

3. Remove seed halves from the solution and wash several times in cool water. Enough water should be retained after the final washing to completely cover the seed. Examine seeds for color changes.

Actively respiring parts of the germ will become red or deep pink. The more staining, the higher the degree of enzyme activity. When the entire germ of the seed is red, the seed is alive and capable of germinating.

NOTE: TZ changes to red whenever it contacts living tissue. In living tissue, enzymes change TZ to formazan, whereas in nonliving tissue, these enzymes are not active.

### Rag Doll Test

- 1. Place two layers of moistened paper towels on a table and evenly space 25, 50, or 100 seeds on top of the towels. (The number will depend on seed availability. Using 100 seeds will make a more dependable test and make it easier to determine germination percentage.)
- 2. Cover the seeds with another two layers of moistened paper towels.
- 3. Fold over each edge of the towels about 2.5 cm. Roll the towels and enclosed seeds into a tube (like a rag doll).
- Place a rubber band around the top and bottom of each rag doll. Insert the rag dolls into plastic bags to prevent moisture loss. Place the rag dolls upright at room temperature. If moisture loss occurs, water as necessary.
- 5. After seven days, count the number of germinated seeds to get the germination percentage. A seed must have a shoot and a radicle to be counted.

Plant a	nd Cr	op Sc	ience:
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## Germination

#### **Answers to Key Questions:**

- Yes. As the seeds absorb water, they begin to expand. Water entering the embryo hydrates proteins and triggers activation, leading to increased metabolic activity.
- 2. The radicle or root is the first structure to emerge from the seed coat. This helps the young seedling absorb water and remain anchored in the soil.
- 3. When the seedling reaches the surface and is influenced by light
- They might not have received enough water, may not have been mature, or may have had damage or disease. Also, environmental conditions might not have been right.
- 5. The larger the seed, the more endosperm or food storage that seedling has until it reaches sunlight to produce its own food through photosynthesis. If you plant a seed too deep, it will run out of food before it reaches the surface.
- 6. Viable seed, moisture, correct temperature, and air. Soil is not necessary for the seed to germinate; however, soil is important for the seedling to grow. Lack of air is the reason crops that have been flooded will not germinate. To be viable, seed must be free from disease and damage.

Do the seeds appear to change in size before the split? Why?
What is the first structure that emerges from the seed? What does it o
At what point in development does chlorophyll firs appear?
Why didn't some seeds germinate?
Why does seed size have an effect on planting depth?
What are essential elements for seed germination

#### **Credits:**

Wisconsin Fast Plants Manual. Madison, WI: Wisconsin Fast Plants Program, University of Wisconsin-Madison Department of Plant Pathology. Burlington, NC: Carolina Biological Supply Company, 1989. Reproduced with the

Plant and Crop Science:	Germination
	permission of Carolina Biological Supply Co., Burlington, NC 27215, and Wisconsin Alumni Reserach Foundation, Madison, WI 53707.
	Osborne, E., J. Moss, and A. Stahl. <i>Biological Science Applications in Agriculture: Plant Science (Teacher's Guide)</i> . University of Illinois, Urbana-Champaign: Agricultural Education, 1993.

## Germination

#### **Supplemental References:**

Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. 800/334-5551 (orders) and 800/227-1150 (technical support).

Wisconsin Fast Plants Manual.

Madison, WI: Wisconsin Fast Plants
Program, University of WisconsinMadison Department of Plant
Pathology. Burlington, NC: Carolina
Biological Supply Company, 1989.

Cooper, E. *AgriScience Fundamentals and Application*. Albany, NY: Delmar Publishers, Inc., 1990.

Osborne, E., J. Moss, and A. Stahl. Biological Science Applications in Agriculture: Plant Science (Teacher's Guide). Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

### **Applications:**

Seed germination is a critical step in seedling establishment. The production of high-quality seed is tedious and expensive. Some seeds for greenhouse crops are difficult to produce and are very costly.

All commercially produced seed is subjected to a trial germination test. Several tests estimate the germination potential of seeds under ideal conditions. The actual germination of seed is affected by seed condition, planting procedures, soil conditions, and other environmental factors.

Growers have direct control over many of these factors and can adjust planting practices to align with planting conditions. First, they must understand how environmental conditions affect seed germination.

#### **Extensions:**

- 1. Use different types of soil.
- 2. Plant seeds at different depths.
- 3. Follow up this activity with the Tropism activity.
- 4. Divide the number of student experiments equally and conduct the following. Have students record results. Afterward, share results and conclusions of each student's experiment. Experiment with at least two petri dishes in each control area.
  - a. Continue to observe the seedlings. Record germination percentages and average root and hypocotyl lengths over a week's time. Plot these averages versus time (days).
  - To determine the effect of light on germination and seedling development, completely cover a specified number of petri dishes with aluminum foil so they receive no light.

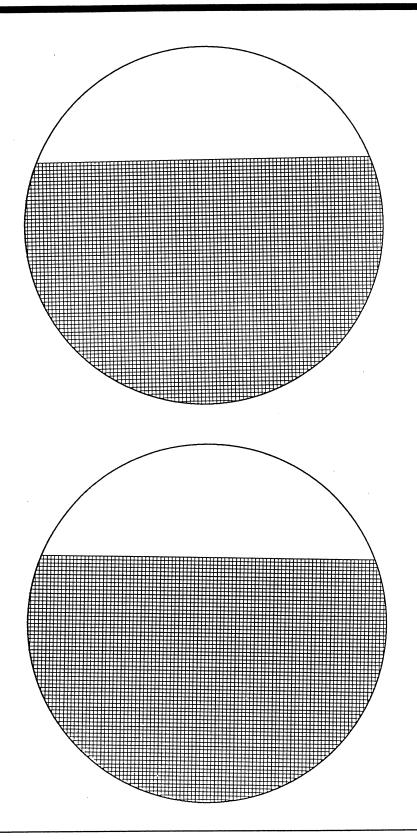
Plant	and	Crop	Science:	
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## Germination

Compare the rate of germination, color, and size of seedling development.

c. To determine the effect of temperature on germination and seedling development, prepare the following temperature tests: Incubate at freezer temperature, refrigerator temperature, room temperature, and at incubator temperature (warmer than room temperature). (A small light bulb in a box makes a good incubator.) Be sure to record the different temperature results.

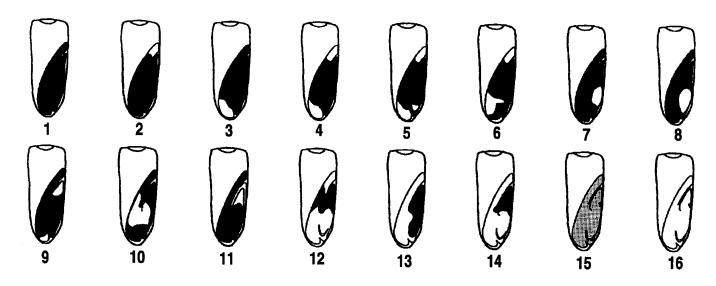
# Germination



## Germination

### The Tetrazolium Test for Seed Viability in Corn

Black areas indicate stained and living tissue; white areas represent unstained and dead tissue.



- No. 1 GERMINABLE. Entire embryo stained bright red.
- No. 2-4 GERMINABLE. Extremities of scutellum unstained.
- No. 5-6 GERMINABLE. Extremities of scutellum unstained; non-critical portions of radicle unstained.
- No. 7-8. NON-GERMINABLE. Area where seminal roots originate is unstained.
- No. 9 NON-GERMINABLE. Plumule unstained.
- No. 10 NON-GERMINABLE. Central portion of scutellum and area of seminal root development unstained.
- No. 11 NON-GERMINABLE. Plumule and radicle unstained.
- No. 12 NON-GERMINABLE. Unstained area on lower scutellum and radicle extends into region where seminal roots develop.
- No. 13 NON-GERMINABLE. Scutellum entirely unstained.
- No. 14 NON-GERMINABLE. Scutellum and radicle unstained.
- No. 15 NON-GERMINABLE. Stain very faint pink.
- No. 16 NON-GERMINABLE. Entire embryo unstained.

**Credit:** Osborne, E., J. Moss, and A. Stahl. *Biological Science Applications in Agriculture: Plant Science (Teacher's Guide).* Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

## **Plant Taxonomy**

#### **Student Outcomes:**

Crop Science 3: Identify common plants and weeds in Missouri.

Plant Science 2: List and describe methods to classify plants.

Forestry 2: Classify Missouri trees by use and grade.

#### Situation/Problem:

Show students a common plant and ask them to write down its name. Discuss the common name of the plant and what people in other parts of the U.S. and the world call it. Does anyone know a name that will satisfy everyone and help them accurately talk about a certain plant?

Relate to students how people in different cultures have names reflecting their heritage and how human names are used for identification, just as plant names identify plants. By knowing scientific plant family names, differences are easier to determine.

#### **Scientific Principles:**

10A-5: Classify selected plants into taxonomic groups (e.g., moss, fern, gymnosperm, monocots, dicots).

#### **Activity Length:**

Preparation time: 10 minutes Class time: One class period or more, depending on the depth that the instructor would like to have students involved Using scientific names helps people speak a universal language, regardless of their country of origin. This activity will explore taxonomy, the science of classifying organisms into systematic groups.

### **Activity Objective:**

- 1. Recognize characteristics of leaves.
- 2. Use characteristics of leaves and a classification key to identify plants.

### **Vocabulary Terms:**

Binomial nomenclature - two-part scientific name made up of genus and species. An abbreviation of the scientist who named the plant often follows the scientific name.

Cotyledon - first leaf to be developed by the embryo; also called the seed leaf.

Cultivar - crop variety that is cultivated and retains its features when reproduced.

Dicots (dicotyledon) - plants with seeds that have two cotyledons or seed leaves, such as beans.

Divisions in classification - kingdom, phylum (or division), class, order, family, genus, species (KPCOFGS).

Monocotyledon - plant having a single cotyledon or seed leaf, such as corn.

Scientific name - two-word name of an organism.

Taxonomy - science of classifying organisms and other objects into systematic groups, such as species, genus, family, and order.

Variety - group of related organisms in a species.

## **Plant Taxonomy**

### **Equipment and Materials:**

Different tree leaves and tree parts to use for identification (at instructor's discretion)

### **Background:**

To identify a leaf, decide which statement in the following key best fits the leaf characteristics. At the end of that statement is a number telling you which statement comes next. If the statement is followed by a name, you have identified the plant. Continue the process until you have identified each leaf. Place the correct name in the blank by its picture and/or plant specimen.

This key is a simplified version of a technical key that agri-scientists use to identify various plant species. This key uses only a few of the phyla of plants and lists characteristics of only those phyla, classes, etc., that are needed in this exercise.

### Phylum of Kingdom Plantae

- A. Pterophyta ferns
- B. Coniferophyta conifers (gymnosperms):
  - 1. If leaf is needlelike, go to 2.
  - 1. If leaf is scalelike, go to 5.
  - 2. If needles are in clusters, go to 3.
  - 2. If needles are not in clusters, go to 4.
  - 3. The needles are in clusters of two and three, slender and flexible, and yellow-green. The tree grows on dry, sandy soils in the southern part of Missouri.

Order: Pinales

Family: Pinaceae (pine)

Scientific name: Pinus echinata Mill.

Common name: shortleaf pine

## **Plant Taxonomy**

4. Needles are not in clusters and are arranged in compact spirals around the twigs; each needle is four-sided and nearly square. The tree produces egg-shaped or rounded cones.

Order: Pinales

Family: Pinaceae (pine)

Scientific name: Picea mariana (Mill.) B.S.P.

Common name: black spruce

5. Needles are opposite each other in rows and usually small and scalelike; it has a scalelike stem; twigs are flattened or angled with a dot on the gland (underneath).

Order: Pinales

Family: Cupressaceae (cypress)

Scientific name: *Juniperus virginiana* L. Common name: eastern red cedar

C. Ginkgophyta - ginkgos: This tree has fan-shaped leaves, slightly wavy on the broad edge, and often two-lobed. This species is the only one of its family worldwide.

Order: Ginkgoales Family: Ginkgoaceae

Genus and species: *Ginkgo biloba* L. Common name: maidenhair tree

- D. Anthophyta flowering plants (Its sex organ is the flower.):
  - \* Class: Monocotyledon Young plant has one seed leaf; leaf veins are parallel. Examples are grasses, sedges, lilies, irises, palms, and corn.
  - 5. If leaf has parallel veins, go to 6.
  - 5. If leaf has veins like a net, go to 7.

## **Plant Taxonomy**

6. The leaf is narrow, parallel-veined, thick, stiff, and bordered by tiny teeth; bayonet-like leaves are crowded and spreading at the top.

Order: Liliales

Family: Liliaceae (lily)

Scientific name: Yucca aloifolia L. Common name: aloe yucca

\* Class: Dicotyledon - Young plant has two seed leaves; leaves have net-like veins. Examples are roses, maples, oaks, and elms.

- 7. If leaf is compound and made of three or more leaflets, go to 13.
- 7. If leaf is simple (one piece), go to 8.
- 8. If leaf edge is smooth, go to 10.
- 8. If leaf edge is not smooth, go to 9.
- 9. If leaf edge is like a saw blade, go to 11.
- 9. If leaf edge has sharp points and deep indentations, go to 12.
- 10. The leaf is oblong, thick, and firm with edges slightly turned under. It has a shiny, bright green color on top and is pale green with rust-colored hairs beneath. The tree produces large, white, fragrant flowers.

Order: Ranales

Family: Magnoliaceae (magnolia)
Scientific name: *Magnolia grandiflora* L.
Common name: southern magnolia

10. The leaf is heart-shaped with a broad, short point, and 5-9 main veins in leaf.

Order: Rosales

Family: Leguminosae (legume)
Scientific name: *Cercis canadensis* L.
Common name: eastern redbud

## **Plant Taxonomy**

11. The leaf is broad with three or five shallow, short-pointed lobes, wavy edges with scattered large teeth, and 3-5 main veins from the base.

Order: Rosales

Family: Platanaceae (sycamore)

Scientific name: *Platanus occidentalis* L. Common name: American sycamore

12. The leaf is elliptical with spiny points; coarse, spiny teeth; and is thick, stiff, and leathery.

Order: Sapindales

Family: Aquifoliacae (holly) Scientific name: *Ilex opaca* Ait. Common name: American holly

13. The leaf is compound with leaflets alternately attached to twig. It has slightly sickle-shaped leaflets that have long points at the tip and a fine, saw-toothed edge.

Order: Juglandales

Family: Juglandaceae (walnut)

Scientific name: Carya illinoensis (Wangenh.)

K. Koch

Common name: pecan

14. The leaf is compound with leaflets in pairs, except at the tip. It is elliptical-shaped without teeth.

Order: Rosales

Family: Leguminosae (legume)

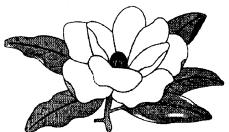
Scientific name: Robinia pseudoacacia L.

Common name: black locust

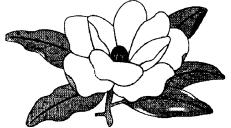
#### **Procedures:**

By the picture of each example, write the phylum, order, family, scientific, and common names. (Remember that

## **Plant Taxonomy**



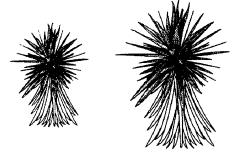
the scientific name is always italicized [underlined] with only the first word capitalized.)



Phylum - \_\_\_\_\_ 1. Order -\_\_\_\_ Family - \_\_\_\_\_\_\_Scientific name - \_\_\_\_\_\_ Common name -



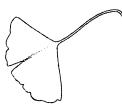
Phylum - \_\_\_\_\_ 2. Order - \_\_\_\_\_\_Family - \_\_\_\_\_ Scientific name -Common name -



Phylum - \_\_\_\_\_ 3. Order -Family - \_\_\_\_ Scientific name -Common name - \_\_\_\_\_

Plant and Crop Science:	Plant Taxonomy
	4. Phylum
	5. Phylum
	6. Phylum
	7. Phylum

Plant and Crop Science:	Plant Taxonomy	
	8. Phylum	
	9. Phylum	
	10. Phylum Order Family Scientific name Common name -	



11. Phylum - \_\_\_\_\_ 



## **Plant Taxonomy**

#### **Answers to Key Questions:**

- Taxonomy is the classification of living things. Taxonomy is also known as systematics.
- Scientific names help people accurately communicate information. Scientific names are used all over the world. They help communicate cultural practices, as well as research results.
- 3. In sequence, they are: kingdom, phylum or division, class, order, family, genus, and species.
- 4. Wheat Triticum aestivum
  Corn Zea mays
  Cotton Gossypium hirsutum
  Soybeans Glycine max
- White oak Quercus alba
   Sweet gum Liquidambar
   styraciflua
   Shortleaf pine Pinus echinata

Key	Questions:
1	What is taxonomy?

- 2. Why is the use of scientific names important? \_\_\_\_\_
- 3. What are the seven levels of the classification system?
- What are the scientific names for the following crop plants? (See the instructor for reference material, if needed.)
   Wheat -

5. What are the scientific names for the following trees?

White oak - \_\_\_\_\_\_Sweet gum - \_\_\_\_\_Shortleaf pine - \_\_\_\_\_

**Credit:** Lee, J. S., and D. L. Turner. *Introduction to World AgriScience and Technology (Activity Manual).* Danville, IL: Interstate Publishers, Inc., 1994.

## **Plant Taxonomy**

#### Supplemental References:

Nebraska Weeds. Lincoln, Nebraska: Weed Division, Nebraska Department of Agriculture, 1979.

Anderson, C. Diane, et. al. Broadleaf & Grass and Grasslike Weed Vegetative Identification Keys. Vocational Agriculture Service, University of Illinois at Urbana-Champaign, 1989.

Herren, R. V., and R. L. Donahue. *The Agriculture Dictionary*. Albany, NY: Delmar Publishers, Inc., 1991.

Lee, J. S., and D. L. Turner.
Introduction to World AgriScience and
Technology (Activity Manual).
Danville, IL: Interstate Publishers,
Inc., 1994.

Settergren, C., and R. E. McDermott. *Trees of Missouri.* University of Missouri-Columbia, 1983.

Whitson, Tom D., et. al. Weeds of the West. Newark, CA: Western Society of Weed Science in cooperation with Cooperative Extension Services, The Western U.S. Land Grant Universities, 1992.

#### **Conclusions:**

In agriculture, plants and animals are identified by their common names. When scientific names are used, agriscientists can communicate more accurately about plants and animals. Many people in agriculture use science and therefore need to know about scientific names. Scientific names are universal; an organism has the same scientific name in all parts of the world.

An example is *Zea mays.* This plant is commonly called corn in North America. However, it is known as maize in other parts of the world. Scientific names help us refer to organisms in a universal language.

### **Extension:**

Collect, identify, and mount plant specimens common in your area.

## **Water Quality**

#### **Student Outcomes:**

Plant Science 4: Describe how pests affect plant growth.

Crop Science 9: Describe methods of plant pest control.

Small Grains 3: Describe the practices necessary to establish a satisfactory stand of small grains.

#### Situation/Problem:

All living things are products of their environments. The disappearance of a plant that is sensitive to salt suggests that a particular soil is salty. In the upper Midwest, excessive grazing of dry prairie regions can be diagnosed by a decrease in little bluestem (a species of grass) or an increase in ragweed, dandelion, and white clover. Common duckweed (*Lemna minor*) can be used as an indicator species to monitor water quality.

### **Scientific Principles:**

8C-1: Hypothesize several ways organisms would respond to drastic environmental changes.

8D-4: Predict the effects of pollution (e.g., acid rain, smog, oil spills, excess pesticides, and fertilizer runoff) on the environment.

9D-4: Predict possible local environmental consequences based on factors such as increased population, amount of available fresh water, and amount and types of pollution.

9K-5: Observe the effects of manmade pollutants on the atmosphere. Have you ever wondered how clean the water is in which you drink, swim, or bathe? Water is such an essential part of our ecosystem that our survival depends on maintaining water quality. In this activity, you will explore ways to test for water quality. You will see how monitoring an indicator species gives us clues about what is happening in the environment.

### **Activity Objectives:**

- 1. Demonstrate how an indicator species can be used to monitor a water supply.
- 2. Test the susceptibility of common duckweed to a variety of contaminants.

### Vocabulary Terms:

Bioassay - use of a living organism to test the effects or presence of a substance

Effluent - liquid or dissolved waste from a commercial, industrial, or agricultural operation.

Frond - leaf structure of duckweed.

Hydrophobic - incompatible with or insoluble in water.

*Indicator species* - plant or animal species that is used to monitor the health of an ecosystem.

Leach - ability of a substance to move through the soil over time.

Leachate - liquid that has percolated through solid waste or other medium and may contain extracted, dissolved, or suspended particles from the medium.

Point source contamination - pollution that comes from an identifiable source or has been concentrated in one area.

## **Water Quality**

#### **Activity Length:**

Preparation time: 90 minutes to construct bottles and for laboratory setup Class time: 10 minutes during four days to collect data

INSTRUCTOR NOTES: Duckweed refers to a group of floating, aquatic plants of the family *Lemnaceae*. Duckweed plants are tiny--2-4 mm in diameter--and each has a simple, two-part structure of a frond and a root. The plants grow in colonies and, after undergoing asexual vegetative reproduction, form aggregates of two or more fronds. New fronds grow roots and eventually break away from the original plant. Duckweed grows very fast, doubling in frond number every 1-3 days.

You may want to substitute substances to be tested in this procedure.

When students count duckweed fronds, it is essential that they count any protruding bud as a frond. This will eliminate individual bias. Demonstrate how to count the fronds to the students.

Each member of the team should count each team dish to ensure accuracy.

If using powdered soap or detergent, mix the 5 percent solution as follows: 5 g powder to 95 ml water.

If using a chemical (2,4-D, Roundup™, etc.), the stock solution should be mixed to twice the normal field concentration, as determined by the label instructions that accompany the chemical container.

It is important that the lab is set up on a Monday so the final observation can be taken on Friday.

### **Equipment and Materials:**

Six teams of 2-5 students each

Six 2-liter plastic soda bottles with caps

Distilled water

100 ml of 5 percent detergent solution

100 ml graduated cylinder

10 ml graduated pipette

48 two-frond colonies of duckweed (Lemna sp.)

Hand lens for counting fronds

Graph paper

Liquid fertilizer (mixed to standard strength)

Scalpel

Felt-tip marker

### **Procedure:**

- Cut the colored base of the bottle to make a pedestal. Cut down the colored plastic to about 7 mm above the bottom. Cut around the base until the excess is removed. Make sure you leave enough of the bottle base so that the bottle sits without rocking. (See Figure 1.)
- 2. Mark cutting lines with the felt-tip marker. Cut A should be made about 7 mm below the shoulder. Cut B should be about 7 mm above the hip.
  - e ------ Cut A

Figure 1

- 3. Cut the bottle as marked in Figure 2. Discard the middle section.
- 4. Label the observation dishes with your team name and the following:

A - Control D - 1x10<sup>-2</sup> X B - 1X E - 1x10<sup>-3</sup> X C - 1x10<sup>-1</sup> X F - 1x10<sup>-4</sup> X

These concentration values are listed in *scientific notation*. This style is a convenient way to express

## **Water Quality**

Make sure the duckweed doesn't stick to the sides of the observation dish.

INSTRUCTOR NOTE: To make stock solution, each team needs 100 ml of a 5 percent liquid detergent and 5 ml of nutrient solution. The solutions can be prepared as follows:

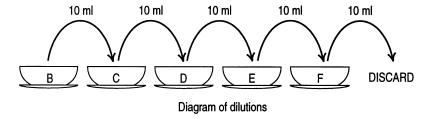
5 percent detergent solution				
No. of Detergent Distilled teams (ml) water (ml)				
1	5	95		
5	25	475		
10	50	950		

very small or very large numbers. A value of  $1x10^{-1}=0.1$ ,  $1x10^{-2}=0.01$ ,  $1x10^{-3}=0.001$ , and  $1x10^{-4}=0.0001$ .

Nutrient solution preparation: 1 T liquid fertilizer per gallon of water (or according to label directions).

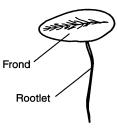


- 5. Fill dish A with 90 ml distilled water. This is the control.
- 6. Fill dishes C, D, E, and F with 90 ml distilled water.
- 7. Fill dish B with 100 ml of the 5 percent detergent solution.
- 8. With the graduated pipette, take 10 ml of liquid from dish B and add to dish C. Mix well. NOTE: It is important to completely rinse and drain the pipette between dilutions so that dilutions will be accurate.
- Take 10 ml of liquid from dish C and add to dish D. Mix well. Rinse pipette.
- Take 10 ml of liquid from dish D and add to dish E. Mix well. Rinse pipette.
- Take 10 ml of liquid from dish E and add to dish F. Mix well. Rinse pipette.
- 12. Take 10 ml of liquid from dish F and discard.



## **Water Quality**

- 13. Add 5 ml of liquid fertilizer solution to each dish.
- 14. Place eight two-frond colonies of duckweed into each dish and cover them. Try to pick plants where the two fronds are about the same size to ensure that they are at approximately the same stage of growth.







Two-frond plant showing new frond bud



Triple-frond plant

- Place dishes under artificial light so they get 24 hours of light a day.
- 16. Observe the dishes every 24 hours for four days (96 hours) for:
  - \* Total number of fronds (Record number each day.)
  - \* Note changes in color.
  - \* Note any changes in appearance (roots broken off, colonies broken apart, etc.).
  - \* Compare general frond size in relation to the fronds in the control dish.

NOTE: Each team member should count the fronds in each dish to ensure accuracy.

Plant	and	Crop	Scier	ice:
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## **Water Quality**

17. After 96 hours, graph the data (i.e., number of fronds) and write your conclusions.

Hours of exposure

Dish	0	24	48	72	96			
Dish A (control)								
Dish B (1X)								
Dish C (0.1X)								
Dish D (0.01X)								
Dish E (0.001X)								
Dish F (0.0001X)								

## **Answers to Key Questions:**

- The detergent will slow the growth of duckweed in weak dilutions and kill the duckweed in stronger solutions.
- 2. As concentrations increase, the duckweed will begin to die.
- 3. Colonies in the control group should reproduce normally.
- 4. The colonies that have stronger dilutions of the soap
- 5. As the amount of leachate is decreased in the later dishes, the plants grow better.

## **Key Questions:**

Day 1

	do you expect the duckweed to do in the ent concentrations?
Which norma	n colonies do you expect to reproduce ally?
	n colonies do you expect to reproduce more

Plant and Crop Science:	Water Quality
6. Yes. As time goes on, less leaching of the contaminant will occur as the water leaching through the soil dilutes it.	6. Does the rate of leaching vary over time?

## **Water Quality**

#### Supplemental References:

"AgriScience Institute and Outreach Program Instructional Materials." Alexandria, VA: The National Council for Agricultural Education, 1992.

Clark, J. R., V. D. Nicholson, R. B. Cherry, D.S., and J. Carino.

Accumulation and Depuration of Metals by Duckweed (Lemna persusilla.) Ecotoxicol Environmental Section 5, 1981, pp. 87-96.

### **Applications:**

Concerns for the quality of surface and groundwater are ever growing as we become more aware of threats to our health and the health of our planet. Agricultural pesticides and fertilizers are often suspect in water quality issues. What are some reasons that water becomes contaminated with agricultural pesticides and fertilizers?

Testing and monitoring water is costly for government and commercial laboratories. Researchers are searching for reliable, accurate testing procedures and indicator species that will make water quality monitoring economically feasible.

Common duckweed (*Lemna minor*), a tiny floating aquatic plant found all over the world, is getting much attention as a water contamination indicator species. Duckweed, because of its broad sensitivity range, can also be used as an indicator species to test landfill leachate, groundwater quality, surface water quality, and agricultural chemical leachate.

Duckweed toxicity testing has been accepted and used by the EPA (Environmental Protection Agency) and private industrial laboratories. However, duckweed does not measure the degree of contamination or the identity of the contaminant. It only suggests that water quality has been affected, and further methods of source contamination testing need to be investigated.

#### **Extensions:**

- Use different leachates on duckweed.
- 2. Design a system to monitor environmental changes using duckweed.
- 3. Use substances other than a chemical detergent, such as coffee, soda, or gasoline.

- Growth Regulators
- Herbicides
- Photosynthesis
- Tropism
- Tissue Culturing

## **Growth Regulators**

#### **Student Outcomes:**

Plant Science 7: Describe how plants reproduce sexually and asexually.

Plant Science 8: Describe how genetics influences plant growth.

#### Situation/Problem:

How many students mow lawns? How many really enjoy mowing lawns? Have you ever wished you had something to spray on the grass to keep it from growing? A small sample of such a growth retardant product exists. Does anyone want to try it out on their lawn? Should it be tested first to see if it really works and to make sure it won't damage the lawn? How can we test this product's ability to reduce the growth rate of grasses?

#### **Activity Length:**

Preparation time: 60-90 minutes Observation time: 20 days (none during days 2-7, 5-10 minutes during days 8-16, and 50 minutes on day 20) In nature, competition between plant species results when one species limits the supply of nutrients, water, light, or other necessities. Some plants produce substances that actively inhibit growth of others, while auxins stimulate growth. Still other regulators can modify plant growth. This activity explores the effects of gibberellic acid, a growth stimulant.

### **Activity Objectives:**

- 1. Investigate the role of one class of hormones, the gibberellins, by treating plants with gibberellic acid (GA) and observing the results.
- 2. Explain how growth regulators affect life and growth processes within a plant.
- 3. Describe the nature and source of plant growth regulators.
- 4. Explain recommended practices for applying and using plant growth regulators.

### **Vocabulary Terms:**

Auxin - substance that promotes the growth of plants; a plant growth hormone.

Cotyledon - first leaf to be developed by the embryo; also called the seed leaf.

Hormone - chemical substance produced in minute amounts in one part of an organism and transported to another part of the same organism, where it has a specific effect.

Internode - stem region between nodes.

*Node* - a region of the stem where one or more leaves are attached in a knob-like enlargement.

## **Growth Regulators**

### **Equipment and Materials:**

Carolina Biological has two hormone kits available, depending on budget and class size:

15-8728, 6 students, \$15.95 15-8730, 32 students, \$47.50 Plant growth regulator - synthetic or natural organic compound that promotes, inhibits, or modifies plant growth.

### **Equipment and Materials:**

Hormone Kit (Carolina Biological, 15-8728 or 15-8730) Rulers

#### **Procedures:**

Day 1

1. Following the kit's "Growing Instructions," plant each of two cells of your quad with three wild-type seeds. Label one, "Wild Type, Water," and the other, "Wild Type, GA." Plant each of the other two cells of the quad with three rosette seeds. Label one cell, "Rosette, Water," and the other, "Rosette, GA."

Follow the procedures in the "Growing Instructions" to the end of the life cycle of the plants, adding the steps listed below.

2. Label one pipette "Water" and the other "GA."

NOTE: There is no activity on days 2-7.

Day 8 to 16 (starting on Day 8 and alternating days until flowering)

- 1. Record plant height (distance along the stem from the point of cotyledonary attachment to the very tip of the plant). Measure to the nearest millimeter and record on Table 1.
- 2. According to the treatment labels, apply one drop of GA or water to each leaf on the plants. Use the proper pipette.

Day 20

Pool the class data and calculate averages for each date. Using class averages, construct two growth curve graphs--one for the wild-type plants and one for the rosette plants (Graph 1).

## **Growth Regulators**

### **Answers to Key Questions:**

- 1. Rapid stem elongation
- Very little effect. It appears there is already enough GA in the wild-type plant, so additional GA has no effect.
- Amounts of GA applied varied with different people. Variations in planting techniques could cause differing levels of growth and development.
- 4. Yes. Addition of GA compensates for something the rosette plant lacks. However, this test does not determine the exact point in the development pathway of the plant where the deficiency occurs. (The GA used is a mixture of several gibberellins.)
- Greater effect might be expected from placing GA in the most actively growing areas of the plant.
- 6. Too little light and nutrient imbalance

### **Key Questions:**

- 1. What is the effect of GA on plant growth in the rosette mutant?
- 2. What is the effect of GA on plant growth in a wild-type plant?
- 3. How can you explain the variation in response found within the class for the treated rosette plants?
- Compare the water-treated plants and the GA-treated plants. Do results prove that the rosette is a gibberellic-acid-deficient mutant? \_\_\_\_\_ Explain. \_\_\_\_\_
- 5. How would varying the application's location affect plant response?
- 6. What environmental factors can affect stem elongation?

**Credit:** Wisconsin Fast Plants: Investigating Plant Physiology. Madison, WI: Department of Plant Pathology, University of Wisconsin-Madison. Burlington, NC: Carolina Biological Supply Company, 1987.

## **Growth Regulators**

Table 1. Plant Height.

Name\_\_\_\_\_ Date of planting \_\_\_\_\_

vame						Date	ot planti	ng		
			Height (in mm)							
			Wild-type plant				Rosette plant			
Age		Water-treated		GA-treated		Water-treated		GA-treated		
<b>.</b>	.0			Class		Class		Class		Class
Date	plaı	nts	Yours	avg.	Yours	avg.	Yours	avg.	Yours	avg.
				-						
	-									
		Γ	<del></del>		<del>-                                    </del>		<del> </del>	1	1 1	
Graph 1	. 22		-				-		-	
	20				+		1			
	18									
	16		<del>                                     </del>		1					
(mr	14									
Height (in mm)	12									
jht (	10 8									
Heiç	6									
_	U		1							



Days after planting

## **Growth Regulators**

#### Supplemental References:

Wisconsin Fast Plants: Investigating Plant Physiology. Madison, WI: Department of Plant Pathology, University of Wisconsin-Madison. Burlington, NC: Carolina Biological Supply Company, 1987.

Osborne, E., J. Moss, and A. Stahl. Biological Science Applications in Agriculture: Plant Science (Teacher's Guide). Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

### **Applications:**

Plant growth regulators have many uses in agriculture today. Auxins are used to stimulate root growth in plant cuttings, promote uniform fruit setting on fruit trees, prevent premature fruit drop, thin fruits, prevent sprouting in woody ornamentals, initiate roots and shoots in tissue culture, and control weed growth (herbicides).

Gibberellin is used to break seed dormancy, increase the size of seedless grapes, lower growing temperatures, cause flowering in long-day plants, cause some lettuce seeds to germinate in darkness, increase the size and crispness of celery stalks, and increase the release of sugars from the endosperm of grains during malting.

Growth inhibitors are used to prolong vegetable storage life, retard shoot growth in potted plants, induce shoot development in tissue culture, reduce wind damage in wheat by shortening straw length, and place nursery plants in a temporary state of dormancy for shipping.

### **Extensions:**

- 1. Do a similar activity using a growth retardant.
- 2. Use more control groups, such as light vs. darkness, amount of water vs. no water, and warm vs. cool temperature.

## **Herbicides**

#### **Student Outcomes:**

Horticulture 3: Identify characteristics and production practices for warm season solanaceous crops.

Plant Science 6: Describe good crop production practices.

Plant Science 8: Describe how genetics influences plant growth.

#### Situation/Problem:

Your neighbor decides to use a weed-and-feed fertilizer/herbicide on his lawn to improve the established lawn and get rid of dandelions. The results are excellent--a beautiful lawn. He notices there a lot of dandelions in the flower garden, also. Since the fertilizer/herbicide worked on the lawn to kill the dandelions, he reasons it should help improve the flower garden, as well. A few days later, the dandelions are dead, but so is the flower garden.

Why did this happen? How can a herbicide kill one species of plant and not another? What would result if a large grain producer used the wrong herbicide on a crop?

#### Scientific Principles:

9K-5: Observe the effects of manmade pollutants on the atmosphere.

10A-5: Classify selected plants into taxonomic groups (e.g., moss, fern, gymnosperm, monocots, dicots).

10C-2: Hypothesize how genetic resistance develops from continued exposure to pesticides or antibiotics.

#### **Activity Length:**

Preparation time: 45 minutes Observation time: two weeks Herbicides play an important part in our lives. Without herbicide use, weeds would do severe damage to crops and significantly lower production yields. How do herbicides know which species of plant to kill?

## **Activity Objectives:**

- 1. Explain the difference between selective and nonselective herbicides.
- 2. Identify factors that affect the genetic resistance of plants to herbicides.
- 3. Describe the effects of different herbicides on selected plants.
- Compare the response of monocotyledon and dicotyledon plants to applications of specific weed killers.

### **Vocabulary Terms:**

Adjuvant - substance added to a herbicide to aid in spray retention.

Cuticle - thin membrane that covers all above-ground parts of a plant.

Epinasty - condition when leaves turn downward due to more rapid growth of upper layers of cells; opposite of hyponasty.

Glycolysis - breakdown of carbohydrates by enzymes.

Herbicide - chemical used to kill unwanted plants (weeds).

Interception - initial herbicide contact on a leaf surface.

Nonselective herbicide - herbicide that will kill any plant that it contacts.

## **Herbicides**

*Phloem* - inner bark of stems that conducts food from the leaves downward to the roots.

Retention - percentage of intercepted spray that is held on the leaf surface.

Selective herbicide - chemical that will kill weeds but results in little or no injury to the crop plant or turf.

Translocation - movement of herbicide throughout the plant.

*Xylem* - woody plant tissue under the bark that transports water and nutrients from the roots upward to the stems and leaves.

## **Equipment and Materials:**

Monocotyledon and dicotyledon seeds (Corn and soybeans or oats and radishes work well.)

Planting flats or small pots

Potted broadleaf plants

Selective herbicide (2,4-D, 2,4,5-T, MCPP, Weed-B-Gon<sup>™</sup> and Grass-B-Gon<sup>™</sup> are readily available at garden stores.)

Non-selective herbicide (Roundup™, Kleenup™)

#### **Procedures:**

#### Selective vs. nonselective herbicides

- 1. Mix monocotyledon and dicotyledon seeds together and plant three flats of the mixture.
- 2. After plants are growing well (about 4" tall), spray one flat with selective herbicide using the label-specified concentration. (Premixed formulas in spray bottles are available in garden stores.)
- 3. Spray the second flat with the nonselective herbicide. The third flat will be used as the control.

		_	_			
Pla	nt	Sc	ie	n	C	e:

4. In Table 1, record observations of all flats twice a week for a period of two weeks. Maintaining normal environmental conditions.

Table 1

	Flat #1 Treatment:	Flat #2 Treatment:	Flat #3 (Control)
Date			

## Herbicide uptake, translocation and action

- Obtain four broadleaf potted plants. A fifth plant can be used as a control.
- 2. On one plant, apply one drop of the selective herbicide to one side of the stem.
- 3. On another plant, apply one drop of the nonselective herbicide to the stem.
- 4. Using one herbicide for each of the other plants, coat a portion of the stem and 1-2 leaves.

1	D	la	ni	t.	S	ci	مز	n	c	0	
	_	ıa	,,,		0	6	6	ш	•	5	

## Table 2

Dates>		
Plant #1 Treatment:		
Plant #2 Treatment:		
Plant #3 Treatment:		
Plant #4 Treatment:		
Plant #5 (Control)		

5. On Table 2, record observations of all four potted plants twice a week for two weeks. Maintain normal environmental conditions.

## Foliar-applied herbicides

- Label one plant of each species as "complete" coverage and the other of each species as "singleleaf" coverage.
- 2. Separate the complete and single-leaf treatment plants into two areas. For the complete coverage group, spray 2,4-D over the entire foliage surface until leaves are wet to the point of dripping.
- 3. For the single-leaf treatment plants, select one of the largest leaves on each plant and mark by placing a small amount of clear tape around the petiole. Isolate this leaf from other leaves with a sheet of plastic wrap or another nonabsorbent material. Do this for one leaf on each of the three plants.
- 4. Spray the selected leaf of each of these plants until the entire surface area of the leaf is covered to the point of runoff.
- 5. Write a brief description of each plant's condition on day 1 of the experiment. Observations should include: species, size, total number of leaves, number of leaves showing some injury or deficiency, nature of injury or deficiency observed, extent of injury or deficiency symptoms on leaves, and other similar factors.
- 6. Maintain the same favorable environmental conditions for all six plants for the next 10 days. Follow a normal daily schedule for watering.
  - NOTE: In subsequent experiments, environmental conditions could be varied.
- 7. Make observations on days 4, 7, and 10. Write a brief description of these observations; include information described in step 5.

Plant Science:	Herbicides
Answers to Key Questions:  Herbicides kill plants by	Key Questions:  1. How do herbicides v

- 1. Herbicides kill plants by interfering with plant processes such as photosynthesis.

  Contact herbicides affect only the treated part of the plant.

  Translocated sprays enter the plant and move within it to the site of action.
- 2. Selective herbicides are intended to control undesirable plants only.
- Selectivity is due to differences in retention, differential wetting, orientation of leaves, location of growing points, uptake, movement, metabolism, timing of application, and method of application.
- Nonselective herbicides kill all vegetation, regardless of the type of plant.
- 5. Contact herbicides do not move into the phloem but diffuse across the leaf membranes.

  Translocated herbicides enter the phloem and are transported throughout the plant. The movement from the xylem to the phloem is due to pH differences between the two tissues. Once ions are in the phloem, they are trapped due to the higher pH.
- 6. Low temperature, low light, and low moisture decrease the herbicide translocation.
- 7. To be the most effective and environmentally safe

<b>y</b>	Questions:  How do herbicides work within the plant to cause injury or death?
	Why are some herbicides selective in terms of classes of plants they affect?
	Why are some herbicides selective in the plants they affect?
	What makes other herbicides nonselective?
	Why do some foliage-applied herbicides remain on plant tissues only where they are applied, where others are translocated throughout the plant?
	Why are climatic conditions important when applying herbicides?
	Why is timing and application method important?

_	_		
P	lant	Science:	
	IUIIL	OUIGIIGE.	

- 8. The rate of application depends upon a number of factors, including weed plant size.

  Economic feasibility is important, as are environmental considerations, effectiveness, crop damage, and yields.
- The size of the plant has an effect on herbicide activity within the plant. Young plants are more actively growing and more susceptible to injury/affects of the herbicide.
- Herbicides penetrate the cuticle to contact live plant tissue. The cuticle is water repellent with a wax coating. It is sponge-like, and its pores open and absorb liquids best under high humidity conditions.
- 11. Herbicides kill plants by:
  1) slowing photosynthesis,
  2) generating toxic molecules,
  3) chemically blocking the plant's ability to metabolize and/or 4) inhibiting plant growth.

effectiveness?
How do plant characteristics and life processes affect herbicide activity within the plant?
How do herbicides enter the plant?
How do herbicides work within the plant to cause injury/death?

**Credit:** Osborne, E., J. Moss, and A. Stahl. *Biological Science Applications in Agriculture: Plant Science (Teacher's Guide).* Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

## **Herbicides**

#### **Supplemental References:**

Cooper, E. L. *AgriScience Fundamentals & Applications*. Albany, NY: Delmar Publishers Inc., 1990.

Osborne, E., J. Moss, and A. Stahl. Biological Science Applications in Agriculture: Plant Science (Teacher's Guide). Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

## **Applications:**

The use of herbicides for weed control is an important practice in many areas of plant growth. More than 100 organic herbicides are available for weed control in the U.S. With this many herbicides on the market, growers must be well informed about the most effective herbicides for a crop situation.

Producers and homeowners must be aware of the diversity of herbicide products so weed control can be effective, economical, and safe. Reading and following the label is essential. Misapplication or poor selection of herbicides can have a big impact, both financially and environmentally. Yields can be greatly reduced, or entire crops can be lost, if weed control is poor.

Herbicides are an important part of agriculture and feeding the world's population. However, herbicide misuse can have a damaging effect on the environment. Application rates, timing, climatic conditions, methods of application, and the size and types of plants are important considerations when applying herbicides.

## **Photosynthesis**

#### **Student Outcomes:**

Small Grains 1: Describe the soil and climatic adaptations and the uses of small grains.

Plant Science 3: Describe factors that affect plant growth and development.

Plant Science 6: Describe basic plant processes.

#### Situation/Problem:

In an SAE program, you decide to grow poinsettias to sell. This year, October and November are extremely dull and cloudy. The crop isn't growing very well and looks like it could be a complete failure. Plants are alive but don't look very salable. Help students analyze what went wrong. The analysis should lead back to photosynthesis.

Despite the poinsettia failure, you decide to grow a crop of geraniums this winter. It normally takes 16 weeks to grow out a crop of blooming geraniums. What can you do to get your crop to bloom in 12 weeks? Can it be done? How and why?

How do plants get their food?

What are the requirements for plant growth?

How important is a knowledge of photosynthesis in producing plants?

#### Scientific Principles:

9D-1: Predict the rate of photosynthesis under various environmental conditions.

9D-2: Predict the rate of respiration under various environmental conditions.

9G-1: Infer how energy can be transformed from one form to another.

Plants convert sunlight into chemical energy in a process called photosynthesis. The rate of plant growth can be controlled by increasing or decreasing one or more of the requirements for photosynthesis. These mini-activities will explore how the process can be manipulated to produce plants when needed.

## **Activity Objectives:**

- 1. Identify components of the photosynthesis reaction.
- 2. Write the chemical reaction for photosynthesis and discuss the components that are products of the reaction.
- Predict the effect on photosynthesis when one or more of the necessary inputs are unavailable to the plant.

### **Vocabulary Terms:**

Chlorophyll - substance present in all green plants. Chlorophyll transforms light energy from the sun into chemical energy to manufacture plant food from carbon dioxide, water, and essential soil minerals.

Chloroplasts - minute objects within plant cells that contain the green pigment, chlorophyll.

Photosynthesis - process by which green plants, using chlorophyll and the energy of sunlight, produce carbohydrates and release oxygen.

Respiration - chemical process that takes place in living cells, whereby food is "burned" to release energy and waste products (mainly carbon dioxide and water).

## Rate of Photosynthesis

## **Equipment and Materials:**

Anacharis (Elodea) from aquarium supply Sodium bicarbonate (baking soda) Four test tubes

## **Photosynthesis**

10A-7: Describe the significance of the light and dark phases of photosynthesis.

10C-6: Design an experiment in which the growth of plants is affected by photosynthetic condition.

10D-8: Describe general ways in which human activities affect environmental quality.

#### **Activity Length (per mini-activity):**

- Rate of Photosynthesis (30 min.)
- Sugar Production and Photosynthesis (20 min.)
- Photosynthesis and Starch (30 min.)
- Light and Photosynthesis (10 min.)
- Carbon Dioxide and Photosynthesis (30 min.)
- Chlorophyll and Photosynthesis (20 min.)
- Water and Photosynthesis (20 min.)

Test tube rack
Bright light source
Four glass stirring rods or cut pieces of glass tubing

#### **Procedure:**

- 1. Fill four test tubes with water to within 4 cm of the top.
- 2. Cut four pieces of *Anacharis* that have been growing in bright light and are cut about 6 cm long.
- 3. Wind the pieces around a glass stirring rod or piece of glass tubing; immerse each in a test tube.
- 4. Add a small amount of sodium bicarbonate to each test tube.
- 5. Place one test tube under a bright light, the second in daylight, place the third in diffuse or dim light, and the fourth in total darkness.
- 6. Wait 15 minutes and observe the cut ends of each plant.
- 7. Count the number of bubbles given off of each plant in one minute. Record results in Table 1.

Table 1 - Photosynthesis Data Table

Type of light	Bubbles in 1 min.	Bubbles in 5 min.	Ave. per minute
Bright light			
Daylight			
Dim/diffuse light			
Dark			

## **Photosynthesis**

### Sugar Production and Photosynthesis

### **Equipment and Materials:**

Two coleus plants
Clinitest tablets (from drug store)
Two test tubes
Mortar and pestle
Beaker of water
Graduated cylinder
Filter paper
Funnel
Labels or wax marking pencil

#### Procedure:

- 1. Place one plant in the dark for 24 hours and the other plant in sunlight for 24 hours.
- 2. Select one leaf from the plant grown in the dark. With a mortar and pestle, grind it with 20 ml of water.
- Filter the results with filter paper in a funnel and pour the liquid into a small test tube marked "dark."
- 4. Do the same with the plant that was in the sunlight and label it accordingly.
- 5. Place a clinitest tablet into each test tube and shake gently. Compare the colors with the color guide on the tablets.

#### Photosynthesis and Starch

## **Equipment and Materials (per student or group):**

Cornstarch

Test tube with about 25 mm of distilled water

Pinch of sugar

Ethyl alcohol

Hot plate or Bunsen burner

Pan

Petri dish

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## **Photosynthesis**



- Place a pinch of cornstarch in a test tube with about 2.5 cm of water. Shake and add a drop of iodine solution. Note the color that appears.
- 2. Repeat the cornstarch test, but substitute a pinch of sugar for cornstarch. Note the color that appears. \_
- 3. Extract the chlorophyll from the covered leaf (in Light and Photosynthesis activity), the petroleum jelly leaf (in Carbon Dioxide and Photosynthesis activity), and the variegated plant leaf (in Chlorophyll and Photosynthesis activity).

Caution: Alcohol will burn and is likely to catch fire if heated over an open flame. To avoid this, place a beaker of alcohol in the pan of boiling water. Since alcohol boils at a lower temperature than water, it will boil freely before the water is hot enough to boil. The alcohol will turn green as chlorophyll is extracted.

- 4. When it has lost its green color, remove the leaf from the boiling alcohol and place it in a petri dish. Flood the leaf with Lugal's iodine solution.
- Compare the portion of the leaf that was exposed to light with the covered portion. Record your observations.

### Light and Photosynthesis

## **Equipment and Materials:**

Black cardboard Paper clips Potted plant

## **Photosynthesis**

#### Procedure:

- Cut a piece of black cardboard about twice the size of a leaf and fold it in half so that it can be used to enclose the leaf like the covers of a book.
- 2. Cut an irregular hole in the cardboard that is to cover the top of the leaf.
- 3. Place the cardboard around the leaf and fasten with paper clips.
- 4. Put the plant in a sunny location and leave for 1-2 days.
- 5. Remove the cardboard and determine if there is a difference in appearance between the covered and uncovered portions of the leaf.

### Carbon Dioxide and Photosynthesis

## **Equipment and Materials:**

Petroleum jelly

Cornstarch

Test tube with about 25 mm of distilled water

Pinch of sugar

Ethyl alcohol

Hot plate or Bunsen burner

Pan

Petri dish

### **Procedure:**

- Coat both the top and bottom surfaces of one-half of a leaf from your stock plant with petroleum jelly and leave the plant in ample light for 1-2 days.
- 2. Remove the leaf and test for the presence of starch following the same procedure as in Photosynthesis and Starch. The hot water and alcohol will remove the petroleum jelly so it does not interfere with the accuracy of the results.

Plant Science:	Photosynthesis
	Compare the portion of the leaf that was coated with petroleum jelly with the portion that was not.  Record your observations
	Chlorophyll and Photosynthesis
	Equipment and Materials: Variegated plant Cornstarch Test tube with about 25 mm of distilled water Pinch of sugar Ethyl alcohol Hot plate or Bunsen burner Pan Petri dish
	Procedure:  1. Remove a leaf from the variegated plant and sketch which portions contain chlorophyll (green) and which do not.
	2. Remove the chlorophyll from the leaf following procedures in the Photosynthesis and Starch activity.
	3. Test the leaf for the presence of starch.
	4. Sketch the leaf again, shading those areas that show positive starch reaction.
	5. Compare the two sketches.

Potted plant Cornstarch

Test tube with about 25 mm of distilled water

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## **Photosynthesis**

Pinch of sugar
Ethyl alcohol
Hot plate or Bunsen burner
Pan
Petri dish

#### **Procedure:**

- Withhold water from a potted plant until the leaves begin to wilt. Keep the plant in plenty of light so that the leaves remain green.
- 2. Remove a wilted leaf and test for starch following procedures in the Photosynthesis and Starch activity.
- 3. Record your observations.

#### **Answers to Key Questions**

- The rate of photosynthesis was more when the *Anacharis* was exposed to sunlight.
- 2. Sunlight aids in the manufacturing of sugar for the plant.
- 3. The absence of starch in the covered area indicates the necessity of light for photosynthesis.
- 4. The iodine solution will turn dark in the presence of starch. The leaf will have a dark spot in the shape of the irregular hole in the cardboard, which shows that starch was manufactured in the area exposed to sunlight.
- Carbon dioxide was prevented from entering the plant in the area covered by petroleum jelly. The iodine test will be negative

## **Key Questions:**

- What made the difference in the rate of photosynthesis?
- What does this experiment indicate about the presence of sugar in each leaf?
- 3. Why is there a difference between the covered and uncovered leaves?
- 4. What were your findings in the Light and Photosynthesis activity's starch test as far as color and starch manufactured?
- 5. What was the purpose of using petroleum jelly on the leaf? What did the iodine test tell you about the

Plant Science:		Photosynthesis			
	for starch produced in this portion of the leaf and positive for the part left exposed to air.		leaf portion that had petroleum jelly and the portion that was exposed to air?		
6.	The green area of the leaf will show a positive test for starch, indicating the action of photosynthesis in this part of the leaf. The sketches made from the color variations in the leaf correlate with the sketches made from the starch test.	6.	How did the sketch of the variegated leaf compare with the sketch of the leaf in the starch test? What does this tell you?		
7.	Water supplies the hydrogen necessary for photosynthesis. A plant that lacks sufficient water (wilted), even though it receives plenty of light and is still green, will have decreased in photosynthesis. The iodine test will show a lack of starch in the wilted leaf. When water is lacking, photosynthesis will also be suppressed by a deficiency of carbon dioxide. This is caused by the closing of the guard cells, which control the amount of air that passes into and from the leaf.	7.	What effect did the water-deprived leaf have on photosynthesis in the starch test?  Write and explain the equation for photosynthesis.		
8.	(The instructor may need to help students if they haven't had prior instruction.) Photosynthesis is carried out by plants in the presence of sunlight and chlorophyll. Solar energy produces chemical energy, which rearranges the elements of carbon dioxide and water. Sugar and oxygen are produced.  6CO <sub>2</sub> + 12H <sub>2</sub> O + light = C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> + 6H <sub>2</sub> O + 6O <sub>2</sub> Carbon dioxide + water + light = Glucose + water + oxygen				
9.	Sunlight, carbon dioxide, chlorophyll, water, and proper temperature are all important	9.	What environmental factors or substances are necessary for photosynthesis to occur?		

Pla	ant Science:	Pho	otosynthesis
<b>Pl</b> i	factors for photosynthesis to occur.  Photosynthesis occurs in all living plant tissues. The plant uses sugar through respiration, which is the oxidation of food to yield energy for cellular activities.  Yes  Respiration is the opposite of photosynthesis. As photosynthesis manufactures food by the plant, respiration burns that food for use by the plant.		Where in the plant does photosynthesis take place, and how is the energy (sugar) produced used by the plant?  Does photosynthesis occur in non-green plants?  What is the relationship between photosynthesis and respiration?

## **Photosynthesis**

#### **Supplemental References:**

Lee, J. S., and D. L. Turner. Introduction to World AgriScience and Technology. Danville, IL: Interstate Publishers, Inc., 1994.

Osborne, E. W. *Biological Science Applications in Agriculture*. Danville, IL: Interstate Publishers, Inc., 1994.

## **Applications:**

All living organisms require a constant supply of energy from some external source. If any components required for photosynthesis are lacking, the plant growth will slow down or stop.

As a crop producer or greenhouse operator, the fundamentals of photosynthesis are important factors that influence the success or failure of the crop. Researchers are constantly seeking ways to increase plant productivity. Increasing the process of photosynthesis is one focus of research.

## **Tropism**

#### **Student Outcomes:**

Plant Science 3: Describe factors that affect plant growth and development.

Plant Science 6: Describe basic plant processes.

#### Situation/Problem:

When sowing seed, do you have to worry about planting the seed right side up? Why or why not? If a plant were to get disoriented with the roots growing upward and the shoot growing down, what would happen to the plant? Why might a plant become disoriented and grow the wrong way?

#### **Scientific Principles:**

9C-1: Observe tropisms in plants due to stimuli.

#### **Activity Length:**

Preparation time: Three days (Seedlings from Germination activity could be used to shorten this time.)

Class time: One class period for setup; three partial periods for observations When seeds germinate in the ground, it is important that the roots grow downward and the stem upward. What causes the shoot of a plant to grow toward the light and the roots to grow down into the soil? This activity explores the effect of light on plant growth.

## **Activity Objectives:**

- 1. Describe the effects of tropism on plant growth.
- 2. Describe plant mechanisms that enable plants to respond positively or negatively to gravitational and light stimuli.
- 3. Predict plant growth patterns under specified light and gravitational conditions.

### **Vocabulary Terms:**

Amyloplast - colorless plastid that stores starch and influences the direction of plant growth.

*Auxin* - substance that promotes the growth of plants; a plant growth hormone.

Coleoptile - protective sheath surrounding the emerging shoot of grass seedlings.

Cotyledon - first leaf to be developed by the embryo; also called the seed leaf.

*Gravi-tropism* - response of a shoot or root to the pull of the earth's gravity.

Hypocotyl - the short stem of an embryo seed plant; portion of the embryo seedling's axis between the attachment of the cotyledons and the radicle.

Phototropism - plant growth response to sunlight in which the plant or its parts seem to turn to face the light.

Tropism - a plant's growth reaction to various external or internal stimuli, usually in the direction of the stimuli.

## **Tropism**

### **Equipment and Materials:**

Lightproof, 35 mm plastic film canisters

Plastic film sheets (red, blue, and green)

1/4" hand-held paper punch

Clear plastic tape and black electrical tape

2 cm square pieces of blotting paper or absorbent paper towels

Waterproof pen

Turnip seeds or RCBr seeds (available from Wisconsin Fast Plants, Carolina Biological Supply)

3- to 5-day-old seedlings

Corn seeds

Small pieces of cardboard, 13 cm x 13 cm

Chlorine bleach, 5 percent solution

Straight pins

Plastic bags

#### **Procedures:**

### **Phototropism**

- 1. Make a phototropism chamber as follows:
  - a. In the sides of the film container, punch three windows 20-22 mm from the bottom.
  - b. Tape squares of colored plastic over the windows with clear tape. Cover one window with green film, one with red, and one with blue.
  - c. Mark the lid with the waterproof pen to line it up with the windows at the same locations each day.
  - d. Place a square of wet blotting paper in the lid of the film canister. The lid becomes the base of the tropism chamber.
- 2. Place three seeds on the blotting paper or paper towel. Close the chamber and put it, with the lid at the bottom, under the light bank where all three windows receive uniform light.

## **Tropism**

3. After 48-72 hours, open the chamber and observe which way the plants have grown (toward which color).

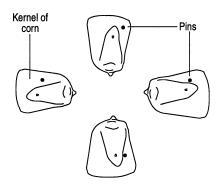
### Gravi-tropism - Experiment A

- 1. Set up a windowless chamber. Mark the top of the lid with an arrow. Place a 2 mm square of blotting paper in the lid and moisten.
- 2. Place three seeds on the blotting paper. Close the chamber and place vertically with the lid at the bottom.
- 3. After two days, observe seedlings and record observations. Close the chamber tightly and place it horizontally with the arrow pointing upward.
- 4. Examine the seedlings on the third day and record observations. Replace the chamber horizontally with the arrow pointing upward.
- 5. Examine the seedlings on the fourth day and record observations.

### Gravi-tropism - Experiment B

- 1. Set up another windowless chamber. Mark the top of the lid with an arrow. Place a 2 mm square of blotting paper in the lid and moisten.
- 2. Cut off a 3- to 5-day-old seedling at the soil level. Stick the cotyledons to the moistened blotting paper. Close the chamber and place horizontally with the arrow pointing upward.
- 3. Examine the orientation of the hypocotyl after 24 hours, 48 hours, and 5-7 days. Record observations. After each examination, close the chamber and place it horizontally with the arrow pointing upward. Observe what is happening to the very tip of the hypocotyl.

## **Tropism**



Gravi-tropism - Experiment C

- 1. Soak corn seeds in a 5 percent solution of bleach for 30 minutes to kill any mold spores attached to the seeds.
- 2. Soak the seeds in pure water overnight.
- 3. Select four seeds and pin to a piece of cardboard so one seed points down, one points to the right, one points to the left, and one points up.
- 4. Label the cardboard and place in a plastic bag with two folded, moist paper towels at the bottom.
- 5. Hang the bag in a dark place to avoid any influence of light.
- 6. After germination has begun (about two days), inspect the cardboard daily.
- 7. Record observations of the direction of growth. Observe both the root and the stem.

#### **Answers to Key Questions:**

- The seedlings grew toward the window admitting the blue light and/or away from gravity.
- 2. a. Light (from the Greek "photos")
  - b. Heavy (from the Latin "grav")
  - c. To turn (from the Greek "tropo")
- 3. It would grow horizontally since the force of gravity's direction would be changing (in relation to the plant).

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1.	What colored window did the seedlings grow toward in the phototropism experiment?
2.	What do the following prefixes mean?
	a. Photo b. Gravi c. Tropism -
3.	In what direction would a seedling grow if a darkened horizontal chamber was slowly, constantly rotated? Why?

Plant Science:					
4.	Plants will grow downward toward a light source. An experiment could be set up with light coming from below the plants so their attraction to light overcomes growing away from gravity. (An experiment lacking gravity is needed for a definite				
	answer.)				

- 5. A mechanism in the plant controls the growth process by releasing hormones, which stimulate growth in specific plant cells. The shoots grow toward light, which is a positive response to this environmental stimuli. The roots have a highly sophisticated, gravity-sensing mechanism that cause them to grow toward the gravity stimuli (downward).
- The coleoptile produces auxin, which is equally distributed to the cells in the plant's tip. In horizontal positions, auxin is found in greater concentration on the lower side of the shoot, creating faster growth on the lower side.
- Plants are positively phototrophic, which causes them to grow toward a source of light.

## **Tropism**

affect	n do you think is the more important factoring the direction of plant growth: light or y? Why?
uprigl	do germinating seeds and plants maintain at orientation for growth? What makes the s grow upward and the roots grow downw
	do plants and germinating seeds respond onal changes in gravity?

**Credit:** Osborne, E., J. Moss, and A. Stahl. *Biological Science Applications in Agriculture: Plant Science (Teacher's Guide).* Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

## **Tropism**

#### **Supplemental References:**

"AgriScience Institute and Outreach Program Instructional Materials." Alexandria, VA: The National Council for Agricultural Education, 1992.

Lee, J. S., and D. L. Turner. *Introduction to World AgriScience and Technology (Activity Manual)*.

Danville, IL: Interstate Publishers, Inc., 1994.

Osborne, E., J. Moss, and A. Stahl. Biological Science Applications in Agriculture: Plant Science (Teacher's Guide). Urbana-Champaign: Agricultural Education, University of Illinois, 1993.

### **Applications:**

How do seedlings know which way to grow to emerge above the soil? Why do plants on a window sill seem to lean toward the light? Downward root growth and upward shoot growth are essential for a plant's survival. The response to gravity and to light are thought to be mediated by plant hormones called auxins, which affect cell elongation.

In roots, perception of gravity appears related to the settling of organelles called amyloplasts. When the plant is turned, amyloplasts sink toward the source of gravity and accumulate on the side of the cell that is currently down. Curving of the roots results from asymmetrical growth as the root elongates. Elongation of plant cells is affected by auxins or plant hormones. Auxins stimulate cell elongation in shoots, but inhibits it in roots. Auxins also allow roots to bend toward the source of gravity and shoots to bend away from it.

The plant's response to light is mediated by the shoot tip. Unequal auxins are transported away from the lighted side and toward the darker side of the shoot. Auxins stimulate cell elongation on one side of the shoot, and the shoot bends toward the light. Root or shoot bending is due to cell elongation in response to differing auxin concentrations.

## **Tissue Culturing**

#### **Student Outcomes:**

Plant Science 7: Describe how plants reproduce sexually and asexually.

Plant Science 8: Describe how genetics influences plant growth.

#### Situation/Problem:

Bring a carrot to class and tell the students it is a very rare carrot. It has been found growing in the desert and is a drought-tolerant carrot. You have an order for 1,000 seed packages of this type of carrot. What methods could be used to produce this number of plants in a short time? What problems/challenges for the grower does this present? Can biotechnology play a part in responding to this request? How?

## **Scientific Principles:**

10C-4: Associate the roles of genetic variation and natural selection with changes in organisms over time.

10D-9: Analyze the risks and benefits of genetic engineering to society.

#### **Activity Length:**

Creating a transfer chamber
Preparation time: 15-30 minutes
Class time: 45 minutes

Tissue culturing a weeping fig Preparation time: 15-30 minutes Class time: 50 minutes

Transferring ficus plantlets to rooting

Preparation time: 15-30 minutes

Class time: 50 minutes

Trees can take years to reach maturity and produce seeds, and some are incapable of producing sterile seed. Tissue culturing is widely used in horticulture to asexually propagate plants that are incapable of producing sterile seed or take a long time to mature. This activity explores tissue culturing and sterile techniques.

### **Activity Objectives:**

- Build a transfer chamber.
- 2. Demonstrate techniques used to maintain aseptic conditions during tissue culture operations.
- 3. List reasons for doing tissue culturing.
- 4. Sterilize plant material.
- 5. Demonstrate sterile techniques.
- 6. Propagate a *Ficus benjamina* (weeping fig) plant from an explant.
- 7. Transfer plants from shooting to rooting media.

### **Vocabulary Terms:**

Agar - sugary gel derived from certain algae; used as a medium for cultures.

Aseptic - being free of infectious microorganisms.

Autoclave - equipment using steam and pressure to sterilize dishes and cultures. (A pressure cooker can substitute for an autoclave.)

Clone - plants produced asexually from a single plant.

Contaminant - growth of any undesirable living organism, such as bacteria or fungus, in a tissue culture.

Disinfect - method of eliminating microbes from within plant tissues.

## **Tissue Culturing**

*Disinfest* - removal of surface organisms from the explant before culturing.

Explant - the part of the plant that is removed and placed in the tissue culture.

*In vitro* - in glass; growing plants in test tubes.

Medium (media, plural) - any material used to provide nutritional support for plants or plant material.

Micropropagation - plant propagation by tissue culturing.

*Plantlets* - small plants, developed by tissue culturing, which are capable of developing into complete plants.

Sterile - free from contamination by living bacterial, fungal, or viral organisms.

Subculture - group of cultured cells or tissues that are transferred to a fresh medium.

Tissue culture - the aseptic growth of cells, tissues, or organs in artificial media.

Totipotency - capability of a single cell to develop into an entire plant under proper conditions.

Transfer - process of moving a piece of plant material from one location to another.

Transfer chamber (transfer hood) - equipment/facility used to culture plants that prevents air movement from contaminating plants.

## **Tissue Culturing**

### Creating a transfer chamber

### **Materials and Equipment:**

Cardboard box (approximate size 30"L x 18"W x 18"H)

70 percent ethanol or isopropanol

Plastic wrap Aluminum foil

Spray bottle Paper towels
Surgical gloves Empty beaker

Sterile petri dish Scalpel

NOTE: Sterile technique is vital to the success of this activity. Thoroughly review sterile technique (Reference 1) with students and have them execute a practice run before attempting the procedures.

Example: Each time students enter the transfer chamber, their hands must be sprayed with alcohol. Any time equipment or supplies are brought into the chamber, they must be sprayed with alcohol. Avoid breathing on the supplies, plants, and equipment. Open the test tubes for a short time only; prevent air currents caused by sudden movements over the opened test tubes, work area, or explants.

### **Procedures:**

1. Remove the box top with the scalpel.

NOTE: To ensure sterility, wear surgical gloves.

- 2. Tightly line the entire inside of the box with aluminum foil. Secure aluminum foil at seams with masking tape, if necessary.
- 3. After lining the box with aluminum foil, spray the entire inside with 70 percent ethanol or isopropanol.
- 4. Stretch plastic wrap over the opening of the box. Secure the plastic wrap at the top of the opening with masking tape. Allow the plastic wrap to hang down over the opening approximately 12".
- 5. Place the transfer chamber in an area free of drafts or air movement.
- 6. Before and after use, the transfer chamber should always be sprayed and wiped down with 70 percent ethanol or isopropanol.

### Tissue culturing a weeping fig

#### **Equipment and Materials:**

Ficus shooting multiplication medium is available from Carolina Biological, 800/334-5551. Order Murashigi Shoot

## Materials and Equipment (per team):

Ficus benjamina plant (weeping fig)
95 percent ethanol or isopropanol, 100 ml
70 percent ethanol or isopropanol in a spray bottle

## **Tissue Culturing**

Multiplication Medium A (#19-5754, 10 tubes for \$14.75).

Two test tubes of shoot multiplication medium 4 x 4" squares of cheesecloth

Forceps

Transfer chamber

Marking pen

Scissors

100 ml distilled water 10 percent bleach, 75 ml Dishwashing detergent

Sterile petri dish

6" strings

Growing shelf space Petri dish

Beaker Parafilm

Scalpel

### **Procedures:**

NOTE: Aseptic technique is critical for success of this activity. As a team, work with a partner with one person spraying tools and supplies and handing them to the other partner, who works in the transfer chamber.

- 1. Prepare the transfer chamber by spraying it with alcohol. Follow aseptic techniques to prepare the transfer chamber and to arrange instruments and materials in the chamber.
- 2. Open the bottle of 95 percent alcohol in the transfer chamber and place the tissue culture tools (scalpels, forceps, and beaker) in the alcohol.
- 3. The ficus plant will contain tiny shoots at the very tip of the branches. With the scissors, cut two pieces of these shoot tips for each student. Use only the very tip, which is called the explant. The explant will be about 1/2" long.
- 4. Surface sterilize the explants in 100 ml of 10 percent bleach and two drops of dishwashing detergent. Loosely tie the shoot tips in the cheesecloth. Tie the ends of the cheesecloth together to form a bag.
- 5. Drop the cheesecloth bag into the bleach solution with the forceps.

## **Tissue Culturing**

- 6. Allow the cheesecloth bag with explants to soak in the bleach solution for 10 minutes.
- 7. Return the forceps to the 95 percent alcohol. Spray hands, arms, and the outside of the beaker containing the explants with 70 percent ethanol. Place the beaker into the transfer chamber.
- 8. While working inside the transfer chamber, pour off the bleach solution into a beaker.
- 9. Add 50 ml of sterile water. Pour off the water and repeat the procedure.
- 10. Use a sterile petri dish as the work surface. Remove the shoot tips from the cheesecloth using sterile forceps and a sterile pair of scissors.
- Carefully pick up the shoot tips (explants) with the forceps and place them upright in the test tube of ficus multiplication medium. Place two shoots in each test tube.
- 12. Switch and let your partner do the same with the other test tube. Be sure to use sterile technique.
- 13. Label the test tubes with your name, the date, and "shooting medium, ficus."
- 14. Seal the cap onto the test tube using parafilm. Stretch the film tightly around the test tube and cap.
- 15. Place test tubes in a rack on the grow shelf.
- 16. Return all tools and supplies to their proper locations.
- 17. Check the cultures daily for contamination. If they become badly contaminated with fungi and/or bacteria growth, sterilize them in a pressure cooker and throw them away.

In approximately four weeks, many new shoots will have developed on the explant. Then, the shoots can be divided and placed in rooting media, using sterile technique.

## **Tissue Culturing**

#### **Equipment and Materials:**

Ficus rooting medium is available from Carolina Biological, 800/334-5551. Order Murashigi Minimal Organic Medium (#19-5744, 10 tubes for \$14.75).

## Transferring ficus plantlets to rooting media

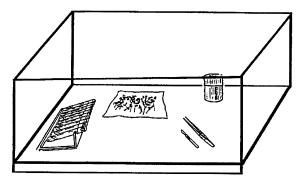
### **Equipment and Materials:**

95 percent ethanol or isopropanol, 100 ml
70 percent ethanol in a spray bottle
100 ml distilled water
Two test tubes of ficus rooting medium
Ficus explants with shoots (from previous activity)
Transfer chamber
Grow shelf space
Marking pen
Forceps
Sterile petri dish
Parafilm

#### **Procedures:**

- 1. Thoroughly wash hands and arms with a bacterial soap; spray with alcohol to prevent contamination. Remember, do not touch anything with your hands except the equipment and materials needed. Always spray your hands, arms, and all tools and supplies immediately before they are placed in the transfer chamber. Work as a team.
- 2. Spray the inside of the transfer chamber with 70 percent alcohol. Using sterile technique, set up the transfer chamber with all the tools and supplies. Arrange the supplies inside the chamber. (See Figure 1.)

Figure 1 - Right-handed transfer chamber layout



## **Tissue Culturing**

NOTE: In approximately two weeks (after roots have developed), the plants can be transferred to pots. Since the plants have been in ideal humid conditions, they must be protected and slowly acclimated. This can be done by creating a terrarium effect for the new plants. Before potting, be sure to wash away all rooting medium from the plants.

### **Answers to Key Questions:**

- These items are essential for keeping plant tissue cultures free of contaminating organisms.
- Treating materials so they are free of contaminating microorganisms that could interfere with plant growth in the culture
- 3. It minimizes the flow of air currents over the sterile area used to transfer plant material. Air currents can contain spores of microorganisms, which can contaminate the culture media.

- 3. While inside the transfer chamber, carefully remove the plant shoots from the medium using forceps. Place them in the sterile petri dish.
- 4. Divide shoots into individual plants with the forceps.
- 5. With the forceps, carefully place 1-2 plants into the test tubes containing rooting media.
- 6. Seal the test tubes with parafilm.
- 7. Label the test tube with your name, date, and ficus rooting medium.
- 8. Place the test tubes on the grow shelf.
- 9. Clean up the area; return all tools and supplies to their proper locations.

## **Key Questions:**

	Vhy are ethanol, bleach, and a transfer chamber nportant parts of tissue culturing?
	Define sterilization.
ν -	Vhat is the function of the transfer chamber (hood)?
_	

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## **Tissue Culturing**

## Answers to Key Questions (tissue culturing):

- If the culture medium develops a fuzzy or slimy appearance, it has been contaminated with fungus and/or bacteria.
- 2. Bacteria, which is orange colored and slimy, and fungus, which is a fuzzy growth
- Opening contaminated cultures allows contaminating spores to become airborne and possibly spread to other cultures.
- 4. They should be autoclaved (or pressure-cooked for 30 minutes at 15 psi) before throwing away to kill the contaminating spores.
- 5. Use 100 ml of bleach and 900 ml of sterile distilled water.
- 6. A 70 percent solution of ethanol or isopropanol is sprayed onto the surface of the transfer chamber, hands, and arms.
- 7. The instruments are soaked in a 90 percent solution of ethanol or isopropanol. Then, instruments are dipped in sterile, distilled water to rinse the alcohol from the instruments.

  (A strong solution of alcohol can kill some plant cells.)
- 8. Tissue culturing enables virusfree stock and mass production of a given plant.

## **Key Questions (tissue culturing):**

- What are the two major contaminants found in tissue cultures?
- 3. Why should contaminated cultures be kept closed?
- 4. How should contaminated cultures be discarded?
- 5. How is one liter of 10 percent bleach solution prepared?
- 6. How are hands, arms, and the inside of the transfer chamber sterilized before beginning to work? \_\_\_\_\_
- 7. What is used to sterilize the scalpel, scissors, and other instruments used to do tissue culturing? \_\_\_\_\_

8. List two reasons a typical grower should consider tissue culture techniques.

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## **Tissue Culturing**

9. The initial cost of setting up a tissue culture facility is high. When plants are propagated asexually, the genetic makeup is the same as the parent plant. These offspring are often called clones. Since these plants are genetically identical, it is possible that the whole population of plants could be wiped out by a given disease.

What are the disadvantages of tissue culturing?					

## **Tissue Culturing**

#### **Supplemental References:**

Plant Tissue Culture Techniques . . . Cloning the Weeping Fig. Tucson: College of Agriculture, University of Arizona, 1992. (This 19:25 min. video illustrates the tissue culturing process and is available for loan to Missouri educators from the Missouri Vocational Resource Center, 800/392-7217, FAX 314/882=9935.)

Kyte, Lydiane. *Plants from Test Tubes: An Introduction to Micropropagation*. Portland, OR: Timber Press, Inc., 1987.

Zeiher, C. A. *Biotechnology Instructional Unit.* Tucson, AZ:
Department of Agriculture Education,
University of Arizona, 1993.

Various mediums and tissue culture kits from Carolina Biological, 2700 York Road, Burlington, NC 27215-3398. 800/334-5551.

Various mediums from Sigma Chemical, PO Box 14508, St. Louis, MO 63178-9916. 800/325-3010.

NOTE: Many starter plants that are already growing in test tubes can be ordered through scientific catalogs.

## **Applications:**

Plant tissue culturing is a form of biotechnology that has had a dramatic impact on the agricultural industry. A tiny piece of plant tissue can produce an infinite number of new plants. Growers can produce large amounts of plants in months, instead of years. Certain plants take up to 10 years to begin producing seed that can then be used in plant breeding efforts. Through tissue culture techniques, plants can now be produced in their younger stages in a matter of months.

Plant tissue culturing also has become an important part of plant breeding programs. It allows scientists to clone the most desirable plants. The result is a more costeffective hybrid, ready for marketing in a shorter amount of time.

Scientists have been cloning plants for some time. Tissue culturing produces the same result more quickly in a virus-free environment. Scientists can select superior plants by observing the genetic makeup of a cell, instead of growing the whole plant.

#### **Extensions:**

Tissue culture other plants such as an African violet, miniature rose, cauliflower, carrot, fern, tobacco, iris bulb, and Venus's-flytrap. Tobacco is a good plant to tissue culture because it is very totipotent and will grow quickly. Venus's-flytraps are popular plants to tissue culture. Miniature roses are fun to culture as a small rose is visible when the plant is growing in the test tube.

## **Tissue Culturing**

## Reference 1--Aseptic Technique by Randy Ryan, Plant Science Dept., University of Arizona

The goal of plant tissue culturing is to establish the growth of plant tissues in vitro (test tubes). If other organisms, such as fungi or bacteria, are introduced with the plant tissue, the contaminants will often overcome and destroy the plant cells. To disinfect *most* external contaminants from plant material, use household bleach and a detergent. This process is commonly called surface sterilization. (Sterile means the lack of any living organism so the culture will have only one organism growing.)

Growth medium for plant cells is a rich source of nitrogen salts, sugars, amino acids, vitamins and other nutrients. Bacteria and/or fungal spores are found in the air in great numbers. To prevent these organisms from becoming established in the culture medium, create a clean environment to prevent their entry. There are several ways to **prevent** contamination from occurring:

- Create a *clean room* where all things and persons entering it are free of dust, mold, and bacteria. Air movement is regulated and filtered. (Example: a surgical suite) Ultraviolet lights can be turned on to kill organisms on room surfaces. (This type of room is overkill for most plant tissue culture needs.)
- A sterile curtain of air keeps bacteria and fungi from settling onto the medium and materials. These units are called laminar flow hoods. A blower forces room air through a High

Energy Particulate Air (HEPA) filter. The sterile curtain of air helps prevent contamination.

• Barrier methods, such as a glove box or transfer chamber, can be used to prevent contamination. Transfer chambers can be constructed using very simple materials, such as a cardboard box and a clear plastic bag. More elaborately designed chambers can be built from rigid plastic sheets.

In general, all these units prevent contaminants from landing on the medium. However, good sterile technique must be employed to keep new contaminants out of the sterile work area. Good sterile technique is the adherence to a group of **rules** that prevent contamination. These four rules are:

- 1. Keep the general working environment as clean as possible, e.g., dust the room and mop the floor the day before you plan to work. Do not work directly below air conditioning ducts.
- 2. Always wash hands and forearms with soap and water. Just before manipulating cultures, spray hands and arms with 70 percent ethanol. Roll up sleeves and tie back long hair. Sweaters can carry in tremendous amounts of dust and mold.
- 3. Clean the work surface with a cleaning agent. (Again, 70 percent ethanol works well.) Spray and wipe clean all objects placed into the sterile work area.

## **Tissue Culturing**

4. Do not move arms or equipment over open containers of medium or sterile explants. Move around these items and arrange the work surface so that sterility is maintained at all times.

Once airborne contaminants are controlled by the previous techniques, be sure to **sterilize** all materials that contact the medium or sterile explant. To sterilize the medium, use one of these methods:

- Boil medium in hot water bath. Boil
   30 minutes for each half liter.
- Set a pressure cooker for 15 psi and 30 minutes to sterilize medium and containers.
- Autoclave a half liter of medium for 20 minutes at 121°C and 15 psi. (An autoclave is a scientific type of pressure cooker that controls steam pressure, temperature, and the cycle length automatically.)

To sterilize glass and metal, items can be baked in dry heat at 320°F for four hours. Plastics can be sterilized by spraying with 70 percent ethanol. Many types of plastics can be boiled or steamsterilized at 15 psi (temperatures of 121°C). Instruments used for manipulating sterile materials should be cleaned before use and disinfected by dipping in 95 percent ethanol and then rinsed in sterile water.

Household bleach, such as Chlorox, is a diluted solution of sodium hypochlorite Na<sub>2</sub>ClO<sub>4</sub> and acts by releasing free chlorine in small quantities. The solution is toxic to plant cells, but with short exposures of 10-

15 minutes, the solution does not penetrate deep within the tissues.

Always use a fresh solution of bleach, as it should not be stored for more than two months. Bleach should smell strongly of free chlorine.

Detergents in the disinfectant act by reducing the surface tension of water, allowing the disinfectant to reach deep into cracks and crevices to kill microbes. In the lab, a pure detergent called Tween 20 can be used instead of household dishwashing detergent.

Contamination can be a severe problem with field-grown material. Other techniques include using a vacuum to help infiltrate the disinfectant into the tissues, treating the explant with heavy metals (such as mercury or silver compounds), and including antibiotics or fungicides in the medium.

Sometimes, the contaminant cannot be eliminated because it lives within the plant cell. These types of organisms are called endophytes. (Endo refers to within, and phyte is the Greek word for plant.) Viruses cannot be eliminated by surface sterilization. These contaminants can be eliminated by other tissue culture techniques, however.

# **Crop Science**

- Fermentation
- Hydroponics

# **Fermentation**

#### **Student Outcomes:**

Crop Science 8: Describe factors related to harvesting and storing quality forages.

Small Grains 6: Determine the most profitable practices and methods of harvesting and storing small grains.

#### Situation/Problem:

Suppose you are a livestock producer feeding silage to your cattle in the winter. This year you noticed an excessive amount of spoilage in the silage. In solving the problem, you must know how silage is processed and stored. What causes silage to spoil? Since silage is about 80 percent moisture, why doesn't it spoil when stored correctly?

In the winter, notice that silage coming out of the silo is warm. Is this a good sign? What causes the higher temperature? If stored hay bales were that warm, they would mold and spoil. Why will the hay spoil, while the silage or haylage is still a good feed? What is the difference between haylage and hay? Are there other agricultural products that use the fermentation process to maintain a quality product?

#### **Scientific Principles:**

8C-1: Hypothesize several ways organisms would respond to drastic environmental changes.

8E-8: Identify methods of determining whether a solution is basic, neutral, or acidic.

8H-7: Describe the reactant and products of a chemical reaction.

8H-9: Design and conduct an experiment which demonstrates physical and/or chemical changes.

The fermentation process has been used for generations in food and feed production. When done properly, fermented foods are tasty and last longer. Improper fermentation results in spoiled food or feed. This activity will use a mini-silo to illustrate the fermentation process.

#### **Activity Objectives:**

- Describe the fermentation process in terms of reactions that occur and the biological inputs into the process.
- 2. Explain how fermentation alters food products and enhances overall quality and taste.
- 3. Predict the extent of fermentation activity, given conditions for storing and processing food products.

## **Vocabulary Terms:**

Aerobic - containing oxygen.

Anaerobic - lacking oxygen.

Anaerobic fermentation - without oxygen, the conversion of sugars to alcohol, lactic acid, or similar compounds.

Bacteria - single-celled microorganisms. Some cause human, animal, or plant diseases, while others are beneficial.

Biochemistry - study of the chemistry of living organisms.

Biotechnology - technology concerning the application of biological and engineering techniques to microorganisms, plants, and animals; sometimes called genetic engineering.

Bunk life - the time that silage and haylage remain free of mold and spoilage after it has been exposed to air.

Clostridia - type of anaerobic bacteria.

# **Fermentation**

9D-6: Identify the control, the dependent, and the independent variables in an experiment.

9E-6: Identify the four basic types of chemical reactions: composition, decomposition, single replacement, and double replacement.

#### **Activity Length:**

Two hours to collect materials and prepare the necessary solutions (Collect plant material 12-24 hours prior to the experiment to allow time for the material to dry.)

One class period to demonstrate and for students to construct the bottle chambers (Allow 24 hours for the silicone in the chambers to dry completely.)

One class period to chop up materials, seal the bottle, and add the indicator solution

If possible, start materials fermenting on a Monday so students can observe the chambers for the remainder of the week. Take 2-3 observations the next week and one the following week.

An additional period might be needed for discussion of the product and the results.

#### **Equipment and Materials:**

To measure temperature changes more accurately, use a 5-gallon bucket with a tight-fitting lid and sampling hole instead of a 2-liter bottle. Adjust other materials as needed.

Dry matter (DM) - material left in a food specimen after all water is removed. DM is the basis on which all feed nutrients are measured.

Enzymes - complex protein molecules produced by the body that speed up chemical reactions, such as starch breakdown.

Facultative anaerobes - organisms able to grow both in the presence and absence of oxygen.

Fermentation - action of certain bacteria and yeasts in decomposing carbohydrates anaerobically.

Lactobacillus - type of bacteria that ferments sugars to lactic acid.

*pH* - measures the acidity or hydrogen ion concentration of a substance. pH values below 7.0 are acid, and values above 7.0 are alkaline. A pH of 7.0 is neutral.

Silage - a crop that has been preserved in moist, succulent condition by partial fermentation in an airtight container (silo). The chief crops stored in this way are corn, sorghum, and various legumes and grasses. The main use of silage is for cattle feed.

Silo - derived from the Greek word meaning "a pit or hole sunk in the ground for storing corn."

## **Equipment and Materials: (per group)**

Two clear, 2-liter plastic bottles with plastic tops Two small test tubes

Two 45 cm long pieces of plastic tubing (Tygon 5/16" O.D., 3/16" I.D.)

Five ears of corn (with husks) or one grocery bag of alfalfa, grass clippings, or other green material

Tube of silicone sealant

Safety razor

Sturdy, large kitchen knife

Cutting board (or a flat piece of wood or tray)

## **Fermentation**

Hole punch

Sharp needle

Heat source to heat a hole poker (needle or paper clip)

Balance and/or scale

Tapered reamer or drill

Mortar and pestle

Scissors

Beaker, 250 ml or larger

Thermometer

Black electrical tape

pH paper (to measure from a pH of 3-7 in increments of 0.5 units or less)

Microwave

Lime water (calcium hydroxide) (optional)

Cabbage juice pH indicator solution (optional)

#### **Instructor Procedures:**

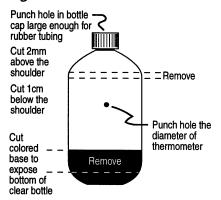
- Remind the students to carefully seal their mini-silos where the plastic tubing enters the bottle top and plastic bottle halves meet.
- Instruct students to work slowly and carefully as they use knives to cut the corn into 1 cm pieces.

## **Procedures:**

Constructing the fermentation chamber (Figure 1)

- Remove label and cut plastic bottle 1 cm below shoulder.
- 2. Cut off outer covering at bottom of bottle, leaving a slightly visible rim on the base. This will make it easier to see into the bottom of the mini-silos.
- 3. Drill or ream a hole slightly smaller than the plastic tubing's diameter in the bottle cap to allow for tight fit of the flexible tubing.
- 4. Punch a very small hole halfway up from the bottle base. (This hole will be for temperature and pH reading.) Cover hole with black electrical tape.
- 5. Insert plastic tubing about 2.5 cm through the top of the cap.
- 6. Seal with silicone sealant where the plastic tubing goes through the top of the cap; let dry.

#### Figure 1



# **Fermentation**

 Push the tubing into the test tube as far as it will go so it stays in place as the gas collects.

To test for CO,

- 1. Attach a test tube to the outside of the plastic bottle with silicone sealant.
- 2. Fill the test tube with cabbage indicator solution or limewater to within 2.5 cm of the top.

INSTRUCTOR NOTE: When carbon dioxide bubbles through limewater, a calcium carbonate precipitate forms. Limewater forms the white precipitate when acidified.

3. Place plastic tubing all the way to the bottom of the test tube.

INSTRUCTOR NOTE: Alfalfa will work the best. Cut alfalfa the night before and allow it to wilt overnight.

## Setting up the experiment

- 1. Cut the corn, alfalfa, grass clippings, or other green plant material into 1 cm pieces; divide into two portions. You will need enough to pack the two silos tightly (approximately 2 x 500 grams) with 150 grams left over. With corn, include the kernels, husk, and stalks (if available); do not attempt to cut up the cob.
- 2. With the 150 grams of extra material, determine the dry matter (DM) content and initial pH of the sample. DM content is the most important outcome in silage production. A loss of DM indicates a loss of food value. If you are not going to measure DM immediately, store the sample in a sealed plastic bag to minimize moisture loss.

To measure DM, microwave the sample for two minutes, stirring if necessary to prevent burning of edges. Weigh the sample and record the weight. Microwave for another 30 seconds and weigh again. Continue until the weight is constant.

# **Fermentation**

Calculate DM as follows:

DM% = ending weight (grams) x 100

To measure initial pH, grind 50 mls (milliliters) of the material with a mortar and pestle; take the pH of the extracted liquid.

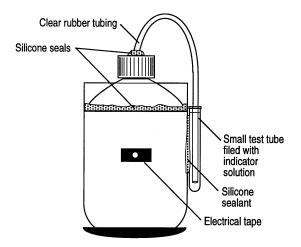
- 3. Firmly pack material into the bottle silos by pushing down with your knuckles as you rotate the bottle. Do this until 500 grams of material is in each of the two bottles. It is very important to pack the material as tightly as possible to eliminate air pockets.
- 4. Place the tops of the bottles into the bottoms and push downward as far as they will go. (See Figure 2.)
- 5. While holding firmly in place, seal off where the top and bottom plastic meet with silicone sealer.
- 6. Place the bottle caps with attached rubber tubing on top of the bottles; screw them on tightly.
- 7. Using a sharp needle, poke 30-50 holes in one bottle base to allow for an aerobic (with oxygen) environment. The other bottle will remain anaerobic (without oxygen).
- 8. Record data every day for the first week and on Monday, Wednesday, and Friday of the following week. One data point will be taken in the third week. Measure and record the following on Graphs 1 and 2.
  - \* pH of the material Open the sampling port on the side of the bottle and insert pH paper. You may need to rub the paper around a bit to get moisture on it. Remove the paper and record the pH.

# **Fermentation**

The sample may be too dry to get a reading with pH paper. To get a reading, grind silage with distilled water and take a reading.

- \* Temperature of the material Insert a thermometer into the center of the silo through the sampling hole and record the temperature. Remove the thermometer and tightly reseal the hole with a new piece of electrical tape.
- \* Presence of CO<sub>2</sub> Check for the presence of CO<sub>2</sub> by bubbling the CO<sub>2</sub> through water, cabbage juice, or limewater. (See Figure 2.)

Figure 2



9. After three weeks, measure the DM content of the anaerobic silo only. The silo exposed to oxygen may have undesirable fungi and bacteria growing in it and should not be opened. Dispose of it.

## Answers to Key Questions: Key Questions:

 Hypothesis may contain a statement that the student believes might happen. Example: Bacteria allowed to grow in a controlled environment will help preserve food.

•	State your hypothesis.	•	78.00

Crop	Science:
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# **Fermentation**

Graph 1 - Daily pH changes in mini-silos

рΗ

Day

Graph 2 - Daily temperature changes in mini-silos

Temp.

Day

- Temperature and pH will vary during the experiment according to the dependent variable, the days.
- 3. The pH went down to about 4.0.
- What are the dependent variables of the experiment? \_\_\_\_\_

3. What happened to the pH as the days increased during the experiment?

Cı	op Science:	Fer	mentation
4.	As the number of days increased, the pH decreased.	4.	Explain your pH observations from Graph 1.
5.	Temperature increased.	5.	What happened to the temperature as the days increased during the experiment?
6.	As the number of days increased, the temperature decreased.	6.	Explain temperature observations from Graph 2
7.	The silage took on a slight color change (to a darker green) because some liquid accumulated at the bottom. There was no mold growth in the silage.	7.	What changes did you observe in the silo during the experiment? What may have caused these changes?
8.	(Depends on the student's hypothesis)	8.	Was your hypothesis supported? Explain.
9.	Bacteria convert complex compounds, such as sugars, into simpler products during the anaerobic process.	9.	What are the reactants of anaerobic fermentation? _
10.	Alcohol, lactic acid, and carbon dioxide are products of the fermentation process.	10.	What are the products of anaerobic fermentation?
11.	Many beneficial types of bacteria are growing and reproducing.	11.	What organisms caused the changes occurring in the mini-silos?
12.	Yes. Through the fermentation process, bacteria are growing and reproducing.	12.	Would you describe your mini-silo system as alive and dynamic? Explain
13.	Making sauerkraut, yogurt, pickles, beer, wine, and sourdough breads are all processes that require fermentation.	13.	What other processes that are helpful to people use anaerobic fermentation?

		1	
14.	Fermentation is the anaerobic process by which bacteria convert complex compounds, such as sugars, into simpler products, such as alcohol, lactic acid, and carbon dioxide.  Fermentation inhibits the growth of other nonfermentative bacteria and fungi, therefore preventing food from spoiling.	14.	What is fermentation?
15.	Fermentation is promoted by the moisture in the silage, anaerobic conditions within the silo, filling a silo rapidly, compactness of the plant material, and tightly sealing the silo. With time, oxygenconsuming microorganisms in a packed silo create an increasing anaerobic environment. Under these conditions, beneficial bacteria present on the crops begin to multiply. As the bacteria convert sugars to lactic acid, acetic acid, and ethanol, the pH drops. The fermentation process takes 1-3 weeks.	15.	What conditions promote fermentation? Why?
16.	If carried out correctly, it preserves valuable nutrients so they can be eaten in the off season or when fresh foods are not available. Bacteria convert complex compounds, such as sugars, into simpler products, such as alcohol, lactic acid, and carbon dioxide. Fermented products are quite palatable (tasty) to humans and prevent the food from spoiling by inhibiting the growth of undesirable bacteria and fungi.	16.	Why is fermentation desirable in the processing of some plant products?
17.	Many harmful bacteria cannot tolerate low pH. In a pH range of 3.7-4.1, silage will be preserved almost indefinitely.	17.	How does pH affect fermentation?

**Fermentation** 

Crop Science:

# **Fermentation**

## Supplemental References:

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Gillespie, J. R. *Animal Nutrition and Feeding*. Albany, NY: Delmar Publishers, Inc., 1987.

McDonald, Peter. *The Biochemistry of Silage*. Chichester, England: John Wiley and Sons, Ltd., 1981.

Osborne, E., J. Moss, and A. Stahl. Biological Science Applications in Agriculture: Plant Science (Teacher's Guide). Urbana-Champaign: Agricultural Education, University of Illinois, 1993. **Credit:** "AgriScience Institute and Outreach Program Instructional Materials." Alexandria, VA: The National Council for Agricultural Education, 1992.

#### **Applications:**

Throughout history, humans have harnessed fermentation to produce and preserve food. Microbial fermentation is important for many of today's foods, such as yogurt, breads, cheese, wine, and beer, as well as in agricultural situations to store high-quality feeds. Producers ferment corn plants to produce silage and hay to produce haylage. Succulent forages are therefore preserved and stored for use throughout the year.

The production of a high-quality silage product requires that plant respiration, protein degrading, enzyme activity, undesirable anaerobic bacterial growth, and aerobic microbial growth be limited. An essential way to limit these processes is to create anaerobic conditions within the silo. If left exposed in an aerobic environment, harvested plant material will decay into a useless, inedible, and often toxic product. This decay is caused by aerobic microbial activity.

Filling a silo rapidly, making sure the plant material is very compact, and tightly sealing the silo keeps the oxygen level to a minimum. The amount of moisture in the silage is another important factor. Because temperatures of 95-99°F encourage the growth of clostridia, maintain temperatures higher than 99°F.

Continued fermentation research includes the isolation and engineering of new, mutant forms of superhomofermentative bacteria as silage inoculants. Also, scientists look for strains of bacteria that may work especially well on particular crops.

#### **Extension:**

Using the same procedure, build a silo from a 5-gallon bucket instead of a 2-liter bottle. Take periodic pH and temperature readings.

# **Hydroponics**

#### **Student Outcomes:**

Horticulture 1: Describe preparations necessary for fruit and vegetable production.

Horticulture 3: Identify characteristics and production practices for warm season solanaceous crops.

Plant Science 3: Describe factors that affect plant growth and development.

#### Situation/Problem:

How many acres would it take to grow enough food to support one person? How much would that soil weigh?

How can we grow food in small spaces to sustain a person for long periods of time?

#### **Scientific Principles:**

9C-2: Infer from examples of environmental conditions the ways in which living things would adapt in order to survive.

10E-1: Compare and contrast organic and inorganic compounds.

#### **Activity Length:**

Class time: 50 minutes

NOTE: This activity is designed to help students design and build their own hydroponic systems. The types of systems discussed in the procedure can be altered to fit student needs. Students can build their own units according to available budget and the type of system that best fits their needs.

What do plants need to grow? Can plants grow without soil? In this activity, students will explore hydroponics, the growing of plants in nutrient solutions without soil.

## **Activity Objectives:**

- Compare the growth of plants grown hydroponically with those grown in soil.
- 2. Identify necessary components in a hydroponic solution for successful plant growth.
- 3. Explain the advantages of hydroponics as compared to growing plants in soil.
- Design and build a hydroponic system for home use.

#### **Vocabulary Terms:**

Aeroponics - when plants are suspended and a mist of nutrient solution is sprayed on the roots.

Gravel culture - where plants are grown in channels that are sub-irrigated from the bottom or in open systems that are trickle irrigated. The gravel serves only as a plant support media.

pH - measures the acidity or hydrogen ion concentration of a substance. pH values below 7.0 are acid, and values above 7.0 are alkaline. A pH of 7.0 is neutral.

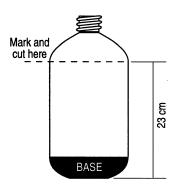
Sand culture - open system (where nutrient solution is not caught and recycled). Plants are grown in nutrient-free sand and fed by trickle irrigation.

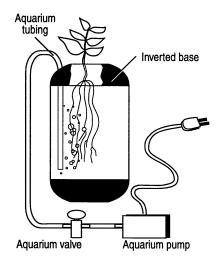
Water culture - growing plants hydroponically.

#### **Equipment and Materials:**

Electrical conductivity (EC) meter Two-liter soda bottles (2) Felt-tip marker Sharp knife

# Figure 1 - Solution culture system (Credit: "Pardon Me, but Your Roots are Showing."





# **Hydroponics**

Scissors

Aluminum foil

Plastic aquarium tubing

Plastic soda straw

Aquarium pump with three-way valve

**Plants** 

Plastic bag

Water (either tap or distilled)

Hydroponic nutrient solution (can be ordered from science education suppliers or hydroponic suppliers)

#### **Procedures:**

System construction

- Fill a pair of two-liter soda bottles with hot tap water to soften the glue so the labels can be easily removed. Use bottles with black bases because they exclude light.
- 2. Twist or pry off the base of one bottle and invert it to form the system lid.
- 3. Mark the other bottle 23 cm from the base using a felt-tip marker. With a sharp knife, puncture the bottle at the mark, then cut off the top with scissors. This detopped bottle is the system reservoir.
- 4. Cut a hole in the lid with a cork borer or knife so plants can be inserted.
- 5. To prevent algae growth, exclude light by wrapping aluminum foil around the middle of the reservoir.

#### Aeration

- 1. Insert a length of plastic aquarium tubing through an existing hole in the lid.
- 2. Slip a plastic soda straw over the tubing to give it rigidity.

# **Hydroponics**

3. For aeration, attach an aquarium air pump to the tubing. Use a three-way aquarium valve to adjust the airflow to bubble gently.

NOTE: You can run more than one hydroponic system off each aquarium pump by making T joints off the valve.

#### **Plants**

Foliage houseplants are good for solution culture projects because they tolerate low light in homes and classrooms, are readily available, and root quickly from cuttings. Shoot cuttings of wandering jew, coleus, piggyback plant, pothos, and common philodendron will root in the solution culture system. If the tap water is very low in calcium, add 2 mmol dm<sup>-3</sup> CaCl<sub>2</sub> or CaSO<sub>4</sub> since roots require calcium in the external solution. A plastic bag over the cuttings will prevent dehydration. Vine or rosette types of plants are preferred because they do not require staking.

#### Nutrient solution

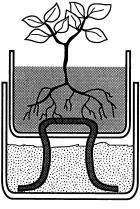
Read the label on the type of nutrient solution you are using to determine mixing requirements. The solution can be prepared from tap water unless the water quality is poor or nutrient-deficient solutions are desired. Use distilled water in those cases.

#### Maintenance

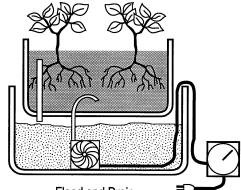
- 1. Add water whenever the solution level drops by more than one-third.
- 2. Change the solution weekly or biweekly, or use an electrical conductivity (EC) meter to estimate the ion concentration.
- 3. When the EC has dropped to half the original value, replace the solution. Generally, deficient solutions do not need to be changed since slower plant growth will not deplete the ion concentrations.

# **Hydroponics**

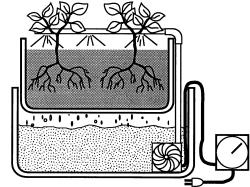
Figure 2 - Other hydroponic systems



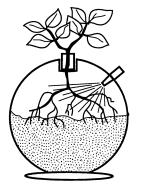




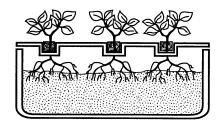
Flood and Drain



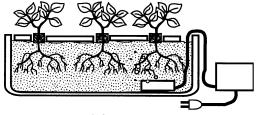
Top Feed



Aero-hydroponic



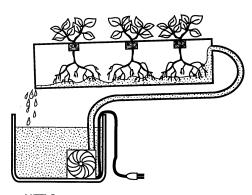
Non-circulating System



Raft System



Aeroponic



NFT System

# **Hydroponics**

Other systems to build (See Figure 2.)

- Wick Nutrient solution is drawn up into the planting medium through fiber wicks immersed in the reservoir below.
- 2. Flood and drain These rely on the periodic flooding and draining of a medium-filled growing bed with nutrient solution.
- 3. Top-feed or drip A timer-controlled pump delivers nutrients regularly to the base of the plants by thin tubing or drip emitters. The nutrient solution is returned to the reservoir by gravity.
- 4. Aero-hydroponic (water culture) These media-less systems require constant contact between root structures and the nutrient solution to prevent drying and rapid root death.
- 5. Non-circulating The plants are supported above the nutrient reservoir with the root tips hanging in the nutrient solution. An air space between the plants and the surface allows some roots to specialize in oxygen uptake, while the lower roots take up nutrients and water.
- 6. Raft Plants are supported by collars or rock wool cubes pressed into holes cut in a floating raft, usually constructed of rigid foam board. The board and plants float on the surface of the nutrient reservoir. Since the roots are constantly immersed in the solution, mechanical aeration is necessary.
- 7. Nutrient Flow Technique (NFT) Nutrients are delivered to the roots by continuous flow in tubes, gutters, or troughs. Plants are supported from above with collars, cups, or rock wool cubes. The solution is collected and returned to the reservoir for reuse.

# **Hydroponics**

- 8. Aerohydroponic The lower roots are suspended in a constantly moving nutrient solution. Similar to aeroponics, the upper roots are sprayed or misted with nutrient solution.
- Aeroponics Plant roots are suspended in an enclosed container and sprayed or misted with nutrient solution. Aeroponics is the ultimate in hightech growing technology.

NOTE: The best system depends on the grower and that person's unique growing environment.

#### **Answers to Key Questions:**

- Soil contains the necessary nutrients for a plant to survive. Think of soil as the cupboard containing the food supply. With hydroponics, plants are given the proper amounts of nutrients directly, without the soil acting as the cupboard.
- The pH is the degree of acidity or alkalinity. It affects the availability of certain nutrients for the plant. Wrong pH levels can poison the plant with the nutrients or restrict the plant's availability to use certain nutrients.
- Oxygen is an essential element for plants to survive. Roots need air to breathe and prevent suffocation. Some plant roots do not need as much oxygen as other plants.
- Nutrients are essential for plant growth and production.
   Deficiency effects are the coloring of the leaves or lack of fruit or vegetable development.
   Plant leaves will yellow from lack of proper nutrients.

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1.	Why are plants able to survive without soil?
2.	What effect does a nutrient solution's pH have on plant growth?
3.	Why is oxygen pumped into the nutrient solution in most hydroponic systems?
4.	What effects do nutrient deficiencies have on plants?

- 5. Plant support is lightweight because there is no soil, nutrients are used more efficiently, plants can be grown year-round with no interruption or need to rotate crops, crop diseases and bug infestations are avoided, minimum physical effort and attention is needed, there is more control over the crop, and less space is required.
- A container to grow the plants, an inert material to support the roots and stems of the growing plants, a nutrient solution to feed the plant, and air and light supplies

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What are the basic red	quirements to start a

#### **Credits:**

Hershey, D. R. "Pardon Me, but Your Roots are Showing." *The Science Teacher* 57(2) (February 1990): 42-45. Reprinted with permission from NSTA Publications, Jan. 1993, from *The Science Teacher*, National Science Teachers Association, 1840 Wilson Blvd., Arlington, VA 22201-3000.

Parker, D. "Systems for Beginners: Hydro 101." *The Growing EDGE* 5(1) (fall 1993): 61-66. (For more information, call 800/888-6785.)

# **Hydroponics**

#### **Supplemental References:**

Carpentier, D. R. "Hydroponics on a Budget." *The Agricultural Education Magazine* January 1991: 63(7): 15, 16, 23.

Hershey, D. R. *Hydroponics for Teaching*. College Park, MD: University of Maryland, 1992.

Hershey, D. R. "Pardon Me, but Your Roots are Showing." *The Science Teacher* 57(2) (February 1990): 42-45.

Parker, D. "Systems for Beginners; Hydro 101." *The Growing EDGE* 5(1) (fall 1993): 61-66. (For more information, call 800/888-6785.)

Hydroponic Society of America, PO Box 3075, San Ramon, CA 94583. 510/743-9605.

#### **Conclusions:**

Hydroponics is another way to grow vegetables in areas where good soil is scarce. Scientists predict that one day our cities will have huge towers of hydroponic farms that will make fresh vegetables available every day of the year. Work is underway now to use soilless farming on space shuttle flights.