Chapter 11
FOREIGN EXPLORATION AND IMPORTATION OF BC AGENTS

I. Introduction

A. In classical biological control there are several steps involved from the initiation of the search for natural enemies to the final evaluation of BC agents in the area of release. **These may be divided into:**

1. Foreign exploration
2. Quarantine handling
3. Colonization
4. Establishment
5. Evaluation.

B. Foreign exploration is most often in acted when outbreaks of exotic pests occur due to the lack of their natural enemies to control them. The importation of beneficial insects is the most widely practiced and potentially the most rewarding approach to biological control.

C. The objective of foreign exploration is to detect, select, and export natural enemies which show promise as biological control agents and to provide information facilitating their establishment in the country of introduction.

D. **The selected agents should be imported in**:

1. Healthy condition
2. Suitable stages
3. Sufficient quantities to insure safe arrival at their destination.

E. The foreign explorer should also collect data for later comparative evaluations of the natural enemies in their original and adopted ecosystems (improve understanding of biological control itself).

F. Organization of a foreign exploration program is dictated by the objectives of the project. It is necessary that these objectives be well defined. **Needed information to define objectives includes:**

1. Type of target organisms (plant or animal)
2. The organism's noxious status worldwide
3. Results of previous attempts at BC, if any
4. The quantity and quality of information available on its natural enemies
5. The level of financial, collaborative, and logistical support available. These factors will determine the scope and depth of the program.

G. Various phases of a foreign exploration program (pioneer type) are diagrammed on next page. **They may be organized in the following manner**:

1. Planning and preparation of the program
a. Accumulation and evaluation of available information
b. Selection of target organisms and exploration areas
2. Inventory research and investigations on selected species
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b. Comparative analysis of natural control factors
c. Detailed studies on selected species
3. Importation of natural enemies
a. Final selection of agents for introduction
b. Preparation of the material for shipment
4. Analysis of final results of project

H. Under actual conditions several phases usually proceed concurrently. Information obtained in one phase can be extremely useful in other phases.

II. Accumulation and Evaluation of Available Information

A. This information should be obtained prior to the actual search for natural enemies:
1. Taxonomic position, life history, and economic importance in country of origin
2. Native geographical distribution
3. Total present distribution
4. Host plant distribution
5. Probable center of origin of the organism and its close relatives
6. Coextensive occurrence of related species
7. Occurrence and distribution of related and ecologically similar species in regions where the target organism does not occur but where exploration for an enemy agent seems desirable because of climatic similarities to infested areas of country of introduction
8. Available records of natural enemies and other mortality factors.

B. Correct identifications of pests and natural enemies cannot be stressed enough. Incorrect identifications or nomenclatural inconsistencies can greatly impede progress. Wrong identifications could result in foreign exploration in the wrong geographical areas.

III. Selection of Target Organisms and Exploration Areas

A. Logistical Aspects: several problems may be encountered which modify exploration schemes. These include:
1. Inaccessibility of a search area because of national or international considerations, unavailability of transportation, or inclement weather
2. Lack of laboratory facilities within the search area
3. Restrictive customs procedures barring importation of specialized equipment into the search areas
4. Maintenance of cultures or collections during extended research periods
5. Quarantine restrictions on material imported for testing purposes

B. Target Organisms: first choice of natural enemies should be those dominant species occurring at low densities in the native home of the pest. If no promising control agents of the target species can be found (or if control of a native species is desired) then species closely related to the target organism (pest) should be used as sources of natural enemies.

C. Exploration Areas: several criteria must be considered in selection of exploration areas:
1. Searches should initially be undertaken in areas where target species is native because chances are increased for finding rich, diversified complex of natural enemies. However, suitable control agents may be found outside native areas due to the spread of natural enemies with the host or new natural enemies adopted the target species as a host when it spread to new areas.
2. The climate and environmental conditions should be similar to area of intended introduction (enhances chances of natural enemy establishment).
3. At least a part of the search area should include or be as near as possible to the center of the target species and its close relatives (if climatically similar to area with pest problem) because the diversity of complexes of specialized natural enemies tends to be a function of the duration of association of an organism with a given ecosystem.
4. Faunal and floristic history, vegetation, and habitat structures should present maximal diversity in the area selected (important when cannot determine native range or origin of target species)

5. Exploration areas should be as wide and diversified as possible to maximize chances of finding numerous and ecologically differentiated natural enemies of broad genetic variability, and of acquiring information on the ecology of individual species under different conditions.

IV. Inventory Research and Investigations on Selected Species

A. Inventory Research: objectives of this phase are to provide:
1. A survey (or inventory) of the available natural enemies
2. Criteria for the selection of suitable BC agents within the foreign areas being explored. Searching for natural enemies may involve several strategies:
a. Collecting in high host density areas where natural enemies are easy to find (not the best idea)
b. Collecting in areas where conditions are suitable for target species but it occurs at low densities due to actions of natural enemies
c. Artificially concentrating host densities in the field by the "exposure method"
B. Signs which assist in the recognition of areas where control of target species by natural enemies is good includes:
1. Scarcity in areas suitable for target species
2. Colonial or clumped type host distribution which is associated with highly effective natural enemies
3. Occurrence of localized outbreaks of target organisms associated with pesticide usage
4. Abnormal abundance of target species where it is protected from natural enemies by ants, dust, or litter. As much information as possible should be recorded on the habitats where natural enemies were found. Status of the target species should be observed in different localities.
C. Comparative Analysis of Natural Control Factors - has a central role in any foreign exploration program:
1. Results in selection of species for detailed studies as candidate BC agent
2. May provide additional data and feedback on selection of exploration areas
D. Effect of natural enemies: usually determined through in depth biological studies that are impossible to conduct on exploration trips. Conventional methods may be used to determine the following parameters:
1. Host range: range of acceptable host species, estimated by the number of supraspecific systematic categories
2. Host preference: indicates the acceptance of the target host relative to other acceptable hosts
3. Constancy: the percentage occurrence of a natural enemy in all samples taken in the exploration area. Measure of frequency of association between enemy and host
4. Abundance: designates the numerical occurrence of one species within individual samples (expressed as "apparent percentage parasitism")
5. Intrinsic competitive capacity: indicates outcome of intrinsic competition with other natural enemies
E. Interactions among natural enemies: well adapted natural enemies with good searching abilities coexist with less specialized, but intrinsically superior species (often capable of both primary and secondary parasitism) has been referred to as "balanced competition". If analysis shows this to be true then the foreign explorer should recommend that species are introduced in a predetermined sequence starting with the intrinsically inferior natural enemies

F. Hyperparasites and other secondary enemies: must be eliminated from material to be shipped. These organisms can reduce the effectiveness of a natural enemy. When in an environment without these the natural enemy may be greatly improved.

G. Factors other than natural enemies affecting target species: comparative analysis may reveal important control factors in the exploration area that are not common to area of introduction

H. Detailed Studies on Selected Species - can be conducted prior to introduction

1. Reproductive capacity and impact on host: determine fecundity of natural enemies and number of hosts killed by single individual
2. Adaptation to different climates: natural enemies must be effective in climatic conditions of intended area of introduction
3. Searching ability: this parameter could be estimated by looking at parasitism at various levels of host densities
4. Host selection: can be empirically defined through field observations and laboratory tests. Most important in species imported as biological control agents of weeds, but is becoming more important in BC of arthropods (consult notes on environmental impacts) due to concerns about non target, endangered species. Limited host range is desired in BC agents of arthropods, and it is absolutely necessary for BC agents of weeds.
5. Synchronization: this is a complex area, but is a must for effective colonization. Especially important when moving natural enemies between northern and southern hemispheres.
6. Genetics: investigations of genetic variability may aid in selection of enemy strains suitable for target areas

V. Importation of Natural Enemies

A. Selection of Agents for Introduction: most crucial phase in any BC project. Is entirely an empirical process. The following criteria are important:

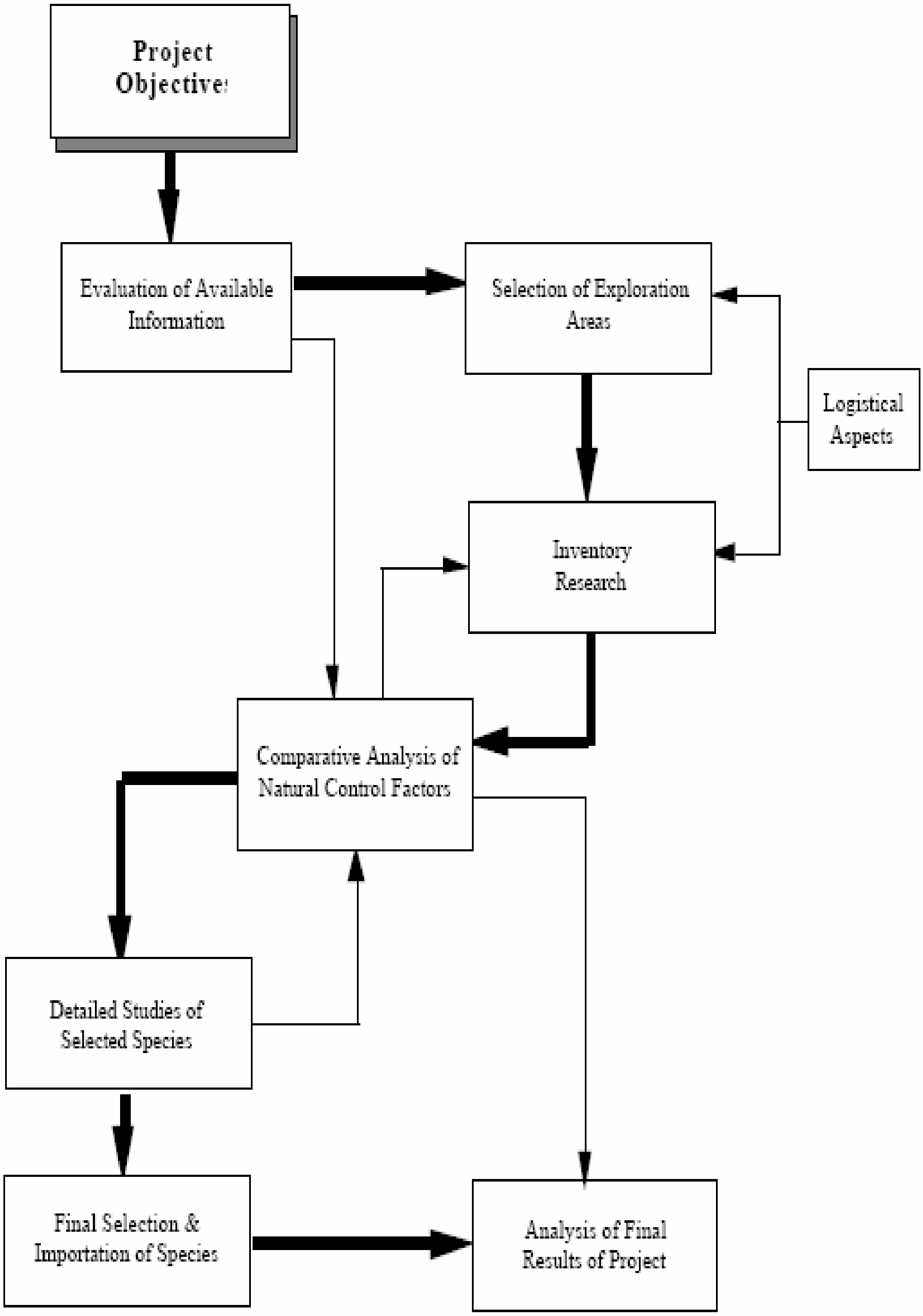
1. The candidate species must not develop harmful effects in the country of introduction (top priority in weed control, and becoming more important for arthropod control)
2. It should possess a good searching capacity and be able to operate effectively at low and high host densities
3. It should be well adapted to the climatic conditions, host habitats, and other ecological factors in release area
4. A relatively high host specificity or the capability of developing a high host preference in the situation is desirable
5. Its life cycle should be well synchronized with its host, thus allowing exploitation of the host
6. High reproductive capacity, preferably combined with short generation time and a high "effective rate of oviposition"
7. In some instances it may prove useful that the agent is able to integrate itself into the system of mortality factors already existing in the target country. Remington (1968) warns against introducing material from laboratory rearing started from just a few individuals. He suggests that a large sample from a large, central, wild source population in an environment most similar to that of the region of intended establishment should be introduced. He also suggests introductions with individuals from many wild source populations is also good. Multiple species should be introduced.

B. Preparation of the Material for Shipping

1. Laboratory Propagation: If possible, it is desirable to set up a temporary lab facility for propagation of field collected material. This enables the explorer to increase the numbers of individual natural enemies for shipment.
2. Timing of Shipments: Natural enemies must be shipped so that any immediate releases to be made will be insured of potential success by the availability of the preferred host stages at the release sites. Problems of synchrony are encountered when shipping material from the southern to northern hemispheres. Those receiving natural enemies in quarantine facilities must also be ready to rear natural enemies.
3. Screening for Parasites and Diseases: Great caution must be exercised to prevent the shipping of hyperparasites and unwanted pathogens with the natural enemies. Shipping of adult natural enemies is best in reducing hyperparasites. The use of sterile techniques may be necessary to insure that no pathogens are transported.

C. Shipping Methods

1. Satisfactory transporting of natural enemies is considerably facilitated by air freight facilities, but still requires considerable planning and ingenuity. The optimal conditions of humidity and temperature vary with different natural enemies; hence, pilot tests should be made to determine the best shipping method for the species concerned. Food should be provided for natural enemies.
2. Shipping containers should be well insulated, sturdy, light, and easy to handle.
3. Shipment packages should bear instructions for the postal authorities as well as a importation permit. When sending shipments by air freight, affixing instructions concerning special handling, transfers, etc., may facilitate quick transportation.



Information flow in foreign exploration programs (modified from Zwölfer et al. 1976).

Chapter 12
ESTABLISHMENT AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

I. Post-Importation Procedures

A. After natural enemies are shipped to the area intended for introduction, four additional phases of BC procedure must be dealt with before the process is complete. These are:

1. Quarantine;
2. Colonization & Establishment;
3. Evaluation; and
4. Cost / benefit analysis

II. Quarantine

A. Philosophy of quarantine: prevention of the introduction of undesirable species of phytophagous insects, weeds, hyperparasites, plant pathogens
and/or any other type of undesirable organism.

B. Pest organisms do not respect political borders and only their environmental preferences restrict organisms to certain geographic areas. These areas do not usually coincide with each other. Quarantines defend political borders.

C. Travelers to Hawaii are subject to quarantine check with respect to organisms they consciencously or unconsciencously bring into the state.

D. All insects imported into Hawaii for scientific reasons must be accompanied by importation permits allocated by the Federal Government (see Fig. 18.1). These permits are obtained after approval of state (e.g., Quarantine Branch, Hawaii Dept of Agriculture) and federal government agencies (e.g., USDA Animal & Plant Health Inspection Service [APHIS] and U.S. Fish and Wildlife Service).

E. Shipments which are suitably packaged and have the correct importation permits move easily through quarantine inspections in route to the quarantine facilities at the Hawaii Department of Agriculture, Honolulu (parasitoids, predators & insect and plant pathogens) or the Federal Facility, Volcano Nalt. Park (phytophagous agents for weed control)

F. In quarantine, everything used in shipping insects is either destroyed or autoclaved. Any live host material is destroyed. Only predators and parasitoids are kept and these are cultured to insure no hyperparasites are present in the shipment.

G. Only until the natural enemies are absolutely identified and their potential for becoming a pest species is known to be zero are the imported species released from quarantine for the establish phase. Phytophagous insects imported for weed control remain in quarantine until researchers are absolutely positive that they will not attack any other host plant of value except the plant species targeted for control. In the last few years, host specificity testing has become more commonplace for natural enemies of arthropods as a result of community fears of non-target impacts on desirable species (e.g., endemic species).

III. Establishment

A. The first step in establishment of a species in an area is to colonize it. Colonization may be defined as the process of field release and manipulation of imported natural enemies so as to establish them and favor their increase and dispersal in a new environment.

B. Successful colonization results when a natural enemy has been permanently established in one locality. This locality serves as a locus for natural enemy spread and as a field colony and a source of material for further distribution methods.

C. Initial colonization may be attempted by two methods which are:

1. Direct releases from quarantine; and
2. Insectary propagation.

D. Direct Releases: these are not encouraged by many BC researchers.

Many scientists insist that insectary propagation offers some distinct advantages. When the direct release method is used, repeated introductions from abroad are required followed by periodic releases.

Direct releases may be the best method when:

1. Funds are lacking for insectary propagation;
2. Rearing of the host or natural enemy is difficult and;
3. Adequate facilities are lacking for propagation.

E. Insectary Propagation offers some advantages which include:

1. Provision of adequate numbers of natural enemies for releases (insures greatest latitude in the timing and geographic coverage of releases);
2. Insectary culture insures vigorous stocks for releases; and
3. Provision of an opportunity for detailed studies of biology and host relationships of natural enemies.

F. In earlier years in the USA, upon receipt of natural enemies (and subsequent check on their potential as pests) usually a few specimens were directly released from the initial stocks imported. This is not generally practiced in the USA now, but may be in foreign countries. In some cases low numbers of individuals may be enough to achieve establishment. If establishment is achieved, then time and labour will be saved with respect to insectary propagation. However, trial releases are generally doomed to failure. There are a few cases where success was achieved (Establishment of the lace bug, *Teleonemia scrupulosa*, as a BC agent on the weed *Lantana camara* - established in Hawaii with 6 individuals).

G. Many ecological factors determine the success or failure of colonization efforts. **Reasons for failure of colonization**:

1. Failure to adapt to climatic conditions
2. Lack of alternative hosts in new environment
3. Native natural enemies (although ineffective) may (due to their high numbers) compete with introduced species and thus prevent the limited numbers of insects from establishing a permanent colony

H. **Lack of establishment of species may be due to**:

1. Unsuitability of host plants as shelters for new natural enemies
2. Adequate food sources unavailable
3. Unsuitability of attractant stimuli during host habitat finding phase by adult parasitoids
4. Certain host stages or species may prove physiologically unsuitable
5. Highly developed dispersal habits may retard or hender establishment of natural enemies due to problems in individuals finding each other to mate (when population densities are small)

I. The critical test in the ability of a natural enemy to become established is whether it has the ability to survive periods of climatic extremes (e.g., summer heat and winter cold).

IV. Evaluation of Established Natural Enemies

A. Three reasons to evaluate BC agents

1. To show the value and short comings of existing natural enemies
2. To provide insights into the principles of population ecology relating to the interplay of biotic and abiotic factors
3. To demonstrate the effectiveness of natural enemies

B. Two distinct questions may be asked in evaluation of natural enemies.

These are:

1. Does regulation of the host population by the introduced natural enemies actually occur?
2. How does the regulation of the host occur?

C. Experimental proof of regulation is needed

1. Sometimes new pest invasions and outbreaks eventually subside to lower levels even when no BC agent is introduced for control (e.g., two-spotted leafhopper in Hawaii)
2. Increased levels of parasitization or predation by newly established natural enemies is not the best measure of effectiveness

a. It has been shown that percentage parasitization cannot be equated with level of control exerted (see Fig. 18.2)

b. It is the number (not the percentage) of survivors that escape natural enemy action that determines subsequent pest density

D. Legner (1969) stated that the "ultimate and probably only reliable method for judging a parasite's effectiveness is the reduction in host equilibrium position following liberation". This requires an evaluation of a parasite's effectiveness by using "before" and "after" introduction density comparisons.

E. Hassell and Varley (1969) state that "laboratory studies can go some way to predict which species (among several introduced) may be successful, but an introduction provides the only real test".

F. Experimental (comparison) methods of evaluation - most of these methods can be employed with either newly introduced, exotic natural enemies or indigenous natural enemies. These include:

1. Addition Method
2. Exclusion Method
3. Interference Method

V. Addition Method

A. This is essentially a "before" and "after" comparison. Set up a number of plots (e.g., 20) and introduce natural enemies into 10 plots and leave others as controls. Check them at a later time to see the impact.

B. Plots must be set up prior to introductions of natural enemies. Plots must be distant from each other to prevent invasion of check plots by introduced natural enemies.

VI. Exclusion Method (also known as Subtraction Method)

A. This technique involves the elimination and subsequent exclusion of resident natural enemies from a number of plots which can then be compared with a like number of otherwise comparable plots where the natural enemies are not disturbed.

B. Significantly different "before and after" pest population densities indicating different equilibrium levels for the two groups of plots serve as a direct measure of the control and regulating effectiveness of the natural enemies.

C. Experiments must be designed to be biologically realistic. One should not alter parameters such as temperature and dispersal area which may exert an appreciable influence on the results. The techniques work best with insects with low powers of dispersal.

D. Natural enemies are mechanically excluded from hosts by use of exclusion cages (this is often referred to as the "paired cage" technique). These take several different forms. The general process is:
1. Select sites for the studies.
2. Clean up sites with insecticides or by hand removal of pests and parasitoids.
3. Re-introduce pests into cages.
4. Close cages up and let pests become established
5. Open one cage up to natural enemies
6. Take census counts during the study

VII. Interference Method

A. This method involves greatly reducing the efficiency of natural enemies in one set of plots as contrasted to another set having natural enemies undisturbed.
B. Any increases in pest density in the interference plots, relative to the normal biological control plots, demonstrate the effectiveness of the natural enemies.
C. Such comparisons reveal only a part of the total extent of host population control because the natural enemies are not entirely removed and may be producing some limiting effect.
D. The percentage parasitism may commonly be as great in the interference plots as in the normal activity plots. The density of the pest population at equilibrium is determined by the rate at which premature mortality of a pest increases with pest density. If the rate of parasitization increases rapidly as the host population starts to increase, the host equilibrium density will be low. If it increases slowly, the density will be high. Because the equilibrium mortality level (mortality = natality) is reached quickly at low levels in the unexcluded plots and not in the latter, this illustrates the difference between an effective and ineffective natural enemy.
E. The above explanation also explains why pesticides (an interference measure) can cause pest upsets when effective natural enemies are inhibited and why parasitoids may be even more abundant in a chemically treated habitat and yet unable to control the pest, or maintain it at a low density.
F. The various techniques in the Interference Method include:
1. Insecticide check method
2. Hand-removal method
3. Biological check method
4. Trap method
G. Insecticide Check Method: Selective toxic materials are used to interfere with the natural enemies. Usually these are insecticides such as DDT, Methomyl, or Carbaryl. Can also be substances such as talc or road dust? However, some compounds induce the condition of hormoligosis which is the stimulation of an insect to produce more eggs by sublethal doses of insecticides.
H. Hand-Removal Method: Natural enemies are periodically removed from area. Intensive labor required and time consuming. Used in studies on regulation of spider mite mite densities by natural enemies on foliage of strawberries and papaya. Too expensive with respect to "manpower".
I. Biological Check Method: Interference with ants that naturally interfere with natural enemies which attack honeydew producing insects. Ants may be eliminated by insecticides (e.g., Amdro®) or barriers such as "Tack Trap". Restriction of the Argentine ant, *Iridomyrmex humilis* Mayr, resulted in control of California red scale. In Hawaii, suppression of the big-headed ant, *Pheidole megacephala*, in pineapple plantings led to reductions in pineapple mealybug densities.

J. The Trap Method**:** A large area is selected as the test site. Insecticides are applied around the outside borders thus creating a wide band of insecticide treated foliage which must be crossed by organisms either leaving or entering the test site. Nothing is modified directly in the test area, thus natural enemies are not inhibited. This technique is most successful with fairly sessile pests which have rather mobile natural enemies. By use of several different selective chemicals applied at required intervals one could make this a fairly sophisticated technique.

VIII. Life Tables and Population Models

A. The techniques discussed above only tell us how effective are natural enemies, but they do not tell us why they are effective nor how we can change the system to improve their effectiveness.

B. The use of life tables and population modeling allows one to break apart the systems in which we are interested and to analyze the components.

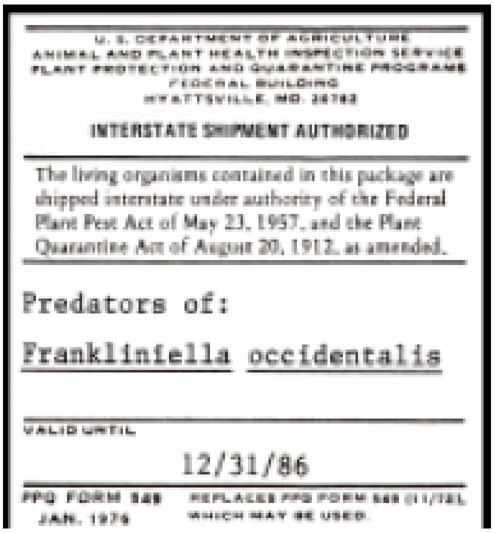
C. Comparison methods tell us if natural enemies are effective whereas population modeling can tell why they are effective.

IX. Cost / Benefit Analyses

A. Financial evaluation is beneficial to a program to view the economic benefits of biological control and should be implemented after establishment, implementation, and adoption of the natural enemies as a control factor.

B. Typically, classical biological control has a much better return on the financial investment (as much as $30 to every $1 invested) than chemical control, and often it keeps on providing benefits without additional inputs.

C. Consult notes on economics of biological control for more information.


U. S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE PROGRAMS
FEDERAL BUILDING
HYATTSVILLE, MD. 20782

INTERSTATE SHIPMENT AUTHORIZED

The living organisms contained in this package are shipped interstate under authority of the Federal Plant Pest Act of May 23, 1957, and the Plant Quarantine Act of August 20, 1912, as amended.

Predators of:

Frankliniella occidentalis

VALID UNTIL

12/31/86

PPQ FORM 548 JAN. 1976 — REPLACES PPQ FORM 548 (11/72), WHICH MAY BE USED.

Fig. 18.1. Example of importation permit issued by USDA APHIS. Natural enemy shipment containers should display an official permit.

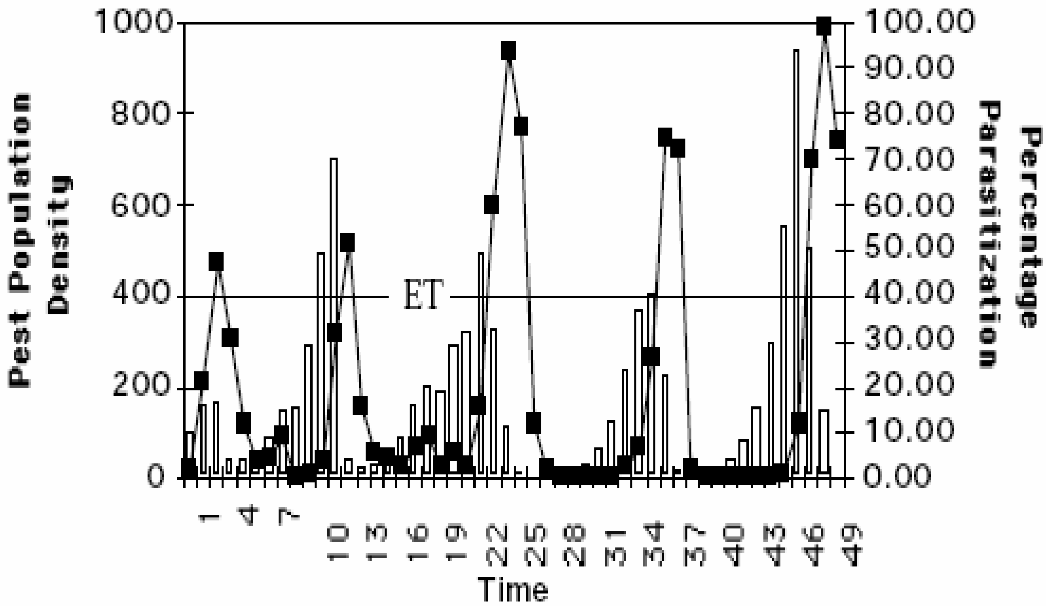


Fig. 18.2. In the above graph, the pest density (–■–) [left axis] and percentage parasitization (bars) [right axis] is shown. Note that if a economic threshold (ET) were set at a density of 400 pests, then all pest populations would be above the ET even though as much as 70% parasitization had been achieved.