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# Douglas-Fir Tussock Moth Handbook

# Techniques for Monitoring the Effects of Insecticides on Forest Fauna



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by Patrick J. Shea,[1] Richard C. Reardon,[1] and Stamford D. Smith[2]

In 1974 the U.S. Department of Agriculture initiated the Combined Forest Pest Research and Development Program, an interagency effort that concentrated on the Douglas-fir tussock moth in the West, on the southern pine beetle in the South, and on the gypsy moth in the Northeast. The work reported in this publication was funded in whole or in part by the Program. This manual is one in a series on the Douglas-fir tussock moth.



**Pesticide Precautionary Statement**

This publication reports research involving pesticides. It does not contain recommendations for their use, not does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

**Caution:** Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

## Introduction

Information from many sources, published and unpublished, was used in preparing this handbook. The principal source of information was a study in northeastern Oregon conducted as part of the U.S. Department of Agriculture's Expanded Douglas-Fir Tussock Moth Research and Development Program. The purpose of that study was to determine the effects of three chemical insecticides on forest fauna. Results of that study are not reported here, but information about the techniques used for monitoring was drawn primarily from that work.

This handbook describes some methods and techniques for evaluating or monitoring the effects of insecticides on nontarget organisms (organisms other than the insect pest to be controlled) during research studies or during pilot control or operational spray programs in forests (fig. 1). Resulting data are used to determine whether an insecticide harms various components of the ecosystem and may be provided to the Environmental Protection Agency (EPA) for registration purposes. The Forest Service uses such data to decide on options for managing outbreaks of forest insects.

[1] Supervisory Research Entomologist and Research Entomologist, U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station, Davis, Calif.
[2] Associate Professor, Central Washington University, Ellensburg, Wash.

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Figure 1.—Monitoring is done during pilot control or operational spray programs in forests.

Nontarget monitoring studies are usually conducted during pilot control or operational spray programs. Since most candidate insecticides have already undergone extensive safety testing by this time, the goal is usually to test for unforeseen side effects. The sampling methods depend on which faunal groups are most likely to be affected. Selection of nontarget faunal groups for monitoring depends on: (1) chemical and physical properties of the insecticide being applied, (2) assessment of hazard in preliminary studies, and (3) laboratory evaluation of potential acute and chronic effects (National Academy of Sciences 1975). Because many variables interact with insecticides under field conditions (Tucker and Crabtree 1970), the selection of faunal groups to be studied should be made carefully. Insecticides may cause two types of selective toxicity—ecological and physiological. Ecological toxicity refers to the faunal species encountering the insecticide. Physiological toxicity refers to the morphology and biochemistry of the species and is related to the action and structure of the insecticide. For example, organophosphate and carbamate insecticides often cause cholinesterase inhibition,[3] and appropriate methods are needed to detect this effect on faunal groups exposed to spraying. The persistence of the particular insecticide will determine whether short- or long-term effects or both should be anticipated.

[3] Cholinesterase inhibition is the inactivation of the neural enzyme cholinesterase resulting in acetylcholine accumulation and stoppage of nerve transmission.

# Considerations in Sampling

Monitoring is done in the research study area, the general area designated for pilot control or operational spraying, or in other designated study areas such as adjacent downwind sites where the insecticide might be expected to drift. Study areas should be large enough to minimize movement of species in or out of the area, should be replicated, and should be sufficiently separated to avoid interaction of faunal populations. Each study area should be mapped and habitats described with their locations shown on the map. Within habitats, sampling stations or collecting sites are selected, with strata designated by the faunal groups to be studied.

A study area should have the following features: (1) a substantial population of the target insect; (2) one or more permanent streams with riparian vegetation; (3) accessibility by road; and (4) absence of major disturbances, such as logging and road construction, at least for the year of study and longer if indirect and long-term chronic effects are to be determined. In that case, the faunal groups should be monitored for 1 to 3 years after spraying.

Nontarget monitoring studies must be done in coordination with other studies aimed at measuring effects of the insecticide on target insects. The success of spray treatments for control of the target insect should be weighed against effects on nontarget fauna. Then, depending on the degree of hazard posed to forest stands, tradeoffs can be adequately considered.

# Sampling Faunal Groups

The faunal groups discussed in this handbook include small mammals, passerine birds, terrestrial arthropods, aquatic insects, and fish. All except terrestrial arthropods are included in the general wildlife effects guidelines set forth by EPA,[4] which include species that are important components of forest ecosystems or that can serve as indicators of damage to the ecosystem.

Techniques to detect fluctuation in quality and in numbers of individual species or related species are described and discussed for each faunal group. These techniques will usually provide an indication of acute (immediate) effects. More sophisticated methods are needed to detect chronic effects over an extended period.

## Small Mammals

Small mammals, such as deermice (*Peromyscus maniculatus*), are useful for evaluating the impact of insecticides because they are often plentiful, easily trapped, and have a relatively high reproductive rate (fig. 2). Their close relationship to laboratory animals used in establishing toxicity profiles can also add to their relevance as subjects for field investigations. Densities of small mammals, however, are difficult to estimate (Nikiforov 1967).

Mammals are trapped before and after spraying to get an estimate—or census—of the population. Both treatment and check areas must be

[4] Federal Register, Monday, July 10, 1978. Part II. Registration of Pesticides in the United States.

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Figure 2.—Small mammals are useful for evaluating the impacts of insecticides.

3A

3B

Figure 3.—A live trap is used to collect small mammals (A) and weight of the animal is determined (B).

sampled adequately for 3 to 7 days before insecticides are applied. Trapping is done on a grid system or along line transects in similar or comparable habitats. The data can be used to estimate population numbers, mortality, and movement of animals out of the study area.

The capture/recapture technique is most often used to census a population. This involves repeated live trapping of mammals in the same area (figs. 3A and 3B). During the first trapping, animals are tagged or otherwise marked for future identification (Taber and Cowan 1969). Species, sex, weight, age class, and physical and reproductive condition of each animal are recorded. Since it is impossible to trap all the animals during the first trapping effort, second and subsequent trappings should yield both marked and unmarked animals; the last few trappings should have a higher percentage of marked animals. Detailed discussions of capture/recapture studies are found in Smith and others (1972) and Otis and others (1978).

Studies can also be conducted to determine the reproductive success of small mammals in both sprayed and untreated areas. Animals are dead trapped (the trapping device kills the animals) in areas away from the capture/recapture plots, both before and after spraying. Uteri are removed and examined for numbers of embryos and placental scars. Scars are classified as old or new. At least 10 females should be collected during each sample period. With this technique, mean litter size and trends in litter size can be determined and compared. Martin and others (1976) discuss some reliability problems when only scars are used to estimate litter size. Carcasses of animals can also be used for analysis of insecticide residues.

**Passerine Birds**

Passerine birds, such as the dark-eyed junco (*Junco hyemalis*), are important in forest ecosystems. Many species are wholly or partially insectivorous and play a role in regulating forest insects.

Several techniques for taking a bird census are available. Censuses must be conducted in similar habitats in both treated and untreated areas. The number of birds and species can vary greatly, depending on the habitat. It is not appropriate, for example, to draw conclusions about effects of insecticides if one set of data is obtained from a closed stand of Douglas-fir and another from the interface between forest and riparian zone.

*Spot mapping* (Williams 1936) is one of the most widely used techniques for taking a bird census; it is sometimes referred to as the international mapping method (Robbins 1970, Franzreb 1977). A grid system is established in both treated and untreated areas. The grid consists of parallel lines that form a square or rectangle. Stakes or flags are set out along intersecting lines and identified by a number and letter code.

Census walks (fig. 4) are conducted in early morning (daylight) when birds are very active. The census taker, using accepted symbols (Robbins 1970), records the location of each bird seen or heard on a gridded map of the plot. After several censuses, the breeding territory of individual pairs is determined and plotted. Censuses should be conducted in both treated and untreated plots before the insecticide is applied and after spraying until the breeding season is over and defended territories begin to collapse. Data are expressed as number of breeding pairs per hectare.

For the spot mapping technique, the grid should be constructed so a stream with riparian vegetation bisects the plot and is bounded on both sides by forested slopes. This increases the probability of observing many birds of different species and thus increases the representativeness of the monitoring effort.

The *fixed station* census provides an index of the relative abundance of birds (Richmond and others 1979). There are at least two variations of this method. In one variation, census stations are established inside both treated and untreated areas and birds seen or heard are counted for 5 minutes. Counts are repeated several times before and after the insecticide is applied. In the other variation, a small (0.2 hectare (0.49 acre)) square or circular plot is established around the census station, and only birds heard or seen inside the plot are counted. In both variations, results are expressed as mean number of birds identified per station.

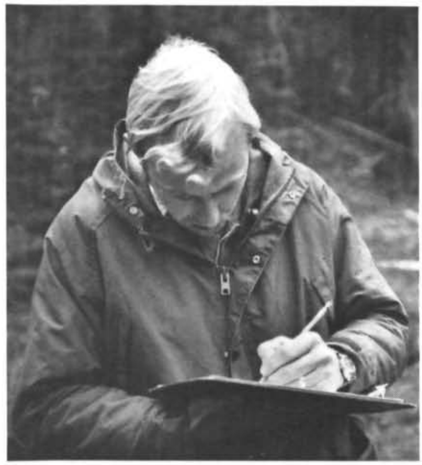

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Figure 4.—Census walks are done in the early morning.

The *line transect* method is the simplest, but generally the least sensitive, technique for detecting effects of insecticides on birds. Songbirds are counted either by sight or song along a preestablished line, usually 1 or 2 kilometers (0.62 to 1.24 mi) in length (Baird and others 1978). Census walks are taken for several days, usually 5, before insecticides are applied and then again several days after spraying. Walks are taken at the same time of day, preferably in early morning. Results are expressed as number of birds observed per kilometer. Counts tend to be variable and consequently are insensitive to other than catastrophic events, such as a mass killing of birds, induced by insecticides.

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Figure 5.—Nest boxes can be used to supplement nesting sites for study of some cavity-nesting species.

*Nesting studies* can be a sensitive indicator of the effects of insecticides on birds. Nests located in both treated and untreated areas are checked periodically to determine clutch size, hatching success, and fledgling development. Artificial nest boxes (fig. 5) can be used to supplement nesting sites for some cavity nesting species (Richmond and others 1979). It is important that there is enough time before treatment to find sufficient nests for an adequate sample. Nests and their contents should be handled as little as possible. Ground nests are particularly vulnerable to predators, so care must be taken not to disturb the surrounding vegetation.

*Search for casualties* should be conducted after spraying; this can be done in conjunction with census and nesting studies. The number of person-hours spent searching for dead birds and any unusual behavior—such as birds unable to maintain a perch, erratic flying behavior, and occurrence of canopy feeding species foraging on the forest floor—should be recorded.

Unusual behavior may be related to application of carbamate and organophosphate insecticides, most of which inhibit cholinesterase enzymes. Techniques have been developed (Dieter and Ludke 1975; Zinkl and others 1977, 1979) to assess the effects of these types of compounds. Cholinesterase levels in brains of birds collected in both treated and untreated areas can provide data on the duration and magnitude of exposure. Birds can be collected either by mist netting or shooting. A diversity of species should be collected with at least three individuals of each species per sampling period. The specimen must be placed on dry ice immediately to prevent deterioration of the cholinesterase enzyme and analyzed within 24 hours of collection.

All these techniques have advantages and disadvantages. For example, the spot mapping technique, by repeated censuses, allows the investigator to become familiar with specific individuals and thus detect subtle changes in behavior or in bird replacement. It is, however, very subjective, relying heavily on the skill of the observer. Also, establishment of plots is time consuming. With the fixed station technique, care must be taken not to include individuals from more than one station. A general problem is that birds that flock can artificially inflate the count.

**Terrestrial Arthropods**

Many techniques are available to collect the numerous species of arthropods found in forests; but there are few analytical methods to evaluate the effects of insecticides on these species because invertebrate populations can fluctuate widely; their distributions are irregular; and there is a lack of information on population dynamics for many species. Southwood (1966) discusses three methods of estimating population density: (1) absolute estimates—numbers per unit area of habitat; (2) relative estimates—population density measured in relative terms as, for example, by counting numbers per unit of time; and (3) population indices—relative abundance of products created by arthropods—such as frass, webs, nests, or the intensity of effects on plants or host insects.

*Relative estimates* are most commonly used to estimate population density of insects. These are obtained by numerous techniques in two broad groups: (1) catch per unit effort (for example, sweep-netting, shaking, and beating methods or visually searching an area for a given length of time) and (2) trapping (for example, Malaise traps, pitfall traps, sticky traps) (Ruesink and Kogan 1975). Interpretation of data is difficult because of changes in numbers of insects with time, differences in their behavior during development, changes in activity, and differences in responsiveness by sex and developmental stage to trap stimuli. Nevertheless, relative estimates are useful for comparing the numbers, biomass, and diversity of species from year to year and for estimating emergence dates.

In some situations (for example, beating methods used by the Canadian Forest Insect and Disease Survey), long-term fluctuations in composition and abundance of species can be used to estimate absolute populations.

Sampling should be done at predetermined intervals before and after insecticides are applied and at the same locations in subsequent years. Several sites should be sampled and more than one technique used to estimate population density. Seasonal timing depends on the arthropod species and toxicity and persistence of an insecticide.

Three groups of terrestrial arthropods —parasites[5], predators, and pollinators—demonstrate varying degrees of susceptibility to insecticides. Parasites and predators are part of the natural enemy guild that regulates insect populations; they can be affected directly by an insecticide or indirectly by effects on their hosts or prey and also by toxic residues passed along food webs (Croft and Brown 1975). The short-term effects of nonselective insecticides include reductions in both numbers of a species and the diversity of species (Brown 1978). The long-term cumulative effect of nonselective insecticides is likely to be fewer parasites and predators, which might result in secondary pest outbreaks. If applied only 1 year, the effects on the parasite complex are likely to be minor and temporary in subsequent years (Carolin and Coulter 1971).

Pollinating insects are exposed to insecticides by bodily contact with the residues on plants or pollen or by imbibing contaminated nectar and water. Bart and Hunter (1978) provide an annotated bibliography on the ecological impacts of selected insecticides.

[5] The term "parasites," refers only to parasitic insects and not to parasitic bacteria, fungi, protozoa, or nematodes.

**Parasites**

Two orders of insects, Hymenoptera and Diptera, contain species important for biological control of forest defoliators. Hymenoptera are dominant in numbers of parasitic species, with the families Ichneumonidae and Braconidae in the superfamily Ichneumonoidea and several families in the Chalcidoidea being the most common. In Diptera, the families Tachinidae and Sarcophagidae contain many beneficial species (Clausen 1940). Effects of insecticides on these parasites can be deduced by rearing or dissecting host larvae and pupae and by monitoring adults.

**Immature stages.**—Monitoring the parasites of major forest insect pests is feasible because their life histories are known. Host insects are available from sampling done before and after spraying to evaluate effectiveness of an insecticide against a target insect; the host insects can be reared or dissected for recovery of parasites. Sampling techniques include pole pruning to remove foliage samples (Leonard and Simmons 1974) and beating lower branches (Harris and others 1972). "Apparent parasitism"[6] can be calculated for each parasite species both before and after spraying (Williams and others 1979) and for sprayed and unsprayed areas (Leonard and Simmons 1974).

[6] This term refers to the proportion of hosts attacked by a parasite and is determined from a sample drawn from the host population at a particular time.

Monitoring the effects of insecticides on parasites of insect hosts other than the target host, particularly those parasites of insect associates feeding with the target host, requires recognition of these associates in larval and pupal stages. Descriptions of larvae and pupae of some of these associates are available, but training in recognition is necessary because of changes in their appearance during development.

**Adult parasites.**—Immediate effects of an insecticide can be determined by caging adult parasites within the spray areas or by exposing adults to contaminated foliage and observing their behavior and mortality (Price 1972). Short-term effects are measured by using drop cloths (fig. 6) to collect dead adult parasites (Varty 1975) or by using various traps to intercept parasites in flight. Trapping usually produces more insects and is statistically more reliable than using drop cloths. If the flight period of a particular parasite overlaps the time of insecticide application, numbers recovered per unit of time before and after spraying can be compared.

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Figure 6.—Drop cloths are used to collect dead adult parasites.

The Malaise trap (fig. 7) (Townes 1962) is often used to collect adult parasites of forest defoliators; Lepidoptera are also commonly caught. The trap intercepts insects in flight. On entering the trap, the insects contact a barrier and are funneled upward into a collecting chamber. Traps should be positioned along natural flightpaths, such as old roads or dry streambeds; they can also be located along vertical strata in the forest canopy (Reardon and others 1977). Efficiency of trapping is affected by temperature, precipitation, and air movement (Matthews and Matthews 1971). Traps should be emptied at least once a day for 2 weeks before and after the insecticide is applied and then weekly until no parasites of interest are found. Catches should be sorted and counted or weighed by species. Data are expressed as number or weight caught per unit of time and plotted over days of trapping.

McPhail (baited) traps, sticky traps, window traps, and light traps are also used to collect adult parasites. All have major inherent disadvantages (Southwood 1966). Sweep nets are occasionally used to catch parasites—usually to determine when trapping should start—but they are more often used for predators.

### Predators

Many species of predaceous arthropods occur in forests. Some occupy hidden niches on the boles and limbs of trees or in the forest litter and are extremely difficult to monitor. Others are exposed on foliage or bark or wander about the forest floor; these are susceptible to monitoring. Predators of forest defoliators are usually exposed, at least in the adult stage.

The orders Phalangida (harvestmen) and Araneida [Araneae] (spiders) include many predaceous species common in forests. These arthropods are considered important predators because of their abundance and documented predatory activities (Loughton and others 1963).

Three families of Coleoptera (Cicindelidae—tiger beetles, Carabidae—ground beetles, and Cleridae—checkered beetles) and three families of Hemiptera (Phymatidae—ambush bugs, Reduviidae—assassin bugs, and Pentatomidae—stink bugs) include species that are predaceous in both the immature and adult forms. Hymenoptera have many predaceous species, including members of Vespidae—yellowjackets (genera *Vespula* and *Dolichovespula*) and Formicidae—ants. In Europe, several species of red wood ants (*Formica rufa* group) are recognized as potentially effective predators (Adlung 1966); in western North America, carpenter ants (*Camponotus* spp.) are common predators.


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Figure 7.—A Malaise trap is often used to collect adult parasites of forest defoliators.

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Figure 8.—Yellowjackets can be studied by using traps baited with heptyl-butyrate.

Immediate effects of insecticides on predaceous species inhabiting trees can be evaluated by collecting dead or dying predators on drop cloths under the canopy dripline (Varty 1977). Species inhabiting the ground surface stratum can be monitored with area samplers. For species inhabiting both arboreal and ground surface strata, immediate effects can be determined by exposing caged predators directly to the insecticide or to prey or foliage treated with insecticide (Doane and Schaefer 1971).

Short- and long-term effects are best evaluated using techniques appropriate for the predator being monitored. For yellowjackets, this includes observations at baiting stations using fish, other baits, or heptyl-butyrate baited traps (fig. 8); direct observation of flightline activity; and analysis of nests. For ants, techniques include observations of worker ants foraging on the ground or entering trees to forage (Roush and Akre 1978) and direct observation and opening of mounds to locate ants and broods, per Markin and Kitayama.[7]. Ground beetles can be counted in artificial niches such as burlap bands on tree boles (Doane and Schaefer 1971).

[7] Study plan. Impact of the insecticide Orthene on an ant population of an Idaho coniferous forest. Davis, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station; 1977. 14 p. Unpublished.

Additional techniques, not directed toward a particular predator, include taking branch samples with pole pruner or beating lower branches (Varty 1977), sweep-netting, and pitfall trapping. Beating provides general information on the relative abundance of different predators. Sweep-netting should be used only to provide supplemental data because accuracy varies with the habitat, distribution of species, weather, and the person using the net (Southwood 1966). The pitfall trap (fig. 9) captures only actively moving arthropods and is of little value for measuring species composition or abundance (Southwood 1966). For population estimation, Gist and Crossley (1973) found pitfall trapping valid only for mark-recapture studies.


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Figure 9.—The pitfall trap is used to capture actively moving arthropods.

**Pollinators**

The most important pollinators in forest ecosystems are wild bees—bumble bees (*Bombus* spp.) and solitary bees (carpenter, cuckoo, leaf-cutter, mining, and yellowfaced). Honeybees are less important as pollinators in forests, although they are useful indicators of poisoning by insecticides.

**Wild Bees.**—The social bees (bumble bees and honeybees gone wild) are easier to monitor than the solitary bees. For the latter, additional information is usually needed on biologies, flight times, and flower associations before estimates of population density can be meaningful (Varty 1977).

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Figure 10.—Standard Langstrom hives can be used to study short- and long-term effects of insecticides on honeybees.

The immediate effects of insecticides are determined by exposing bees in cages to direct application of insecticides or to the residue on plants or pollen (Waller 1969). Estimation of short- and long-term effects can be based on many techniques. These include cone traps (Miliczky 1978), Malaise traps (Varty 1977), sweep nets (Varty 1977, Schmidt and others 1978), tallies along transect lines (Varty 1977), foraging activity around specific flowering plants (Doane and Schaefer 1971, Robinson and Johansen 1978[8]), and pollination studies.

[8] U.S. Department of Agriculture Douglas-Fir Tussock Moth Research and Development Program. Impact of chemical control application in the forest on beneficial insects. Davis, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station; 1977. 21 p. Unpublished.

**Honeybees.**—The domestication of honeybees simplifies their use as subjects of monitoring. Their biology and habits are well known.

Immediate effects of insecticides can be determined in the same way as for wild bees (Plowright and others 1978). Short- and long-term effects are studied using colonies of honeybees placed in standard Langstrom hives (fig. 10) in each study area.

Short-term effects of an insecticide are determined by various techniques. One is to place drop cloths beneath plants in bloom and record dead or dying bees. Another is to fit hive entrances with collecting devices, such as the Todd dead bee trap (fig. 11), and record daily death rates. In addition, bees leaving or entering the colony can be counted and pollen carriers recorded (Anderson and Atkins 1968, Buckner and others 1975). Also, populations of foraging bees can be estimated by visually examining an area with plant species in flower and counting the visiting bees for 1 minute.

Long-term effects are related to the ultimate condition of the colony. This can be determined by counting the number of brood frames covered with bees and measuring brood area; honey stores; pollen stores; survival of the queen, drones, eggs, and larvae; and mortality within the hives (Anderson and Atkins 1968; also see footnote 8).

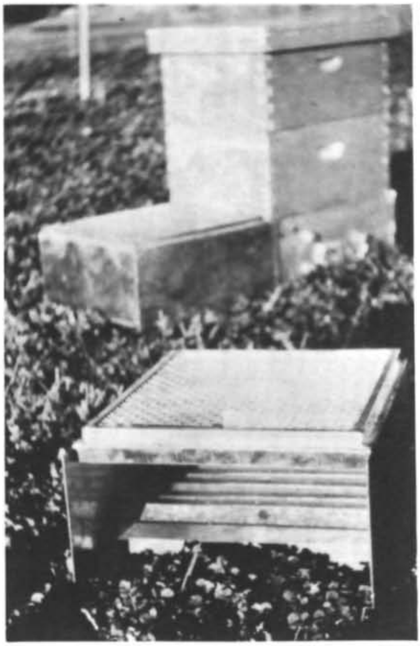

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Figure 11.—The Todd dead bee trap can be used as a collecting device at the entrance to hives.

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Figure 12.—Surber or modified Surber samplers rely on the current to carry bottom-dwelling insects into a net.

## Aquatic Insects

The insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), Odonata (damselflies and dragonflies), and Trichoptera (caddisflies) are exclusively aquatic. A few families in the Neuroptera and in the Diptera are also aquatic. All are important sources of food for fish.

### Streams and Creeks

Two basic methods, Surber sampling and drift sampling, are best for monitoring the short-term effects of a spray program on stream ecosystems. They are frequently used together. Adult emergence traps may provide supplemental information. Use of artificial substrates has some promise in measuring long-term effects.

Surber (fig. 12) or modified Surber samplers (Merritt and Cummins 1978) rely on the current to carry bottom-dwelling insects into a net. A prescribed area of stream bottom is selected and the bottom worked with the hands to dislodge insects that are then swept into the net.

In each study area, a riffle that has a relatively uniform bottom composition and is long enough to allow about 10 samples in each of the upstream and downstream portions is selected. Prespray samples should be taken in the downstream section of the riffle and postspray samples in the upstream portion of the same riffle. Each sample is preserved in 70-percent ethyl alcohol, and specimens are sorted in the laboratory. Specimens are identified to family, and the number of specimens in each family is counted. Average counts for each riffle are obtained separately for

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Figure 13.—An adult emergence trap is a modified A-frame anchored to permanently placed wooden beams that parallel the stream.

prespray and postspray samplings and subjected to a one-way analysis of variance. Populations in streams before and after spraying are then compared with populations in untreated streams. Only catastrophic effects are usually detected because numbers and kinds of bottom-dwelling insects vary between samples and between riffles.

Drift-net sampling provides a more immediate evaluation of an insecticide's acute spray effects than Surber sampling. Three drift samples should be taken: one prespray, one the day of spraying, and one 3 to 5 days after spraying. A sample is taken by placing a drift net into a stream for 10 to 15 minutes. Samples should be taken at approximately 0800, 0900, 1000, 1200, and 1400 hours on each day of sampling.

Adult emergence traps (LaGasa and Smith 1978) capture emerging adult insects from a riffle. Data provide useful information on species; reproductive potential of major benthos components; and, when used for several years, the long-term impacts of an insecticide.

The adult emergence trap is a modified "A-frame," completely sealed around the bottom (fig. 13). Each trap is 10 by 10 feet at the base, has cross-stream supports, and is anchored to permanently placed wooden beams that parallel the stream. The superstructure of the trap is made from prefabricated triangular end panels connected at the top. Four upright supports complete the basic framework. The end panels and the rest of the trap are covered with alternating sections of clear plastic and galvanized window screen. A hinged door at one side of the trap permits access to the inside, and a plank walkway permits movement within the trap to collect insects with a suction device without disturbing the creek bottom. The upstream and downstream lower edges have a screen apron that floats on the surface of the water. Collections should be made at least every other week. At each collection, the observers clean out the traps, wait 48 hours, and clean them again.

Several artificial substrate samplers have been developed. One uses wire baskets that are filled with washed substrate from the sample area and then anchored to the bottom of the stream. The baskets remain in place for about 6 weeks, after which they are retrieved and the benthic organisms removed.

This method is useful only to study long-term effects on particular aquatic insects. Weber (1973) provides an analysis of the pros and cons of artificial substrates. Advantages are that effects due to differences in substrates are reduced; population estimates are relatively precise; comparable data can be obtained in different stream environments; and samples contain little extraneous material. Disadvantages are that a long exposure period is required before results are obtainable; samplers and floats may be hazards to navigation; samplers are vulnerable to vandalism; effects of pollution on the substrate are not measured; and data are restricted to the insect community developed during the sampling period.

**Ponds**

Stillwater habitats are difficult to sample, especially for macroinvertebrates. If vegetation is absent, adequate samples can often be taken with a Peterson or Ekman dredge. These sampling devices, described by Weber (1973) and Lind (1974), are designed to collect a sample of bottom sediment (muck). Each sample is sorted or sieved, and the organisms are removed and preserved like they are for Surber samples. The data are handled in the same way as for Surber samples.

An Ekman dredge is most efficient if the bottom of the pond is composed of mud, silt, or similar soft sediments. The Peterson dredge works better in bottoms of gravel or plant litter. The dredge is quite heavy and can penetrate such substrates, but requires a winch for operation. The steps in sampling involve selecting sampling sites, taking samples with the dredge, sieving contents through a No. 30 screen, preserving specimens in alcohol or formalin, and separating and counting insects in the various taxa. Sampling is more difficult if considerable vegetation is present, making quantitative sampling nearly impossible.

Some have attempted to sample vegetative areas by sweeping through the vegetation and water with aquatic insect nets at constant depths for set distances. If this is done correctly, organisms in pond vegetation can be compared before and after spraying. Usually, however, a visual inspection of ponds will show obviously high numbers of dead or dying fish, frogs, salamanders, or other organisms. If dead vertebrates are found, some should be preserved for analysis in a laboratory.

An extensive discussion of techniques for sampling and evaluating aquatic organisms is provided by Weber (1973).

**Fish**

Because of their mobility and heterogenous distribution, fish populations are difficult to sample quantitatively. Both active and passive collecting techniques have been used, but many of the methods discussed by Weber (1973) are not applicable for sampling in small streams and ponds. Active sampling methods that may be useful include electrofishing, chemicals, and hook and line; passive methods involve gill nets or similar nets and traps. Sampling populations before and after spraying most likely will indicate only catastrophic effects. The most effective procedure is to cage a known number of fish (rainbow trout are often used in cold water streams) and record mortality after spraying. Fish are also caged in unsprayed streams or ponds to estimate mortality that might be caused by caging. Specimens from sprayed areas should be frozen and submitted promptly to a laboratory for analysis.

Physical and chemical parameters of the streams and surrounding environment are often measured while sampling is being done. Water and air temperature should be recorded frequently. Features of water chemistry that are most frequently measured are dissolved oxygen, pH, hardness, and turbidity. Aquatic vegetation and size and composition of the substrate should be recorded (Lind 1974, Weber 1973). Chemical analyses are most readily made with a "field lab," such as a Hach Portable Engineer Laboratory. Data recording sheets should be used to insure that all information needed for analysis is obtained.

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