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Section I: Biological control of weeds

N.E. REES, P.C. QUIMBY, Jr. and B.H. MULLIN

Rees and Quimby are with the USDA, Agricultural Research Service, Range Weeds & Cereals Research Unit, Montana State University, Bozeman, MT. Mullin is with the Montana Department of Agriculture, Helena, MT

(*Article begins on following page.)

Biological Control of Weeds

N.E. Rees, P.C. Quimby, Jr., and B.H. Mullin

Biological control, or *biocontrol*, is the intentional use of living organisms to reduce the population of a pest. It may include the use of insects, nematodes, mites, plant pathogens, and vertebrates. Often more than one biocontrol agent is introduced on a weed. Each enemy detrimentally affects the weed to some degree. This effect may be obvious, such as when the plant is defoliated, or it may be subtle, such as when slight damage caused by the biocontrol agent allows secondary organisms (such as pathogens) to inflict greater damage.

Because the majority of the noxious weeds in the United States are introduced without their natural enemies, and many of these weeds are minor members of the plant communities in their native lands, insights about weed management may be found by studying these plants in their native homelands. Studies reveal which organisms are associated with the target plant, which of these damage the weed, and which damage other plants. From these results, potential biocontrot agents are selected and tested to determine their host range. Those that have a very limited host range under starvation feeding trials are approved for release into the United States. (**Appendix 2** explains how agencies find, screen, and import biocontrot agents.)

The eventual impacts of a biological control agent on its target plant will be the result of: 1) the density of weeds compared to the density of the agent,- 2) the effect of the local biotic and abiotic conditions on the agent and on the weed; 3) the plant's reproductive ability (seeds only or seeds and vegetative reproduction); 4) the agent's ability to stress the plant each year and the plant's ability to maintain and replace root reserves; 5) the plant's ability to recover from the effects of the biocontrol agent; and 6) the interactions of multiple biocontrot agents attacking a single weed species.

Both advantages and disadvantages are associated with the use of biocontrol agents. One advantage is that once a biological control agent becomes established it usually will reproduce, increase its numbers, and continue to attack the target organism, generally without additional costs to the land manager. Second, biocontrol agents move to host plants anywhere within their climatic range, readily crossing ownership boundaries and some geographical barriers. Third, approved biocontrol agents are selective. Host weeds are attacked without damage to the surrounding vegetation. Finally, properly tested biological control agents are not a source of environmental contamination.

A disadvantage of biocontrol is that it often takes many years for the populations of the introduced agents to increase to levels that permanently decrease the pest plant population. A limited number of eggs are laid by insects and the initial population build-up appears slow. However, insect numbers increase exponentially. As biocontrol agent populations gradually increase, the weed population will gradually decrease and may be unnoticed by the land manager. Photopoints can help document the seriousness of the original weed problem and the change in the weed population over time. A second disadvantage is that some biocontrol agents may be subject to predators. Third, environmental conditions (shade versus sun, low versus high rainfall, sandy versus clay soils) often exclude some biocontrot agents from certain locations. Finally, biological control agents usually do not eradicate weed populations. Use of multiple control methods is important when implementing any management system.

Using biological control agents to help manage weed problems is more effective when the land manager understands the target weed biology, the biological control agents available and how they impact the weed, and the environmental and management restrictions in the area.

Understanding Basic Concepts

A discussion of terminology and concepts is important to understanding the practice of biological control.

Plants and animals attempt to survive as individuals and to perpetuate their species. People regard an organism as "beneficial"" when it produces food, fiber, or other human benefits; it is a "pest" when it competes with us for food or fiber, or affects our personal comfort; and it is ignored when it does not benefit or harm us. When organisms feed on both desirable *and* pest plants we have a conflict of interest, a condition that can only be resolved by risk:benefit analyses to satisfy economic pressures and federal laws, such as those preserving native plants and protecting rtre, endangered, or threatened species.

The term "control" does not imply the complete eradication of the species in a specific area. The environment, weed infestation, land condition, and land use requirements of the land manager will determine the level of control needed and the control tool or tools chosen.

When we use natural enemies to reduce pest populations, we refer to the natural enemies as "biological control agents," or sometimes "biocontrol agents." "Biological control" can be defined as the use of living organisms to depress the population of a pest. However, biological control could be more accurately called "biological suppression," i.e., reducing the population of the target weed to an acceptable level. Often the goal of those who use biological control agents on rangelands is to suppress the alien weeds and at least partially restore the native plant communities.

Basic plant biology

An understanding of basic plant biology will allow the land manager to better choose appropriate control tools in a long-term management program. Understanding the life cycle of the weed will help land managers determine when the plant is most vulnerable to a particular control method.

Included here are illustrations of components common to many plants. The glossary in the **Endnotes** of this guide may also be helpful in defining botanical and biological terms.

Annual weeds germinate from seeds, flower, produce seeds, and die in one year or less. Most annual plants are easiest to control as seedlings. Prevention of seed production is the most effective control. Annual plants are not well adapted to a biological control program.

Annual weeds are classified as summer annuals or winter annuals. *Summer annual*s germinate early in the growing season, grow and produce seed during the summer, mature, and die in the fall. Lambsquarters, pigweed, ragweed, and foxtail are examples of summer annual weeds. *Winter annuals* complete their life cycle from fall to spring or early summer. They germinate in the fall and overwinter as seedlings or rosettes. They usually produce seeds in spring and die by midsummer. Yellow starthistle is an example of a troublesome winter annual weed.

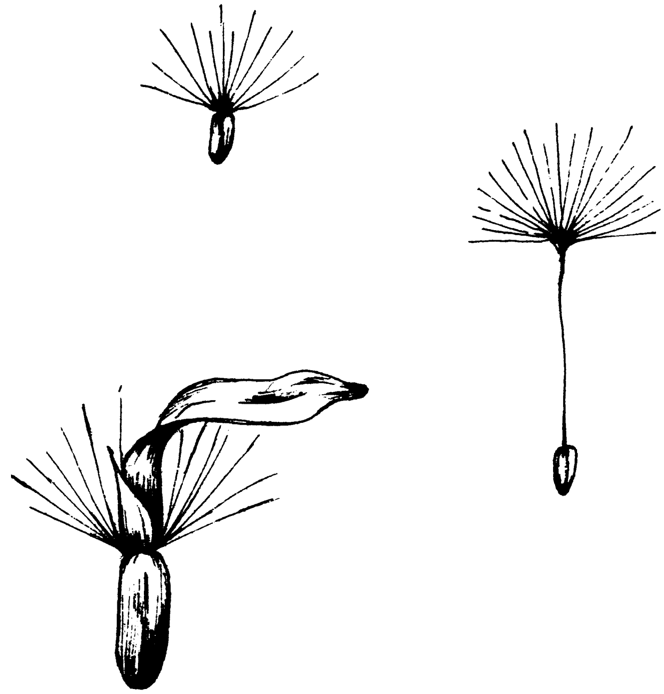
Biennial weeds complete their life cycle in two years. During their first season of growth they develop a deep root system and a low-growing rosette of leaves. They are dormant over the winter in the rosette stage and send up flowering stalks, or *bolt*, early in the second spring of the life cycle. During the second year they flower, produce seeds, mature, and die. Burdock, houndstongue, bull thistle, and musk thistle are examples of common biennial weeds. Control is most effective during the first season of growth when the plant is in the rosette stage and prior to the development of viable seed.

Perennial weeds live for more than two years. Most reproduce by seeds and many are able to spread vegetatively by underground stems, or *rhizomes*, by tubers, or by an extensive root system. They are classified according to their method of reproduction as simple or creeping.

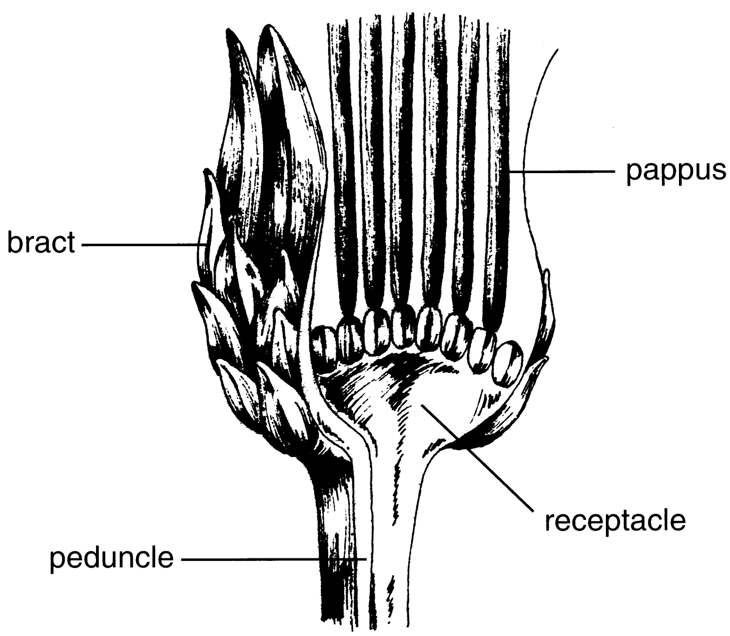
Simple perennials spread by seed. They cannot naturally spread vegetatively, but can produce new plants if injured or cut. The roots are generally fleshy and may grow very large. The plant grows for many years from the crown area of the plant. Examples of simple perennials are dandelion and St.Johnswort.

Creeping perennials reproduce by creeping roots, by creeping aboveground stems, or stolons, or by rhizomes. Examples include field bindweed, Canada thistle, hawkweeds, and quackgrass. Some weeds propagate by means of tubers, which are modified rhizomes adapted for food storage. Yellow and purple nutsedge are examples.

Once an area is infested, creeping perennials are probably the most difficult group to control. Plowing and tilling may spread these weeds. Continuous and repeated cultivations, mowing, and persistent herbicide applications are often necessary for control. Perennial weeds are the most logical target for a biocontrol program.

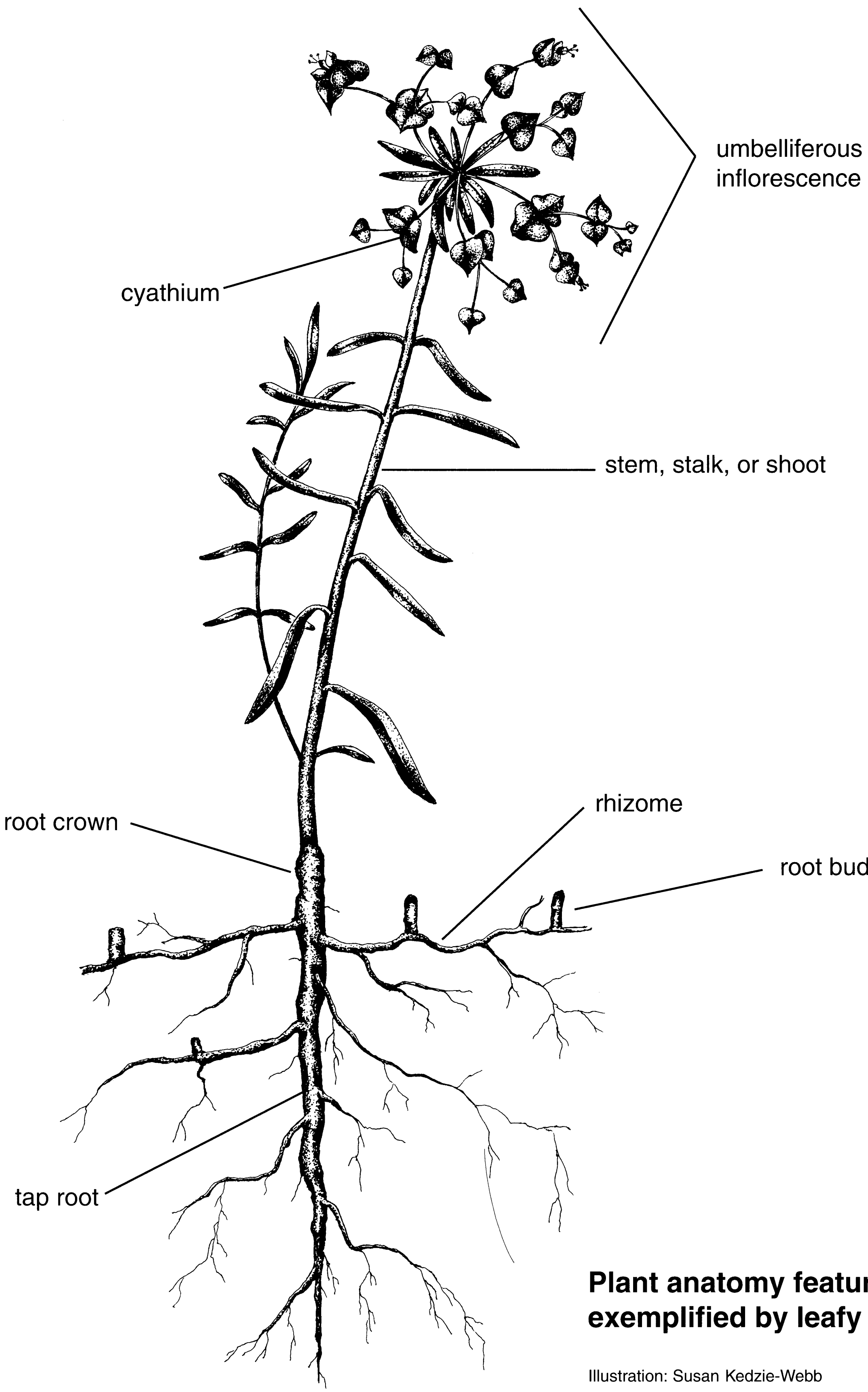


The pappus is a feathery tuft attached to the seed. It can take many forms.



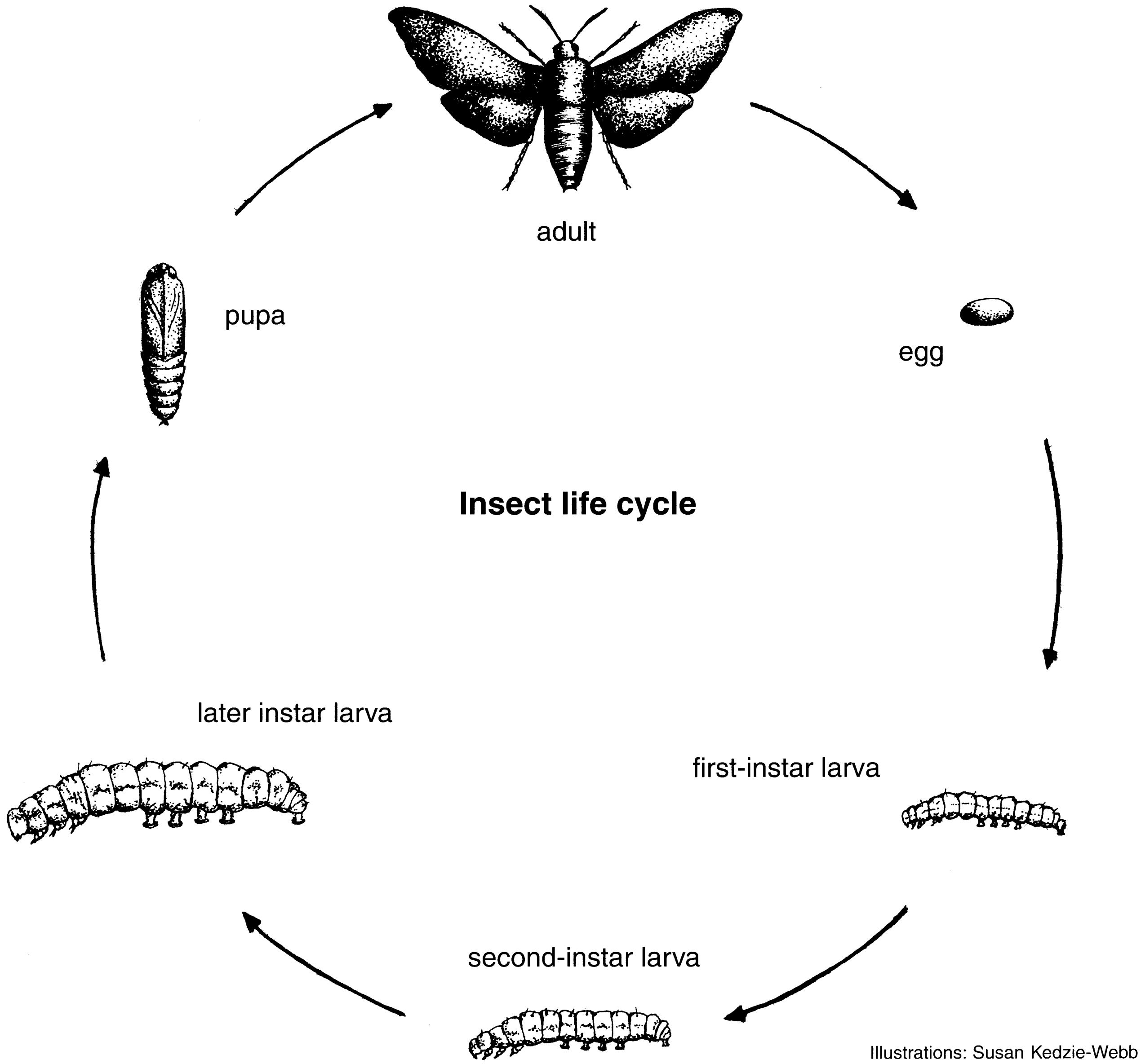
Capitulum, or seed head

Illustrations: Susan Kedzie-Webb



Plant anatomy features, exemplified by leafy spurge

Illustration: Susan Kedzie-Webb



Basic insect biology

Insects are a very large and diverse group of animals and comprise the majority of weed biocontrol agents used today. Before beginning to work with insects you should know a little about insect anatomy. Insects have several characteristics that distinguish them from other animals. These adult chartcteristics are:

*An exoskeleton;

* A segmented body of three parts: head, thorax and abdomen;

*Three pairs of legs.

Insects have several different types of mouth parts: chewing (grasshoppers and beetles); piercing-sucking (aphids and mosquitoes); sponging (house flies); rasping (fly larvae); and rasping/sucking (thrips).

Insects develop and grow through a series of molts. The transformation of an immature to a mature insect is termed metamorphosis. There are two main types of metamorphoses. In an incomplete metamorphosis insects go through an egg stage, a nymph or immature stage (which resembles an adult, but lacks fully developed wings), and an adult stage. In a complete metamorphosis the insects go through an egg stage, a larval stage or stages (in which they do not resemble adults), a pupal stage, and an adult stage.

Insect body parts

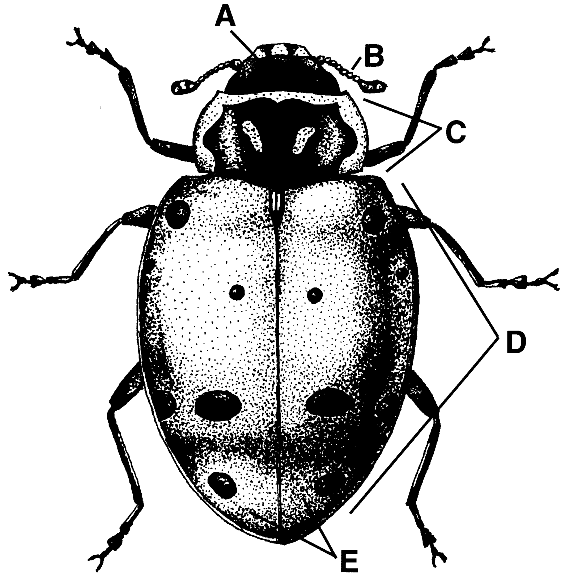
A - head

B - antennae

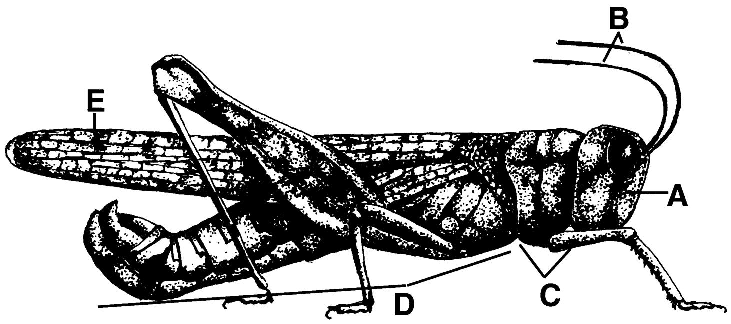
C - thorax

D - abdomen

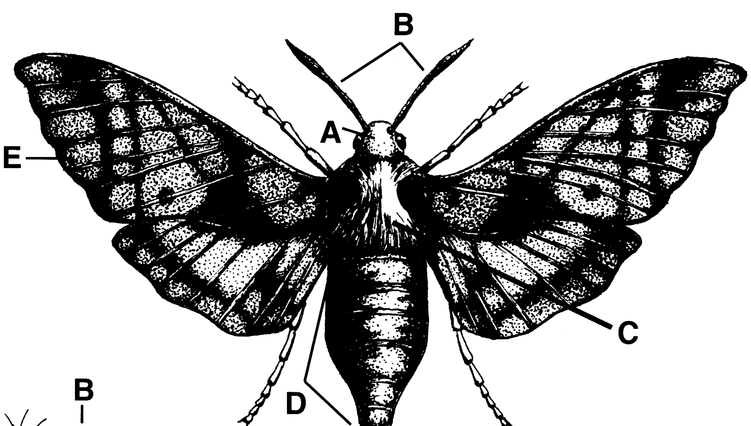
E - wings



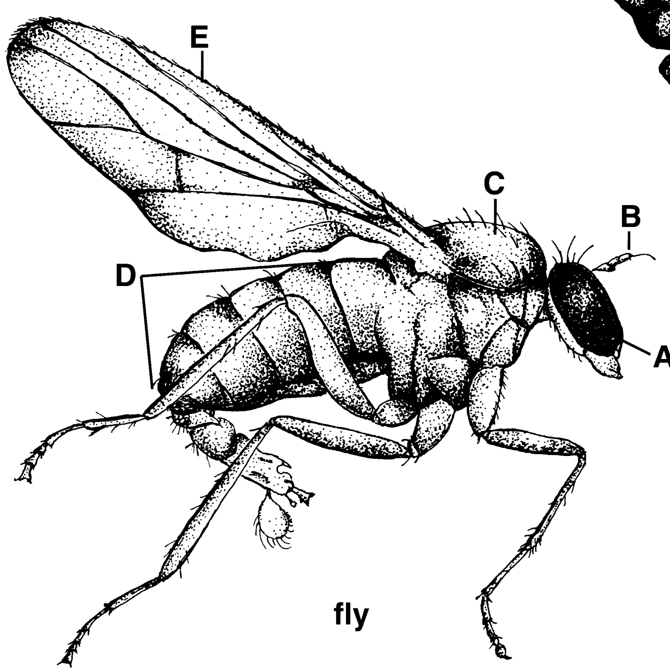
beetle



grasshopper

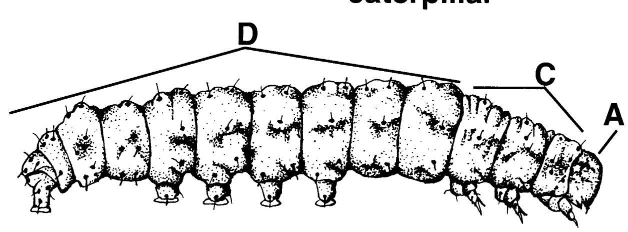


moth



fly

caterpillar



Insect points of attack

Each specialized biological control agent has co-evolved with its plant host. Limited numbers of an agent will generally have little effect on the vigor and survival of the host, but as the number of biocontrol agents increase in relation to the number of available plants, there will be an increased impact on the plant population. This may result in visible symptoms, such as damaged leaves, flowers, stems, or roots; wilting; discoloration of foliage; dropping of leaves; reduced numbers and viability of seeds; smaller plant size and vigor; retarded growth or flowering periods; and sometimes plant death. Injured plants may also attract and be vulnerable to attack by other insects, mites, nematodes, and pathogens. Multiple attacks may reduce plant reproduction and may even cause plant death.

The following is a list of biocontrol insect agents categorized by their feeding strategies:

Gall-producing insects: Adult female gall-producing insects sting the plant and insert an egg into plant tissues; larvae secrete chemicals into the plant tissues. Both affect plant growth and development and cause the plant to change its cell structure and cell function near the gall producer, thus creating an enlargement (gall) of the plant at that point. Nutrient-rich cells within the gall are consumed by the organisms for growth and survival. In many cases, the biocontrol agent causes the plant to produce or assists in producing hardened chamber walls that protect the biocontrol agent from potential predators and parasitoids. The presence of limited numbers of gall organisms is often not lethal to the plants, since fresh plant cells must be maintained during the gall producer's development. However, the galls influence the plant by acting as a nutrient sink, causing the plant to direct nutrients to the tissues rather than into seeds or plant growth. This demand reduces the vigor of the plant and may reduce seed production.

Defoliators: Some defoliators partially or completely consume leaves, flowers, bracts and sometimes the stems, while others mine these areas, removing layers of outer plant tissues. In this manner, the defoliator obtains nutrition while reducing the plant's ability to produce sugars for the root system. Reduction of these sugars may suppress the plant's ability to grow and survive.

Sap suckers: Insects and mites with piercing-sucking mouth parts feed on a plant similar to the way mosquitoes feed on animals. Reducing nutrients in the plant's circulatory system may reduce the plant's vigor, thus weakening it and making it vulnerable to attack by other organisms. In addition, viruses, bacteria, and fungi arc sometimes transmitted from one plant to another by these arthropods.

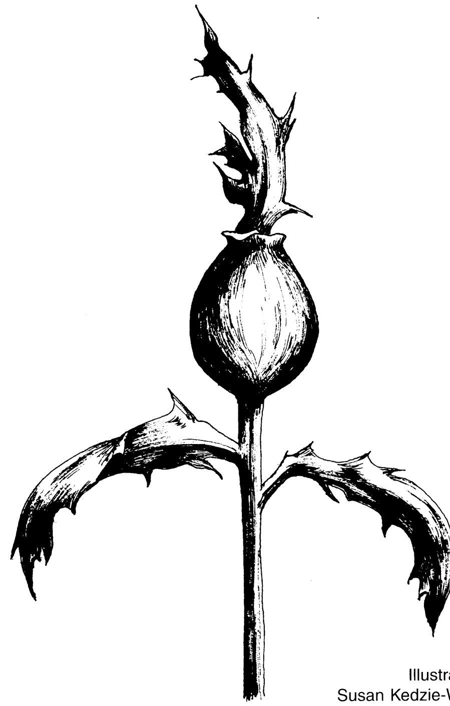


Illustration: Susan Kedzie-Webb

Insects can cause tissue enlargement, or galls, on plants. Above is an example of a gall on Canada thistle.

Seed-attackers: Damage from biocontrol agents that attack the seeds or seed-producing tissues may be direct such as when the agent consumes some or all of the seeds, or it may be indirect, such as when seed-producing tissue is damaged and nutrients are diverted from producing healthy seeds. This greatly reduces the viability of the seeds.

Stem-dwellers: Stem-dwelling organisms are protected from potential parasitoids and predators while they mine within the tissues. These tissues arc generally alive during larval development of the insect. In some cases, secondary damage from pathogens or destructive arthropods caused after the stem-dweller leaves its host may be greater than the direct damage it causes.

Crown- and root-burrowers: Many species of insects burrow into the plant's crown and root. Their feeding activity reduces the root reserves and sometimes reduces the plant's ability to either translocate nutrient reserves or replenish nutrients.

Root feeders: Organisms that feed upon and in the root hairs and young roots reduce the plant's ability to take up moisture and nutrients and to replace depleted root reserves. Wounds caused by these agents may allow entry by soil microorganisms and secondary diseases may further weaken the plant.

Basic disease biology

There are an estimated 100,000 parasitic plant diseases. Most occur rarely or on a very limited scale. Plant diseases have been studied as biological control organisms and show much promise for the future.

Plant disease is the result of the right combination of a susceptible host plant (in this case, the target weed), a virulent pathogen (the disease-causing agent), and suitable climatic and environmental conditions. Variations from normal climatic conditions are often responsible for sudden outbreaks of diseases that would not normally occur. This may be the case when some weeds are seriously injured in a limited location or a particular year.

Plant diseases are either non-parasitic or parasitic. *Non-parasitic* (physiological) diseases include: 1) nutrient deficiencies or excesses; 2) environmental extremes; 3) air pollution and pesticide injury; 4) drought; 5) genetic abnormalities; and 6) other physiological disorders. Non-parisitic diseases are not predictable, repeatable, nor can they spread from plant to plant and so are not useful as biological control tools.

Parasitic diseases are caused by living organisms that can multiply and spread from infected to healthy plants. Organisms commonly causing parasitic diseases are fungi, bacteria, viruses, and mycoplasmas. To date the most common plant pathogens used for biological control of weeds are fungi. Some research is being conducted on the use of bacteria for biocontrol of weeds.

Fungi are simple microorganisms lacking chlorophyll. They obtain nutrients from living plants and animals or decaying organic matter, and enter plants through wounds, natural openings, or by penetrating directly through the plant tissue. Fungi cause local or general disintegration of plant cells or tissue, stunting, or abnormal vegetative growth. They may affect plant growth by removing or blocking nutrients to the plant or by producing toxins that can affect the structural and metabolic actiity of the plant. Fungi life cycles range from simple to very complex.

Bacteria are commonly found one-celled organisms that can be seen only with high-powered microscopes. Bacteria reproduce rapidly through simple cell division, and enter plant tissue through wounds or natural openings in the plants. Once inside they multiply rapidly, causing cell death, abnormal plant growth, or tissue breakdown. Some produce toxins. Bacteria are spread effectively by cultivation, rain, flowing water, wind, dust, and transport of diseased plant material.

Viruses are complex macromolecules composed of ribonucleic acid with a protective protein coat. They resemble chromosomes found in all living cells and can only function and reproduce in a living cell. Viruses divert normal growth and development processes in plants, causing stunting, streaking, yellowing, mosaics,ringspot, or streaking. They may be spread by direct contact between healthy and diseased tissue or by vectors, such as insects or nematodes.

Mycoplasmas are bacteria-like organisms without cell walls. Most cause "yellows" or "witches'-broom" symptoms in plants. Leafhoppers are common insect vectors.

Some insect biological control agents may cause wounding of the target weed that allows secondary infection by disease organisms. Additional research is needed to understand this interaction and to develop additional plant disease agents for biological control of weeds.

Other biocontrol agents

Mites are similar to insects but have two body parts. Mature mites have four pairs of legs, although immature mites have three pairs of legs. Eriophyid mite adults and immatures, however, have only two pairs of legs. Plantfeeding mites may attack the plant bud, form galls, cause cupping or curling of the leaf, discolor the fruit, or retard the plant's growth. High temperatures and humidity provide favorable conditions for mite populations.

Nematodes are microscopic, unsegmented roundworms that live in water or soil. They can injure plants by sucking plant juices through hollow, spcar-like mouth parts. Nematode feeding lowers natural plant resistance, results in gall formation, reduces plant vigor and yield, and allows easy entrance for fungi and bacteria. Nematode-damaged plants are often more susceptible to winter injury, drought, disease, and insect attack.

Some nematodes enter plant tissues to complete their life cycle, while others remain outside the plant with only their feeding parts attached to the roots. All plant parasitic nematodes reproduce by laying eggs. Nematodes generally require several years to build up damaging populations in the soil. They are easily spread by moving infested soil, plant parts, or contaminated objects. Identification of nematodes is difficult and must be done in a laboratory equipped with extraction equipment and microscopes.

Integrating Weed Management Methods

Integrated weed managment (IWM) is a systems approach to the management of undesirable plants. IWM is defined in the Federal Noxious Weed Act as "a system for the planning and implementation of a program, using an interdisciplinary approach, to select a method for containing or controlling an undesirable plant species or group of species using all available methods, including education; prevention; physical or mechanical methods; biological control agents; herbicide methods; cultural methods; and general land management practices." It is a multidisciplinary, ecological approach to managing unwanted plant species - weeds.

Management techniques

Integrated weed management involves using the best control techniques described for the target weed species in a planned, coordinated program to limit the impact and spread of the weed. The control methods selected should be determined by the objectives for the land, the effectiveness of the control techniques on the target species, environmental factors, land use, economics, and the extent and nature of the weed infestation.

Prior to deciding which control measures are most appropriate, a land manager should: 1) inventory and assess the land to identify the target weed species and determine the size of the infestation; 2) assess non-target vegetation in the management area; 3) determine soil types, climatic conditions, and important water resources; and 4) determine the limitation of various control methods.

An accurate assessment of the target infestation will help determine the most appropriate control method or methods for the weed species. A small patch of leafy spurge could be economically and easily controlled with a residual herbicide application and continued vigilance of the landowner. A large, dense infestation of leafy spurge may require establishment of a grazing program or biocontrol agent, combined with a herbicide containment program.

All control options have limitations. If complete eradication of a weed is necessary or possible, biocontrol agents arc not a good choice. if soils, proximity to water, the presence of threatened or endangered species, or other environmental concerns preclude use of a herbicide, mechanical or biological controls may be the best choice.

Weed control methods

Prevention: Preventing the establishment or spread of weeds by seeds or vegetative propagules should be the first line of defense in developing a weed management program. There are many potential weed threats to lands, and land managers need to be aware of the threats, identify unknown species, and implement effective controls immediately.

Quarantines and embargoes can be effective in isolating and preventing the spread of weeds into new areas. Weed-free hay programs and certification programs for agronomic crops are designed to stop movement of new species into areas where they are not currently found. State seed laws are enacted to insure the purity of seed for planting, thus helping to prevent the spread of weeds.

Education: Weed identification and the early detection of new invader species benefits the prevention program. Publications and computer programs arc available that offer excellent information and color pictures of important weed species in the western United States. Ongoing weed management education of weed district and federal agency personnel and private land-owners is an important part of a weed program. This includes yearly training seminars for land managers and state pesticide applicator certification programs.

Educational efforts targeting the general public emphasize what weeds are of concern and why they are a threat. These programs have included brochures, bumper stickers, radio announcements, and tours of areas with serious weed problems. They all emphasize the effect of noxious weeds on the land.

Physical or Mechanical: The use of physical methods to eliminate weeds can be effective on small infestations. Handpulling, hoeing, tilling, mulching, burning and mowing are all commonly used methods. Physical/mechanical methods are labor intensive for perennial, deep-rooted weeds, making them expensive treatments that are often not effective.

Handpulling and hoeing are most effective where there is a limited weed infestation and soil types allow for the complete removal of plant material. Small infestations of spotted knapweed have been effectively pulled in areas with loose soil near water where other methods were unacceptable. Pulling is not recommended in dense infestations where native vegetation is unavailable to replace the pulled plants.

Tilling is generally limited to cropland situations. Canada thistle has been effectively controlled when tilled every 21 days throughout a growing season. Some rhizomatous weeds, such as field bindweed, are spread rather than controlled by tillage. Mulching is most effective against annual weeds.

Burning can be an effective set-up treatment for herbicide applications by removing old vegetation that would interfere with herbicides as they are applied. Burning should be timed properly to minimize damage to non-target plant species.

Mowing reduces seed production in some plants. It is most effective on annual and biennial plants. Timing and frequency of mowing varies with each species. It has been shown that mowing can increase seed production in diffuse knapweed, while spotted knapwecd can show a seed decrease when mowed in the flowering stage.

Biological: Biological control involves using living organisms, such as insects, pathogens, or grazing animals, to suppress a weed infestation. Biological control attempts to recreate a balance of plant species with their natural enemies. Since many serious rangeland weeds are introduced species, they have few established natural enemies on this continent. Classical biocontrol focuses on introducing natural enemies from the weed's area of origin to local plant populations. Biocontrol methods generally suppress host weed populations, but may not contain or eradicate them. it is most effective on dense weed infestations over large areas. This publication is designed to aid the implementation of biological control in weed management programs.

Chemical: Herbicides are categorized as selective or non-selective. Selective herbicides kill a specific type of plant, for example, 2,4-D kills only broadleaved plants. Herbicides are also selective based on the amount used. Spotted knapweed generally is controlled using less herbicide than leafy spurge; the lower herbicide rate causes less impact on non-target broadleaved species.

The most commonly used herbicides for noxious weed management are 2,4-D, picloram, dicamba, clopyralid, and glyphosate. With the exception of glyphosate, they are all auxin-type compounds that are selective for broadleaved plants, making them effective tools in some environments for controlling weeds while maintaining valuable grass forage species. Glyphosatc is non-selective; care must be taken when it is used around desirable grass and non-target plants.

Be aware of important licensing and training recltiirements when using herbicides. Environmental concerns make it critical to follow all label and site directions.

Cultural: Cultural methods of weed control that enhance the growth of desirable vegetation may help slow weed invasion. Plant competition, smother crops, crop rotation and allelopathy are methods often most suited to cropland agriculture. When dealing with weeds, it is important to maintain healthy native or desirable vegetation to allow adequate competition once the weeds have been suppressed. Fertilization and reseeding with competitive, adapted species may be necessary in areas without a residual understory of desirable plants.

Long-term land management: Follow-up management is especially important on range and pasture lands. It determines the longevity of control obtained by the methods used. Because noxious weeds have persistent growth characteristics and seeds can remain viable in the soil for years, long-term control programs must be implemented.

Long-term management includes continued cultural, mechanical or biological control practices, or retreatment with herbicides to maintain low weed populations. Range improvements, such as grazing systems, cross fencing, and water development, will help retard reinvasion of many weed species. Degraded sites with no desirable species should be reseeded to a competitive plant species as a part of the management program.

Integrating biocontrol with other control methods

A weed infestation may increase in density and area faster than the newly released biocontrol agent populations, therefore other control methods must be used in conjunction with the release of biocontrol agents. The perimeter of the infestation may be sprtyed to keep the weed from spreading while the main infestation is grazed by sheep or goats, if appropriate, to suppress the weed population and reduce seed production. As biocontrol agents increase in density and begin to occupy more area, herbicide use or grazing animals may be reduced to provide more resources for the biocontrol agents. Eventually, a spray program might be reduced to occasional spot treatments. When the target weed species has been eliminated from the area, the control program may be discontinued. However, the land manager must continue monitoring the area for weeds for five or more years.

Timing of herbicide applications may be an extremely important factor in the interaction of biocontrol agents and the host plants. Herbicides should be applied when their effects on the host plants will not interfere with the life cycle of the biological control agents. Indirect effects of herbicide applications might become apparent if the sprayed weed dies or the foliage becomes unpalatable before the biocontrol agent has completed its development.

Research continues on the interactions between biological control agents and other weed management techniques.

Suggested Reading

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Managing Insect Biological Control Agents

Once biological control agents have been cleared for release into the United States, it is important that they be handled, released, and managed in a manner that allows them to survive in their new environment. This section deals specifically with the collection, redistribution, shipping, release and monitoring of insect biological control agents. Specific information regarding the handling and distribution of disease organisms can be found in the descriptions of specific diseases in **Section II.**

Redistribution

Once a biological control agent becomes established and increases enough to be easily collected, it is advisable to redistribute the species to new locations, especially if the agent is poor at self-dispersal. Redistribution expands the agent's distribution and reduces the likelihood that the entire colony will be destroyed by some disaster. Different species are collected in various ways, such as with a sweep net or black light, by hand collecting, or by rearing from galls. Collections should be made with a minimum of stress to the agent and its time in captivity should be minimized. Collected females may deposit eggs in their container, so the longer they are left in the container, the more eggs that will be wasted.

Collections can be made in cooperation with local weed districts or federal agencies, or by individuals or commercial collectors. The Animal and Plant Health Inspection Service (USDA-APHIS) is the federal agency generally responsible for redistribution of certain biocontrol agents. They have established nurseries, or *insectaries*, to increase insect numbers and to help states and local weed districts distribute insects.

Make sure that all insects to be transferred to the new location are the proper species. If any doubt exists, have the agents identified prior to release by an experienced entomologist or by the Systematic Entomology Laboratory, USDA-ARS, 10300 Baltimore Ave., Room 101A, Bldg. 046, BARC-West, Beltsville, MD 20705-2350. It is also advisable to have the insects checked by an insect pathologist to ensure that the colony is disease-free. To find an insect pathologist in your area, check with your state university, the Extension Service, your state Department of Agriculture, or with USDA-ARS or USDA-APHIS field offices.

Redistribution of introduced biological weed control agents should be documented whenever the distribution is a significant distance [generally more than 25 miles (46 kilometers)] from where they were collected, particularly if distributed to another county or state. The USDA Form AD-943, *Biological Shipment Record - Non-Quarantine* (see **Appendix 3**), is available for these shipments. This form indicates what information should be recorded and provided to the scientists responsible for evaluating the biological control program. Shipment forms are available from USDA-ARS Biological Control Documentation Center, Insect Biocontrol Laboratory, Rm. 2, Bldg. 004, BARC-West, Beltsville, MD 20705-2350.

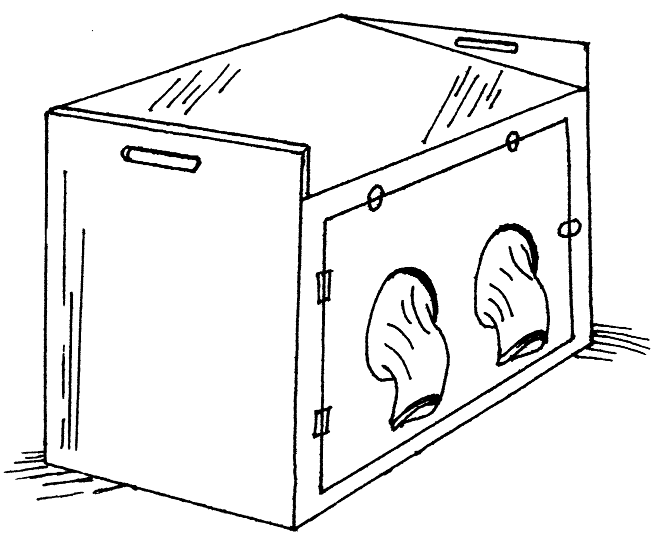
Collection

When collecting agents, be sure you can accurately identify the agent. Contact your local weed district or state Department of Agriculture if you have specific questions. Get permission from the landowner with the insectary site before collecting. Commercial collectors are a source for those who do not wish to collect for themselves. Many states maintain lists of commercial sources of biocontrol agents.

Hardy insects, such as adult beetles, can be collected in large numbers using a sweep net. Do not confine biocontrol agents to the net bag for prolonged periods. Some flies, moths, butterflies, soft-bodied larvae, and nymphs can be collected with a sweep net if they are removed from the bag after each swing. Very delicate flies can be collected by gently brushing the net over the foliage or knocking any material clinging to the foliage into the net bag and then transferring the flies to a collecting jar or tube. Sheets can be placed on the ground under large bushes and the bushes hit with sticks to dislodge insects, which can then be collected by hand.

Sorting the biocontrol agents from other insects and debris is extremely important. Spiders and other predators will feed on collected agents if confined in the sweep net or shipping containers. Sorting will also keep potential pests from being moved to other areas.

Biocontrol agents that are collected in seed heads or roots can be reared in a box with a window until they can be sorted. Generally, a sleeve box (a box with a window, screen back, and openings with sleeves for arms) can be constructed without too much effort. Again, this will help ensure that parasitoids and predators will not be transported with the agents.



Sleeve box

Shipping

Before moving collections of biocontrol agents across state borders, check with your state Department of Agriculture concerning regulations and restrictions. States generally require a permit for the importation or movement of biocontrol agents within or into the state. Generally a USDA-APHIS-PPQ Form 526 permit (see **Appendix** 3) is required. It is the responsibility of the party receiving the biocontrol agents to obtain any permits, although sometimes suppliers will provide this service. Contact your local weed district, cooperative extension agent, or Department of Agriculture for assistance, if needed. These agencies also like to be kept informed and are often willing to work with you on redistributing biocontrol agents.

After making collections, the biocontrol agents should be sorted from other species, counted, and stored in containers or cages that will allow them to exercise and feed until they're shipped. It is best to ship or transfer insects quickly and release them as soon as possible after collection. This will prevent excessive loss of insects due to confinement and loss of eggs laid in the container. Biocontrol agents can be refrigerated for several weeks if absolutely necessary, but they must be provided with intermittent pcriods of warm temperatures to allow feeding and exercise.

Shipping insects is sometimes difficult, especially if they must travel through commercial shipping companies (U.S. Postal Service, United Parcel Service, Federal Express).They must be packaged so that they will not be damaged and they must be kept cool until released. The best shipping containers for insects and mites are nonwaxed cardboard ice cream cartons that "breathe." Lids should be taped around the edges to prevent escape; make sure that the sticky part of the tape is not exposed to the insects or mites. Plastic or glass containers are not recommended because they allow moisture to build up which increases biocontrol agent mortality.

Biocontrol agent shipping containers should have enough host plant material to serve as a food source, a moisture source, and a substrate that allows the biocontrol agents to climb and spread out. Do not ship parts of the plant that might reproduce at the new location, such as root material or seed.

Cartons containing insects and fresh plant material can be stored briefly in refrigerators no colder than 40° F (4° C) or in cool ice chests until shipment/transfer. Ice chests with ice packs work well for shipment. It is best when they can maintain the desired temperature for 24 to 36 hours. Ice packs should not touch the insect container. A layer of newspapers will help prevent condensation on the shipping containers. Make sure that cartons and ice packs are securely positioned within the shipping box. Shipments should be made within 24 to 48 hours of collection.

When insects are carried in containers for short distances, make sure they have something to walk on (plant material, wadded-up tissue paper, or a towel). If they become trapped on their backs they will die in a relatively short time. Supply water in moist cotton or a sponge to adult insects that do not feed on foliage. Be careful that excess water does not escape and drown the agents. Flies and moths can be fed a honey-water mixture from a piece of sponge affixed to the container lid.

Releasing

Several factors are important in selecting sites for releasing and establishing insect biocontrol agents. The site should be free from chemical treatments such as insecticides and herbicides, or at least free from the future use of these chemicals when they would interfere with the biocontrol agent's life cycle. The field should also be free from grazing animals during the establishment of the biocontrol agent, although grazing by wildlife probably won't threaten the establishing colony. Fencing the release site may be the best method to avoid disturbance and allow agents time to establish.

The release site should provide elements of the environment that are necessary for the survival of the biocontrol agent, including: 1) a sufficient supply of the target plant; 2) adequate moisture; 3) sufficient flowering plants for adult nectar feeders; 4) wind breaks for delicate insects; 5) sunny areas for heat-loving insects; 6) shrubs and trees for insects that use them for protection and rest during the day; and 7) hiding places that are relatively free from parasitoids and predators such as ants, rodents, and birds.

Specific site requirements are known for some agents, while more research is needed for others. Refer to the "Habitat" section for each individual agent in **Section II** for more specific information on biological control agents of interest to you.

Generally, 100 to 500 insects are released on a host plant population of moderate density. Tall, dense stands of plants sometimes provide too much shade, while moderately dense stands allow more insect movement and provide more sunlight, thus improving the chances for establishment. Releases should be made in areas free from grazing, flooding, or general traffic. Herbicides and insecticides should not be sprayed nearby because chemical drift could jeopardize biocontrol agent survival. The initial release site should be protected for five to 10 years. Secondary sites may not need as much protection once the biocontrol agents are established.

If the agent is a strong flyer, has difficulty finding a mate, or is released in very low numbers, cages may be needed. Flying insects require a large, tall cage in which they can fly to attract mates, visit multiple host plants, and exercise. Small insects that do not readily disperse can be confined in much smaller enclosures. A "cage effect" results from limited space, from cage walls on which the insect can climb reducing the time spent on or near the host plant, from higher humidity, and from the unnatural and flexible cage fissures that affect survival of the agent. Larger cages produce fewer cage effects.

Cage fabric is also an important factor in insect survival. For example, metal fabric holds the heat of the sun and, although the temperature within the cage may be acceptable, the metal may be many degrees warmer and thus harm the resting agent. Fiberglass fabric cages can be damaged by chewing insects such as grasshoppers and crickets. Saran® fabric cages are excellent, but become discolored and weakened within a few years depending upon use and care. Long-term maintenance of cages is important.

Release data should be gathered and entered on an appropriate documentation form (see Appendix 3).

Obtaining landowner consent

Always obtain the consent of the landowner to release the biocontrol agent if you do not own the land where you are making a release. A written, cooperative agreement allows you maintain control of the agents and protects the agent from chemical spraying or other cultural practices such as grazing, mowing, and plowing. (See the sample agreement in **Appendix** 3.) These agreements also protect the landowner. They can be written for a limited duration: five years is generally the shortest time a biological control agent requires to build sufficient numbers to survive most natural or chemical disasters. A period of 10 years is more realistic.

Monitoring release sites

Once a biological control agent has been released, specific information about what is happening in the field should be collected. Questions to be answered include: 1) Is the agent established? 2) Are numbers increasing or declining? 3) is the species affecting the target plant as expected? 4) Is the weed population increasing or declining? If the weed population is declining, what new plant species is dominating the area? 5) What are the biotic and abiotic forces acting on the biocontrol agent? 6) How does the agent relate to other established organisms? 7) Has the population reached a level conducive to redistribution?

Monitoring the release site is the most effective way to answer these questions. The sampling method for monitoring depends on the way the agent uses its host, the suspected density of the agent at the time of sampling, the life cycle of both agent and host, the desired accuracy of the data to be obtained, and the amount of effort, labor, and money that can be expended on sampling.

Active, attractant, and passive methods of sampling insects are described below.

• Active Sampling

Before-and-after photographs: This is one of the simplest methods for recording biocontrol results. Photographic records from consecutive years, taken at approximately the same time each year from the same location and with the same horizon, may be compared to determine the change in the plant community. This method does not provide quantitative information about the density of the weed, density of the biocontrol agent, or the type of damage inflicted on the plant, but does display the conditions at the time of the photograph. Unlike memory, permanent photographic records are reliable and display visual differences.

Observation: Observation is often the easiest but least statistically reliable method of sampling. It provides limited information about the occurrence of the weed and the agents at the site, agent activity during the observation period, the type and amount of damage inflicted on the host, and how this damage has affected the plant.

A better method combines observations within a defined boundary, such as rings or staked plots of a known size, with counts of a particular species in that area. To collect data that can be evaluated, observations must be repeated many times. Additional information can be obtained by sorting the data by location of the agent that is, on stems, leaves, flowers, etc., or on the soil surface. Data that should be recorded includes: the time of day that the counts were taken, air and ground temperatures, wind velocity, sun or shade, moisture conditions and levels, and plant density and conditions. Sufficient samples allow determination of whether the biocontrol agent is bunched or uniform throughout the area and the type of conditions it favors within the ecosystem.

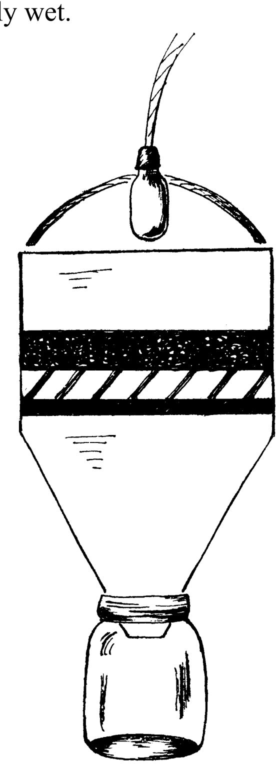
Daubenmire frame and ring sampling: When specific areas are to be sampled and specific data are required, squares, rectangles, or circular sampling grids can be used. Plant sampling frames of specific size, such as Daubenmire frames, can be placed on the ground and all plant material within counted, measured, identified, clipped, sorted, and weighed to determine the plant composition, canopy cover, and biomass of the area. Samples taken periodically over an extended area will provide data on shifts in plant densities and plant composition. Samples for which the exact sample area, time, and conditions are known can be statistically analyzed. The results can provide a reliable measure of biocontrol agent impacts.

Paired samples of treated areas versus untreated controls provide data showing the effect of particular treatments. Radial sampling over time at predetermined distances from a release point provides population expansion data.

Sweep net sampling: Sweep net sampling is conducted when the agent is on or is found near the host. This sampling method is generally restricted to times when the agent is feeding on the flowers, leaves, or stems, or when the adult agent is attempting to lay eggs. Therefore, it is important to be familiar with the agent's life history and habits.

Sweep net sampling can be somewhat quantitative if it is done by the same individual and in the same manner. Otherwise, the results may reflect the sweeping style of each sweeper instead of the actual variation in the population of the agent. Agent density can be assessed by comparing the number of insects collected with the number of sweeps taken or the period of time spent sweeping. Amount and type of vegetation, excitability of the agent, aspect of the terrain, air and ground temperature, and wind velocity all may influence the number of organisms collected. Results can be standardized by comparing the number of insects swept per given area with numbers of insects observed or collected per given area by a more precise means of sampling.

Berlese funnels: Berlese funnels have a heat source at the top that causes soil-inhabiting organisms to try to escape the heat. A screen or wire mesh funnel holds the soil. As the organisms move downward, they are funnelled into a preservative-filled collecting container. This device is effective in collecting many soil-inhabiting organisms. For best results, soil should not be excessively wet.



Berlese funnel

Dissection: When biocontrol agents attack within a plant, plant material must be collected and dissected. This provides information about: the infestation rate of the host population; the number of agents per host; the stages of development of both agent and host; the species composition of agents when numerous agents are involved; where the agent resides within the host; and the amount of damage inflicted by the agents. If the sample size is sufficient, data will provide accurate indications of the agent and weed population densities.

Suction or vacuum: A gasoline-powered suction machine (D-VAC®) can be used to collect live insects from plants in the field. Unless all plant material is strongly agitated in the suction area and the soil area also vacuumed, many specimens may be missed because some species are very difficult to dislodge from debris and vegetation. Therefore, this is an unreliable method of determining density unless the vegetation is checked afterward to confirm that all organisms have been collected.

Vacuums can also be used to catch flying insects, but the height of the vacuum mouth and time of day will affect which species are collected.

Digging: Various stages of insects and nematodes are found in the soil or in the root system of the target weed. Mesh screens can be used to sift the soil and expose the agent. Water may be used to float the specimens. Root dissections can reveal the biocontrol agent in its natural surroundings.

Water sorting: Mites, nematodes, and other small organisms are often sampled by dissolving small samples of the soil in water and checking the fluid under a microscope. By knowing the volume of soil washed and the number of organisms obtained, one can calculate the density of the population. Samples are often taken at continuous levels to determine at what depth in the soiol the species is most concentrated.

• Attractant traps

Black tight traps: Black or ultraviolet light traps are commonly used to attract flying insects. These traps are usually operated at night. Not all types of insects are attracted to the light because all species do not detect the same wavelengths of light. Moths and butterflies, flies, some beetles, and lacewings are most often collected.

Species that are attracted to black light can be collected from long distances. Insects fly against the trap baffles, fall into a funnel, and are collected in the lower trap where they are either killed by chemicals or cold, or held until they can be examined. A black light placed against a white bed sheet is sometimes used. The specimens land on the sheet and then can be hand-collected. Either method may be used to determine what is in the vicinity. However, unless testing has previously determined the ratio of collected organisms to those available per given area, this method cannot be considered quantitative.

Pheromone traps: Pheromones are chemical attractants or odors that are usually species specific. Commonly, pheromones are sex attractants and lure only males or females. Minute quantities of pheromone are carried for great distances on the wind. If the area that the pheromone covers can be calculated, the approximate density of a population can sometimes be determined. This method can be used to attract biocontrol agents into cages without harm.

Entrance traps: Entrance traps are screen traps with a passage that allows the organism to enter, but not leave. These traps are often baited with some type of food.

• Passive sampling

Pitfall traps: Pitfall traps use containers that are buried with the opening flush with the soil surface. They are charged with a preservative chemical (such as antifreeze) for killing the captured specimens. A pitfall trap collects insects (and, in some cases, small rodents) running or walking on the ground and flying insects attracted by the odor of the preservative. Moths, butterflies, and some flies may be difficult to identify after being trapped in the preservative, but most specimens remain pliable many days after their capture. Again, this is a general method of collecting organisms and does not provide an accurate indication of species density.

Sticky traps: Sticky traps are similar to the old sticky fly traps. A strip or board is covered with a non-drying sticky material and placed in the vicinity of the species. Sometimes a food attractant (such as sugar) is added or a bright yellow or orange color is used to attract certain flying species. The main problem with these traps is that to properly identify species, the

Making Biological Control Work in the Field

Biological weed control programs are a desirable part of a land management program. To prevent problems that can waste time, money, and scarce biological control agents, the land manager needs to have the proper knowledge and experience to develop and implement a program. Valuable knowledge includes: 1) which species attack your target weed; 2) which species will do best in the environment of your target site; 3) knowing whether the agents you want to release have already been established nearby; 4) knowing whether agents are free from pathogens or parasites; 5) knowing of potential predators in the area; and 6) proper identification of both the biocontrol agent and the host plant.

The following precautions can help prevent problems when developing a biological control program:

1) Learn as much as possible about biological weed control and how it works prior to initiating a program on your land. Know which biocontrol agents feed on your target weeds and determine which of these agents will work best under your land management and environmental conditions. Understand how to integrate biocontrol into your land management practices. Know how to monitor the biological agent population and its effect on the weed. Much of this information can be learned from local weed control departments, state universities, state and federal Departments of Agriculture, some offices of the Bureau of Land Management, Forest Service, Bureau of Indian Affairs, USDA-APHIS, and from the USDA-ARS Rangeland Weeds & Cereals Research Unit in Bozeman, MT.

2) Work with personnel from your local weed control department and keep them informed of your biocontrol agent releases and their locations. This allows them to protect the agents from potential spray programs and to report the biocontrol programs to the state Department of Agriculture. Local weed departments can also supply you with updated information as it becomes available and assist in the success of your integrated program. 3) Obtain proper, healthy species of biocontrol agents. You may request from any biocontrol agent supplier a pathologist's or taxonomist's written statement certifying the health and species of the agents you receive. Or, you may consult a pathologist or taxonomist yourself. (See **Redistribution** on p. 17.)

4) Select the best biocontrol agents for your habitat and release those agents on the areas of your property that best meet their requirements. Protect the release area from grazing, chemical sprays, and general disturbance until the insects are well established (possibly up to five years).

Within the core area, the agents can increase in number and area occupied and migrate to other weed-infested areas. When numbers warrant (generally after three or four years), you can collect from this core area and redistribute to more distant locations. Removal of these "starter colonies" at this time should not noticeably diminish the effect of the established colony.

5) Continue your current weed management program outside the biocontrol agent release areas while you arc waiting for the biocontrol agents to establish and multiply. For biocontrol agents that don't disperse rapidly, determine annually how far the colony has spread and calculate a buffer zone. Beyond this zone, continue other weed control programs. Although the biocontrol agent population may increase each year, the weed also will increase each year. Using faster-acting management techniques around the infestation's perimeter allows the agent to catch up and suppress the weed over time.

6) Photograph the colony site at the same time each year from the same location and with the same horizon in the viewfinder. These photographs will document the extent of the weed infestation and the yearly impact of the biocontrol agents.