



Wyoming  
Demonstration  
and Research  
Pest Control:  
Category 910

## **Wyoming Agricultural Pest Control: Categories 910**

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# PREPARATION FOR YOUR EXAM

If you are preparing to take the Wyoming Commercial Pesticide Applicator Exam for category 910, review this manual several times. Please read and respond to the learning objectives in each section.

Exam questions may come from any section of this manual — this includes the glossary.

It is important that you take note of the following:

- You may bring a basic hand-held calculator with you to use during the exam (cell phones and other communication devices are prohibited — **you will be failed** if using your cell phone during the exam).
- Exams are closed book. You will not be allowed to refer to any notes, manuals, or other unauthorized training materials during the exam.
- You must pass each category with a 70% or better to be issued a license.
- Exams can be taken at any University of Wyoming County Extension office — please call your local Extension office to make an appointment.



# ABOUT THE WYOMING COMMERCIAL PESTICIDE APPLICATION AND SAFETY TRAINING GUIDE FOR DEMONSTRATION AND RESEARCH PEST CONTROL

Persons certified in Demonstration and Research Pest Control (Category 910) are allowed to make applications of pesticides in the course of conducting field research or demonstration. No license or certification will be issued in this category unless the applicant also obtains licensing or certification in the specific category listed in these rules, which is appropriate to the research activity.

## INFORMATION ABOUT THIS TRAINING GUIDE

This manual is the basis for the certification exam in Category 910: Demonstration and Research. The educational material provides practical information to prepare you to meet the exam requirements. It is designed for research scientists, Extension Agents, Extension Specialists, industry representatives, employees of federal and state government as well as other professionals who conduct research in categories covered by the Wyoming state statutes using unregistered experimental pesticides or demonstrations with registered pesticides. It will help you learn about the differences between research and demonstration, laws pertaining to conducting research and demonstrations with pesticides, good lab practices, and applying research and scientific methods when studying pesticides. You will also learn about safely using pesticides when conducting research and how to calibrate some of the equipment used in small plots.

## HOW TO PREPARE FOR THE EXAM

This study guide **DOES NOT** include the knowledge you need to pass other category certification exams which are required when conducting research and demonstrations using pesticides [such as Ag Insect Pest Control (901B) or Agricultural Weed Control (901A)].

Topics from the Wyoming Pesticide Applicator Certification CORE manual which may be included on your exam include: first aid, personal protective equipment (PPE), protecting the environment, pesticide movement, surface and groundwater protection, endangered species, application methods and equipment, equipment calibration, pesticide use, pesticide formulations, pesticide applications, and area measurements. The resource for ordering the Wyoming Pesticide Applicator Certification CORE manual can be found at the University of Wyoming Pesticide Safety Education Program website, <https://uwyoextension.org/psep/commercial-applicators/training-materials/>.

## USING THIS GUIDE

At the beginning of each chapter, learning objectives highlight the key information you should understand and be familiar with before taking the Demonstration and Research exam.

A list of additional resources is included at the end of each chapter and is compiled at the end of the manual. These are included to provide you with

additional information above and beyond what is presented in this study guide.

A glossary of terms used in this guide is found near the end of the guide. Terms that appear in the glossary are in boldface type the first time they are found in the guide or when the definition is given. Items in the glossary may be found on the exam, so the glossary is an important study resource.

# CHAPTER 1: INTRODUCTION TO DEMONSTRATION AND RESEARCH

## LEARNING OBJECTIVES

After reading this section, you should be able to:

- A. Describe the types of people who might conduct research and demonstrations.
- B. Discuss the types of pesticides and how they are used in research or demonstration.
- C. List the types of research sites and exclusions.
- D. Describe the basic differences between demonstration and research activities.
- E. Name the types of demonstration and research and how they differ.
- F. Explain how to apply the scientific method to pesticide research trials.
- G. Describe the advantages and disadvantages of demonstrations and research plots.
- H. Describe the factors in deciding whether to conduct field research off-station versus on-station.
- I. List the advantages and disadvantages of on-station research.
- J. List the advantages and disadvantages of off-station research.
- K. Describe the desirable characteristics of a farmer-cooperator when considering off-station research.
- L. Explain how Integrated Pest Management (IPM) fits into Demonstration and Research, including the use of economic thresholds versus action thresholds.

## WHO CONDUCTS DEMONSTRATIONS AND RESEARCH EXPERIMENTS?

People who are included in the Demonstration and Research Pest Control category are working with either restricted-use pesticides, unregistered pesticides or **off-label** uses of a pesticide.

A **restricted-use pesticide** (RUP) that is classified under the provisions of FIFRA can only be sold to or used by certified applicators. A pesticide, or some of its uses, is classified as restricted if it could cause harm to humans (pesticide handlers or other persons) or to the environment (non-target organisms or potential contamination of water sources). RUPs may be used in either demonstrations or research.

**Unregistered pesticides**, are usually pesticides that are under development that have not yet received an EPA registration number because the manufacturer must obtain data to submit with the pesticide registration packet. Unregistered pesticides are often tested through research experiments to determine efficacy, residue levels, timing of application, etc.

Research is sometimes conducted that would be considered an off-label use. This includes applying to crops that are not included on the label (to establish tolerances, or allowable residue levels), at rates above the label rate (to determine if **resistance** may be developing), or to determine a crop tolerance at two-times rates or for a prohibited application method or for more applications than

allowed by the label. Off-label uses are generally limited to research experiments.

People licensed in Category 910 are typically demonstrating to the public the proper use and techniques of applying restricted-use pesticides or supervising such demonstrations. They are often Extension specialists, Extension agents, vocational agriculture instructors, college and university instructors, and industry representatives who demonstrate pesticide products as well as others who demonstrate methods used in public programs, such as County Weed and Pest Districts.

This category also includes those who conduct field research with restricted-use pesticides, field research with experimental (unregistered, unnumbered) pesticides, and investigate off-label uses such as applying to an unlisted crop, changing the type of application equipment, timing of application, or applying higher rates than the label. This group typically includes state, federal, university, industry, commercial applicators, and research scientists.

Genetically Modified (GM) crops are under the oversight of USDA during the experimental phase. These include crops which are herbicide-tolerant or contain plant-incorporated-pesticides (PIP). While herbicide-tolerant crops are regulated in the experimental phase, they are deregulated upon commercialization. However, the herbicide must be registered by U.S. EPA and Wyoming for use on the GM crop. After the experimental phase of development of a PIP crop, the final crop is not labeled as a pesticide by U.S. EPA and does not require pesticide registration in Wyoming.

Excluded from federal and state regulatory requirements are persons conducting controlled laboratory-type research involving RUPs as well as doctors of medicine and doctors of veterinary medicine applying pesticides as drugs

or medication during the course of their normal practice.

## **DEMONSTRATION VERSUS RESEARCH EXPERIMENTS**

Research experiments and demonstrations are very different activities that have distinct goals and are designed, analyzed and reported differently.

### **DEMONSTRATIONS**

The goal of **demonstrations** is to show how a product or method works under local conditions. Demonstrations are usually used to acquire experience with new technology and expose others to new technology. Demonstrations involve application equipment and use of registered pesticides or restricted-use pesticides. Demonstrations can be categorized as either method demonstrations or result demonstrations. Demonstration trials are often large scale, strip trial experiments which may or may not be replicated.

#### **Method demonstrations**

**Method demonstrations** show how to do something. For example, how to calibrate or properly clean a sprayer.

#### **Result demonstrations**

**Result demonstrations** show, by example, what happens with the practical application of new information, principles, or comparisons that support a practice or recommendation. For example, application of a herbicide at different growth stages or comparing a pesticide application with or without an **adjuvant**. Yield data is not necessarily measured or analyzed.

An effective result demonstration requires a clear-cut and simple objective and a uniform field site that is easily accessed. Observations and notes

should be made throughout the season or duration of the demonstration, which may prove useful to explain unexpected developments. Field days are often used to show off the results.

### RESEARCH EXPERIMENTS

**Research** is a systematic investigation that includes research design, testing and evaluation. Research experiments are often small plots in a replicated design. This allows for the data to be analyzed statistically. The goal of research experiments is to generate data that can be used to:

- Support new pesticide uses or methods such as new rates, sites, equipment or frequency of application.
- Add new target pest species to the label.
- Support existing knowledge.
- Close gaps in existing information.
- Develop new information.

Most research experiments follow the **scientific method** because it allows a researcher to use a logical problem-solving approach to answer a question. If the researcher encounters a problem in the experiment, or the experiment fails, the scientific method provides clues or remedies to make logical changes in the experiment. Before the scientific method was applied to research, experimenters used trial and error which led to repetition of results, misleading results, and incorrect conclusions.

The scientific method also allows a framework for a scientist to repeat or replicate another scientist's experiment. If an experiment cannot be replicated, then the conclusions drawn from the original experiment are suspect.

The scientific method involves the following steps:

1. Identify the problem, purpose or research question.

2. Do background research to learn what others have discovered about your topic.
3. State the **hypothesis** to determine how you think your question should be answered.
4. Design the experiment.
5. Collect, analyze and interpret the data.
6. Draw a conclusion based on your research and the data you collected. (Accept, reject or alter the original hypothesis.)
7. Communicate your results to others who are interested in the topic.

The goal of conducting research using the scientific method is to predict future responses. This requires well-designed experiments and statistical analysis. More details on these topics are included in Chapters 5 through 7, which discuss research experiments and scientific method in more detail.

### ADVANTAGES AND DISADVANTAGES OF DEMONSTRATIONS AND RESEARCH PLOTS

#### Demonstration plots

There are many advantages and barriers to consider when conducting method demonstrations (such as safe and effective use of pesticides or use of spray equipment). You need to be concerned whether language barriers, literacy barriers or cultural barriers exist. For purposes of this category and study guide, the focus is on result demonstration plots. It is important to understand the advantages and disadvantages of conducting result demonstration plots.

Advantages to using results demonstration plots include:

- Allows producers to see improvements on their own farms.
- Does not require replication as it is intended only to gain experience with new technology or practices.

- Allows for comparisons over multiple sites to see the effects under several environments.
- Provides opportunities to connect with producers during field visits and field days.
- Cost of inputs may be lower (producer may provide seed, fertilizer, water, etc. and manage the site).

Some disadvantages to using results demonstration plots include:

- Requires relatively uniform field conditions.
- Less control over site management.
- Disseminating single-plot data can lead to serious errors if producers use it to make decisions, especially farm-wide decisions.
- May be difficult to obtain field history.
- May be difficult to get producers to visit the site.
- Obtaining yield data may not be possible.

## RESEARCH PLOTS

Most agricultural research is done on agricultural experiment stations (**on-station**) or other facilities specifically intended as research sites. However, some research experiments are conducted off-station **on farms** or other sites such as open space or rangeland. There are advantages and disadvantages to both types of sites.

### On-station Research

Advantages to using on-station research plots include:

- Produces results recognized by scientific communities.
- Results are suitable for making decisions.
- Smaller plots generally minimize within-field variability.
- Easier access than traveling to off-station sites.
- More control over plot management.

Some disadvantages to using on-station research plots include:

- Requires more planning and are more complicated to conduct.
- Required equipment may be limited in availability, or specialized and expensive.
- Because pest infestations are often localized, plots may need to be inoculated or infested.

### Off-station Research

Advantages of conducting off-station research include:

- Access to specific pest infestations at naturally occurring levels.
- Access to particular soil types or other physical characteristics not on-station.
- Allows for larger plots.
- Producer may provide access to specialized or commercial-scale equipment.
- Ability to analyze systems that involve multiple interactions for pest management.
- Allows researcher to study long-term effects or a particular production history.
- Producers can provide reality checks as to whether the research will be adopted.

Some disadvantages of conducting off-station research include:

- May be a limited ability to control the experimental conditions such as timing of management practices thus increasing the statistical variability.
- Greater risk of total loss of the experiment due to pest infestations, drought, or other physical or biological stresses that might be controlled on-station.
- The research site may not be a priority for management operations (irrigating,

managing other pests, harvesting, etc.) compared to the rest of the farm.

- If larger plot sizes are used, within-field variability may increase.
- Lack of control may result in accidental over-spray of other pesticides and loss of the trial.

### DECIDING WHETHER TO CONDUCT FIELD RESEARCH ON-STATION VERSUS OFF-STATION

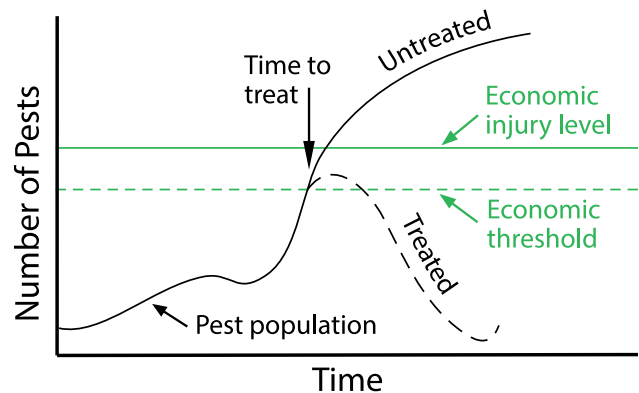
Questions to consider when deciding whether to conduct research on- or off-station include:

- How much control is needed over management practices?
- If conducting off-station research, how involved will the producer be?
- Do you lack necessary equipment or need access to commercial-scale equipment?
- Will the research question be better answered by on-farm research?
- Will the results be better seen or better observed by farmers?
- Does the research treatment(s) need to be tested under a range of conditions?

If you consider conducting on-farm research, look for cooperators who recognize the value of on-farm research and may already be conducting their own research. The cooperator should be someone who has demonstrated that they are willing to adopt new technology or techniques and are willing to make a long-term commitment of land and efforts. The researcher should have well-defined expectations of the cooperator regarding the commitment for land and their role. And, of course, the researcher should always be open to input from the cooperator — that's a real benefit of on-farm research.

## INTEGRATED PEST MANAGEMENT IN DEMONSTRATION AND RESEARCH

Understanding an entire crop-pest ecosystem is not a simple task. When conducting research or demonstration experiments in pest management, it is important to minimize some of the background effects (such as weed competition interfering with insect studies that require yield data) that may be mitigated by using **Integrated Pest Management (IPM)**. Researchers should attempt to use cultural and mechanical methods to manage pests that may interfere with the study. For example, if conducting insect pest research, using a herbicide-tolerant crop may reduce the weed competition 'noise'. IPM strategies can also be included as part of a pest management study. For example, the use of various mulches was investigated to see if it could be used to reduce thrips populations, and subsequent Iris Yellow Spot Virus (IYSV).



To make a control practice profitable, or at least break even, it is necessary to set the economic threshold (ET) below the economic injury level (EIL). Image: National Pesticide Applicator Certification Core Manual.

When conducting demonstration-type experiments, yield data is often not collected so there is little to no concern about using economic thresholds. **Economic thresholds** may or may not be part of a research-type experiment. **Action**

**thresholds** may be more frequently used for demonstration and research experiments. This may include a set of conditions such as the proper weather conditions at a susceptible crop stage for a disease to develop. Or an action threshold might be based on the presence of a particular weed species in a crop. Whenever possible, IPM should be part of the experimental design.

## CHAPTER 1 ADDITIONAL RESOURCES

Baldwin, K. 2004. *A Field Guide for On-Farm Research Experiments*. North Carolina State University Cooperative Extension. [www.sare.org/on-farm-research-field-guide](http://www.sare.org/on-farm-research-field-guide)

Nielsen, R.L. 2010. *A Practical Guide to On-Farm Research*. Agronomy Dept., Purdue University. <https://www.agry.purdue.edu/ext/corn/news/timeless/OnFarmResearch.pdf>

*Wyoming Pesticide Applicator Certification Core Manual, Second Edition*, 2015. [https://uwyoextension.org/psep/wp-content/uploads/2012/09/Core\\_manual\\_10\\_24\\_16-1.pdf](https://uwyoextension.org/psep/wp-content/uploads/2012/09/Core_manual_10_24_16-1.pdf)



## CHAPTER 2: LAWS AND REGULATIONS

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Describe how FIFRA, FFDCA and WPS apply to research and demonstration.
- B. Explain when a Federal EUP is required and exemptions to the EUP.
- C. List the labeling requirements of EUPs.
- D. Explain who has to comply with WPS and how it applies to REIs and required training for handlers or workers when working with research and demonstration.
- E. Describe how research and demonstrations relate to tolerances and exemptions from tolerances.
- F. Explain the regulations in Wyoming that pertain to demonstrations and research including applicator licensing, recordkeeping and local regulations.
- G. List the type of pesticide application records that must be kept for demonstrations and research and the retention period in Wyoming.
- H. Describe how research experiments may be used to support Section 18 and 24c registrations.
- I. Describe when crop destruct is required, grazing restrictions may exist or treated seed crops must be destroyed or labeled properly.
- J. Know who is liable for pesticide applications made under demonstrations and research.
- K. List the requirements for proper storage and transportation of pesticides used for demonstrations and research.

Laws governing the use and users of pesticides are designed to protect humans and the environment. Federal and state laws and regulations govern the manufacture, sale, transportation, and use of pesticides. The United States Environmental Protection Agency (EPA) is the primary regulatory agency as this authority comes from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Pesticide applicators in Wyoming must comply with the federal regulations and regulations under Wyoming state statutes. This chapter provides a review of laws and regulations as they affect applications made for research and demonstration purposes. If you need more information on the federal and state laws and regulations beyond what is presented here, refer to the Additional Resources, page 20, and the Wyoming Pesticide Applicator Certification Core Manual, <https://uwyoextension.org/psep/commercial-applicators/training-materials/>.

### REGULATIONS AFFECTING DEMONSTRATION AND RESEARCH

#### FEDERAL INSECTICIDE, FUNGICIDE, RODENTICIDE ACT (FIFRA)

At the national level, the United States Environmental Protection Agency (EPA) is the primary pesticide regulatory agency. The EPA's authority comes from the **Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)**.

Originally passed in 1947, this law has undergone several amendments and updates including the 2015 Revision to the Worker Protection Standard and in 1996, the Food Quality Protection Act. Based on FIFRA, the EPA establishes regulations for pesticide registration and labeling, certifies commercial and private pesticide applicators to use restricted-

use pesticides, enforces the Worker Protection Standard, and develops pesticide residue tolerance levels on or in food and feed. These regulations also set standards for experimental use of pesticide compounds. Other federal agencies, including the U.S. Department of Agriculture, the National Institute for Occupational Safety and Health, the U.S. Fish and Wildlife Service, and the U.S. Food and Drug Administration may also monitor and regulate some activities involving pesticide use, including research and demonstration activities.

If you are conducting pesticide research and demonstration in Wyoming, you are required to comply with the federal laws and regulations regarding pesticide use. Some examples include:

- Follow all of the label requirements on the pesticide container or any supplemental labeling.
- Comply with the Worker Protection Standard.
- Obtain the proper pesticide applicator license to conduct experimental pesticide use.
- Keep pesticide application records.
- Store, transport, and dispose of pesticides and pesticide containers properly.
- Protect people, animals, and the environment.
- Follow the specific laws and regulations in Wyoming that cover the types of activities used for research experiments and demonstrations, including experimental uses of unregistered products or uses that are not allowed by the label of pesticides registered in Wyoming.

### **EXPERIMENTAL USE PERMITS**

EPA requires that a pesticide product undergo extensive chemical, toxicological, crop residue, and efficacy testing before being registered as a pesticide. Some testing is done under field

conditions using commercial application equipment to fully understand the pesticide's chemical properties, safety, and efficacy.

A federal or state Experimental Use Permit (EUP) is required for experimental use:

- on more than 10 acres of land, or
- more than 1 surface acre of water, or
- of pheromone used at rates <150 grams ai/acre/year if the site exceeds 250 acres, or
- testing of pesticides indoors (e.g. cockroach or termite control).

This permit is required for entities that want to conduct experimental use on more than 10 acres or more than 1 surface acre of water. Experiments with pheromones used at rates less than 150 grams ai/acre/year require a Federal EUP when the site exceeds 250 acres. EUPs are required for testing of pesticides indoors. This includes testing of pesticides for use in domestic dwellings and institutions (such as control of cockroaches and other insect pests), and for field-testing of swimming pool sanitizers and disinfectants under actual use conditions.

Federal EUP regulations require pesticide products shipped or used under federal EUP be labeled with directions and conditions for use. In most cases, this labeling will include the following:

- a prominent "For Experimental Use Only" statement,
- the federal EUP number,
- the name, brand, or trademark
- the name and address of the permit holder, or producer, or registrant,
- the net contents,
- an ingredient statement,
- any appropriate limitations on entry of people into treated areas,

- the establishment registration number except in cases where application of the pesticide is made solely by the producer,
- the directions for use,
- in addition to these items, when a federal EUP is used under federal conditional registration, the labeling must include the following statement: “Not for sale to any person other than a participant or cooperator of the EPA approved Experimental Use Program,”
- Warning or Caution statements, and
- supplemental labeling — in the case of a registered pesticide permitted to be used under an experimental use.

### EXEMPTIONS FROM FEDERAL EUP REQUIREMENTS

EPA will generally NOT require an EUP for an experimental substance or mixture of substances if the EUP is limited to:

- Laboratory and greenhouse tests.
- Limited replicated field trials when testing is on:
  - plots of land 10 acres or less in size. HOWEVER, if testing involves more than one pest being investigated at the same time, the test plot still may NOT exceed more than 10 acres.
  - water bodies one surface acre or less in size. However, bodies of water involved in or affected by the tests may not be used for irrigation, drinking water supplies, or body contact through recreational activities. In addition, pesticides may not be tested in waters that contain or that affect any fish, shellfish, or other plants or animals that may be taken for food or feed unless a tolerance or exemption from tolerance exists for the test product. Please refer to 40 CFR 172.3(c)(2), <https://www.gpo.gov/fdsys/pkg/CFR-1996-title40-vol11/pdf/CFR-1996-title40-vol11-sec172-3.pdf>

[gov/fdsys/pkg/CFR-1996-title40-vol11/pdf/CFR-1996-title40-vol11-sec172-3.pdf](https://www.gpo.gov/fdsys/pkg/CFR-1996-title40-vol11/pdf/CFR-1996-title40-vol11-sec172-3.pdf)

- Animal treatment uses, but animals must NOT be used for food or feed unless a tolerance or exemption from tolerance exists for the product.

### WYOMING DEPARTMENT OF AGRICULTURE EUPS

Experimental use of pesticides refers to formal research efforts conducted to scientifically assess the pest control potential of a registered pesticide or an experimental pesticide. Experimental pesticides include:

- unregistered pesticides,
- unregistered uses of registered pesticides, and
- pesticides or pesticide uses being evaluated under an Experimental Use Permit issued by the U.S. Environmental Protection Agency (EPA) or the Wyoming Department of Agriculture.

The EPA or state may grant an Experimental Use Permit (EUP) to researchers wishing to gather data necessary to grant registration under Section 5 of FIFRA for:

- a pesticide not registered with the EPA, or
- a new use of a registered pesticide (i.e., one not previously approved).

The EPA has determined an EUP is not required when:

- experimental work is limited to laboratory or greenhouse tests, and
- the researcher neither intends nor confers pest control benefit to those conducting it.

For limited replicated field (or other) tests, conducted only to determine a chemical’s pesticide

potential, its toxicity or other properties, in which the persons conducting the test do not expect to receive any benefit in pest control from its use, the EPA has determined that an EUP is not required for:

- **Land use** — the cumulative area treated per site, per crop, per experimental compound is less than 10 terrestrial acres (up to 250 acres for pheromones), provided:
  - When testing for more than one target pest occurring at the same time and in the same locality, the 10-acre limitation must encompass all of the target pests.
  - Food or feed crops involved in or affected by tests (including crops subsequently grown on this land, if such crops may reasonably be expected to contain residues of the compound) must be destroyed or consumed only by experimental animals, unless an appropriate tolerance or exemption from a tolerance has been established.
- **Aquatic use** — tests involving use of a particular experimental compound are conducted on a total of not more than one surface-acre of water, provided:
  - When testing for multiple target pest species occurs at the same time and in the same locality, the one surface acre limitation encompasses all target pest species.
  - The water involved in or affected by the tests will not be used for irrigation, drinking water supplies or body-contact recreational activities.
  - The tests may not be conducted in waters which contain or affect any fish, shellfish, other animals, or plants take for recreation or feed unless an appropriate tolerance or exemption from a tolerance has been established.

- **Animal treatments** — tests are conducted only on experimental animals. No animals receiving test treatments may be used in food or feed unless an appropriate tolerance or exemption from a tolerance has been established.

To apply for a Wyoming EUP the following information must be submitted to the *Wyoming Department of Agriculture (WDA)*, 2219 Carey Avenue, Cheyenne, WY 82001-0100.

- name of the experimental compound and its EPA registration number if federally registered,
- name and mailing address of the experimental compounds manufacturer,
- activity of the compound (e.g., insecticide, herbicide, fungicide, etc.),
- amount of experimental compound used,
- total area treated including the number of replicate applications,
- name of crop treated,
- location of the treated area, and
- agency and contact person responsible for the experimental use study.

The WDA may issue a state-specific experimental use permit to:

- Any person for the purpose of gathering data necessary to support FIFRA section 24(c) registrations.
- Any agricultural research agency or education institution conducting experimental-use work within Wyoming for any purpose not directly intended to result in the registration of a specific pesticide product.

EUP permits are issued with an authorization letter that outlines the requirements and restrictions

for the Wyoming EUP. In such cases, the WDA approved EUP labeling must be followed.

Use of a pesticide under an EUP must be consistent with the terms of EUP, including any additional restrictions imposed by WDA, and the experimental protocol.

All food or feed derived from a pesticide's experimental use must be destroyed or fed only to experimental animals for test purposes, unless an appropriate tolerance or an exemption from a tolerance has been specifically granted for residues of pesticide on the food or feed crop(s).

An experimental pesticide may be used only in accordance with its experimental use permit or any federally registered use permitted by its labeling. If an experimental pesticide does not have federally registered uses, at the study's conclusion, return any excess compound to its original provider.

A final report must be submitted to WDA at the end of trial period.

## RESTRICTED ENTRY INTERVALS

Knowing what **restricted entry interval (REI)** applies when conducting research or demonstration trials can be confusing. If the product is registered, it will have an AGRICULTURAL USE REQUIREMENTS and a NON-AGRICULTURAL USE REQUIREMENTS box under DIRECTIONS FOR USE on the pesticide product label. (Example to the right.) Use the REI that applies to the type of research or demonstration trial that is being conducted. For example, a trial involving agricultural production would use the REI under the AGRICULTURAL USE REQUIREMENTS box while a trial involving a non-agricultural use (for example, right-of-way or turf), would follow the REI under NON-AGRICULTURAL USE REQUIREMENTS. When using multiple products

with different REIs, **always apply the longest REI to the entire trial to avoid confusion!**

### Agricultural Use Requirements

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE) and restricted entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 4 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Chemical-resistant gloves made of any waterproof material
- Shoes plus socks

### Non-Agricultural Use Requirements

The requirements in this box apply to uses of this product that are NOT within the scope of the Worker Protection Standard for agricultural pesticides (40 CFR Part 170). The WPS applies when this product is used to produce agricultural plants on farms, forests, nurseries, or greenhouses.

Keep people and pets off treated areas until spray solution has dried.

Examples of AGRICULTURAL USE REQUIREMENTS and NON-AGRICULTURAL USE REQUIREMENTS boxes.

If an unregistered experimental pesticide has been applied to a treated area, refer to the product supplier for guidelines on re-entry times and PPE that should be provided. At the very minimum, a safety data sheet will contain information on hazards, first aid and protective equipment. Applicators and handlers should at least use the following protective equipment:

- protective eyewear,
- long-sleeved shirt,
- long pants,
- chemically-impervious gloves,
- chemically-impervious boots, and
- other protective clothing indicated on a technical bulletin or Safety Data Sheet.

Certain pesticides may have additional regulations regarding worker exposure, restricted-entry intervals, and other restrictions or limitations of use.

### **WORKER PROTECTION STANDARD (WPS)**

Worker Protection Standard may apply if the research involves pesticides intended for use when producing agricultural commodities on farms, in forests, nurseries or enclosed space production facilities. There is an exemption from the Federal Worker Protection Standard when conducting research on *unregistered pesticides* because they do not have an EPA-approved label. This exemption DOES NOT include research on an *unregistered use* of a registered pesticide product or maintenance pesticide applications applied to agricultural plants subject to research.

WPS applies if you use a WPS-labeled pesticide product (one that contains an AGRICULTURAL USE REQUIREMENTS box under DIRECTIONS FOR USE on the pesticide product label) and employ workers or handlers. When conducting research or demonstrations, your test area may include plots that are exempt from WPS (unregistered pesticides) AND plots that require you to comply with WPS. To avoid confusion, you should treat the entire area as if WPS applies.

If you are using a WPS-labeled pesticide in research or demonstration plots, some (not all are listed) of the things you are required to comply with include:

- annual training of workers and handlers and associated recordkeeping,
- notification of applications which may include posting of pesticide applications with Restricted Entry Intervals (REI) greater than 48 hours,
- maintaining central display, including application and hazard information safety data sheets, of WPS-labeled pesticides,

- Personal Protective Equipment requirements,
- minimum age (18 years or older) for early entry workers and handlers, and
- compliance with respirator requirements if required by the label or documents provided with the experimental compound.

### **ENTERING FIELDS UNDER AN REI OR WHEN REI IS UNKNOWN**

If WPS applies, and someone must enter a treated area during a REI when conducting research and demonstrations, the person(s) must be treated as an early entry worker or a handler and be provided the protections provided under WPS. This includes:

- ensuring that the minimum age requirement (at least 18 years) is met;
- providing PPE and instructions required under WPS to early-entry workers;
- reading the pesticide label to review statements related to human hazards or precautions, first aid and user safety; and
- making decontamination supplies available.

For more information on how to comply with WPS, including training materials for workers and handlers, refer to Additional Resources, page 20.

You must be aware of and follow all regulations that are relevant to your trial. When you work with pesticides in experimental trials, you are responsible for ensuring the legal and safe use of the materials you use.

### **FEDERAL FOOD, DRUG AND COSMETIC ACT (FFDCA)**

#### **TOLERANCES AND RESIDUE TESTING**

Under the Pesticide Amendment to the Federal Food, Drug and Cosmetic Act (FFDCA), the authority to establish a legal **tolerance** for each

pesticide applied to food or feed is under the authority of EPA. Anyone or any company that registers pesticides under FIFRA, or seeks a tolerance or tolerance exemption for a pesticide under FFDCA, must submit data in order to establish that level. The data are often obtained by manufacturer contracting with universities or private companies to collect those data using Good Laboratory Practice (GLP). More information on GLP is provided in Chapter 4, page 29.

If your trial uses a product that is a federally registered pesticide for your crop and you have used the product according to its label, it is presumed to meet the required residue tolerance without further testing. If you treat crops at higher than labeled rates, by a different application method, or use shorter pre-harvest intervals (PHI), generally you must either destroy the crop or have the crop analyzed to confirm that it is within the labeled tolerances before you allow it to enter the market.

## RECORDKEEPING REQUIREMENTS

Accurate and legible records must be maintained for each pesticide application made by a licensed commercial applicator business or registered limited commercial or public applicator. The records must be kept for two years, and must include:

- Name and address of person for whom the pesticide application was made.
- Location where application was made, if different from above. The location should be fully described.
- Target pest i.e. the specific pest for which the application is made. A general term is acceptable only if the pesticide label refers to that general term e.g., “broadleaf weeds.”
- Site, crop, commodity or structure treated.

- The EPA registration number of the specific pesticide applied. The pesticide product brand name and manufacturer address may also be included.
- Dilution rate: the amount of formulated product or active material per unit of volume of the carrier. If not diluted, “no dilution” or “ready to use (RTU)” should be used.
- Application rate: the total gallons or pounds of the final tank mix applied per unit of area or volume.
- Carrier, if other than water.
- Date of application and time (within ½ hour) application was started or stopped.
- Name of person who made the application.
- Endangered species protection bulletin (ESPB) for the county and month in which the application was made for any pesticide product used, when required by the label. One ESPB may be applied to multiple applications. (Refer to EPA website on Endangered Species in Additional Resources, page 20, for more information.)

## FEDERAL EUP RECORD REQUIREMENTS

All producers of pesticides produced under a Federal EUP must maintain records as outlined in 40 CFR, Part 169 and Part 172. Permittees must also comply with the surveillance and reporting requirements in 40 CFR 172.8 which includes:

- (a) The permittee shall supervise the test program and evaluate the results of testing at each site of application. It will further be the responsibility of the permittee to report immediately to the Administrator, or to any person designated by the administrator, any adverse effects from use of, or exposure to, the pesticide.



- (b) The permittee shall submit the following reports to the Registration Division during the experimental program.
  - (1) [Reserved]
  - (2) A final report shall be submitted within 180 days after the expiration of the permit, unless a request for extension of time is approved, and shall include:
    - (i) All data gathered during the testing program; field notes need not be submitted but must be maintained and submitted upon request;
    - (ii) A description of the disposition of any pesticide containers and any unused pesticides including amounts disposed of and the method and site of disposition; and
    - (iii) The method of disposition of affected food or feed. The data under paragraph (b)(2)(i) of this section above may be submitted as part of an application for registration submitted within 180 days after the expiration of the permit, provided that the final report shall include a statement that such application has been made, and the date of such application.
- (c) In addition to the reporting requirements provided for elsewhere in this part, in the case of any meat-producing animals or birds that receive a direct treatment or application of any experimental use pesticide, the name and location of the packing plant where the animals will

be processed shall be sent to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, DC 20250, at least 10 days before the animals are to be shipped for slaughter. This requirement may be waived, on request, by the USDA. These provisions do not exempt treated food-producing animals and their products from compliance with other applicable inspection requirements.

- (d) Failure to submit required reports may constitute grounds for revocation of the permit.
- (e) For the purpose of supervising the use of experimental use pesticides, the Agency may require the permittee or any participant to give reasonable advance notification of the intended dates, times, and sites on which such experimental use pesticide will be applied.
- (f) The permittee or participants in the experimental use program will permit any authorized representative of the Agency, upon presentation of official identification, entry, at any reasonable time, to any premises involved in the testing program to inspect and to determine whether there has been compliance with the terms and conditions of the permit.

These records can be very important if there are problems associated with the application. Good records might be important to your defense in any legal action. Additional recordkeeping is also important when conducting research and demonstration trials as it may explain some of the



unexpected results. For these reasons, consider keeping additional records of:

- posting requirements;
- weather records which might include wind speed and direction, relative humidity, temperature. Record time of measurements and where measurements were taken (height, location) as well as manufacturer information for instruments used;
- observations used to determine whether a temperature inversion exists; and
- variances due to terrain or vegetation (remember to consider nearby windbreaks or buildings).

## SECTION 18 AND 24(C) REGISTRATIONS

### STATE-ISSUED SECTION 18 AND 24(C) REGISTRATIONS

Data generated locally through field research experiments conducted under this category are sometimes used to support Section 18 registrations and to obtain EPA approval of state 24(c) registrations.

#### Section 18 emergency exemptions

Under Section 18 of FIFRA, EPA can allow state and federal agencies to permit an additional use (not specified by the pesticide's label) during a short-term pest management crisis in a specific locality. Section 18's are referred to as "emergency exemptions".

Section 18 emergency exemptions are used when there are no other federally registered pesticides available to control a serious pest problem and there would be significant economic loss without the use of the Section 18 pesticide.

The request for a Section 18 exemption must be submitted by WDA which may include supporting

data that were generated through research experiments. Research can also be conducted using a Section 18 label, however, a permit is required from the WDA in order to use the Section 18 product and the label must be in the possession of the user at the time of pesticide application.

#### 24c registrations

Under Section 24(c) of FIFRA, Wyoming can register additional uses of a federally registered pesticide. These additional uses are for distribution and use within a particular state to meet a special local need (SLN). Research data can be used to support a 24(c) and research can be conducted under a 24(c).

Although SLNs can be approved for many different reasons and application sites, most involve use on crops. A certain crop grown within Wyoming may be attacked by a particularly damaging pest, or Wyoming officials may expect it to be attacked sometime during the growing season, thereby creating a special pest problem. The pesticide must have an established tolerance associated with the crop, or be exempt from the requirement of a tolerance for that crop. SLN's also may pertain to uses for control of pests peculiar to one or several states.

The official request for a 24(c) registration comes from a pesticide manufacturer or formulator to WDA. Commodity groups, Extension Service personnel, and others can inform the formulator of the need, but the request comes from the formulator. Section 24(c) labels are valid only in the state of issue. The applicator must possess a copy of the Wyoming label when the pesticide is applied. EPA reviews the individual 24(c) registrations and broadly oversees the states' 24(c) registration programs.

## CROP DESTRUCT, GRAZING RESTRICTIONS AND SEED CROPS

### CROP DESTRUCT

One of most important responsibilities for applicators conducting research and demonstration, is to know whether you must adhere to the requirements for crop destruction. The regulations regarding crop destruct must be followed not only because it is the law, but also because failure to do so may result in harm to people, animals or the environment.

Unregistered pesticides and numbered compounds usually lack an established EPA residue tolerance for the active ingredient and crop combination. Registered products used experimentally on crops or in ways not allowed by the label may exceed or lack existing tolerances. The EUP holder must destroy the food or feed item unless:

- a residue tolerance has been established by EPA for the pesticide-crop combination, rate, and use pattern tested; or
- the pesticide you are testing has been exempted from the requirement of a residue tolerance; or
- the pesticide you are testing has a time-limited tolerance established by EPA that is in effect.

**Crop destruct** means to render the crop unusable for food or feed, or to use for research purposes only. Some examples of methods of crop destruction include plowing the crop under or hauling to a landfill for burial.

The crop destruction rule applies to all treatments for crops, including dormant, fallow, and pre-plant treatments. No portion of a crop to which a pesticide product having no established pesticide residue tolerance for the crop has been applied, shall be used or distributed for food or feed.

This restriction pertains to, but is not limited to, green chop, hay, pellets, meal, whole seed, cracked seed, straw, roots, bulbs, foliage or seed screenings. This restriction also includes **grazing** the crop, stubble, or re-growth for 365 days. If you submit a justification for harvest or use, you must include information about the pesticide product's applicable residue tolerances.

### Documentation of crop destruct

Food or feed plant parts are never allowed to enter the food or feed chain without complying with established tolerances. Documentation of crop destruct becomes very important when there could be a trace-back of contaminated food and feed items to the trials you are responsible for. Documentation must include the date and the crop destruct method. Photos of the destruction process are also recommended.

### GRAZING RESTRICTIONS

If a crop has been treated with a pesticide that does not have a tolerance established for forage or for meat and milk, the treated site must not be used for grazing of animals for a minimum of 365 days from the date of the last application. The permit holder must ensure that the grower/cooperator is informed of this restriction.

If animals are allowed to graze on land that has been treated with a pesticide that does not have a tolerance for such, the animals may be harmed or may have pesticide residues in the animal's meat or milk. In this situation, the animals, meat, milk or other contaminated commodity may not be consumed or marketed for any purpose.

### TREATED CROPS GROWN FOR SEED

If you grow a specialty seed crop (other than grass grown-for-seed) for a research experiment or demonstration, you need to know whether pesticides used on specialty seed crops have established pesticide residue tolerances. If a

tolerance is lacking or the labeled rate is exceeded, you need to inform your seed conditioner which pesticides were applied to your crop. It is your responsibility to ensure no portion of a treated seed crop is used or distributed for human food or animal feed.

## LIABILITY ISSUES

In general, when a researcher contracts with a manufacturer to field test unregistered pesticides, they may sign a waiver with the manufacturer. However, the applicator assumes personal responsibility for accidents and injuries that arise as a result of each pesticide application. The applicator may be subject to fines, jail sentences and loss of their applicator license if they are negligent in the application of pesticides or have broken state or federal laws. Also, the applicator may be held responsible in lawsuits for personal injury or damages. If you are working for someone else, the applicator's actions may result in lawsuits against or fines to the employer. If someone brings a negligence claim against the applicator, accurate records can help with the defense.

## STORAGE AND TRANSPORTATION

Many environmental and human health hazards can be prevented by safely handling and transporting undiluted pesticides and properly disposing of unused pesticides and their containers.

When you use registered pesticides in your trials, follow the requirements in regulations and on the label about how to store and dispose of unused pesticides and empty pesticide containers. If your trial involves an unregistered material, follow the storage and disposal guidelines on the product's Safety Data Sheet or technical bulletin.

## SERVICE CONTAINERS

**Service containers** are any container, other than the original labeled container, that holds pesticides (either in concentrated or diluted form), that is of a size and capacity that permits it to be carried or moved by only one individual, unaided by any tool or apparatus. Various manufacturers design service containers for applying, storing, or transporting pesticide concentrates or diluted pesticide preparations.

If you are using a service container, label it prominently with the following information from the original container label:

- the common name of each active ingredient, or the chemical name if there is no common name;
- the EPA registration number;
- each and every human hazard signal word shown on the original container label; and
- the name of the licensed commercial applicator business, or registered limited commercial or public applicator.

When you use an unregistered material under a Federal EUP, there are special container labeling requirements that were previously covered in this chapter.

## STORAGE

Store pesticides in their original, tightly closed containers. Protect pesticides from extremes in temperature and from becoming wet. A pesticide storage area should be a separate building, away from people, living areas, food, animal feed, and animals. The area must have good ventilation and lighting. Be sure it is dry and secure, with lockable doors and windows. Post signs around the storage area, visible from any direction of probable approach—especially on all entrances—to warn others that the building contains pesticides.

Product pesticide labels and safety data sheets contain information on how to properly store registered pesticides. Information on proper storage conditions should be provided with the unregistered pesticide through the safety data sheet or technical bulletins.

### TRANSPORTING

Regulations prohibit transporting any pesticide in the same compartment with people, food, or feed. All containers should be secured to prevent spillage onto or off of the vehicle. Many pesticides are subject to state and federal hazardous materials transportation requirements.

If you have an accident involving spilled pesticides, alert the highway patrol, county sheriff, city police, or local fire department at once. (When possible, have the appropriate current telephone numbers in the vehicle with you.) Keep people and vehicles away.

Never leave the scene of a spill until responsible help arrives. In the case of an emergency, you may obtain advice on cleaning up spills by contacting **CHEMTREC (Chemical Transportation Emergency Center) at 800-424-9300**. The manufacturer that provided the product also may be contacted.

To guard against theft and prevent danger to children and animals, never leave pesticide containers unattended. Always keep pesticides away from food and water and away from sources of heat and fire. Never allow paper containers to get wet.

### DISPOSAL OF PESTICIDE AND CONTAINERS

Be sure to safely dispose of pesticides. You can try to return registered products to the manufacturer. Unregistered products should be returned to the manufacturer after the research experiment.

## CHAPTER 2 ADDITIONAL RESOURCES

Electronic Code of Federal Regulations. Select: *Title 40 - Protection of Environment*, Parts 150 - 180 contain Pesticide Regulations.  
<https://www.ecfr.gov/>

EPA website on Endangered Species Bulletins.  
<https://www.epa.gov/endangered-species/endangered-species-protection-bulletins>

EPA website on Experimental Use Permits (EUP).  
<https://www.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-12-applying-experimental-use-permit>

Wyoming Department of Agriculture Statutes, Rules and Regulations, <http://wyagric.state.wy.us/divisions/ts/statutes-rules-a-regulations>

Wyoming Department of Agriculture Pesticide Program, <http://wyagric.state.wy.us/divisions/ts/sections-a-programs/pesticide>

## CHAPTER 3: PESTICIDE-ORGANISM INTERACTIONS

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Describe the factors that affect pesticide penetration into organisms.
- B. Explain the fate of a pesticide once inside the organism.
- C. Define selectivity and its importance in IPM.
- D. Define pesticide resistance and why it is important in research experiments and demonstrations.
- E. Explain how pesticide resistance develops.
- F. Explain the difference between Mode of Action (MOA) and Site of Action (SOA).
- G. State the primary MOA for insecticides, fungicides, and herbicides.
- H. State the most common mechanisms of resistance for insecticides, herbicides, and fungicides.
- I. Describe how IPM strategies can be applied in research and demonstration trials to avoid or delay pesticide resistance.
- J. List the ways pesticides can degrade.
- K. List the factors that affect degradation of pesticides.
- L. Explain biological magnification.
- M. Explain how pesticide interactions may affect efficacy or cause phytotoxicity.

### INTRODUCTION

Federal regulations (40 CFR 171.4(c)(10)) require that persons conducting demonstration and research work with pesticides should demonstrate an understanding of pesticide-organism interactions and their importance in IPM programs. Both the beneficial and harmful effects of pesticides are determined by pesticide-organism interactions.

To be effective, a pesticide must

- come into contact with or penetrate the organism,
- move or be transported to the **site of action**, and
- disrupt or alter a vital function. The way in which the pesticide affects the vital function is called its **mode of action**.

**Penetration, transport**, and mode of action determine how a pesticide interacts with target AND non-target organisms. Pesticide-organism interactions also are involved in the metabolism, accumulation, and elimination of pesticides by the organism, and in biodegradation and biological magnification.

### PESTICIDE PENETRATION INTO ORGANISMS

The terms **contact** and **systemic** relate to how the active ingredient is transferred to the target organism. Contact pesticides must come into direct contact with the organism and do not rely on penetration. Unlike other pesticides that remain on the surface of treated foliage, systemic pesticides

are taken up by the plant and translocated to other sites which were not directly sprayed.

The speed and extent of penetration primarily depends two factors: on the permeability of the organism to the specific pesticide and the chemical nature of the pesticide.

**Permeability** differs significantly among plants and insects and even among different tissues of the same organism. Among animals, tissues of the respiratory and digestive system are usually much more permeable than the skin. In plants, new, succulent growth is more permeable than mature growth and bark. Additionally, waxy leaf surfaces or abundant trichomes (hairs) can limit penetration of the pesticide into the plant.

The **chemical nature** of the pesticide and the type of formulation also affect penetration. For example, emulsifiable concentrates (EC) and ultra-low volume (ULV) formulations are readily absorbed through the skin of humans or animals and may damage plants.

Penetration can sometimes be increased by either incorporating **adjuvant** compounds directly into a formulated pesticide product (by the manufacturer) or adding an adjuvant product to the diluted pesticide mixture in the tank. Examples of adjuvants that can increase pesticide penetration include crop oil concentrates (COC) and methylated seed oils (MSO).

## FATE OF PESTICIDES IN THE ORGANISM

Once inside the organism, the pesticide may undergo one of the following processes:

- translocation,
- storage,
- metabolism, or
- exudation.

### TRANSLOCATION

Systemic pesticides are those in which the active ingredient is taken up by plant foliage or roots, and transported (translocated) to other locations throughout the plant. The ease with which a pesticide moves from the place where it entered an organism to its site of action depends on the mobility of the pesticide molecules and the efficiency of the transporting mechanism of the plant or animal. Systemics move within the vascular tissues of plants, either through the xylem (water-conducting tissue) or the phloem (food-conducting tissue) depending on the characteristics of the material.

Some insecticides and miticides have **translaminal**, or local, systemic activity. These materials penetrate leaf tissues and form a reservoir of active ingredient within the leaf (e.g. the bottom side of the leaf). This provides residual activity against certain foliar-feeding insects and mites.

### STORAGE

Pesticides and their metabolites may be stored or accumulated within an organism which may provide residual activity. Storage of pesticides can occur through a chemical or physical binding of pesticide to plant constituents. Largest amounts are frequently found close to the point of absorption, in areas of intense metabolic activity.

Because pesticide residues may accumulate within organisms, producers must take special precautions during harvest or slaughter. Observing specified intervals between pesticide application and grazing, harvest, or slaughter ensures that the products will be safe for consumption as they will not exceed established tolerance levels. (More information on residue tolerances in research trials is provided in Chapter 4, page 29.)

### METABOLISM

**Metabolism** is the process by which a pesticide or other chemical is changed into one or more different chemicals within a living organism. The metabolic product, or metabolite, may be either more toxic or less toxic than the original pesticide ingredient. Given enough time, an organism may be able to metabolize certain pesticides to less toxic metabolites. Survival may depend on whether or not the organism can metabolize the pesticide into less toxic metabolites before the toxic activity is complete or irreversible.

Metabolism of herbicides is nearly always a detoxification process for the plant, but the products may be biologically active in other systems and still important as residues. Insecticides are also metabolized in plants. Although insecticides and their metabolites are generally not active in plants, they are frequently toxic to other organisms.

### EXUDATION

**Exudation** is the removal of pesticides from inside the plant. Volatile pesticides and metabolites may leave the plant as vapors through the stomates (pores in the leaves). Whether this occurs depends on the pesticide and environmental conditions (i.e. temperature, relative humidity).

## SELECTIVITY OF PESTICIDES AND IPM

Integrated pest management (IPM) makes use of all available control strategies, including cultural, host plant resistance, biological, and chemical controls to manage pests. Pesticides most suitable for IPM are those that combine optimal control of target pests with minimal impact on the activity of non-target organisms such as natural enemies. **Selectivity** is the ability of a pesticide to affect one organism and not another. Products that have a short residual effect on natural enemies are favored for IPM programs. Consider both the short-term and long-term effects of an application when selecting a pesticide treatment.

Non-selective pesticides, especially insecticides and miticides, may leave residues on the plant that are potentially toxic to pollinators, predators, and parasites for days to weeks following application, depending on the persistence of the product. While many pesticides used today are selective, scientific studies should connect the various pieces of information gathered from research to include pesticide effects on natural enemies, in order to determine the most appropriate pesticide to be used in pest management.

## PESTICIDE RESISTANCE AND IPM

**Pesticide resistance** is the heritable reduction in the sensitivity of a pest population to a pesticide that was previously effective at controlling the pest. Pest species evolve pesticide resistance via natural selection: the most resistant specimens survive and pass on their genetic traits to their offspring.

Many insect and mite species have become resistant to pesticides worldwide. In addition, at least 200 species of fungi, more than 200 species



of weeds, and several species of nematodes and rodents also are resistant to one or more pesticides.

Resistance often develops in pest populations that have been treated frequently with pesticides that have a common mode of action. The development of resistance may sometimes be averted or delayed by reducing the number of treatments or alternating the use of pesticides with different modes of action (described in the next section).

Pesticides that are **persistent** (long residual) increase selection pressure as many individuals are exposed over an extended time. On the other hand, short residual activity may allow some individuals to be exposed to a lower dose shortly after application. If these individuals have some level of resistance, a greater proportion will likely survive, also contributing to increased levels of resistance.

### IMPORTANCE OF RECORDKEEPING

Remember that application techniques also play a role in the development of pesticide resistance; therefore, mode of action should be recorded as part of the records. When conducting research and demonstrations, it's important to use the best management practices for the application method (aerial, ground, chemigation, etc.) and to keep records of carrier volume, nozzle, and pressure used as well as the calibration and maintenance of the application equipment used.

## MODE OF ACTION (MOA) AND SITE OF ACTION (SOA)

When discussing resistance, the terms Mode of Action and Site of Action are commonly used and sometimes, interchangeably. It's important to understand the difference between the two terms but they will be combined as MOA for purposes of this study guide.

To help with resistance management, all active ingredients have been assigned a group number based on their MOA. EPA has requested chemical companies voluntarily include a pesticide's MOA group number in a standard format on a label. This numbering system allows a quick, easy determination of whether products have the same or different MOA, aiding the selection of products in a rotation system. Examples of the numbering system are shown for the different types of pesticides. Since it is voluntary, not all pesticide labels will contain the MOA group number. The information can still be found in the websites described in the sections below. Most likely, experimental product information will not include the MOA as this may be proprietary information for the product manufacturer.

### MODE OF ACTION (THE HOW)

All pesticide interactions with the target organism, from application to final effect, are considered the **mode of action**. The mode of action involves absorption into or by the organism, possible translocation or movement in the organism, metabolism of the pesticide, and the physiological response. It describes the biological processes that are disrupted by the pesticide, for example, pigment inhibitor.

### SITE OF ACTION (THE WHERE)

**Site of action** is the specific process in the organism that the pesticide disrupts to interfere with growth and development. The SOA is the most important aspect of pesticides when dealing with prevention and control of pesticide resistances, for example, carotenoid biosynthesis inhibitor.



## INSECTICIDE RESISTANCE AND MOA

Insects develop resistance to insecticides through one of the following mechanisms:

- **metabolic** — insect metabolizes the toxin or metabolizes it quicker than other insects;
- **target-site** — the insecticide can no longer connect at molecular target site;
- **penetration** — the insects' exoskeleton absorbs the insecticide more slowly; or
- **behavioral** — insect detects insecticide and avoids it.

As of 2017, the Insecticide Resistance Action Committee has identified 27 different groups for primary site of action and includes one classification for unknown or uncertain MOA. The MOA for insecticides can be further broken down classified based on SOA. But the MOA can be broadly classified by affecting the insects in one of the following ways:

- nerve and muscle (e.g. pyrethroids, organophosphates, neonicotinoids),
- growth and development (e.g. juvenile hormone mimics, lipid biosynthesis inhibitor),
- respiration (e.g. rotenone, phosphides),
- midgut (e.g. *Bacillus thuringiensis*), or
- unknown (e.g. azadirachtin and sulfur).

GROUP	1B	INSECTICIDE
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Registrants of insecticides do not always include the MOA group classification on the pesticide product label. The IRAC website has various resources including a search tool to browse and filter chemical groups, classes and active ingredients. The website also has a link to download reference material, see Additional Resources, page 28.

## HERBICIDE MOA

There are four main mechanisms by which weeds can become resistant to herbicides:

- alteration of the target site (most common),
- enhanced metabolism,
- over-expression of the target site protein(s), or
- compartmentalization (binding to another substance like a plant sugar molecule) or sequestration (moved from metabolically active regions of the cell to inactive regions).

GROUP	9	HERBICIDE
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In a perfect world, there would be one classification system for herbicide sites of action but that is not the case. In the early 1990s the Herbicide-Resistance Action Committee (HRAC) created a classification system based on letters. The HRAC system is the classification system used in most countries. In 1997, the Weed Science Society of America (WSSA) created a classification system based on numbers which is only used in the United States and Canada. Regardless of which system used, the lists do generally recognize the same MOAs and contain the same list of active ingredients within the MOA.

As of 2017, there are 10 primary herbicide modes of action. These categorize more than 79 active ingredients that affect various sites of action in the weeds farmers want to control. The MOAs target one or more of the following processes:

- photosynthesis inhibitors,
- amino acid inhibitors,
- growth regulators,
- lipid synthesis inhibitors,
- nitrogen metabolism inhibitors,
- pigment inhibitors,
- cell membrane disruptors,

- seedling root growth inhibitors,
- seedling shoot growth inhibitors, or
- unknown mechanism(Nucleic acid inhibitor).

The WSSA promotes voluntary MOA labeling and a MOA classification system, using MOA group numbers, for ease of reference when planning a herbicide program. Many herbicide manufacturers voluntarily include group numbers to help growers and applicators vary herbicide modes of action. Even if the research experiment or demonstration plots are not designed to investigate weed management, the researcher should keep records including the weeds present as well as the name, MOA, and MOA group numbers of the herbicide applied to the site for plot maintenance. For more information, refer to the International Survey of Herbicide Resistant Weeds website, see Additional Resources, page 28.

FUNGICIDE MOA

There are four main mechanisms by which fungi can become resistant to fungicides:

- alteration of the target site (most common),
- detoxification of the fungicide,
- over-expression of the target site enzyme(s), or
- exclusion or expulsion from the target site in the cell by transport pumps.

GROUP	3	11	FUNGICIDE
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As of 2017, the Fungicide Resistance Action Committee (FRAC) lists 11 modes of action plus Unknown Mode of Action. The targets of these MOAs include:

- respiration,
- nucleic acid synthesis,
- amino acid and protein synthesis,
- lipid synthesis or transport function,

- cell wall biosynthesis,
- host plant defense induction,
- signal transduction,
- melanin synthesis in cell wall,
- sterol biosynthesis in membranes,
- cytoskeleton and motor proteins, and
- chemicals with multi-site activity.

STRATEGIES TO AVOID OR DELAY RESISTANCE

When conducting research experiments or demonstrations using pesticides, it’s important to be mindful of strategies to avoid or delay the development of resistance with a goal of keeping the pesticides as viable tools in pest management. While rotating MOA groups is a start, there should be a more integrated approach. IPM strategies are a critical part of demonstration trials and researchers should report the strategies used to maintain the sustainability of the pesticides involved. Although research experiments involved in determining pesticide efficacy do not typically involve IPM strategies, researchers should note complementary strategies that may affect pest management in these trials as well.

DEGRADATION OF PESTICIDES

Several factors can affect not only the efficacy of a pesticide but also the manner and rate of its decomposition in the environment. Once a pesticide has been released into the environment, it can be broken down by:

- exposure to sunlight (**photolysis**),
- exposure to water (**hydrolysis**),
- exposure to other chemicals (**oxidation and reduction**),
- **microbial activity** (bacteria, fungi, and other microorganisms), and
- plants or animals (**metabolism**).

While understanding how pesticides degrade is important, scientists are also concerned with **metabolites** created from degradation of the original active ingredient as some metabolites are more toxic than the original active ingredient. Scientists do experiments to determine how long pesticides, and sometimes their metabolites, last in various environments. They apply pesticides to soils, leaves, and other surfaces and measure the time it takes for half of the pesticide to break down, a measure called the **half-life**. After one half-life, half of the chemical may be broken down. Following another half-life, half of the 50% remaining may be broken down, leaving 25% of the original amount and so on. The half-life can be a useful measure of how long a pesticide may last, but studies have found a wide range of half-lives for the same pesticide under different environmental conditions.

Some of the factors that affect degradation of pesticides include:

- soil microorganisms,
- soil organic matter,
- soil pH,
- soil texture,
- soil moisture,
- temperature,
- humidity, and
- ultraviolet light (affects microbial populations).

## BIOLOGICAL MAGNIFICATION

**Biological magnification** is the tendency for certain pesticides to become progressively more concentrated in each type of organism as they move up the food chain. Biological magnification has been observed to occur with pesticides that are **lipophilic**, poorly metabolized by an organism and are persistent in the environment. An example

of biological magnification is when birds of prey become sick after feeding on animals poisoned by pesticides. Perhaps the most familiar example is the thinning of the eggshells of birds exposed to certain organochlorine insecticides such as DDT. This eggshell thinning may result from a chain of events that began when invertebrates that consumed plants containing DDT residues were, in turn, eaten by rodents, reptiles, amphibians, fish and insectivores, with the residues becoming more concentrated in each species. These intermediate predators in the food chain were eaten by the top predators, which then received yet higher insecticide concentrations. It is important to be aware of such pesticide-organism interactions when working with certain pesticides.

## PESTICIDE INTERACTIONS

Crops may receive several pesticide treatments during a season. Some agricultural chemicals degrade rapidly and do not interact with other chemicals, but some do persist and interact with chemicals that are already present or that are applied later. Research has shown that phytotoxic interactions between major pesticide groups are infrequent, but not rare. Using insecticides with herbicides can increase or decrease the herbicidal activity. Most herbicide-insecticide mixtures increase the injury to the crop.

Herbicidal interaction with fungicides is generally antagonistic. Mixing certain insecticides with fungicides can make the insecticide more toxic to non-target organisms.

Most pesticide interactions involve herbicides, which can interact with other herbicides and with non-herbicidal chemicals. These interactions may not affect a herbicide, or they may make it more or less toxic than normal.

**Synergism** occurs when the plant response is greater than expected (more than an additive effect). A synergist can be an adjuvant such as a crop oil or surfactant. Herbicide synergists are non-herbicides that are not phytotoxic themselves but are used to increase the phytotoxicity of a herbicide by increasing the amount a plant takes up, preventing the herbicide's deactivation, or affecting some more complex process.

**Antagonism** occurs when the plant response is less than expected (less than an additive effect). A herbicide safener (previously called antidote) selectively protects crop plants from herbicide damage without reducing activity in target weed species. They are used commercially to improve herbicide selectivity between crop and weed species and can be applied either as a mixture with the herbicide or as a seed-treatment to the crop seed prior to sowing.

If applying tank mixes of registered products in research experiments or demonstration plots, read the label for directions for each product used in the tank mix. Experimental compounds, on the other hand, should not be tank mixed unless the cooperating company has provided specific information that the mix has already been tested and proven compatible.

## CHAPTER 3 ADDITIONAL RESOURCES

Insecticide Resistance Action Committee. (IRAC)  
Provides educational resources to promote awareness of insecticide resistance and management strategies worldwide. <http://www.irac-online.org/>

Mode of Action Classification: <http://www.irac-online.org/modes-of-action/>

Pesticide Resistance Management. Provides learning materials for pest resistance management. Plant and Soil Sciences eLibrary, University of Nebraska. <http://passel.unl.edu/communities/pesticiderm>

Fungicide Resistance Action Committee (FRAC).  
Provides resources of interest in fungicide resistance and management including a Mode of Action Poster and recommendations for fungicide mixtures. <http://www.frac.info/home>

International Survey of Herbicide Resistant Weeds.  
Resources on herbicide resistance and herbicide resistant weeds globally. Provides ability to look up active ingredients to determine both HRAC and WSSA MOA classifications. <http://weedsience.org/default.aspx>

## CHAPTER 4: GOOD LABORATORY PRACTICES

### LEARNING OBJECTIVES

After reading this chapter, you should be able to

- A. Describe the purpose of Good Laboratory Practice Standards (GLPS) and how it applies to research experiments.
- B. Define the terms as they relate to GLPS: study director, raw data, standard operating procedures, test system, test substance, control substance, carrier, vehicle, protocol amendment, protocol deviation.
- C. Explain the purpose of the GLPS protocol and how it should be conducted.
- D. Explain what GLP records must be kept.
- E. Describe the role of IR-4 in pesticide registration.

### INTRODUCTION

Following **Good Laboratory Practice** (GLP) means more than practicing good science when conducting research experiments. GLPs are federal regulations that apply when conducting laboratory and residue studies to establish tolerances on certain products regulated by the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). The EPA's Good Laboratory Practice Standards (GLPS) compliance monitoring program ensures the quality and integrity of test data submitted to the agency in support of pesticide product registration under FIFRA, section 5 of the Toxic Substances Control Act (TSCA). **GLPS apply to residue and toxicology trials. Most efficacy trials do not require GLPS.**

### FIFRA GLP STANDARDS

**FIFRA GLP** standards cover any application for a research or marketing permit submitted to U.S. EPA. Applications for research or marketing permits include:

- applications for pesticide registrations, reregistrations, or amended registrations,
- applications for experimental use permits (FIFRA Section 5),
- applications for exemption under FIFRA Section 18, and
- petitions or other requests for establishment or modification of residue tolerances or exemption from residue tolerances.

The exhaustive definition of application for research or marketing permit can be found in

the Code of Federal Regulations (CFR), Title 40, Part 160.3 (40 CFR 160.3). GLP standards were written in response to U.S. EPA and Food and Drug Administration investigations during the 1970s that revealed that some studies had been conducted so poorly that the resulting data could not be relied upon in U.S. EPA's regulatory decision-making process. For example, some studies were conducted by under-qualified personnel and supervisors, or the studies were not adequately monitored by study sponsors. In some cases results were selectively reported, underreported, or fraudulently reported. Also, some testing facilities displayed inadequate record-keeping techniques. FIFRA GLP standards first went into effect in 1983 and at that point covered only health effects testing. In 1989, however, FIFRA GLP standards were significantly expanded to include ecological effects, environmental and chemical fate, and efficacy, in addition to health effects testing.

All types of testing used to obtain data in support of U.S. EPA-issued research or marketing permits for pesticide products must now be conducted in accordance with the 1989 amended FIFRA GLP standards. Overall, FIFRA GLP standards are about good recordkeeping. This is because EPA inspectors need to be able, if they decide it is necessary, to completely reconstruct your research experiment to find out how you got your data and how you came to your research conclusions. To do this, they need to be able to answer the basic who, what, when, where, and why questions about your experiment. To produce valid data that can be defended at every point of your experiment, you must fulfill minimum GLP requirements in several research-related areas.

These areas include:

- organization and personnel,
- facilities,
- equipment,
- testing facilities operation,
- test, control, and reference substances,
- protocol for and conduct of study, and
- records and reports.

The following sections cover some of the main points of the GLP requirements. For the detailed requirements and how they apply to your particular study, consult your study director or 40 CFR 160. Any questions about the interpretation of GLP requirements are ultimately determined by U.S. EPA, and a FAQ section is provided on their website, see Additional Resources, page 35.

## GENERAL PROVISIONS

If your study is subject to GLP standards, make sure that everyone involved in the study is aware that GLP standards must be followed. When you begin the study, you must submit a statement to EPA saying the study will be complying with GLP standards, and you must allow EPA representatives to inspect the research facility and GLP records at any time during the study.

## ORGANIZATION AND PERSONNEL

GLP standards contain specific requirements about the personnel involved in conducting a GLP-compliant research experiment. Personnel involved in your study must have sufficient educational and other qualifications, and you must keep a record of each participant's training and experience. You must be sure there are enough personnel involved in the experiment to conduct it properly, and all personnel must take health and clothing precautions to avoid contaminating the experiment. Anyone with an illness that could affect the quality and integrity of the study (for example, when animals are involved) must be excluded from direct contact with the experiment.



## STUDY DIRECTOR

For each study a qualified **study director** must be appointed to have overall responsibility for and control of the experiment. The study director is the person responsible for the technical conduct of the study and the interpretation, analysis, documentation, and reporting of study results. The director must assure that:

- the study's protocol is approved and followed,
- all experimental data are accurately recorded and verified,
- unforeseen circumstances affecting the quality or integrity of the study are noted when they occur and that corrective action is taken, and
- all **raw data**, documentation, protocols, specimens, and final reports are transferred to archives during the study or at the close of the study.

## QUALITY ASSURANCE

A testing facility must have a **quality assurance** unit that is separate from and independent of the personnel who direct and conduct the study. The quality assurance unit exists to monitor each study, conduct inspections, and maintain records to assure the study's manager that the following aspects of the study comply with GLP requirements:

- facilities,
- equipment,
- personnel,
- methods (including shipping methods and carrier),
- practices,
- records, and
- controls.

The quality assurance unit must immediately notify the study director and management about any

problems found during inspection that are likely to affect the integrity of the study. Designated representatives of EPA may ask the testing facility management to certify that quality assurance inspections are being implemented, performed, documented, and followed up on in accordance with GLP requirements.

## FACILITIES AND EQUIPMENT

You must make sure that the location, size, and design of your experiment facilities (e.g., your field) and the way you handle and store your test and control substances (e.g., pesticides) are appropriate to ensure the quality and integrity of your experimental data. The equipment used in the experiment must be adequately inspected, cleaned, tested, calibrated, and maintained.



Photo: Darren Cockrell

## OPERATION OF TESTING FACILITIES

### STANDARD OPERATING PROCEDURES

GLP standards require you to have a set of **standard operating procedures** that everyone involved in the study follows. They must be written and approved by management prior to the initiation of your study and must cover such things as procedures for making experimental observations,

how to collect samples, and how to handle, store, and retrieve experimental data.

During your study, you must periodically review your standard operating procedures and revise them as needed, and you must keep these written procedures immediately available for inspection.

### TEST SYSTEM CARE

**Test system** refers to the object to which you are applying your test or control substance. This could be, for example, an animal, a plant, soil, or water. You must develop standard operating procedures for the care of test systems. This generally involves conducting analyses at the beginning of your study and periodically throughout the course of your research to make sure that the test systems are free of any diseases or conditions that might interfere with the purpose or conduct of your study. You must document these analyses and maintain them as raw data. You must also document the use of all pesticides in your experiment, and if there is a need to use pesticides that are not directly related to your test, you must avoid using any pesticides that could interfere with or contaminate the study.

### TEST AND CONTROL SUBSTANCES

The **test substance** is the substance or mixture that is added to your test system, usually along with a control substance. The **control substance** is any material other than the test substance that is administered to the test system to establish a basis for comparison with the test substance.

### TEST AND CONTROL SUBSTANCE CHARACTERIZATION, HANDLING, AND MIXING

You must document the name, purity, solubility, and stability of each test and control substance you use in your study. GLP-characterized test substances and characterization reports may sometimes be obtained from the manufacturer of the material. Storage containers must be clearly

labeled with the name, expiration date, and storage conditions of each test and control substance, and you must document the stability of your test or control substance under storage conditions. If your study lasts for more than 4 weeks and you use multiple lots of the test or control substance, GLP standards require you to reserve samples of each lot.

You must handle your test and control substances properly, which includes:

- proper storage, including recording of storage temperatures,
- proper distribution practices if the substance is geographically distributed, to preclude the possibility of contamination, deterioration, or damage to the product during distribution,
- maintaining proper identification of the substance through the distribution process, and
- proper documentation of receipt and distribution of the substance, including dates and quantities.

If you mix your test or control substance with a **carrier**, you must test for the:

- uniformity of the mixture,
- concentration of the test or control substance in the mixture,
- solubility of the test or control substance in the mixture, and
- stability of the test or control substance in the mixture.

You must document the expiration date of the carrier mixture. If you use a vehicle, such as an **adjuvant**, to facilitate the mixing of your test or control substance with a carrier, you must document that the vehicle or adjuvant does not interfere with the integrity of your test.



## PROTOCOL FOR AND CONDUCT OF A STUDY PROTOCOL

You must have a written protocol for your study that indicates the objectives and documents all methods used in the conduct of your study. The study director must sign the protocol before you begin your study.

### GLPS STUDY PROTOCOL CHECKLIST

The protocol must contain the following information:

- study title and statement of purpose,
- name of your test and control substances,
- name and address of the person actually conducting the study,
- name and address of the sponsor of the study (if different from the person conducting the study),
- proposed starting and ending dates of the study,
- your justification for choosing the crop or animal (the test system) that is the subject of your study,
- detailed information about the crop or animal that is the subject of your study,
- procedure for identifying the crop or animal that is the subject of your study,
- description of the experimental design, including methods for the control of bias,
- description of any solvents, emulsifiers, or other materials used in the study to solubilize or suspend the test or control substance before mixing it with the carrier,
- route or method you use to administer the test and control substances and your reasons for choosing it,
- application rate and frequency of application,
- type and frequency of tests, analyses, and measurements to be made during the study,

- records to be maintained during the study (sometimes in triplicate),
- date of approval of the protocol by the study sponsor and the dated signature of the study director, and
- statement of the proposed statistical method to be used.

### PROTOCOL CHANGES

All changes in or revisions to an approved protocol and the reasons for those changes must be documented, signed by the study director, dated, and maintained with the protocol. Changes to the protocol that are made prior to the phase of the study in which the change will occur are called protocol amendments. Unplanned events that affect or could affect the integrity of the study must be documented as protocol deviations and immediately reported to the study director.

### CONDUCT OF A STUDY

You must conduct and monitor your study in conformity with the **study protocol**. You must handle specimens (material you take from the test system to analyze) in a way that precludes error in the recording and storage of data.

All data generated during the conduct of your study must be:

- recorded directly, promptly, and legibly in ink
- dated on the day of entry
- signed or initialed by the person entering the data

Any changes in entries must be made in a way that

- does not obscure the original entry,
- must indicate the reason for the change, and
- must be dated and signed in ink at the time of the change.

## RECORDS AND REPORTS

### REPORTING OF STUDY RESULTS

GLP standards require you to prepare a final report for your study. This report must contain the following information:

- your name, your address, and the beginning and ending dates of the study,
- objectives and procedures stated in the approved protocol, including any changes in the original protocol,
- detailed identification and description of the test and control substances,
- description of the methods used in the study,
- description of the crop, animal, or other substance that was the subject of your study,
- application rate or dosage, how the test substance was applied or administered, and the duration of application or administration,
- description of all of the circumstances that may have affected the quality or integrity of the study,
- data, including protocol deviations,
- names of the study director and all supervisory personnel, scientists, or other professionals involved in the study,
- statistical methods used in analyzing the study data,
- description of the calculations or operations performed on the study data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis,
- signed and dated reports by each person involved in the study, including all those who have analyzed or evaluated specimens or data after they were generated,
- location where you will store all specimens, raw data, and the final study report, and

- quality assurance unit's prepared and signed statement.

The final report must be signed and dated by the study director, and corrections or additions to the final report must be in the form of an amendment by the study director that clearly indicates the reason for the amendment. You must maintain a copy of the final report.

### STORAGE AND RETRIEVAL OF RECORDS AND DATA

You must retain all raw data, documentation, records, protocols, specimens, and final reports generated as a result of your study. You should also save any other correspondence or documents that relate to interpretation and evaluation of data, in addition to those documents already contained in the final report.

You must create an indexed archive for storage and easy retrieval of all raw data, documentation, protocols, specimens, and interim and final reports, and you must appoint a person to be responsible for these archives. You must allow only authorized personnel to enter the archives.

### RECORD RETENTION

GLP standards require you to retain study records for lengths of time specified in the regulations.

Items you must retain include:

- all study documentation,
- raw data,
- specimens,
- the study's master schedule,
- copies of study protocols,
- records of quality assurance inspections,
- summaries of study personnel's training, experience, and job descriptions, and
- records and reports of the maintenance, calibration, and inspection of equipment.

Fragile or perishable specimens need be retained for only as long as their quality affords evaluation. Soil, water, and plant specimens can be discarded after quality assurance verification. If the researcher is working with a formulator, the formulator may require longer periods of record retention than GLPS.

## ROLE OF THE IR-4 PROJECT

The **IR-4 Project** (Interregional Research Project No.4) is involved in making sure that pesticides are registered for use on minor crops. Minor use pesticides are those that produce relatively little revenue for private industry so they cannot invest resources to develop the necessary data or collect information to support the use authorization on minor crops and minor uses on major crops. The lack of crop protection products for specialty crops and minor uses on major crops is referred to as the Minor Use Problem and was the basis for the IR-4 Project being formed in 1963. IR-4 helps the minor use community gain access to new pest management technologies. IR-4 also helps public institutions that develop technologies obtain regulatory support.

The IR-4 Project operates as a unique partnership between the **U.S. Department of Agriculture (USDA)** – both the **National Institute of Food and Agriculture (NIFA)** and the **Agricultural Research Service (ARS)**, the **Agricultural Experiment Stations (AES)**, the **U.S. Environmental Protection Agency (EPA)**, the agrochemical industry, commodity groups, and growers.

In recent years, additional partnerships have been formed with **USDA Foreign Agricultural Service (FAS)** which supports international specialty crop export activities, **Animal Plant Health Inspection Service (APHIS)** to work on selected

invasive species, and the **Department of Defense's Deployed Warfighter Protection Program (DWFP)** to provide regulatory support for public health pesticides.

In early 1971, the concept of Crop Groups was formed. This is a model that allows extrapolation of residue data from a few representative crops to many other crops in the same group. This allowed establishment of residue tolerances for the entire group of crops based on the residue values from certain key crops that were similar. More information on IR-4 and the crop groups can be found at the IR-4 website (Additional Resources).

## CHAPTER 4 ADDITIONAL RESOURCES

EPA's Good Laboratory Practices Standards Compliance Monitoring Program. <https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program>

The IR-4 Project. IR-4 Project works towards developing research data to support new EPA tolerances and labeled product uses. <http://ir4.rutgers.edu/index.html>

## CHAPTER 5: PLANNING RESEARCH EXPERIMENTS

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

Describe the elements of research experiments that should be considered when designing field experiments:

- A. Objectives and hypothesis
- B. Resources available
- C. Location
- D. Test population
- E. Treatments
- F. Experimental design
- G. Units for observation
- H. Data to be collected
- I. Statistical analysis
- J. How to report results

### INTRODUCTION

Chapter 6, page 40, provides specific information on experimental design. This chapter provides things to consider before you begin designing the experiment. It emphasizes the importance of proper planning before conducting research experiments. The scientific method provides the steps that help plan a research experiment in a systematic way.

The scientific method involves the following steps:

1. Identify the problem, purpose or research question.
2. Do background research to learn what others have discovered about the topic.
3. State the hypothesis to determine how you think your question should be answered.
4. Design the experiment.
5. Collect, analyze and interpret the data.
6. Draw a conclusion based on your research and the data you collected. (Accept, reject or alter the original hypothesis.)
7. Communicate your results to others who are interested in the topic.

### ELEMENTS OF RESEARCH EXPERIMENTS

#### STATEMENT OF OBJECTIVES

The particular question that will be addressed and the hypothesis being tested must be clearly stated. The first several steps of the scientific method are used to identify the objectives of the experiment and develop the hypothesis. A well-designed experiment should be simple and precise and contain no systematic error (e.g., the plots receiving one treatment should not differ systematically

from the plots receiving another treatment). The researcher should follow the scientific method meticulously when designing an experiment.

### STATING THE HYPOTHESIS

When working with biological systems, formulating a hypothesis becomes one of the most difficult steps in the scientific method. There is natural variation in the environment and the organisms we want to study. And often times, the scientific study of a population (plants, insects, weeds, etc.) is restricted to a very limited subset of observations (e.g. a small plot trial vs all the agricultural fields in the area).

Hypothesis means a working statement you are trying to prove true or false. When you set up a hypothesis test to determine the validity of a statistical claim, you need to define both a **null hypothesis** and an **alternative hypothesis**. Every hypothesis test contains a set of two opposing statements, or hypotheses, about a population parameter. The first hypothesis is called the null hypothesis, denoted  $H_0$  and means that the statement is ‘nullifiable’. The alternative hypothesis,  $H_1$ , is usually just the opposite of the null hypothesis and is usually what you are testing. To determine whether you will accept or reject the hypothesis, a hypothesis test is performed after collecting the data. (Information can be found on the tests to use by referring to the Additional Resources, page 39.)

### IDENTIFY THE RESOURCES AVAILABLE

Before designing the experiment, it’s critical to determine what resources are available. Many experiments have failed due to grandiose designs that did not have the proper resources and proved impractical to complete. Some of the questions to consider include:

- Will this experiment be conducted under laboratory (greenhouse) or field conditions?

- Will there be adequate space at one site or will multiple sites be necessary?
- Is the proper equipment available and in good condition? This includes not only planting and harvesting (if applicable), but also pesticide application equipment?
- Is there enough labor available to make the required observations and collect samples?
- If the test involves insects or plant diseases, will it be necessary to artificially infest? If so, are the resources and standard methods available?

### ASSESS THE LOCATION(S) OF THE EXPERIMENT

If conducting field research, some important things to consider include:

- Will the location be accessible under variable weather conditions?
- What is the rotational history of the site? This is especially important if conducting the experiment using multiple sites for replication.
- Are there concerns about differences in drainage, slope, etc. for the replications?
- What is the soil type and fertilization history? Again, especially important to consider for experimental design at a site or when using multiple sites for replication. For large sites, or multiple sites, consider collecting representative soil samples from within the experimental site.
- Would acquiring a satellite map of the intended site help identify the best location for the experiment?
- Are there any objects (wind breaks, buildings, roadways, etc.) in the vicinity that might affect the growth of the plants or the applications of treatments.

**IDENTIFY THE TEST POPULATION**

Refer back to the objectives of the experiment to consider the population that is the subject of the experiment. It's important to realize that the conclusions made after a statistical analysis cannot be extrapolated from one population to another. For example, if testing only one variety in the experiment, you cannot conclude that all varieties will respond the same.

**SELECT THE TREATMENTS**

The type and number of treatments as well as the number of replicates for each treatment should be determined in advance of choosing the experimental design. In order to minimize the experimental error, obey the two golden rules of experimental design: randomize and replicate. And remember, research trials are often repeated over years to confirm the results.

When determining the treatments and replicates, always consider the availability of labor, costs, and any equipment required to complete the experiment.

**SELECT THE EXPERIMENTAL DESIGN**

While there are many different ways of conducting research with herbicides, the Weed Science Society of America published a special issue, *Research Methods in Weed Science*, which is worth exploring if this is the type of experiment you will be conducting, see Additional Resources, page 39.

Information on some of the most common Experimental Designs is presented in Chapter 6, page 40. However, Chapter 6 is not intended to provide all the information a researcher may need to know in order to choose the appropriate experimental design to fulfill the objectives and test the hypothesis. In addition to the resources provided in Additional Resources at the end of Chapters 5 and 6, pages 39 and 52,

researchers should consult with a statistician, if possible.

**SELECT THE UNITS FOR OBSERVATION**

When choosing an experimental design, consider the experimental unit (plots, pots, etc.) that will be used and how to minimize any variation and non-uniformity between the units. Differences in soil types, moisture availability, weed infestations, etc., should be minimized within a replicate.

Plot size should be determined based on the level of precision the researcher wants for the experiment as well as equipment that will be used. It is important to control the effects of adjacent units on one another. For example, consider how to control drift when applying various rates of a pesticide, or whether movement of insects between adjacent units is a concern. This is usually controlled by the use of border rows, which should be considered in calculations when determining the total area required for the experiment.

**IDENTIFY THE DATA TO BE COLLECTED**

The variables to be measured should be determined before beginning the experiment and should relate back to the objective(s) of the experiment. Focus on collecting data that will explain why the treatments perform as they do. It may include things such as plant height, root/shoot ratio, plant population, date of emergence, tillering/heading/flowering, and yield parameters.

A clear unbiased procedure for collecting samples should be identified before the experiment begins. The researcher should also be aware of the amount of data that will be collected to ensure that enough time and labor is allotted. The method of data analysis will be determined by the type of data that will be collected (continuous measured data, proportional data, count data, etc.). More information on data collection and recordkeeping is available in Chapter 7, page 53.

## CHOOSE THE APPROPRIATE STATISTICAL ANALYSES

Statistics can help objectively test hypotheses provided the hypothesis is concisely defined. An experiment that cannot be objectively analyzed is worthless! This guide is not intended to be a course in statistics but there are numerous resources listed in Additional Resources that provide good information about experimental design and statistical analyses used when conducting agricultural research.

But we will caution you...before undertaking any experiment, practice preventive statistics; i.e., know what type of statistical analysis will be used *before* you begin the experiment. If you haven't already done so during your design phase, you might want to review your plans with a statistician or one or more of your colleagues. They might help identify adjustments that could be made to allow you to learn more from your experiment.

And, remember, statistics do not prove anything - there is always a probability that your conclusions may be wrong!

## CONSIDER HOW TO REPORT THE RESEARCH

If the work was done under contract, then the results will be provided to the contractor. However, if the researcher has responsibility for reporting the results, identify whether the results will be distributed through peer-reviewed publications, field days, news articles, etc. Remember, even reporting research where the null hypothesis is not rejected is important - it's positive evidence that there may not be real differences among the treatments tested!

## CHAPTER 5 ADDITIONAL RESOURCES

- Clewer, A.G. and D.H. Scarisbrick. 2001. *Practical Statistics and Experimental Design for Plant and Crop Science*. John Wiley & Sons.
- Gomez, K.A. and A.A. Gomez. 1984. *Statistical Procedures for Agricultural Research*. 2nd edition. John Wiley & Sons.
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- Little, T.M. and F. Jackson Hills. 1978. *Agricultural Experimentation: Design and Analysis*. John Wiley & Sons.
- Palaniswamy, U.R. and K.M. Palaniswamy. 2006. *Handbook of Statistics for Teaching and Research in Plant and Crop Science*. The Haworth Press, Inc.
- Roma-Burgos, N. and S.O. Duke, eds. *Research Methods in Weed Science*. (2015). Weed Science, (Special Issue 1). <http://www.bioone.org/doi/full/10.1614/wees-13-00-13.1>



## CHAPTER 6: EXPERIMENTAL DESIGN

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Describe the following factors used to control experimental errors:
  - Replication
  - Randomization and methods used to randomize
  - Blocking
  - Local control
- B. List the elements to consider when designing experiments:
  - Experimental units and control plots
  - Selecting treatments
  - Plot size and number
- C. Describe the types of experimental design and when they should be used:
  - Completely randomized
  - Randomized complete block
  - Split-plot
  - Split-block
  - Latin square
- D. Describe a Factorial Experiment and what experimental designs are used.

### INTRODUCTION

When you plan an agricultural research experiment, keep in mind that research experiments always have uncontrolled variables. You are experimenting in a biological world that is constantly changing. You can plant the same crop variety in two adjacent fields but still get differences in yield because of factors you have no control over. The differences, or variability, among experimental units that have been treated exactly alike are called **experimental error**. The goal is to design experiments that accurately distinguish the uncontrolled variables from the effects you are trying to measure or compare. It's important to spend time to properly design experiments to estimate and control experimental error.

Designing an experiment is an extremely important process because errors made in the design can invalidate the results of the entire experiment. Experimental design also is important because the researcher wants to do more than simply describe the outcome. He or she also wants to make inferences about what factors contributed to or caused events, and to do so without ambiguity. For this reason, proper experimental design is critical for ruling out alternatives and producing clear results. The most able statistician cannot validate conclusions from an improperly designed experiment.



Good experimental technique goes a long way toward minimizing error and bias. Every effort should be made to eliminate these problems through appropriate experimental designs. To help eliminate experimental error and bias:

- apply all treatments uniformly,
- measure all treatment effects in an unbiased way,
- prevent gross errors, and
- control external influences so that all treatments are affected equally.

Properly designing and implementing a field trial may seem complex the first time, but it is really a logical process that should not be intimidating. You may need help the first time you design a trial to ensure that you are not overlooking something important, but if you learn the principles involved in the process, you should quickly gain confidence in your ability to conduct experiments on your own.

The following Experimental Design Checklist might be helpful to use when designing an experiment. It's critical to keep in mind the following:

- Ensure that true treatment effects can be separated from random variation between the units AND from the effects of non-uniformity in external conditions.
- Make observations and measurements without **bias**.
- The collected data should be analyzed using valid statistics.

## EXPERIMENTAL DESIGN CHECKLIST

The following **Experimental Design Checklist** can be used in designing an experiment. These items may be addressed in any order.

- ☐ Determine the objective of the test.
- ☐ Select treatments that address the objective. Consider including positive and negative controls.
- ☐ Determine what data should be collected, and when it should be collected, to address the objective.
- ☐ Select the number of replications to use. Consider four replications a minimum.
- ☐ Determine how big individual plots will be.
- ☐ Select an experimental design.
- ☐ If using a design that includes blocks, determine how blocks should be arranged in the field.
- ☐ Depending on the experimental design chosen, randomize treatments or randomize treatments within blocks.

Source: Oregon Department of Agriculture Research and Demonstration Study Guide

## FACTORS USED TO CONTROL EXPERIMENTAL ERRORS

From a statistician's perspective, an experiment is performed to decide whether the observed differences among the treatments (or sets of experimental conditions) included in the experiment are due only to chance, and whether the size of these differences is of practical importance. Statistical inference reaches these decisions by comparing the variation in response among those experimental units exposed to the same treatment (experimental error) with that variation among experimental units exposed to different treatments (treatment effect).

When designing an experiment, there are three important principles to consider:

- replication, to provide an estimate of experimental error;
- randomization, to ensure that this estimate is statistically valid; and
- local control, to reduce experimental error by making the experiment more efficient.

## REPLICATION

**Replication** means that a treatment is repeated two or more times. Its purpose is to provide an estimate of experimental error and a more precise measure of treatment effects. The number of replications required for a particular experiment depends on the magnitude of the differences you wish to detect and the variability of the data with which you are working. While there are statistical formulas that can be used to determine the number of replications based on the desired precision level, in general, field studies use four to five replicates. More replicates may be desired, depending on the type of research being conducted. For example, it is not unusual to have eight replicates when conducting insecticide trials.

## RANDOMIZATION

An important component of good experimental design is **randomization**. Randomization means assigning treatments to experimental units (plots, pots, etc.) so that all units have an equal chance of receiving a treatment. Its purpose is to assure unbiased estimates of treatment means and experimental error.

## LOCAL CONTROL

**Local control** is recommended to reduce experimental error. In general, adjacent plots tend to be similar in environmental conditions (fertility, moisture, slope, etc.). Based on this principle, adjacent plots can be grouped into ‘blocks’ and

the treatments within that block can be compared under similar conditions.

## METHODS OF RANDOMIZATION

There are many ways to randomize samples, treatments and experimental units. In selecting numbers at random, it is not so much the method of producing the numbers that matters but the properties of the numbers produced. They should have the properties we would expect “random” numbers to have.

### LOTTERY METHOD — PULLING NUMBERS OUT OF A HAT

The simplest way is literally to pull the numbers out of a hat. Assign each treatment a number, write the numbers on individual pieces of paper, mix the slips of paper up, and then select the slips one at a time without looking at them first. The order in which the numbers are drawn is the order in which they will be arranged in a block. Repeat these steps for each block in the experiment.

### RANDOM NUMBER TABLES

Another way to select experimental units or assign treatments is to use a table of random numbers. A random number table is a table of digits. The digit in each position in the table was originally chosen randomly from the digits 1, 2, 3, 4, 5, 6, 7, 8, 9 and 0 by a random process in which each digit is equally likely to be chosen. A number itself cannot be random except in the sense of how it was generated. Generating a random number means that all elements of the set were equally probable as outcomes. This is equivalent to statistical independence.

Tables of random numbers are available in most statistics text books. The first step in using a table of random numbers is figuring out how big a number is required. This will depend upon the

number of experimental units and the number of replications. Do not keep using the same part of a random number table over and over again. Change starting points and directions. Pick a starting point at random, either by using the “look away and stick a pin in it” method or by drawing starting row and column numbers from a hat.

After picking the starting point, sets of numbers are gathered from the table either by reading left, right up or down from the starting number. For each value desired, pick the number of digits to match the highest number and ignore any values picked that are higher than that value. For example, to get 10 random numbers between 1 and 60: 1) randomly select a start point in the table, 2) select this digit plus the one next to it, 3) move up, down, left or right, 4) choose the next two digits. Repeat this process enough times to get ten two-digit numbers. If you encounter a value greater than 60, simply ignore it and select another.

### COMPUTER-GENERATED RANDOM NUMBERS

A third way to randomize experimental units and treatments is to use a computer or an ‘app’. Microsoft® Excel contains functions to generate random numbers. There are several websites that will generate random numbers for free as pseudo-random or true random numbers.

#### Pseudo-random numbers

A pseudo-random number is a number belonging to a long sequence generated by a computer that appears to be random but eventually repeats itself exactly. That is why numbers in the sequence are called “pseudo-random” numbers. In the short run, these sequences of pseudo-random numbers have an apparent randomness. These pseudo-random numbers are sometimes random enough if you don’t have millions of units to randomize. An example website is Research Randomizer, see Additional Resources, see page 52.

#### True random numbers

Modern computers do a better job of approaching true randomness than early computers. More sophisticated programs are available to generate better (less predictable) pseudo-random numbers. Some web sites actually claim to produce batches of true random numbers from a hardware-based random number generator. An example website is Random.org. The website also has a link to a downloadable app for a small fee, see Additional Resources, see page 52.

## ELEMENTS TO CONSIDER WHEN DESIGNING EXPERIMENTS

### EXPERIMENTAL UNITS AND CONTROL PLOTS

An **experimental unit** is the smallest unit to which a treatment can be applied at random. Every treatment should have an equal chance of being assigned to any experimental unit. This ensures a valid and unbiased estimate of the experimental error and treatment differences. Remember, it is the experimental unit that gets the treatment.

The experimental units or plots in which the *treatment is not made* are called the control or check. **Control plots** should be included in all experimental field work. Failure to include control plots or not including enough control plots yields questionable results that are usually unacceptable for publication and sales promotion. Check plots should be selected with the same objectivity as other plots. The same variables that may affect treatment plots also may affect control plots. For this reason, the location of control plots within a field should not be selected arbitrarily. Likewise, control animals should not be selected arbitrarily but should represent a random sample of the test population.

## SELECTING TREATMENTS

The objective, or purpose, of the study will determine the treatments included in an experiment. Write down the test objectives so you can precisely define what it is you want to find out. A test may have more than one objective, although multiple objectives should be closely related and clearly defined to distinguish one from another.

The selection of treatments is usually logical if you can define the purpose of the study. You should include ALL treatments necessary to address the experiment's objective. For example, if the purpose of an experiment is to determine which of five insecticides is most effective, then the treatments will include all five of those insecticides and an untreated control. If the purpose is to determine if any of the five insecticides works better than your current choice, then the treatments will include the five insecticides plus the insecticide you presently use and an untreated control. Accurately stating the purpose of the test before the treatments are applied in the field is critical. After the treatments have begun, it will be too late to add other treatments to answer the question you really wanted to address.

The selection of treatments and the experimental design becomes more complicated as the question you are trying to answer gets more complex. It is common to want to test in the same experiment two (or more) things that influence crop production. For example, you may want to test how a particular post-emergence herbicide influences the yield on five different wheat varieties. The specific questions addressed in this case are:

1. What effect does the herbicide have on wheat yield?
2. What effect do the varieties have on wheat yield?
3. Does the herbicide have the same effect on each variety; i.e., are there any interactions?

The third question may not be as obvious as the first two, but it will always be asked or implied if you are testing two or more factors in the same experiment. In this example, you have to determine the effect of the herbicide on each wheat variety and then compare those effects to each other. To do this, the treatment list must include each variety without the herbicide and each variety with the herbicide treatment (a total of 10 treatments). With this list of treatments, you can make the comparisons necessary to answer the three questions. This example is a factorial experiment that employs a factorial arrangement of treatments. This will be discussed in more detail later in this chapter.

Treatment selection may also include additional treatments needed to provide a relative measure of effect. You might consider including a standard treatment to provide a relative measure of how well the other treatments performed. For example, if you wish to test a new nematicide, you should include a treatment with the currently used nematicide and a treatment with no nematicide as a basis for comparison. Without the proper controls, you will not be able to say that the new nematicide worked better than the currently used nematicide or even that the new nematicide worked better than no nematicide. The questions you wish the experiment to answer should indicate what treatments should be included as controls.

It is often desirable to have both a **positive** and a **negative control** in an experiment to assure that the experiment was done properly and the outcome of the experiment is affected by the independent variable. The negative control, one in which no response is expected, helps you determine if the treatments being tested work better than some minimal treatment (or no treatment). The standard treatment helps you determine if the treatments being tested work better than the current standard practice. You may have several control treatments

in an experiment if you currently have several viable options from which to choose. For example, if you currently can choose either of two fungicides to control leafspot, you may wish to include them both as controls in your experiment when you test new products. You do not have to include all currently available options as controls for the experiment, but you can.

### PLOT SIZE AND NUMBER

A **plot** is the area to which an individual treatment is applied. It can be any size, including a single plant growing in a pot or several acres of a field. However, a plot must be large enough to be representative of a much larger area. Plots that are larger than necessary take up more space and require much more work. Plots that are too small may make it impossible to accurately assess the effects of treatments. When deciding how large your plots should be, consider the equipment to be used in planting, harvesting and treatment; the amount of space available for the experiment; the number of treatments; and the biology of what you are studying. Accommodating equipment and space concerns makes it easier to conduct the test.



Small plot planter.

Photo: T. Walker, Colorado State University

If your equipment can plant, harvest and treat four rows at a time, then the logical plot width would be some multiple of four rows (4, 8, 12 rows, etc.) Using any other width (such as six rows) would make it more difficult to conduct the experiment. The plot length is generally more flexible than plot width. If you plan to weigh the harvest from each plot, the scales you have may influence the length plots should be. If your scales are designed to weigh hundreds of pounds, your plots should be large enough to provide a harvest weight that can be weighed accurately on those scales, and increasing the length of plots is an easy way to do that.

The length of plots can be adjusted so that all plots (all replications of all treatments) will fit into the area available for the test. If you have a large area for the test, space may not be an important consideration.

Accommodating biological concerns reduces the chance of overlooking differences among treatments. Equipment and space considerations are usually easy to identify, but biological considerations are not always obvious. To accommodate biological considerations, you should answer two questions:

- How large a plot is necessary to observe the biological effect (disease severity, insect damage, weed frequency, nematode population levels, etc.) that you are studying?
- How large a plot is needed to minimize the influence of a treatment (chemical application) on the plots next to it?

This information will help you determine the minimum plot size necessary to get useful data from the experiment. To get an accurate measurement of the effect of pest management treatments, the plot must be large enough to account for the uneven initial distribution of the



pest (pathogen, insect, weed, etc.). In some areas the pest may be present to begin with, while in others the pest may appear only after it has spread from its initial location. This is very important for pests that spread very slowly (such as most soil-borne organisms). Some diseases and pests are highly mobile and spread very rapidly (such as many insects).

In an insect management trial, measuring the effect of a treatment can be very difficult if the plots are too small because the insects that you see in the plot may have simply spread from the plot next to it. To minimize this problem, consider increasing plot size and then collect data from the middle section of the plot. For example, you might have an eight-row plot but collect data only from the middle four rows. The rows from which data is not collected are often referred to as “**buffer rows**” because they buffer the effect of the neighboring plots. If buffer rows are not used when they are needed, you may fail to detect differences among treatments and incorrectly conclude that treatments were ineffective.



Sorghum test plots.

Photo: Colorado State University Crops Testing

Buffer rows are often used when it is uncertain whether or not treatments can influence nearby rows. A similar concept involves the use of border rows along the edges of your test area. There is often a significant “**border effect**” at the edge of a field, where plants may grow differently than plants not at the edge. Although you may be able to minimize this problem with blocking, it is often better to eliminate the problem by not using the rows at the edge of a field in your experiment.

Once the plots are large enough to be representative of a much larger area, further increasing plot size will not significantly improve the accuracy of the results. For example, in an experiment testing fungicides for control of white mold in bush beans, a plot four rows wide by 100 feet long should be just as good as a plot eight rows wide by 400 feet long. Plots that are larger than necessary may increase the amount of work required for an experiment, but usually will not adversely affect the test results unless they are so large that the plots within a block are no longer uniform. Plots that are too small may prevent the accurate assessment of treatment effects. If you have limited space for an experiment, use more replications to ensure accurate results but consider whether you have the labor force required to sample and, if collecting yield, to harvest the plots. If the number of treatments generates a large block size, consider breaking the experiment into two separate experiments with common treatments.

## TYPES OF EXPERIMENTAL DESIGNS

### COMPLETELY RANDOMIZED DESIGN (CRD)

The **Completely Randomized Design (CRD)** is the simplest experimental design and is frequently used to compare treatments when environmental conditions are fairly uniform. It only uses two basic principles of experimental design: randomization and replication. Each treatment is applied at

random to several experimental units (plants, pots, plots, etc.). CRD is most useful in laboratory and greenhouse experiments.

Although completely randomized designs are flexible and simple, estimating **experimental error** with this design may be less precise than with other designs. A CRD is set up by assigning treatments and controls at random to a previously determined set of experimental units. Any number of treatments may be tested in this design. It is desirable to assign the same number of experimental units to each treatment and control, but it is not essential.

When plots are laid out within a field, the number of plots is determined by multiplying the combined number of treatments and controls by the number of replications desired.

Imagine a trial comparing 3 herbicides and using 4 replicates. The four treatments would include 3 herbicides + one control, replicated 4 times for a total of 12 plots. The treatments are assigned, at random, to the plots. The plot map might look something like the one below.

#### Completely Randomized Design

A	B	C	D
D	A	C	C
B	B	D	A
D	C	A	B

#### Advantages of CRD include:

- Easy to set up and analyze.
- The number of replicates does not need to be the same for each treatment.
- Analysis is straightforward and not complicated by unequal replication or missing data (plots lost due to hail, flood, failure to treat a plot, etc.).

#### Disadvantages of CRD include:

- There is a loss of precision in determining differences among whole-plot treatments.
- It may create a large experiment if there are a lot of treatments, making applications difficult or increasing labor costs.

#### RANDOMIZED COMPLETE BLOCK (RCB)

The **randomized complete block** design is the most commonly used design in agricultural field research. RCB is the simplest design for a comparative experiment using all three basic principles of experimental design: randomization, replication, and local control. It is used to account for natural variability that would otherwise obscure treatment differences.

In this design, the treatments are assigned at random to a group of plots called a block; a block is a grouping of single occurrences of each treatment. More information on blocking is below. Because adjacent plots are more likely to produce similar yields or have similar pest infestations or similar fertility than those separated by some distance, the block is kept as compact as possible. This is accomplished by placing the plots, usually long and narrow, close together. The number of treatments also should be as small as possible.

#### Randomized Complete Block

Block 1	A	B	C	D	E
Block 2	C	A	D	E	B
Block 3	E	B	D	C	A
Block 4	D	A	C	E	B

Treatments are both replicated and blocked, which means that plots are arranged into blocks and then treatments are assigned to plots within a block in a random manner (as in the diagram). This design is most effective if you can identify the patterns of non-uniformity in a field, such as changing soil types, drainage patterns, fertility gradients,

direction of insect migration into a field, etc. If you cannot identify the potential sources of variation, you should still use this design for field research but make your blocks as square as possible. This usually will keep plots within a block as uniform as possible even if you cannot predict the variation among plots.

### Blocking

**Blocking** refers to physically grouping treatments together in an experiment to minimize unexplained variation in the data you collect (referred to as experimental error). This allows the statistical analysis to identify treatment differences that would otherwise be obscured by too much unexplained variation in the experiment. Variation in an experiment is of two types—variation for which you can account in the statistical analysis and variation that is unexplained.

The goal in blocking is to allow you to measure the variation among blocks and then remove that variation from the statistical comparison of treatment means. If you can anticipate causes of variation, you can block the treatments to minimize variation within each block and remove some variation from the statistical analysis. The mathematics of how blocking allows you to reduce unexplained variation is beyond the scope of this manual.



Blocking can be used to account for drainage differences.

Photo: Dariusz Banaszuk, shutterstock

In agricultural research, field plots are almost always blocked even when no obvious differences are present in the field. It is much better to block when you did not really need to than not to block when you should have blocked. Blocking is a very powerful tool that is most effective if you can anticipate sources of variation before you begin an experiment. For example, in a herbicide trial, one side of a field may have a history of more severe weed problems than another. If you just scattered your treatments randomly through the field, a lot of the variation in the data you collected could be due to the increased weed pressure on one side of the field. Such variation would make it difficult to determine how well each treatment worked. Because you know one side of the field will have more weeds, you can remove that source of variation from the statistical analysis by blocking and improve your chances of identifying differences among treatments.

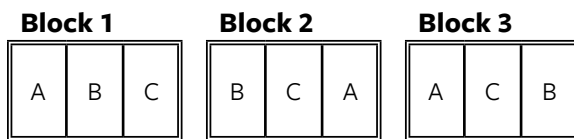
The process of blocking follows a logical sequence. First, you determine that there is something (weeds, drainage, sun/shadow, water, soil type, etc.) that is not uniform throughout the experimental area (field, greenhouse, etc.) that may influence whatever you are measuring (yield, plant height, etc.). Then you arrange your treatments into blocks so that the area within each block is as uniform as possible.

Though the area within a block should be relatively uniform, there may be large differences among the blocks; but that is what makes blocking effective. Your goal is to maximize the differences among blocks while minimizing the differences within a block.

The shape of blocks is not important as long as the plots within a block are as uniform as possible. Ideally, the only differences among plots within a block should be the treatments. Blocks in field experiments are usually square or rectangular,



but they may be any shape. Blocks in the same experiment do not necessarily have to be the same shape but may be an additional source of variability. The shape of individual blocks will be determined by variations in the field that you are trying to minimize. If you are not sure what shape your blocks should be, square or nearly square blocks are usually a safe choice.



Side-by-side blocks

Source: Oregon Department of Agriculture Research & Demonstration Study Guide

Blocks may be arranged through the field in many ways. If the field is wide enough, an easy way to arrange blocks is to place them side-by-side all the way down the field. But blocks do not have to be contiguous and may be scattered through the field in any way that is convenient for you. Note that each treatment occurs only once in each of the four blocks. Treatments are assigned at random to plots within each block, with a separate randomization made for each block. Crop rows should run perpendicular to the fertility gradient to minimize experimental error.

#### Advantages of RCB include:

- The design is very flexible so no limit to the number of treatments or replications.
- Analysis is straightforward and can use missing plot estimates to account for missing data (plots lost due to hail, flood, failure to treat a plot, etc.).
- Allows for sampling to take place over several days as long as sampling is carried out block by block.

#### Disadvantages of RCB include:

- Avoid large blocks which increases within-block variability.
- If plots are uniform over the whole site, CRD is more efficient.

#### SPLIT-PLOT (SP)

The use of **split-plot** designs started in agriculture where experiments were carried out on different plots of land. Split-plot designs have two types of experimental units, whole plots and subplots. The smaller experimental units, the subplots, are nested within the larger ones, the whole plots. An example would be an experiment to evaluate both pesticide performance and crop management practices (e.g., tillage, row spacing, crop variety), such as the effectiveness of three herbicide treatments in no-till and conventional tillage. To simplify the experiment, tillage treatments are established as whole plots. Each whole plot is divided into four subplots and the herbicide treatments (three herbicide treatments plus a control) are randomized within each whole plot.

The split-plot design also can be used when some constraint prevents you from randomizing the treatments into a randomized complete block design. Such a constraint might be equipment limitations or biological considerations. For example, the equipment you have may make it difficult to put out a soil fumigant in randomized complete blocks, but you may be able to put out the fumigant if all treatments within a block that get the fumigant are clustered together rather than scattered throughout the block. You can use a split-plot experimental design to work around this limitation as long as you are able to randomize the other factors. There are other situations when this design is appropriate, but a constraint on randomization is the most likely constraint to occur.

**Example**

Suppose you want to test the effect of five fungicides to control stem rust on two varieties of perennial ryegrass. In this test, you would have a 2 x 5 factorial arrangement of treatments: The two factors would be varieties (two levels of this factor) and fungicides (five levels of this factor). Because a factorial arrangement of treatments is not an experimental design, you still have to select an experimental design that best meets your needs.

If you are able to randomize varieties and fungicides within a block, then you should pick a randomized complete block design, see page 47. If there is some reason why you cannot completely randomize the treatments within each block, then you may be able to use a split-plot design to work around that limitation.

For example, you may have a six-row planter but only enough space in the field to put out four-row plots. To resolve this dilemma, you could plant all of the plots that have the same wheat variety together within a block and then randomize the five fungicide treatments within each wheat variety. In split-plot designs, the terms “whole plots” and “sub-plots” refer to the plots into which the factors are randomized.

As the names imply, whole plots (wheat variety) are subdivided into subplots (fungicide treatment). In the figure, a whole plot is divided into subplots, or fungicide treatments, designated as subplot 1, subplot 2, subplot 3 and subplot 4. The subplots represent different fungicides (four levels of a second factor). Each whole plot serves as a block for the subplot treatments. To assign treatments in a split-plot design, start by identifying where each block will be. The whole plot treatments will be the

Whole plot variety 1		Whole plot variety 2	
subplot 1	subplot 2	subplot 4	subplot 3
subplot 3	subplot 4	subplot 1	subplot 2
subplot 2	subplot 4	subplot 2	subplot 1
subplot 3	subplot 1	subplot3	subplot 4
Whole plot variety 2		Whole plot variety 1	

Whole plots allow two factor testing. In this diagram the whole plots (factor 1: wheat variety) are subdivided into subplots (factor 2: fungicide treatments).

treatments that you are unable to randomize into a randomized complete block design. The subplot treatments can then be randomized within each whole plot treatment.

**Advantages of split-plot include:**

- It simplifies experiments where large equipment is used.
- It can be used for experiments involving varieties, planting date or harvest date.
- The interaction between the main plot and subplots is more precisely estimated than in a randomized complete block design.

**Disadvantages of split-plot include:**

- Analysis is more complicated than randomized complete blocks.
- Main plot treatments are estimated with less precision than a randomized complete block design.
- Missing plots complicate the analysis.

**SPLIT-BLOCK (SB)**

The **split-block** design is a variation of the split-plot design. Subunit treatments are applied in strips across an entire replication of main plot treatments. This arrangement often facilitates physical operations in the subunits but sacrifices precision in comparing the effects of the subunit treatments.

In a split-block design, two sets of treatments are randomized across each other in strips in an otherwise RCB design. It is used where logistics make it necessary to run treatments completely across each block. The number of blocks is the number of replications. This design is useful in orchards and vineyards where pesticide applications are made with air blast sprayers. However, it can be used anywhere that treatments have to run completely across each block.

### LATIN SQUARE

The **Latin Square** design uses a double block design by placing treatments in two different ways—by columns and rows. A Latin Square design may be useful if there are variations (such as fertility) in the field in two directions.

A	B	C	D	E
B	C	D	E	A
C	D	E	A	B
D	E	A	B	C
E	A	B	C	D

Latin Square

Every treatment occurs once in each block (row) and once in each column. A Sudoku puzzle is essentially a Latin Square design (refer to the diagram).

#### Advantages of Latin Square include:

- Variability across the experimental area is measured and removed in two directions.
- This design is used in small experiments where there are four to eight treatments.

#### Disadvantages of Latin Square include:

- The number of treatments must equal the number of replications.
- This design is difficult to manage with a large number of treatments as the number of replications must equal the number of treatments.

### FACTORIAL EXPERIMENTS

The Completely Randomized Designs, Random Complete Block designs and Latin Squares are frequently used for experiments that only consider single-factors (like comparing pesticides for pest control). All other factors were held constant; planting date, seeding rate, variety, etc.

A **factorial experiment** is one in which the treatments consist of all possible combinations of the selected levels in two or more factors. A factorial experiment allows for the researcher to see if there are differential effects, or interactions, of one factor on another. One way to identify factorial designs is by the number of factors involved (for example: varieties, herbicides and fertilizer levels). Although there is no limit to the number of factors, two-factor and three-factor designs are most common. Split-plot and Split-block designs, and variations on these designs, are typically used for factorial experiments.

#### Advantages of factorial experiments include:

- It allows for a broader interpretation of results. If two or three factors, such as pesticides or varieties, are included in the design, then the researcher can examine differences between these specific subsets.
- The simultaneous effect of the factors operating together (interactions) can be tested. For example, an experiment looking at five insecticides applied to two varieties of wheat.

- Factorial designs use one analysis to answer all the questions rather than multiple analyses.

**Disadvantages of using factorial experiments include:**

- The experiment can get large very quickly with several levels of several factors.
- The results may be misinterpreted if main effect means are reported but not interactions.

## **CHAPTER 6 ADDITIONAL RESOURCES**

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## CHAPTER 7: DATA COLLECTION AND RECORDKEEPING

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Describe the difference between biased and unbiased data collection.
- B. Define the following terms used in data collection: variable, value, population, sample, subsample and bias.
- C. Describe how random sampling should be conducted.
- D. Explain the recordkeeping requirement and what additional records might be kept.

### INTRODUCTION

While it is much more common for people to collect too little data than to collect too much data, anyone just learning to conduct research and demonstration experiments tends to collect too much information, some of which may not be useful in answering the objective of the experiment. It's important to focus on the objectives of the experiment to determine the right data to collect. For example, if the objective of a wheat rust fungicide trial is, "to evaluate the ability of five fungicides to reduce rust incidence and severity," then collecting data on rust incidence and severity as well as wheat yield should seem obvious. Collecting data on rainfall and temperature, which strongly influence rust on wheat, may be worthwhile because it can help you explain your results. But collecting data on the physical properties of the soil does not seem to be related to the objective. It is useful to ask yourself, "How can this data be used?" If you have trouble answering that question, then collecting the data may be a waste of time.

So, how much data is enough? The answer is that you want enough data to fully address the test's objective. If you understand the biology of the organisms involved and how your data addresses the test objective, then you should be able to tell if you are collecting enough data. You should take photographs of any differences among treatments that are easily visible. To most farmers, a picture is more convincing than a graph or data table.

Deciding what data to collect is only part of the process. You also have to decide when to collect that data and whether you need to collect the same type of data on more than one occasion. For

example, in a nematicide trial, it is not sufficient to collect nematode population data at harvest; you must also collect data at planting to ensure that the plots started out equal. It is usually a good idea to collect nematode population data in the middle of the season also because even with effective treatments, nematode populations can sometimes increase to the level of the untreated control by the end of the season. The biology of the organisms involved will determine when and how frequently data should be collected.

### ISSUES TO CONSIDER IN DEVELOPING A SAMPLING PROTOCOL

- What and where to sample?
- What is the destructive stage of pest (and virulence of different stages)?
- What is the susceptible stage of the plant?
- What pest stage is most susceptible to management tactics?
- What are the most efficient ways to sample while maintaining good estimates of pest densities or plant damage?
- When to sample (how often)?
- What is the severity of plant response to pest attack?
- What is the epidemiology of the pest population (how quick does it increase)?
- What is the lag time necessary between sampling and implementing a management tactic?
- What is the lag time necessary for a management tactic to be effective?

### BIASED SAMPLING

Data collected from a sample that is not representative of the population will not accurately reflect one or more population characteristics. These are called **biased samples**. A biased sample is one in which not all members of a population are equally likely to be chosen. A biased sample will

always produce biased data. Another kind of bias is called statistical noise. It is the inherent variability from one experimental unit to another. Problems with statistical noise can be lessened by enlarging the sample.

A famous example of a biased sample occurred in the 1936 presidential election polls. One large poll (2,000,000 people) predicted that Landon would defeat Roosevelt. A smaller poll (300,000) predicted that Roosevelt would win. The smaller poll, taken from U.S. census lists, was correct because of its unbiased sample. The larger poll had drawn its sample from telephone directory lists of middle- and upper-income citizens, most of whom voted for Landon. Thus, the larger sample was biased toward more affluent voters because those without telephones had no chance of being chosen.

### UNBIASED SAMPLING

It is critical to collect unbiased data. If you know what treatment was in a plot, or which plots were the untreated controls, your evaluations (disease severity ratings, insect damage ratings, etc.) may inadvertently be influenced. Your subconscious may slightly increase the ratings for untreated lots and decrease it for the plots with treatments that you think should work well. You will probably not even be aware that it is happening, but these subtle influences can change the data enough to affect your ultimate conclusions from the test. If you do not collect unbiased data, you cannot be certain that your conclusions are correct.

The only way to ensure that the data collected is unbiased is to do so without knowing what the treatment was in a specific plot. That would be difficult to do if the treatment were written on a stake in front of each plot. Instead, use some type of code on the plot stakes so that you have to decode the stake number to determine what the

treatment was. You can make up any code you like as long as the person collecting the data cannot tell from the plot stake what the treatment was. For example, you can number the plots sequentially (1, 2, 3, etc.) and have a sheet of paper listing what treatment was applied to plot 1, plot 2, etc. When you collect the data, you write down your observation for plot 1 and later look at your list to see what treatment was in that plot.

## SAMPLING DESIGN

In addition to choosing an experimental design, you must also plan how to collect data from the experiment. To understand sampling, you must understand the following terms: variable, value, population, sample, subsample and bias.

### VARIABLE

Research measures some attribute of the experimental unit, such as the size of plants, the number of organisms, the weight of animals, the yield of crops, the amount of damage, or anything else. Because all experimental units are different, what is being measured is called a **variable**.

### VALUE

Each measurement recorded for a variable (e.g., the number, height, weight, yield, amount, etc.) is called a **value**.

### POPULATION

A **population** is a set of elements about which a researcher wants to make inferences. Elements may consist of people, plants, animals, objects, etc. Researchers draw conclusions about an entire population from inferences made during observations of some population characteristics. The size of a population is the number of observations possible in it. Sometimes a population is too large or difficult to observe in its entirety, so

a portion of the whole population is observed. This set of observations is called a sample.

Population distributions have three general types of dispersal patterns: random, uniform (sometimes called spaced) and clumped (sometimes called contagious). Random sampling designs allow researchers to select members of the population for sampling with equal probability.

### SAMPLE

A **sample** is a small part of a population intended to be representative of the whole. In research, a sample is a subset of an entire population or process, the elements of which are selected in an intentional and predetermined way. Scientific standards demand that a sample be selected in such a way that it won't present an incorrect or biased view of the population. If statistical inference is to be used, there must be a way of assigning known probabilities of selection to each sample. If the sample is selected in such a way that each member of the set has an equal probability of being selected, the sample is called a random sample.

### SUBSAMPLE

**Subsampling** is a measurement that does not include the whole experimental unit. Subsampling is often desirable in recording the effects of a treatment in an experiment. For example, when entomologists sample ten corn plants per plot (where the plot contains more than ten corn plants) to estimate resistance to corn borers, each of these plants is a subsample. Or if there are four plants to a pot, and the pot is the experimental unit, and each plant is recorded individually, then the individual plants are subsamples.

### SUBSAMPLE VERSUS REPLICATIONS

To tell the difference between a subsample and a replication, remember that an experimental unit is defined as the smallest unit to which a treatment



can be applied at random (meaning that each unit is chosen independently of any other unit).

It's important to not confuse subsamples with replications. A replicate is the experimental unit to which a treatment is applied.

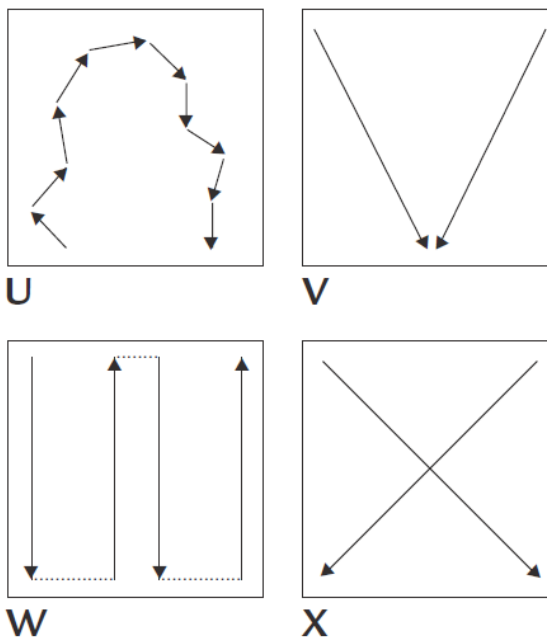
Assume you have 16 pots of greenhouse-grown corn to use in testing three insecticides and an untreated control. Let's assume you choose the four pots on the nearest table and sprayed them with an insecticide. They would become one experimental unit, not four replications of a treatment. To be replications, the pots needed to be chosen at random from among the 16. Thus, there are four possible sampling units in this experiment. If the weight of the plant was the desired measurement, up to four subsamples could be taken for each experimental unit.

## RANDOM SAMPLING

Most agricultural research involves taking samples of representative units from a population and conducting a statistical analysis to make inferences about the larger population from which the samples were taken. In order to make reliable inferences, the samples must be representative of the entire population. This is typically accomplished using **random sampling** where every member of the population has an equal chance of being included in the sample.

There are standard patterns that can be used for random sampling in experimental plots or fields.

Using the patterns will help keep the samples random and avoid bias as you measure treatment effects. Common sampling patterns include use of U, V, W or X shapes (see diagram). To be sure that your sample is representative, the path should cover the entire population where you are sampling.



Examples of pathways for random sampling.

Source: University of California Research and Demonstration manual

Avoid sampling in one area or along the plot edge as it can produce misleading results. Control plots should be sampled in the same way as treated plots.

To avoid introducing new variables due to the sampling procedure, ensure that one person takes all the samples from block (RCB and split-plots) rather than multiple people sampling the same block. If sampling must occur over multiple days, be certain to sample by blocks.

## METHODS FOR FUNGICIDE, BACTERICIDE, AND NEMATODE FIELD TRIALS

There is not a single tool or method for evaluating trials when working with plant diseases as it is dependent on the crop and the pathogen of interest. When conducting research involving plant diseases, it pays to invest time to do background research to see how data was collected from other experiments involved in the same crop or pathogen. A few examples of how data might be collected is included here. The University of Wyoming has extension specialists and researchers that may also be able to



provide guidelines. When rating diseases, video and digital cameras can be used to record differences between treatments. The use of computers to conduct image analyses is becoming more frequent.

### FOLIAR PATHOGENS

Evaluations of **foliar pathogens** often involves the use of a scoring guide. For example, rust diseases can be rated based on incidence in the field as well as severity on the leaf. International leaf scoring scales for leaf disease severity is available for several crops, an example can be seen at right, see Additional Resources, see page 60.

### SOIL-BORNE PATHOGENS

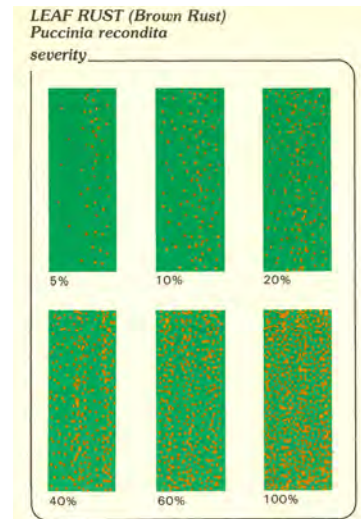
Evaluation of **soil-borne pathogens** presents special challenges including developing methods of determining pathogen populations prior to treatment applications as well as post-treatment. Assessments typically involve digging up a specified number of plants with an emphasis on leaving the root ball intact. Disease incidence (number of plants infected) and severity can then be assessed after washing roots. Severity is reported through a root rating scale, many of which are determined by the researcher. Root rating scales typically range from 1 (no disease) to 9 (severely diseased), depending on the pathogen, for example see the Fusarium Root Rot Rating Scale right.

### NEMATODE TRIALS

*Note: Category 901B (Ag Insect Control) is required if you are conducting demonstration and research using nematicides, not Category 901C (Ag Plant Disease Control), as many nematicides are also labeled for insect control.*

**Nematode** populations fluctuate throughout the year so, in general, sampling is recommended prior to establishing the field trial, midpoint through the trial and a final sampling at termination of the trial (just prior to or shortly after harvest). Nematode distribution in the field is always patchy

due to cropping history, weed hosts, chemical use and environmental factors, so sampling is typically done using a systematic zigzag pattern. Avoid sampling under excessively dry, wet or frozen soil conditions. Composite soil samples should be



Leaf Rust Scoring Guide  
Source: CIMMYT

Fusarium Root Rot Rating Scale	
Score	Phenotypic description
1	No visible disease symptoms.
2	Approximately 5% of the hypocotyls and root tissues covered with lesions.
3	Light discoloration either without necrotic lesions or with approximately 10% of the hypocotyls and root tissues covered with lesions.
4	Approximately 17.5% of the hypocotyls and root tissues covered with lesions.
5	Approximately 25% of the hypocotyls and root tissues covered with lesions but tissues remain firm with deterioration of the root system and heavy discoloration symptoms may be evident.
6	Approximately 37.5% of the hypocotyls and root tissues with lesions.
7	Approximately 50% of the hypocotyls and root tissues covered with lesions combined with considerable softening, rotting, and reduction of the root system.
8	Approximately 62.5% of the hypocotyls and root tissues covered with lesions.
9	Approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system.

Example of root rot scale.

Source: <http://www.scielo.br/pdf/tpp/v37n6/a03v37n6.pdf>

taken within the plot, from around the plant root (rhizosphere) at a depth representative of the root zone of the crop.

## METHODS FOR INSECTICIDE FIELD TRIALS

The method used to sample for insects will depend on the target insect, the stage of crop growth and the amount of labor available. While there are numerous methods used, the protocol you choose may be derived from materials you uncovered during your preliminary background research. More information on collecting insects can be found in the Additional Resources, see page 60. A few techniques that may result in collecting quantifiable numbers of insects (versus absence or presence) are described here:

### Sweep nets

Used for sweeping vegetation. Allows for quantification if the area swept per sweep and the number of sweeps per sample is constant in each plot. Samples can be dumped into a plastic bag or container and refrigerated or frozen to count later.

### Beat sheet or tray

Foliage is struck aggressively with a stick so insects are jarred loose and fall onto the sheet or tray.



Suction sampler  
Image: T. Walker

### Suction sampling or aspirator

These devices are useful to collect very small insects (such as mites). An aspirator is a small device made up of a collecting vial sealed

with a stopper and two tubes. Insects are sucked through a collection tube as the operator gently sucks through the suction tube. A suction sampler can be used to quantify insects if collections are timed as the area which is suctioned is constant between plots.

### Physical collections

This method relies on physically collecting plant samples (tillers, leaves, etc.) and visual counts to determine the number of insects on the plants.

### Pitfall traps

These traps are used to collect ground dwelling insects and arthropods. It uses a container holding preservatives which is placed into a hole in the ground and the insects fall in. These collections are not quantifiable but may provide information on other insects in the plots.

### Berlese funnels

A Berlese funnel uses a heat source such as a light bulb which is placed over a sample placed on wire mesh. As the sample warms and dries, the insects migrate down where they fall into a collection container. This method is based on specific avoidance behavior triggered by heat or dryness so it can be used to extract insects that are mobile and do not desiccate easily.



Berlese funnels  
Image: D. Cockrell

## METHODS FOR HERBICIDE FIELD TRIALS

Trials involving herbicides used to be very basic, primarily involving efficacy studies. However, weed science studies have become more complex,

ranging from investigating basic processes of living organisms down to what is happening at a molecular level. Some current field research includes looking at crop-weed interactions, measuring herbicide volatility, herbicide degradation or fate and weed communities. It is difficult to characterize all the different methods used to study control of weeds using herbicides.

However, *Research Methods in Weed Science*, see Additional Resources, page 60, is a consolidated reference, authored by experts in various fields of weed science, which presents updated protocols for designing and conducting weed science experiments as well as analyzing research data. Uncertainties and differing opinions abound regarding various approaches to the planning and implementation of weed science experiments. Having one reference containing recommended protocols for different experiments is invaluable to students and young weed scientists.

## STATISTICAL ANALYSIS

To make inferences about the entire population based on the data gathered in the experiment, you must determine if observed differences are truly different or if they are a result of random variation. This is where statistics come into play.

After collecting data from a properly designed experiment, you will usually need to analyze the data with appropriate statistical calculations. Statistical analysis methods are selected based on the experiment's objectives and design. The type of statistical analysis used to analyze single-factor experiments would differ from the analysis used for factorial experiments. Proper statistical analysis can be done if your experiment was designed according to the principles outlined in this publication; proper analysis can be complicated greatly if these principles were not followed.

It is probably best to have help in making statistical calculations. Professional statisticians and other scientists may be willing to help you with the statistics if you involve them early in the process (well before you lay out plots). They can also check your proposed design for flaws and omissions. If you want to do the work yourself, some simple statistics can be calculated by hand but most people will make the calculations with the help of computer software. Specialized statistical software is available, but most spreadsheet software can calculate simple statistics. Although a full review of statistical methods is outside the scope of this manual, many texts are available to help you with the statistical analysis see Additional Resources, page 60.

## RECORDKEEPING

In Wyoming, records of RUP or experimental pesticide applications made for research or demonstration purposes must be maintained for 2 years. If the research or demonstration was conducted under contract with a manufacturer, there may be additional recordkeeping requirements. The records serve as a permanent record of the pesticides applied, application dates, types of equipment used, weather conditions and the location of each pesticide application based on the plot map.

The information necessary to meet the recordkeeping requirement was covered in Chapter 2, Laws and Regulations, page 9. In addition to the required records, consider keeping the following information which may help explain research results:

- Crop and variety planted.
- Cropping history including planting date and stages of development, especially when sampled.

- Pesticide(s) used including brand name, percent active ingredient, formulation, manufacturer and purchase date.
- Adjuvants and carrier (water or fertilizer) used.
- Water quality and soil characteristics.
- Equipment used including nozzle size, pressure, speed.
- Dates of equipment calibration and results.
- Weather conditions including wind direction and speed, temperatures during application, and relative humidity.
- Cost of applications. (This is what growers always want to know.)
- Notes on application, including any problems with equipment or changes in plot plan.

## WATER QUALITY

Before the research or demonstration trial begins, consider obtaining an analysis of the water that will be used in the spray tank. Water quality parameters such as pH and dissolved minerals, especially calcium and magnesium, can interact with the active or additive ingredients of the pesticide product and may adversely affect the application.

## TRAINING RECORDS

You may also want to keep records on how others assisting with the research were trained and protected:

- posting requirements and how met,
- handler training records, and
- fieldworker training records.

## CHAPTER 7 ADDITIONAL RESOURCES

Cereal Rusts, <https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/cereal-rusts/>

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## CHAPTER 8: EQUIPMENT AND CALCULATIONS

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Describe how errors in calibration calculations involved in small-plot research are magnified compared to large field-scale operations.
- B. Describe how to measure liquid and dry materials used in research plots.
- C. Describe non-powered or hand-powered and powered equipment used for demonstration and research experiments.
- D. Explain the importance of determining actual speed and how to calculate speed.
- E. Explain how to select nozzle type and size based on flow rate or droplet size.
- F. Explain how to check nozzles for uniform output.
- G. Considerations for calibration of equipment used for small plots.
- H. Describe how to calibrate a backpack sprayer.
- I. Describe how to calibrate a boom sprayer using the nozzle method and ounce method.
- J. Describe how to calibrate granular equipment.

### INTRODUCTION

Accurate calibration and precise measuring and mixing of pesticides is critical when conducting research experiments and demonstrations. The chances of misapplying the correct rate of the pesticide are greatly increased when making applications to small areas. For example, adding two extra ounces to a 100 gallon tank for a general field application may not necessarily be significant. However, if you add an extra two ounces to two quarts of water for a small plot treatment, your results will be highly inaccurate.

### MEASURING PESTICIDES

Many pesticides used today are applied in smaller quantities such as grams or ounces per acre compared to former application rates which were frequently in pounds, pints or quarts per acre. Precise measurement of pesticides is necessary to prevent over- or under-applying the desired rates. Small plot demonstration and research experiments require that you use techniques that allow you to accurately measure small amounts of materials.

Some important things to remember about measuring pesticides:

- A liquid ounce is a *volume* measurement, while a dry ounce is a measure of *weight*. Before you measure, make certain you know whether the measuring device is intended for fluid or dry ounces.
- Purchase measuring containers with graduations that are easy to read. Avoid containers that include both metric and English units as it can be confusing.

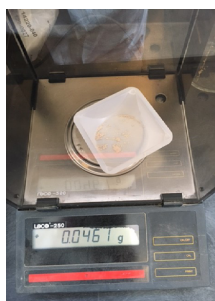


- Replace measuring containers when numbers and markings are difficult to read. Avoid writing on or adding marks to the container.

**Remember: Rough estimates or ‘rounding off’ is not acceptable when measuring any pesticide for research or demonstration plots!**

## DRY MEASUREMENTS

Dry materials should be measured on properly calibrated scales that can measure in milligrams, grams, or ounces. Although many dry pesticide materials come with measuring tubes, the margin of error can range from  $\pm 5\%$  to as high as  $\pm 25\%$  so they are not accurate enough for research purposes.



Use a scale to accurately weigh dry materials.  
Photo: C. Arrieta

## LIQUID MEASUREMENTS

Pouring the right amount of liquid into a measuring device is pretty straightforward until you realize that not all measuring devices are accurate. It's easy to check the accuracy of any liquid measuring device using a graduated cylinder as long as you remember the following equivalent:

$$\begin{aligned} 8 \text{ fluid ounces} &= 1 \text{ cup} \\ &= 236.5882 \text{ milliliters} \end{aligned}$$

So measure 237 milliliters using the graduated cylinder and pour into your measuring device. If the water level is at the 8 ounce mark, you know your container is accurate! If it is above or below that mark, find a new measuring device!

Measuring small amounts of liquid pesticides can be challenging. In order to be accurate, measure small amounts of liquid pesticides using disposable syringes, pipettes or graduated cylinders. NEVER use your mouth to pipette materials as you could draw the pesticide into your mouth or inhale potentially harmful fumes. Rather, use a suction bulb or a pro pipette to measure materials from the container.



Use a safety pipette to accurately measure small quantities of liquid pesticides.  
Photo: C. Arrieta

When adding liquid materials to the carrier (water, fertilizer, etc.), remember to include the pesticide as part of the total volume for the spray tank. For example, if you are mixing up 1 gallon (128 ounces) of total spray material and must add 2 ounces of pesticides, then you would only use 126 ounces of water.

$$\begin{aligned} 2 \text{ ounces pesticide} + 126 \text{ ounces water} &= \\ \text{total volume of 128 ounces} & \\ &= 1 \text{ gallon} \end{aligned}$$

More information on measuring pesticides can be found in Additional Resources, page 78.

## SELECTING APPLICATION EQUIPMENT

Although there may be more complex application devices used in research, most of the equipment used for non-experimental applications can also be used for research and demonstration purposes. This section describes some of the common types of equipment used.

## NON-POWERED OR HAND-OPERATED EQUIPMENT

Several types of non-powered or hand-operated equipment are often used in research and demonstration equipment. These low-pressure sprayers do not usually have agitators so they must be manually agitated occasionally if using formulations that require agitation including wettable powders, emulsifiable concentrates or flowables. When purchasing these types of sprayers, pay close attention to the outlet to the spray wand as you may have to mix more spray solution than required to keep the boom fully charged. Some types of equipment used for research experiments are described below.

### COMPRESSED AIR SPRAYERS

These sprayers typically hold diluted spray solutions in a 1- to 3-gallon tank that has an air pump in the top and a wand with a nozzle for directing the spray. Their best use is for spot treatment of small areas. The tank has to be pumped up frequently to maintain pressure and the tank must be shaken to agitate the chemical. Most have adjustable nozzles to control the spray pattern and droplet size.



Hand-pump sprayer  
Photo: krolya25, shutterstock

### HAND-OPERATED BACKPACK SPRAYERS

Hand-operated backpack sprayers have a hydraulic pump that is operated by pumping a hand lever in an up and down motion. They typically hold 3 to 5 gallons of spray mixture, delivering 0.1 to 2 gallons per minute (gpm) with pressures as high as 100 pounds per square inch (psi). The sprayer can be equipped with a single nozzle on a wand or a small boom. As these sprayers require hand-pumping, applicators sometimes find it challenging to maintain a consistent pressure.

## POWERED EQUIPMENT

Powered application equipment is more commonly used for both small plot research and demonstration experiments as it allows for applications to plots ranging from small to very large in size. There are a variety of configurations that can be used (spray booms, spray guns, etc.) but they often require more maintenance to keep it operating properly.

### HAND-HELD SPRAYER GUNS

Hand-held spray guns typically operate off a truck- or trailer-mounted pump and spray tank. They are sometimes used to apply insecticides and fungicides to trees, vines and shrubs as they apply a high-pressure stream that can penetrate dense foliage and reach the upper levels of trees. Battery-powered hand-gun sprayers are sometimes used in greenhouse applications. The sprayer usually has a handle, valve and nozzle (or small boom with multiple nozzles). Developing a consistent walking speed, arm motion, and uniform spray pattern are the keys to successful application with a hand-gun sprayer.

### POWERED BACKPACK SPRAYERS

Older versions of powered backpack sprayers consisted of a backpack sprayer powered by a small gasoline engine that drove a pump to spray



pesticides through a handgun or small boom. Newer, light-weight versions are powered by a rechargeable battery.

Powered backpack sprayers typically hold 3 to 6 gallons of spray solution and operate at psi ranges of 35 to 50, depending on the model. These sprayers usually come with a cone nozzle but are easily adaptable to spray booms. When completely full, they can weigh as much as 65 lbs so applicators must be fit enough to carry them. Look for backpack sprayers that have a design where the tank outlet and pump can deliver most of the spray solution to avoid having to mix more solution than required.

### CARBON DIOXIDE (CO<sub>2</sub>) SPRAYERS

Carbon dioxide (CO<sub>2</sub>) are commonly used by researchers applying pesticides over small plots. The typical sprayer setup includes a boom with a hand valve, pressure gauge, and CO<sub>2</sub> cylinder(s). Researchers use 2-liter plastic bottles or 3-gallon cylinders to hold the spray solution. CO<sub>2</sub> sprayers can be carried on an aluminum backpack frame or housed on a pull-type sprayer, called a rickshaw.



Rickshaw sprayer  
Photo: D. Cockrell

### ATV OR GATOR-MOUNTED SPRAYERS

Sprayers can be attached to either ATVs or Gators to spray larger research or demonstration plots. The travel speed of the ATV or Gator should be determined, or if it has a speedometer, verified under field conditions. A pressure gauge should also be visible to the operator.

The sprayer is usually equipped with a 15 to 30 gallon polyethylene tank with or without agitators, spray boom and either CO<sub>2</sub> tanks for pressure or an electrical pump which connects to the ATV or Gator power source.



ATV sprayer with boom  
Photo: T. Walker

### Injection Equipment

Fertilizer injectors are devices used to apply various materials like water-soluble fertilizers, plant growth regulators, and pesticides. They can be used to conduct research or demonstration experiments applying both starter fertilizers with fungicides or insecticides at planting or applied later in the crop cycle.



Fertilizer injector  
Photo: Centennial Ag Supply, Inc.

If the equipment will be used frequently for injecting pesticides, the unit should contain no plastic parts, because wettable powders and emulsion formulations are harmful to PVC plastics. Just like other mechanical devices, proper and frequent maintenance and calibration are crucial steps to ensure optimal injector performance.

## MISCELLANEOUS SPRAY EQUIPMENT

### SPRAY BOOTH

Spray booths or chambers offer a very controlled environment in which to conduct pesticide research experiments. The booth often allows the applicator to control such variables as pressure and speed as well as the ability to flush nozzles and change spray bottles easily. In general, the booths use CO<sub>2</sub> or compressed air to pressurize the nozzle boom.



Spray chamber  
Photo: P. Westra

### AERIAL EXPERIMENTS

Anyone considering conducting research using aerial application equipment should participate in an Operation S.A.F.E. Program to have a spray pattern and droplet size analysis. More information is available in Additional Resources, page 60.

### Spray pattern testing

Special flight line equipment is used to collect a sample of the spray pattern. The spray tank of the aircraft (airplane or helicopter) is filled with dye and the pilot flies over a collection string while spraying at desired altitude and speed. The dye makes the application pattern visible to a device called a **fluorometer**, which measures the amount of dye on the string. More recently, specialized **spectrometers** have been designed to analyze the amount of dye on the string. Special computer software uses the data from the fluorometer to determine the spray pattern characteristics. Results include a diagram of the spray pattern uniformity, the optimal effective swath width for both race track and back and forth flight paths, and a numerical calculation of pattern uniformity.

### Droplet size testing

Water sensitive or Kromecote™ cards placed in the plant canopy are used to sample the spray droplets produced by the spray pattern. Kromecote™



Kromecote card in canopy  
Photo: WRK of Arkansas

cards are preferred if the atmospheric relative humidity or humidity in the canopy is high. Kromecote™ cards can be purchased from any print shop that produces glossy business cards and can be pre-printed with information that should be recorded (crop, location in canopy, date, etc.) about the research project. Software, see Additional Resources, page 60, is used to analyze these cards, and calculates several valuable statistics used to describe the droplet spectrum produced by the aircraft. These statistics include volume median diameter (VMD), percentage of spray volume contained in droplets smaller than 100 and 200

microns, relative span, estimated gallons per acre (GPA), and percent coverage.

### Drift towers

Drift towers can be placed downwind from pesticide applications to determine drift by placing droplet testing cards on the tower. The drift distance, the distance the collected drops had to travel to reach the tower collectors, is one of the most important items and varies with wind direction. Both horizontal and vertical drift can be measured using drift towers.

### UNMANNED AIRCRAFT SYSTEMS (UAS) FOR RESEARCH AND DEMONSTRATION

An unmanned aircraft system (UAS), sometimes referred to as a drone, is an aircraft without a human pilot on board which is controlled from an operator on the ground. Their use has increased dramatically. They can be used in agriculture to make applications of fertilizers or pesticides to sloping terrain or inaccessible sites where it is not practical to use conventional equipment or send persons out with backpack equipment. In April 2016, the FAA gave clearance for use of Yamaha's RMAX drone which can be used to apply both fertilizers and pesticides. Since that time, other models of drones capable of performing spray applications have been developed and given FFA clearance as well.

All drones must be registered with the Federal Aviation Administration (FAA). 14 CFR Part 107 governs all rules and regulations associated with drones weighing between 0.55 lbs to 55 lbs. A link to the rules as well as more information on UAVs at FAA can be found in the Additional Resources.



Assorted spray nozzles

## CHOOSING NOZZLES

Nozzles come in a variety of types and sizes and are produced by several manufacturers. The best nozzle for your application will maximize efficacy, minimize spray drift and produce the desired application rate (GPA) and spray droplet size.

To select the best nozzle for the application, consider the following:

- sprayer operation (application rate, pressure, travel speed),
- type of chemical (herbicide, insecticide, fungicide, etc.),
- timing of application (pre- or post-emergence or soil incorporated),
- mode of action for chemical (systemic or contact),
- application type (broadcast, banded, directed, air-assisted),
- target crop and canopy, and
- spray drift risk.

Contact manufacturers to find nozzles that are designed for the target and application. Remember, you need to include the type and size of nozzles used in addition to application rate, spray pressure and travel speed when reporting your experimental results.

Selecting a nozzle requires choosing the appropriate type and then determining the size of the nozzle needed. Many nozzle manufacturers

have smartphone apps or nozzle selection guides or calculators available on their websites to help you choose. Some nozzle manufacturers include:

- CP Products - (both aerial and ground nozzles—<http://www.cpproductsinc.com/site/>)
- Greenleaf Technologies—<http://www.greenleaftech.com/>
- Hypro Shurflo—<http://hypro.pentair.com/en>
- Teejet Technologies—[http://www.teejet.com/spray\\_application](http://www.teejet.com/spray_application)

### CHOOSING NOZZLE TYPE

Choosing a nozzle type should be based on the type of application equipment used, chemical mode of action, target crop or pest and the risk of spray drift.

### SELECTING NOZZLE SIZE

Steps to select the proper nozzle size:

The following steps must be taken to determine the nozzle flow rate (gallons per minute—gpm):

- Step 1. Select the application rate in gallons per acre (GPA). This is a management decision you will have to make based on pesticide label recommendations, field conditions and water supply.
- Step 2. Select a practical and safe ground speed in miles per hour (mph).
- Step 3. Determine the spray width per nozzle (W). For broadcast applications, W = nozzle spacing (distance between two nozzles on the boom) in inches. For band spraying, W = band width in inches. For directed spraying, W = row spacing in inches (or band width) divided by the number of nozzles per row (or band).

- Step 4. Determine the flow rate (gpm) required from each nozzle by using the following equation:

$$\text{gpm} = (\text{GPA} \times \text{mph} \times \text{W}) \div 5,940$$

*(5,940 is a constant to convert GPA, mph, and inches to gpm)*

- Step 5. Select a nozzle size from the manufacturer's catalog that will give the flow rate (gpm) determined in Step 4 when the nozzle is operated within the recommended pressure range. If a nozzle of this size is not available, change the travel speed in the equation above and determine the new flow rate required.

### Example

You want to spray a pre-emergence herbicide at 15 GPA, using a speed of 8 mph. The distance between the nozzles on the boom is 20 inches. The herbicide label requires a spray quality of "Medium." What should be the flow rate of the nozzle you will choose?

Since this is a broadcast application (pre-emergence), W is the distance between nozzles (W = 20 inches). Filling in the variables yields the following calculation:

$$\text{gpm} = (15 \text{ GPA} \times 8 \text{ mph} \times 20 \text{ in}) \div 5,940$$

$$= 0.4 \text{ gpm}$$

This means, to apply 15 GPA at a speed of 8 mph with this sprayer setup, we need to select a nozzle with a flow rate of 0.4 gpm.

Now go to the nozzle catalogue, and find a nozzle that provides a flow rate of 0.4 gpm, while operating the sprayer at an applicable pressure and traveling at 8 mph.



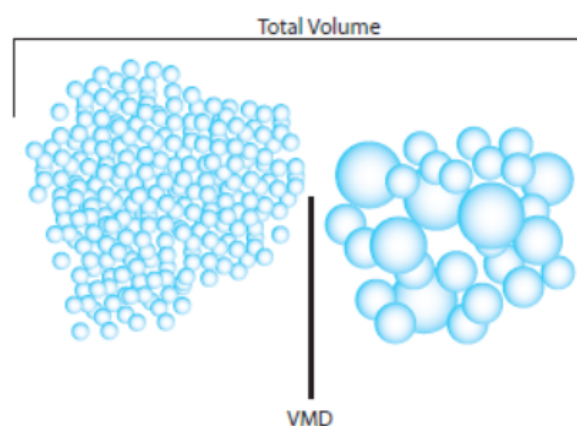
## CHOOSING A NOZZLE BASED ON DROPLET SIZE

It's critical to understand the principles of droplet size to select nozzles that provide adequate coverage yet have adequate drift reduction properties. Spray droplets are measured in microns. Research shows that droplets smaller than 150–200 microns are more likely to move off-target and should be avoided for most applications. Because of their light weight for the droplet surface area, these droplets take much longer to fall and can move greater distances. Particles less than 50 microns in diameter can remain suspended in air for long periods until they evaporate.

All nozzles produce a range of droplet sizes. To measure the range of droplets produced by a nozzle, the term **volume median diameter**, or VMD, is used. The VMD represents the droplet size where half of the spray volume is contained in droplets larger than the VMD, and half of the volume is in droplets smaller than the VMD. (See illustration below.)

The American Society of Agricultural and Biological Engineers (ASABE) developed a standard to measure and interpret spray quality from tips which uses six droplet size classifications for agriculture and horticulture.

Product labels sometimes recommend or require using a nozzle which produces a specific droplet size. The registrant should recommend the desired droplet size when working with unregistered products. The nozzle manufacturer's chart can be used to select a nozzle based on flow rate or droplet size. Remember that changes in spray pressure may result in a shift of the droplet size category. ASABE S572.1 provides a general description for using various droplet sizes, see table below.



VMD is the droplet size at which 50% of the spray volume is in droplets larger than the VMD and 50% of the volume is in droplets smaller than the VMD

Image: Adapted from Matthews, 1992

### American Society of Agricultural and Biological Engineers droplet size classifications

Very Fine	Fine	Medium	Coarse	Very Coarse	Extremely Coarse
<b>Fine</b> Sprays provide enhanced retention for directed spraying on the target including: <ul style="list-style-type: none"> <li>Foliar-acting weed control</li> <li>Contact-acting fungicides and insecticides</li> </ul>		<b>Medium</b> Sprays are the most widely used spray type. <ul style="list-style-type: none"> <li>Used by default by most applicators when spray quality is not defined by the label</li> <li>Systemic-acting fungicides, insecticides and herbicides</li> </ul>		<b>Coarse</b> Sprays are used with systemic, residual, and soil-applied herbicides.	

Most agrochemical applications recommend a fine, medium, or coarse spray.

Source: ASABE S572.1 Droplet Size Classification

## CALIBRATION

The correct **calibration** of equipment and the accurate measuring and mixing of pesticides are extremely important in demonstration and research pest control work. In small plots, the hazards of application may be reduced and the chances of non-target pollution minimized, but the probability of applying a pesticide at the wrong rate is generally greater than in large areas.

Because demonstration and research plots are often relatively small, hand-held equipment is usually used for pesticide application. Both hand-operated and powered equipment must be calibrated to apply pesticides precisely so that research results will be accurate. The use of a **metronome** is recommended to maintain a constant walking speed when applying pesticides by hand.

Before calibration, conduct a visual check of the spray pattern on the ground to determine whether the nozzles have uniform output. An inexpensive flowmeter can be used to determine whether the nozzle output is within the manufacturers rated flow rate (from their nozzle chart). One example of a flow meter is the SpotOn Sprayer Calibrator by

Innoquest, Inc (more information in the Additional Resources). Replace a nozzle if the amount it delivers varies more than 5 percent from the average output of all the nozzles on the boom.

## CHECK NOZZLE DISCHARGE AND UNIFORMITY

It's important to check the condition of the nozzles prior to calibrating. While stationary, start the sprayer using the pressure that will be used during the application and observe the spray pattern from each nozzle. Large variations in spray pattern can be observed visually but for more accurate pattern check, use a digital flow meter to determine flow rate (gpm) from each nozzle and a patternator (see photos below). A patternator is a device used to visualize the nozzle pattern. Poor spray patterns are often due to worn nozzles, or clogged nozzles and strainers. Use a soft brush to clean nozzles and strainers and recheck the pattern.

**Note: Any calibration should be done using water only. As sprayer equipment may have residues that remain, even after cleaning, personal protective equipment should be worn (minimum of long sleeve shirt, long pants,**



Patternator showing good nozzle pattern.  
Photo: Innoquestinc.com



Patternator showing defective nozzle pattern.  
Photo: Innoquestinc.com

shoes, socks and perhaps goggles or safety glasses).

## CALIBRATING BACKPACK SPRAYERS FOR SMALL PLOT WORK

Calibrating backpack sprayers generally requires three pieces of information:

- the walking speed of the operator,
- the width of the swath, and
- the collective nozzle output per minute.

There are numerous resources on how to calibrate a backpack sprayer or handgun. Even if you have a boom attached to the backpack sprayer, you can use the same method to calibrate.

But a calibrated backpack sprayer doesn't help someone design their small plot trials. Here are the important questions to answer:

- How long do I spray before I have sprayed my target volume?
- If I walk at speed X, and I want to spray volume Y, which nozzles should I use?
- The pesticide I'm testing has a rate based on an acre or hectare. How do I know how much I need on my plot?
- I'm spraying trees, bushes, or vines. Do I use a dose based on the planted area, or based on the size of the canopy?

The website, Sprayers 101, <https://sprayers101.com/>, has developed a small plot calculator in Excel that can help answer these questions. You can download it from the site listed in Additional Resources. There is also a resource on how to calibrate motorized backpack sprayers (Jensen, et al., Additional Resources, page 78.)

## CALIBRATION OF LARGE HYDRAULIC BOOM SPRAYERS

There are many different methods to calibrate a boom sprayer but do not use shortcuts when calibrating sprayers used for research and demonstrations. Regardless of the method used, sprayer calibration requires that the following information is available:

- traveling speed of the sprayer,
- pressure settings,
- nozzle flow rate, and
- nozzle spacing or spray width.

Should any of the above factors change, the sprayer must be recalibrated.

## DETERMINING GROUND SPEED

Accurate calibration requires that the actual speed of the sprayer must be determined under field conditions. Because of wheel slippage and rough surface conditions, the actual speed is often lower than the tachometer or speedometer readings. To accurately determine travel speed, mark off 220 feet. Drive the distance in the field at the throttle setting, pressure setting, using a sprayer loaded at least 1/2 to 2/3 full. Also engage any incorporation equipment (disks, planter, etc) that will be used during spraying. Record the time. If the surface and soil conditions of the field are variable, repeat the measurement in several areas.

Use the following formula for calculating speed:

$$\text{MPH} = \frac{150}{\text{time (seconds) to travel 220 feet}}$$

## NOZZLE METHOD

The nozzle method of calibration is a quick and accurate way to calibrate any sprayer as long as the ground speed is known and can be accurately controlled. It can be used to calibrate in the shop



or in the farmyard and is valuable as a quick check for nozzle wear. By using this method it is possible to accurately predict the spray rate at any controlled speed.

The nozzle method requires checking only one nozzle on the sprayer, but assumes all nozzles are delivering the same amount. Be sure to check that all nozzles are delivering at nearly the same rate when using this method of calibration (nozzle flow rate measurement devices are available for purchase, which greatly helps with quickly checking each nozzle). Nozzles must be replaced when their flow rate is greater than or equal to 10% above or below the manufacturer's specifications.

The nozzle method using a constant is based on the formula:

$$\text{Spray rate (GPA)} = \frac{\text{One nozzle output (in oz per min)} \times 46.4^*}{\text{One nozzle coverage (in)} \times \text{Speed (mph)}}$$

*\*Note: This constant applies when delivery is measured in ounces.*

$$\text{Constant} = \frac{43,560 \text{ sq ft/acre} \times 12 \text{ in/ft}}{88 \text{ ft/min}^{**} \times 128 \text{ oz/gal}} = 46.4$$

$$^{**}88 \text{ ft/min} = 1 \text{ mph}$$

*Constants to use when the delivery is measured in units of volume other than ounces:*

- Pints: 742.6
- Quarts: 1,484.8
- Gallons: 5,940

**Steps:**

1. Set the pressure the same as what will be used in the field and collect the water from 1 nozzle for exactly 60 seconds. Measure water carefully.
2. Measure coverage of a nozzle in inches. On a boom sprayer, the coverage is the same as the nozzle spacing on the boom.
3. Multiply the amount (ounces of water) collected in 1 min from Step 1 by 46.4 (the constant).
4. Multiply the forward speed that the sprayer will use (mph) by the nozzle spacing (inches).
5. Divide the answer obtained in Step 3 by the answer in Step 4. This is the gallons of water the sprayer is delivering per acre.

**EXAMPLE:** A sprayer has 16 nozzles spaced 18 inches apart and the boom covers a 24-foot swath. When operated at 40 psi, 1 nozzle delivers 40 oz. of water in 1 minute. The sprayer is to be operated at 4 mph. What is the application rate?

**Steps:**

1. 40 oz. per min (measured)
2. Nozzle spacing = 18 inches
3.  $40 \text{ oz} \times 46.4 = 1,856$
4.  $4 \text{ mph} \times 18" = 72$
5.  $1,856 \text{ divided by } 72 = 25.8 \text{ GPA applied}$

**Caution:** Be sure all nozzles are delivering at nearly the same rate ( $\pm 5\%$  of the average) when using this method of calibration.

### OUNCE CALIBRATION METHOD

Nozzle manufacturer manuals include tables to show spray volumes (GPA) for various nozzles operating at specific pressures and consistent ground speeds. Use this information to initially set up the sprayer and then use the Ounce Calibration Method to evaluate and fine-tune the sprayer for accurate application.

The following method to calibrate a boom sprayer is referred to as the ounce method, or 1/128th acre method because it is based on the fact that there are 128 ounces of liquid in 1 gallon and you will determine how long it takes to spray 1/128 of an acre per nozzle, then collect the liquid from a nozzle for that period of time. This method is quick, easy, and does not require a lot of calculations.

**Materials required:**

- stopwatch,
- containers to collect nozzle discharge,
- container that measures ounces,
- tape measure, and
- marking flag

The ounces of liquid caught from one nozzle **EQUALS** the application rate in gallons per acre (GPA).

### Procedure

1. Fill the sprayer at least  $\frac{1}{2}$  to  $\frac{2}{3}$  full with clean water. Measure the distance between the nozzles and determine the distance to drive using the table below.

Nozzle Spacing (in.)	Travel Distance (ft.)
18	227
20	204
22	185
24	170
26	157
28	146
30	136
32	127
34	120
36	113
38	107
40	102

2. Measure out the distance in the field. Using your normal spray speed and pressure, determine the time it takes to travel the distance in seconds. Repeat at least three times and average the results.
3. While the sprayer is stationary, run the sprayer at the same throttle setting and sprayer pressure, catching the output from each nozzle for the time determined in Step 2. Find the average output, in ounces, by adding individual nozzle outputs and dividing by number of nozzles. If any individual output is 10 percent higher or lower than the average output for all nozzles, clean the nozzle or replace, and repeat this step.

The final **average output in ounces** = **application rate in gallons per acre (GPA)**.

### EXAMPLE

1. The sprayer is set up with 20-inch nozzle spacing, so the chart indicates that a distance of 204 feet must be marked off.
2. The course was driven three times, recording the throttle and pressure settings. The average time was 31 seconds and pressure was 40 psi.
3. In a stationary position, the sprayer was brought to 40 psi and nozzle outputs collected for 31 seconds. The average nozzle output was 15 ounces. (remember that any nozzle that is  $\pm 10\%$  of the average should be replaced and the output remeasured to determine the average. In this example, any nozzle that measure 13.5 ounces or 16.5 ounces, should be replaced).
4. The final average output, 15 ounces = 15 gallons per acre (GPA).

### USING OUNCE CALIBRATION METHOD FOR BAND SPRAYING

While a broadcast application covers the entire acreage sprayed, a band application sprays strips or only a portion of the field. However, the same method can be used to calibrate band sprayers. A band application only covers a portion of the field so the amount of area that is treated is reduced but the total acres sprayed with the same volume as a broadcast application will be greater.

After performing an ounce calibration on the sprayer, multiply the answer (broadcast spray volume or GPA) by the appropriate conversion factor in the table that follows to determine the band rate.

<b>Conversion Factor</b> <b>to convert broadcast gallons per acre to</b> <b>band (gallons per treated area)</b>				
<b>Band width</b> <b>(in.)</b>	<b>Row spacing (inches)</b>			
	20	30	36	40
8	2.5	3.8	4.5	5.0
10	2.0	3.0	3.6	4.0
11	1.8	2.7	3.3	3.6
12	1.6	2.5	3.0	3.3
13	1.5	2.3	2.8	3.1
14	1.4	2.1	2.6	2.9
15	1.3	2.0	2.4	2.7
16	1.2	1.9	2.3	2.5

## CALIBRATION OF GRANULAR APPLICATORS

Granular applicators need to be calibrated only to adjust the rate of flow or delivery. To keep the amount of pesticide released uniform, the travel rate is kept at an even pace. Whether hand-carried or vehicle-mounted, the speed of the equipment determines the amount of pesticide applied per unit area. The speed used during calibration should be the same as the operational speed, and for greatest accuracy the calibration should be conducted at a site similar to the operational target site. Companies manufacturing granular applicators include rate-guide charts in the operator manuals. These charts show the proper settings for a desired application rate. These settings are usually reliable, but make a field check to insure accuracy.

### FIELD CALIBRATION FOR AGRICULTURE

1. Measure and mark 300 feet in the field.
2. Fill one hopper with granules.
3. Disconnect the delivery tubes from the applicator.
4. Catch the material from the applicator in a suitable container (bucket, plastic bag, plastic sack, etc.) while driving the tractor over the 300 foot measured course at the speed that will be used in the field.
5. Measure the weight of the granules discharged by the applicator in pounds.
6. Calculate the square feet of the test area. For insecticide and herbicide applications, multiply the row width in feet times the distance covered (300 ft).
7. Calculate the rate per acre. Multiply the pounds collected (Step 4) by 43,560 and divide the answer by the square feet of the area (Step 6).

$$\text{Pounds per acre} = \frac{43,560 \times \text{pounds applied over test area}}{\text{area of measured course in sq ft}}$$

8. Adjust applicator and repeat the process until the desired rate is obtained. After one hopper has been adjusted, the other hoppers may be calibrated by adjusting each one to discharge the same amount of granules over the 300 foot course.

**EXAMPLE:** An application is applied at 5 lbs. of pesticide over a test area. The swath width is 15 ft. and the test run is 300 ft. long. How many lbs. per acre is the application?

$$\text{Pounds per acre} = \frac{43,560 \text{ sq ft/acre} \times 5 \text{ pounds}}{15 \text{ ft (swath width)} \times 300 \text{ ft (distance covered)}}$$

$$\text{Pounds per acre} = 48.4 \text{ lbs/acre}$$

### SHOP CALIBRATION

Power Take-Off (PTO) driven granular applicators can be calibrated in the shop by calculating the distance that will be traveled in 1 min, then collecting granules for 1 min with the applicator running at field speed.

**EXAMPLE:** The application will be applied at 3 mph in the field and the amount of granules collected in 1 min at this speed is 1/8 lb per tube on a 3 row distributor. The band width will be 12 inches. The application rate will be:

$$\frac{43,560 \text{ sq ft} \times (1/8 \text{ lb} \times 3 \text{ rows})}{1 \text{ acre}} \\ \frac{(88 \text{ ft}^* \times 3 \text{ mph}) \times 3 \text{ ft}}{1 \text{ mph}}$$

Which breaks down to:

$$\frac{43,560 \text{ sq ft}}{1 \text{ acre}} \times (1/8 \text{ lb} \times 3 \text{ rows}) = 43,560 \times (.125 \times 3) = 16,335$$

$$\frac{(88 \text{ ft}^* \times 3 \text{ mph}) \times 3 \text{ ft}}{1 \text{ mph}} = 88 \times 3 = 792$$

$$= \frac{16,335}{792}$$

$$= 20.6 \text{ lbs formulation per acre}$$

$$^*88 \text{ ft at } 1 \text{ mph} = \text{feet per minute}$$

## CALIBRATING FOR GRANULAR INSECTICIDE APPLICATIONS

The labels for some **granular insecticides** recommend applying a certain number of ounces of formulated product per 1,000 feet of row. Calibration is complete when the desired amount of formulated product is collected in the prescribed distance.

Many companies offer calibration tubes for their materials which can be attached to the distributor tube and will give a direct reading for that particular formulation. *These tubes should only be used for the chemical they came with as the manufacturer will provide the appropriate tubes with the next batch, taking into account the current volumetric weight of the product.*

## CALIBRATING ROTARY AND DROP SPREADERS

The delivery rate of granular spreaders can be altered by a number of factors, including temperature, humidity, rate adjustments, and differences in the size or type of granules. Even a different lot number of the same granular formulation can make a difference. Recalibrate your spreader any time any of these conditions changes. The operator of the spreader also affects the delivery rate, so the person who will operate the device should perform the calibration procedure.

When you are using a spreader regularly, it is a good idea to calibrate the device weekly, even if none of these conditions change, because the delivery rate can drift out of calibration through everyday use (and abuse) of the spreader.

A few different methods for calibrating granule sprayers exist. The “Drop Spreader Calibration” resource listed at the end of this chapter, page 78, is different from the method listed below. Take a look at a few methods before deciding which

to use. Use the method that makes the most sense for you and works for you.

Granular calibration method:

1. Measure a known area. Use the formulas covered earlier in this chapter on how to calculate the target area. For calibrating granular spreaders, a rectangular target area is probably the easiest to work with.
2. Set up a collection device. This can be a tarp laid out over the target area or a catch container mounted on the spreader. If you use a catch container, be sure that the device does not interfere with the delivery rate.
3. Apply at proper speed and gate setting. The rate at which granules flow out of the spreader depends on the size of the gate opening. A larger opening allows more granules to flow, so changing the size of the gate opening significantly increases or decreases the delivery rate. The speed at which the spreader moves also affects the delivery rate. When travel speed increases, less material is applied per unit area, and when speed is reduced, more material is applied.
4. Collect and weigh the amount of chemical applied over the target area. The delivery rate is the weight of material collected per the size of the target area. For example, if the target area was 300 square feet and the amount of chemical applied was 2.5 ounces, the delivery rate is 2.5 oz/300 sq ft.
5. Convert the delivery rate to the units specified on the label. Using the same example described above, a delivery rate of

2.5 oz/300 sq ft converts to 8.3 oz/1,000 sq ft or 22.6 lbs/acre.

## CHAPTER 8 ADDITIONAL RESOURCES

Deveau, J. *Calibrating Backpack Sprayers for Small Plot Work*. <http://sprayers101.com/backpack/>. Includes an Excel calculator for calculating nozzle output, time required to spray plot for desired output, etc.

*Drop Spreader Calibration*, UW PSEP Pesticide Fact Sheet #205. <http://webdoc.agsci.colostate.edu/cepep/FactSheets/205DropSpreaders.pdf>

Jensen, Uyeda, et al. 2015. *Sprayer Calibration Using the 1/128th Method for Motorized Backpack Mist Sprayer Systems*. University of Hawaii. <http://sprayers101.com/wp-content/uploads/2017/03/886.pdf>

Operation S.A.F.E. Program (Self-Regulating Application and Flight Efficiency). <http://www.agaviation.org/opsafeprogram>.

Spot-On Sprayer Calibrator. Materials for determining flow rates, spray patterns and droplet coverage. Also tools for cleaning tips. <https://innoquestinc.com/product-category/agriculture/ag-spray/>

Summary of Unmanned Aircraft Rule (Part 107). FAA News. Federal Aviation Administration, Washington D.C. [http://hse-uav.com/faa\\_part\\_107/FAA\\_Part\\_107\\_Summary.pdf](http://hse-uav.com/faa_part_107/FAA_Part_107_Summary.pdf)

Sumner, Paul E. 2012. *Granular Applicator Procedure*. University of Georgia Cooperative Extension. <http://extension.uga.edu/publications/detail.cfm?number=C818>

*Unmanned Aircraft Systems*. Federal Aviation Administration. <https://www.faa.gov/uas/>

Whitford, F., et.al. 2012. *Measuring Pesticides: Overlooked Steps to Getting the Correct Rate*. Purdue Pesticide Program. PPP-96. <https://ppp.purdue.edu/wp-content/uploads/2016/08/PPP-96.pdf>

WRK of Arkansas. Manufactures collectors and spectrometers for spray pattern analysis and develops software for droplet size analysis. <http://www.wrkofar.com/WRK-Products-Services.html>

Zhu, Heping, et.al. 2011. *A Portable Scanning System for Evaluation of Spray Deposit Distribution*. Computers and Electronics in Agriculture 76 (2011) 38-43.

Free droplet size analysis software. DepositScan available at: <https://www.ars.usda.gov/midwest-area/wooster-oh/application-technology-research/docs/depositscan/>



## CHAPTER 9: SAFE USE OF PESTICIDES

### LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- A. Explain how to use the information on labels and safety data sheets to mitigate pesticide exposure.
- B. Describe common symptoms of poisoning.
- C. Describe general first aid for poisoning based on route of exposure, including who to call for help.
- D. Explain methods to protect employees and the public from pesticide exposure.
- E. Describe Best Management Practices (BMP) to protect other non-target (drift, volatility, etc.) organisms.
- F. Describe Best Management Practices (BMP) to prevent groundwater and surface water contamination.
- G. Describe Best Management Practices (BMP) for protecting pollinators.
- H. Explain how to properly dispose of excess registered pesticides, experimental products, and containers.

### INTRODUCTION

Pesticide labels are intended to mitigate hazards to the applicator and others, as well as the environment, when using the product. Registered pesticides have labels and supplemental labeling that contain instructions on the precautions to use when handling the product and how to respond to emergencies. Additionally, all registered pesticides have a safety data sheet (SDS) which contains additional information about hazards, first aid, accidental release measures, toxicity and ecological information.

If an unregistered product is being used, the primary source of information to mitigate hazards will be the safety data sheet. Before handling the product, be certain you have read and understand the safety data sheet. The SDS should be available throughout the handling process as well as the application.

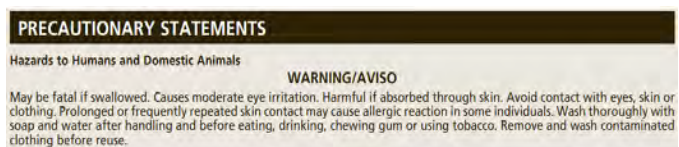
### MINIMIZING PESTICIDE EXPOSURE: LABELS AND SAFETY DATA SHEETS

The most likely time for a serious pesticide exposure is during mixing and applying. However, accidents can happen any time, including in storage or during transport. It's important to follow all safety guidelines and be prepared to respond in the event of an accident or exposure.

Pesticides can enter the body through the skin, the eyes, lungs or mouth. Wearing personal protective equipment (PPE) is critical to minimizing exposure. When working with registered products, applicators are required to wear the PPE listed on the product label but can use additional PPE

found on the SDS, if desired. The label also contains information on the restricted-entry and harvest intervals to follow to protect workers and consumers.

It's important to read the section of the label on HAZARDS TO HUMAN AND DOMESTIC ANIMALS found under PRECAUTIONARY STATEMENTS. This section not only describes the required PPE but also the routes of exposure that are of concern. (See example statement below.)



If the applicator is working with unregistered products, the SDS is the only source of information on hazards of working with the chemical, how to minimize exposure, and protect others and the environment. The SDS contains information in the following sections:

- (i) Section 1, Identification; Product identifier and common name of chemical.
- (ii) Section 2, Hazard(s) identification; Hazard classification (ex. flammable, category), Signal word, Hazard statement, pictograms or hazard symbols, precautionary statements. OSHA only recognizes two signal words (DANGER, WARNING) while EPA recognizes three signal words (DANGER, WARNING, CAUTION) so there may be inconsistency between signal words on the label and the SDS.
- (iii) Section 3, Composition/information on ingredients; Information on

chemical name, common name, the exact percentage of all ingredients [active ingredients and other (inert) ingredients]. Other ingredients are often considered proprietary information so they are not required to be listed.

- (iv) Section 4, First-aid measures; Information on first aid instructions by route of exposure, most common symptoms, and recommendations for immediate medical care and special treatment needed, when necessary.
- (v) Section 5, Fire-fighting measures; Recommendations for fighting a fire caused by the chemical, or the chemical in storage including special protective equipment or precautions for firefighters.
- (vi) Section 6, Accidental release measures; Information on appropriate response to spills, leaks, or releases including containment and clean up practices.
- (vii) Section 7, Handling and storage; Guidance on safe handling practices and conditions for safe storage of the chemical.
- (viii) Section 8, Exposure controls/personal protection; Although this section contains information on personal protective measures, if using a registered pesticide, you must wear the PPE required by the label. This section can be useful for PPE for spill cleanup or equipment cleanup.
- (ix) Section 9, Physical and chemical properties; Physical and chemical properties of the substance. Information

that would be useful for the applicator includes freezing point which can be used when determining where to store the product.

- (x) Section 10, Stability and reactivity; Describes the reactivity hazards of the chemical and chemical reactivity.
- (xi) Section 11, Toxicological information. Identifies toxicological and health effects, including likely routes of exposure (inhalation, ingestion, skin, and eye contact); delayed, immediate, or chronic effects from short- and long-term exposure; **LD<sub>50</sub>**; and **LC<sub>50</sub>** description of symptoms of exposure, whether the chemical is a carcinogen or possible carcinogen.
- (xii) Section 12, Ecological information; (non-mandatory under OSHA) Information on the environmental impact of the chemical. May include toxicity data on aquatic or terrestrial organisms (such as bees), potential to move into groundwater, etc.
- (xiii) Section 13, Disposal considerations; (non-mandatory under OSHA) Guidance on proper disposal of the container and/or chemical.
- (xiv) Section 14, Transport information; (non-mandatory under OSHA) Information on transportation hazard and transporting in bulk.
- (xv) Section 15, Regulatory information; (non-mandatory under OSHA) Information from manufacturer relating that the product is registered by EPA and is subject to labeling requirements under

FIFRA. Contains hazard information that is required on the pesticide label, which may differ from the hazard information from the SDS (especially signal words).

- (xvi) Section 16, Other information; including date of preparation or last revision.

## SYMPTOMS OF PESTICIDE EXPOSURE

Preventing exposure should be a goal of every applicator regardless of whether they are working with registered pesticides or unregistered pesticides. Read the pesticide label or the SDS to determine the most common routes of exposure for the product you are working with. Some SDSs also include commonly observed symptoms that may occur when working with the product.

Symptoms of exposure for many pesticides include skin rashes, headaches, or irritated eyes, nose, or throat. They may go away quickly and sometimes are mistaken for allergies, colds or flu. Symptoms of an exposure to more toxic pesticides may include blurred vision, dizziness, heavy sweating, weakness, nausea, stomach ache, vomiting, diarrhea, extreme thirst, or blistered skin. Some materials may also cause restlessness, anxiety, unusual behavior, convulsions, or unconsciousness. These symptoms can be confused with hangovers, flu, morning sickness, or even heat stress. Do not try to diagnose yourself or a co-worker. Seek medical attention if a pesticide exposure was possible and let the medical professional determine the cause.

**First aid** is the help you provide to a person exposed to pesticides before they reach professional help. The SDS often includes a phone number to call in the event of a suspected exposure. This can be extremely helpful for emergency first aid as well

as for medical providers if the exposure occurred while working with unregistered pesticides. So keep the SDS readily available. If working with a registered pesticide, the Poison Control Center may offer information on first aid for a pesticide exposure. This number works across the United States, and should be readily available:

**Poison Control Center**

**1-800-222-1222**

Available 24 hours, 7 days a week, 365 days a year

## **ROUTES OF EXPOSURE AND FIRST AID**

Become familiar with how to prevent exposure through the following routes and, if an exposure occurs, emergency first aid. First aid information can be found on the pesticide label and on the SDS.

### **SKIN EXPOSURE (DERMAL)**

**The most common route of pesticide exposure is through the skin** as applicators do not always cover their face, hands and forearms when working with pesticides. The easiest way to prevent skin exposure is to wear the label-required PPE or, if working with unregistered pesticides, a minimum of a long-sleeve shirt and long pants. Coveralls should be worn if the signal word is WARNING or DANGER.

#### **First Aid**

- Leave the contaminated area to prevent further contamination.
- Prevent further exposure by removing clothing and thoroughly washing the affected areas, including hair. Use soap or detergent and large amounts of cool or tepid water (hot water opens the skin pores and may increase absorption through the skin).

- Wash hands before using the toilet when working with pesticides (the groin area is a very vascular area with a high rate of absorption).
- Get medical attention and bring the pesticide label (if it exists) and SDS for the medical personnel.

### **EXPOSURE THROUGH EYES (OCULAR)**

Ocular exposure is a very rapid route for pesticides to enter the body. Always wear protective eyewear when mixing, loading, adjusting, cleaning or repairing contaminated equipment and during ground applications when not protected by an enclosed cab.

#### **First aid**

- Immediately flush the affected eye or eyes with a gentle stream of clean water. If running water is not available, with the head tilted to the side, slowly pour clean water from a glass or other container onto the bridge of the nose and across the cheek. Do not allow the water to cross the bridge of the nose and run across the other eye. If wearing contacts, ALWAYS flush the eye(s) for five (5) minutes before removing the lens. It's important to dilute the pesticide immediately rather than remove the contact lens. Do NOT reuse the contact lens from the affected eye.
- Get medical attention.

### **LUNG EXPOSURE (INHALATION)**

- Lungs also rapidly absorb some pesticides and transport it to other parts of the body in the oxygenated blood. Avoid breathing dusts or vapors while mixing and spraying. Consider wearing a respirator even if the label does not require it but be aware that it places an additional strain on the heart and lungs. If applying pesticides to agricultural commodities for research

or demonstration purposes, the Federal Worker Protection Standard contains specific respirator requirements when the label requires a respirator. These include a medical evaluation, annual fit-testing, and annual training on use and maintenance of the respirator. (See Additional Resources for more information, page 88.)

Voluntary use of a respirator does not require following these regulations but it is probably a good safety practice.

### First aid

- Leave the contaminated area or remove an exposed person from the area. (However, remember to wear PPE if having to retrieve a person from an enclosed area where you suspect an inhalation exposure.) Get fresh air immediately! Minimize physical exertion by the exposed person to avoid placing additional strain on lungs and heart.
- Loosen clothing to ease breathing and release any pesticide vapors that may have been trapped.
- Perform rescue breathing if person is not breathing.
- Watch for signs and treat for shock. Inhalation exposure often leads to shock. Have the person lie down and try to keep them calm.
- Seek medical attention.

### EXPOSURE BY MOUTH (ORAL)

Ingestion of pesticides may occur if spray materials or dusts splash or blow into your mouth during mixing or application. Ingestion may also occur if you eat, drink, smoke, chew gum or tobacco before washing hands after working with pesticides. Especially avoid chewing gum when working with pesticides. Keep all food and drinks away from areas where pesticides are being mixed or applied. Never put pesticides in food or drink containers.

And do not mix pesticides with containers that someone may use later for food storage, preparation or serving.

### First aid

- Follow the pesticide label or call Poison Control for guidance on how to treat someone who has swallowed pesticides.
- Depending on the instructions, you may be asked to:
- Dilute the swallowed pesticide if the person is conscious. **DO NOT** give liquids to an unconscious or convulsing person.
- Induce vomiting — **ONLY IF THE LABEL DIRECTS YOU TO OR POISON CONTROL RECOMMENDS IT.** Pesticides that are corrosive or petroleum-based can cause respiratory or lung damage, especially during vomiting. **IF UNCERTAIN, DO NOT INDUCE VOMITING.**
- Get medical care.

## PROTECTING EMPLOYEES

### PROTECTING WORKERS AND HANDLERS

While this category is part of Wyoming Commercial Pesticide Applicator certification, Worker Protection Standard provides specific information and protections to workers, handlers and others when WPS-labeled pesticide products are used on agricultural establishments in production of agricultural plants. These protections often exceed the requirements for commercial applicators. Consider this regulation as good practices even if it does not apply to your situation. This regulation is too extensive to describe in detail here. Refer to the How to Comply Manual for more information. (See Additional Resources, page 89.)

**While unregistered pesticides are exempt from WPS, it may be difficult to separate the treated**

areas under WPS from those that are not. In this case, it is best to treat the entire area as if it is under WPS.

### NOTIFICATION OF APPLICATIONS

Under the **Workers Protection Standard (WPS)**, if you are conducting research on or demonstration on an agricultural commodity, you must notify employees of the application and instruct them not to enter a treated area until the restricted-entry interval (REI) has expired. You may choose to do this either orally or by posting unless the label specifies the method you must use.

Sometimes labels indicate the treated site must be posted with a warning sign. Under WPS, any outdoor application of a pesticide that is subject to WPS which has a REI greater than 48 hours, **MUST** be posted with a warning sign.

A WPS-labeled pesticide applied in enclosed space production (such as a greenhouse) which has an REI greater than 4 hours **MUST** also be posted. Post warning signs prior to, but no earlier than 24 hours before the scheduled application.



Hinged WPS Warning Sign

The warning sign must remain posted throughout the application and the REI. Remove or cover the warning sign within three days after the end of the REI (or at the end of the application if there is no REI). Signs may remain posted after this time but only if the posted area is treated as if it were still under an REI by:

- instructing workers to not enter the treated area, and
- ensuring workers do not enter the treated area, other than permitted early-entry

activities (after providing additional information and supplies).

### PESTICIDE SAFETY TRAINING AND DECONTAMINATION SUPPLIES

Additionally, under WPS, any worker entering the field or enclosed space production facility in the 30-day period after the REI has expired must be provided annual pesticide safety training. Workers must also be provided with soap, single-use towels, and one gallon of water per worker at the beginning of the work period for decontamination. If a worker must enter the field under an REI for purposes of research sampling or other activities, they are considered an early entry worker and must be provided with additional information and decontamination supplies.

Under WPS, handlers must also have annual pesticide safety training and decontamination supplies. Handlers must be provided with soap, single use towels, a clean change of clothes (in case of contamination necessitating removal of clothing), and three gallons of water per handler at the beginning of the work period for decontamination. Emergency eye flushing supplies must be provided at any site where handlers are mixing or loading a pesticide that requires protective eyewear or mixing or loading any pesticide using a closed system under pressure. Additionally, when applying a pesticide that requires protective eyewear, one pint of water must be immediately available to each handler in a portable container.

### PROTECTING THE PUBLIC

#### DURING THE APPLICATION AND REI

When making pesticide applications for research or demonstration, keep all animals and people out of the treated area during an application and until the REI has expired. Consider posting research plots



with a warning sign to discourage unauthorized entry. In general, when conducting research using a registered product for a use not allowed by the label, you should post the research area according to the longest REI on the label. If the pesticide product labeling does not provide an REI, do not allow entry until the spray has dried or dust settled. When using pesticides with different REIs in the trials, follow the longest REI.

### **CROP DESTRUCT**

Commodities (food and feed) which are treated with a registered product for a use not allowed by the label or any experimental pesticide for which there is no pesticide residue tolerance, or any genetically modified organism which is not cleared for release into the food chain, must be destroyed. The treated commodities cannot enter the channels of trade or in any way be made available for use as a human or animal food or feed. Destroy all parts of the treated commodity potentially suitable for use as human or animal food or feed which was not removed from the site for research purposes. Co-workers, family, friends and others must not be allowed to take samples from experimental plots for personal consumption. It's primarily a matter of not knowing enough about the experimental products to warrant exposing someone to potential hazards associated with them. More information is available in Chapter 2: Laws and Regulations, page 9.

In addition to posting during an REI, consider posting treated plots that require crop destruction.

Should you choose to post, prior to the application, post a sign at the experimental plot with the words, "Warning-Crop Destruct, Do Not Pick." The signs should be in English and Spanish, and of a size so the wording is readable to a person with normal vision from a distance of 25 feet. Signs should remain in place until treated crop is destroyed. The

crop should be harvested and destroyed, usually by burning or burial at a designated site.

## **PROTECTING THE ENVIRONMENT**

Always read the pesticide label and SDS for environmental hazards. These include hazards to endangered species, pollinators, wildlife, groundwater and surface water. The SDS may be the only source of information about these hazards for unregistered pesticides. The following information is some **Best Management Practices (BMPs)** to consider to protect the environment.

### **BMPs FOR MITIGATING OFF-TARGET MOVEMENT**

Pesticide drift due to spray drift or volatilization in outdoor applications can affect both unprotected people and non-target organisms. While pesticide drift is difficult to predict, there are some methods that applicators can use to mitigate the likelihood of drift occurring. These include:

- Choose the appropriate nozzle for the pesticide you are using. Chapter 8, page 67, provides information on how to choose nozzles based on droplet size if drift is a concern. Avoid nozzles that produce a large number of droplets smaller than 200 microns.
- Avoid applications when conditions indicate or favor a temperature inversion. For more information on inversions, including how to determine the potential by taking measurements at the application site, refer to the publication from NDSU. (See Additional Resources, page 88.)
- Avoid spraying when wind speeds are less than 3 mph (unless you can determine a temperature inversion does not exist). The upper limit for wind speed at the research or demonstration site will vary depending



on the pesticides used and the size of plots to be treated.

- Appropriate buffer zones should be used to accommodate particulate (spray) drift produced during the application. Size of the buffer zone will vary depending on the pesticides used, the sensitivity of the area and other drift management practices being used.
- Know whether the pesticides included in the project are prone to volatilize. Vapor pressure is one factor in volatilization. Pesticides with higher vapor pressure are more volatile. If available, information on vapor pressure can be found in the Physical and Chemical Properties section of the SDS. However, temperature, relative humidity and soil properties all play a role in volatilization. A free model has been created that may be useful to predict the potential for volatilization of the pesticides in the project which includes these other factors. (See Additional Resources, page 88.)
- Include measurements made on existing environmental conditions at the time of application. Measure as close as possible to nozzle release height (boom height). Record data at the start and finish of the application, or more often if conditions change. Include information on average wind speed (over a 1-2 minute time span) and direction (in degrees magnetic, 0-360°), temperature and humidity (for temperature inversion potential). This information may prove valuable to explain unexpected research results.

### **BMPS FOR PREVENTING GROUNDWATER AND SURFACE WATER CONTAMINATION**

Prevention of groundwater and surface water contamination is very important because once water is polluted, it is extremely difficult and costly

to clean up, and sometimes, impossible. Pesticides can enter groundwater through leaching or by direct entry through wells or other structures that are in direct contact with the aquifers. Surface water contamination can occur through soil erosion and runoff. Some management practices that can help minimize groundwater and surface water contamination include:

- To avoid groundwater or surface water contamination, it is critical that you know the properties of the pesticide you are using. This includes water solubility properties, soil adsorption coefficient (K<sub>oc</sub>) and half-life. The National Pesticide Information Center (NPIC) offers a Herbicide Properties Tool that will provide information on the properties of specific active ingredients. It also provides a Groundwater Ubiquity score that can help access pesticide leachability (see Additional Resources, page 88).
- Know the depth to groundwater at the application site. This information can be found through the National Water Information System or Groundwater Watch websites (see Additional Resources, page 88).
- Become familiar with the soil types at the research or demonstration site. In general,



Field mixing

leaching of pesticides is greater in sandy soils, well-drained soils or soils low in organic matter. The Web Soil Survey, see Additional Resources, page 89, provides soil data for the specific research or demonstration site.

- Locate the mixing or loading site away from wells, streams and lakes. Maintain a distance of at least 100 feet (check the pesticide label for more specifics) between the mixing and loading site and wellheads, ditches, streams or other water sources.
- Whenever possible, measure, mix, and load over an impervious surface, such as a concrete pad, which prevents spills from soaking into the ground. If you are not using a pad, move the mixing and loading steps from place to place to avoid chemical buildup from accidental splashes or spills.
- Be prepared for spills and have a “spill kit” readily available near the mixing loading area. Never leave a tank while it is being filled, and pay constant attention during filling to prevent overfilling and spilling of the pesticide on the ground.
- Do not contaminate surface water when cleaning containers and spray equipment. Rinsates from container rinsing should be part of the spray solution. However, if this is not possible, apply the rinsate to a labeled site. Locate equipment cleaning areas away from wells, streams and lakes.
- Protect nearby water sources from contamination by pesticides when spraying or cleaning equipment. Avoid creating ‘puddles’ that contain pesticide spray material. In addition to occurring during applications, it may occur during equipment calibration or cleaning.
- Inform neighboring beekeepers of the application if there is concern that the pesticide products will impact bees. Attempt to identify and confirm the location of hives near the treatment site. Apiaries can be located on the voluntary sensitive site registry, FieldWatch®. Applicators must register to be able to see all hives, including ones not visible on the public map. (See Additional Resources, page 88).
- Use appropriate buffers between treatment areas and pollinator habitat or hives. Do not spray when the wind direction is blowing toward pollinator habitat or areas where hives are located.
- Check the weather forecast before application and be mindful of changing weather conditions during application. National Weather Service (NOAA) can be used to generate a local forecast. Enter the desired location, then ‘Get Detailed info’ link under the current temperature. Scroll down that page towards the bottom to find ‘Tabular Forecast’ under the Additional Forecasts and Information section. Ideal weather conditions include:
  - Wind speeds 3 mph to <10 mph, not gusty or dead calm. (Wind speeds below 3 mph are common in temperature inversions.)
  - Temperatures below 90°F and,
  - relative humidity above 50%.

### **BMPs FOR PROTECTING POLLINATORS**

EPA has been working aggressively to protect bees and other pollinators from pesticide exposure. Pesticide Stewardship has information on how to protect pollinators during various types of application (see Additional Resources, page 88). Some BMPs to protect pollinators when conducting research or demonstrations include:

## **DISPOSAL OF PESTICIDE AND CONTAINERS**

### **Undiluted pesticides**

Be sure to safely dispose of pesticides. You can try to return registered products to the manufacturer. Unregistered products should be returned to the manufacturer after the research experiment.

### **Diluted pesticides**

If you have leftover spray mixture of a registered pesticide, you may legally use it on another labeled site. If there is no other appropriate site, or if you are using an unregistered compound, the leftover spray mixture must be sent to a Class 1 hazardous waste disposal site. There are private companies that specialize in collecting and transporting pesticide wastes to Class 1 disposal sites. See the UW PSEP website for more information.

Never indiscriminately dump excess pesticide. Such dumping is illegal and a potential source of environmental, groundwater, and surface water contamination. People convicted of dumping are subject to large fines and possible jail terms.

### **Pesticide container disposal**

Refer to the registered pesticide container for information on disposal. In general, the container should be triple-rinsed (rinsates added to the spray mix) and punctured before disposal in the landfill or burned. Refer to the technical bulletin or request guidance from the manufacturer for unregistered pesticides.

## CHAPTER 9 ADDITIONAL RESOURCES

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Web Soil Survey. Website - USDA Natural Resources Conservation Service (NRCS) <https://websoilsurvey.nrcs.usda.gov/app/>

Wyoming Environmental Pesticide Education Program (UW PSEP). Information on various regulations including Worker Protection Standard training materials, How to Comply manual, etc. <https://uwyoextension.org/psep/>

## GLOSSARY

**ACTION THRESHOLD** — the number of pests or level of pest damage before action is required.

**ALTERNATIVE HYPOTHESIS** — the opposite of the null hypothesis and is usually what you are testing.

**ANTAGONISM** — when the plant response is less than expected (less than an additive effect).

**BIAS** — process where the scientists performing the research influence the results, in order to portray a certain outcome.

**BIASED SAMPLE** — one in which not all members of a population are equally likely to be chosen.

**BIOLOGICAL MAGNIFICATION** — the tendency for certain pesticides to become progressively more concentrated in each type of organism as they move up the food chain.

**BORDER EFFECT** — the edge of a plot or field where plants may grow differently than plants not at the edge.

**BUFFER ROWS** — rows from which you do not collect data because they buffer the effect of the neighboring plots.

**CARRIER** — liquid or powder substance combined with the active ingredient in a pesticide formulation. May also apply to the water or oil that a pesticide is mixed with prior to application.

**CONTROL SUBSTANCE** — any material other than the test substance that is used in the test system to establish a comparison with the test substance.

**CROP DESTRUCT** — to render unusable for food or feed, or to use for research purposes only.

**ECONOMIC THRESHOLD** — the density of a pest at which a control treatment will provide an economic return as the value of the crop destroyed exceeds the cost of controlling the pest.

**EXPERIMENTAL ERROR** — the difference among experimental units treated alike.

**EXPERIMENTAL UNIT** — the smallest unit to which a treatment can be applied at random.

**FLUOROMETER** — a device used to determine the presence and amount of fluorescent dye for spray pattern testing.

**HYPOTHESIS** — an educated guess or proposed explanation made on the basis of limited evidence. Serves as a starting point for further investigation.

**INCIDENCE** — the number of plants with disease out of a given number of plants.

**LC50** — concentration of a chemical in air that kills 50% of the test animals during the observation period.

**LD50** — the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals.

**LIPOPHILIC** — fat-loving. Chemicals which dissolve in and are stored in fatty tissues.

**METRONOME** — a device that produces an audible beat at regular intervals, often set in beats per minute.

**MODE OF ACTION** — the biological processes that are disrupted by the pesticide.

**NULL HYPOTHESIS** — typically states that there will be no differences between treatments.

**OFF-LABEL USE** — applications to crops not listed on the label, rates above listed label rates, a prohibited application method, or more applications than allowed on the label.

**POPULATION** — set of elements about which a researcher wants to make inferences.

**RANDOMIZATION** — assigning treatments to experimental units (plots, pots, etc.) so that all units have an equal chance of receiving a treatment.

**RANDOM SAMPLING** — every member of the population has an equal chance of being included in the sample.

**RAW DATA** — information that is gathered for a research study before that information has been transformed or analyzed in any way.

**REPLICATION** — a treatment is repeated two or more times.

**RESISTANCE** — heritable reduction in the sensitivity of a pest population to a pesticide that was previously effective at controlling the pest.

**RESTRICTED ENTRY INTERVAL (REI)** — the amount of time that must pass after a pesticide application is completed before a person can enter the treated area without additional personal protective equipment.

**RESTRICTED-USE PESTICIDE (RUP)** — a pesticide that is classified for restricted use under the provisions of FIFRA. A pesticide that can be sold to or used by only certified applicators.

**SAMPLE** — a small part of a population intended to be representative of the whole.

**SCIENTIFIC METHOD** — a systematic method or procedure to formulate a hypothesis and test it.

**SELECTIVITY** — ability of a pesticide to affect one organism and not another.

**SEVERITY** — often used to describe the percent of disease on a leaf or plant.

**SITE OF ACTION** — the specific process in the organism that the pesticide disrupts.

**SPECTROMETER** — an apparatus used for measuring and recording fluorescent dye deposition to determine spray distribution from boom sprayers (commonly used in aerial application pattern testing).

**STANDARD OPERATING PROCEDURES** — procedures for making experimental observations, how to collect samples, and how to handle, store, and retrieve experimental data.

**STUDY DIRECTOR** — the person responsible for the technical conduct of the study and the interpretation, analysis, documentation, and reporting of study results.

**SUBSAMPLING** — measurement that does not include the whole experimental unit.

**SYNERGISM** — when the plant response is greater than expected (more than an additive effect).

**TEST SUBSTANCE** — substance (e.g., a pesticide) that is the subject of your application for a research or marketing permit.

**TEST SYSTEM** — the object to which you are applying your test or control substance.

**TOLERANCE** — the maximum amount of pesticide residue which is allowed on the crop at the time it is harvested or offered for sale.

**TRANSLAMINAR** — localized, systemic activity of some pesticides.

**UNREGISTERED PESTICIDES** — pesticides that are under development that have not yet received an EPA registration.

**VOLUME MEDIAN DIAMETER (VMD)** — the droplet size where half of the spray volume is made up of smaller droplets and half the spray volume is made up of larger droplets.



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