ROLE OF QUARANTINE FACILITIES IN BIOLOGICAL CONTROL

GARY R. BUCKINGHAM
Agricultural Research Service
U.S. Department of Agriculture
c/o Florida Biological Control Laboratory
P.O. Box 147100, Gainesville, FL 32614-7100

ABSTRACT

Quarantine facilities play an important role in classical biological control programs. Imported natural enemies can be identified, cleaned of diseases and hyperparasites, and studied in a secure quarantine facility without risk to the environment. Personnel at the facilities advise other researchers about regulations and techniques for importation, document results of the importations through shipment record forms, annual reports, and voucher specimens, and conduct research on biologies and host ranges of natural enemies. Not only does the quarantine facility help allay the fears of the public about the dangers of importing foreign organisms, it also protects the researcher from blame for accidental introductions not associated with the researcher's project.

RESUMEN

Las unidades cuarentenarias juegan un papel decisivo en los programas de control biologico clasico. Los enemigos naturales importados pueden ser identificados, asi como tambien sus enfermedades o hiperparasitos pueden ser estudiadas en las unidades de cuarentena sin ningun riezgo a el medio ambiente. El personal engargado de estas unidades, aconseja a los investigadores acerca de los reglamentos y tecnicas de importacion, y documenta los resultados de las importaciones, a traves de registros de envio, reportes anuales, especimenes tipo, e investiga la biologia y el rango de hospederos de los enemigos naturales. La unidad cuarentenaria ayuda no solamente a aliviar el temor publico en lo referente a las consecuencias de la importacion de organismos foraneos, sino que tambien proteje al investigador de culpabilidad por la introduccion accidental de organismos no asociados con el projecto de investigacion.

Classical biological control programs involve shipments of natural enemies between countries usually with insect and plant hosts included. Often the hosts are present in the receiving country, but not always. Sometimes natural enemies are collected from insects or plants closely related to the pest rather than from the pest itself. In these cases the host of the natural enemy might become a new pest if it were inadvertently released. In addition, the host material often includes natural enemies of the desired natural enemy.

To house these natural enemies and their hosts upon arrival, a network of quarantine facilities has been organized. These facilities are not part of the traditional quarantine inspection system that prohibits entry of unwanted guests into the country or into a state. They are operated by or for researchers not by or for inspectors. This distinction has occasionally been overlooked by foreign agricultural officials surprised and disappointed that they were not visiting an inspection facility when they visited our quarantine facility at the Florida Biological Control Laboratory (FBCL), Division of Plant Industry, Florida Department of Agriculture and Consumer Services, in Gainesville. Approximately 15 facilities that process insect shipments are present throughout the

United States. Some of the larger or longest established ones in addition to the FBCL are in Riverside and Albany, California; Newark, Delaware; Stoneville, Mississippi; Columbia, Missouri; Bozeman, Montana; and College Station and Temple, Texas.

Role of the Quarantine Facility

The quarantine facility plays an important role in the "biological control drama". Like an effective actor, it dominates the second act of a three-act improvisational play. During the first act, it whispers advice to other actors from the wings. Bursting upon stage in the second act as a sword-slashing swash-buckler, it protects the stage from all intrusions. With the trained memory of a seasoned actor, it documents the improvised movements and dialogue from the second act, saving them for future playwrights and actors. Finally, during every performance it researches new movements, gestures, and dialogue to improve itself and to ensure success of the final act.

Of course, biological control is not an improvisational play that can be manipulated as the actors and director wish, but it does have many similarities. For example, it plays to both empty and full houses; it has limited engagements and record-breaking long runs; it has road tours; and its existence is often dependent on the financial support of a "producer" and the interest of the ticket-buying public. Like a classic Shakespeare play, classical biological control provides generations of followers with a performance that changes subtly with each new theater group and new director.

Departing from the drama analogy, I believe the principal functions of a quarantine facility and its personnel can be characterized as follows:

1. Advise foreign explorers and other researchers about regulations governing importation of natural enemies and about shipping techniques.
2. Protect the environment from unwanted introductions of pests and of natural enemies of biocontrol agents.
3. Document introductions of biocontrol agents into and out of quarantine for the benefit of researchers, administrators, and the historical record.
4. Conduct basic research, for example, studies of biologies of natural enemies, of host ranges of natural enemies especially weed control agents, and of exotic insects for projects not associated with biological control.

In this paper I discuss these functions to aid those readers who wish to construct a quarantine facility, to modify a laboratory room for quarantine use, or to import natural enemies through an existing facility. If classical biological control is to develop fully in the Caribbean area, quarantine facilities will undoubtedly be needed. Further information about quarantine procedures can be found in the paper by Rose & Harrison (1990).

Advise Researchers

As interest in classical biological control grows, more and more researchers who are inexperienced in foreign exploration or in importation of agents need advice before beginning a project. The quarantine facility provides a highly visible and ready source of information about regulations, permits, shipping labels and techniques, etc. This will be especially true in a country or state with a new quarantine facility and new emphasis on natural enemy importations.

In the United States, introduction of insects and pathogens for control of agricultural pests is regulated by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) and by the Departments of Agriculture of the individual states. The researcher completes a form (Form PPQ 526) requesting permission to introduce the natural enemy. This form must be submitted to state officials for approval before it is forwarded to APHIS. It includes the names of the pest and its natural enemies, the source of the material to be introduced, the names of the researchers, and information about the project and future disposition of the natural enemies. If the request is approved, APHIS sends distinctive shipping labels to the researcher along with a signed copy of the form. These labels are placed on the outside of the package by the foreign explorer or collaborator to alert customs and agricultural inspectors at the port of entry. I usually recommend that researchers also place a copy of the permit in an envelope addressed to the plant quarantine inspector and attached to the outside of the package. National regulations concerning introductions of biocontrol agents are currently being examined for possible changes. It behooves all countries to have a stream-lined permitting process that protects against unsafe introductions yet facilitates movement of packages through ports of entry.

In addition to information about regulations, quarantine personnel are often asked about shipping techniques and clearance procedures at ports of entry. Whenever possible international shipments that cannot be hand carried should be sent by air freight or by courier service rather than by international air-mail, unless the postal service's Express Mail International service is available and has been previously tested. A shipment of alligatorweed flea beetles (*Agasicles hygrophila* Selman & Vogt) that I sent from Florida to Beijing, People's Republic of China, was received by the scientist within 3 days using Express Mail International service. This was faster than two preceding attempts to have the flea beetles hand carried to Beijing. Within the United States, various express delivery services and the postal service's Express Mail system offer convenient, dependable overnight service to most locations.

Shipping containers come in all shapes and sizes. The container with the natural enemies should be enclosed in a cloth bag and packaged inside another container to prevent escape in case the outside package is damaged. Information about shipping containers can be obtained from Bartlett & van den Bosch (1964), Boldt & Drea (1980) and Hendricksen et al. (1987).

Researchers wishing to import specific natural enemies should make sure that quarantine personnel have authoritatively identified specimens ahead of time for comparison in quarantine with the living material. This greatly accelerates the release from quarantine and can increase the chance of successful colonization if the biology of the identified natural enemy has been previously reported and can be consulted immediately by quarantine personnel. This provision of specimens to quarantine personnel ahead of time can not be over-emphasized. Natural enemy identification is extremely difficult without specimens for comparison.

Protect the Environment

Quarantine facilities are foremost a filter system used to prevent plant pests and natural enemies of biocontrol agents from entering the country along with the flow of biocontrol agents. They protect not only the environment, but also the researcher whose work is viewed as a threat by many people. It is not unusual to discover new immigrant insects during a project because of the increased sampling in a crop or habitat. If the project were not conducted in quarantine, the researcher might be showered with blame for the unwanted "introduction", thus jeopardizing the project and possibly future projects. This protection is overlooked by some researchers who utilize the quarantine facility merely because regulations require it.

Facilities differ greatly in size and complexity, however they generally share several safety features. Doors remain locked and admittance is restricted to authorized personnel. Some facilities have recently installed push button locks with codes that can be changed monthly or when projects finish. There is at least one anteroom and often two.

In the FBCL facility, the first anteroom is illuminated continuously and the second which opens into quarantine has no light. White laboratory coats are donned in the darkened anteroom. Light is provided by a small window between the rooms which is also a baffled insect trap to catch insects that might have been carried on the laboratory coats. Negative air pressure inside the facility helps prevent insects on or near the door from being sucked outside when the door is opened. Floors, walls, ceilings, and windows in quarantine facilities must be carefully sealed to prevent insect escape. Air intakes and exhausts are either covered with fine mesh metal screens or the air-handling system is recycled through large, very efficient filters as it is at the FBCL. All waste material leaving quarantine is autoclaved or sterilized in another manner. Many facilities have tanks for treatment of waste-water. Greenhouses must have wire-reinforced glass or very strong tempered glass. Because the greenhouses are tightly sealed a cooling system is essential. When the air-conditioning system disfunctions at the FBCL, we run a lawn sprinkler on top of the greenhouses to cool them below lethal temperatures.

Some organizations may need and could support large quarantine facilities like those at Stoneville, Mississippi (313 m^2, two 25 m^2 greenhouses) and at the FBCL (173 m^2, four 18 m^2 greenhouses). However, countries or organizations with lesser needs and smaller budgets can have safe, efficient quarantine rooms like those at the University of California, Albany, California (60 m^2) and at the Center for Biological Control in Central America (CBCCA), El Zamorano, Honduras (32 m^2) (R. Cave, personal communication). With care, an existing laboratory room could be renovated as a quarantine room. Detailed descriptions of the preceding quarantine facilities are reported in Leppla & Ashley (1978). Organizations wishing to build large facilities are cautioned to consider carefully the large maintenance and energy costs that these facilities require and to provide for these costs. Insect colonies can not be moved to another building in case of equipment failure and if they die out they can not be replaced readily as can native or adventive insects used in most research programs.

Equally as important as the facility for protecting the environment and perhaps more important are the personnel who work in the facility. Each facility has a quarantine officer who is responsible for enforcing rules and ensuring maintenance. At smaller facilities the quarantine officer often handles most of the material, but at large research facilities this is usually not possible. The quarantine officer must ensure that the identity of a known biocontrol agent is confirmed either by a comparison with specimens or by a specialist. This is true even with pure colonies from other research laboratories. A shipment of laboratory parasitized fly puparia sent to the FBCL from an overseas research program yielded a pure colony of parasites, but the wrong species. A detailed examination of the shipping containers showed that the permitted species had emerged (and died) earlier in transit. The "pure" colony had contained two species. Even the most careful rearing programs occasionally have contaminated colonies especially when rearing difficult to distinguish microhymenoptera.

The quarantine officer or other personnel must have sufficient taxonomic training to immediately separate individuals by species for rearing when field collected host material is received. The parent generation is often dead before an identification is obtained from specialists outside quarantine. Schlinger & Doutt (1964) provided a key to the families of entomophagous insects which is a handy reference for quarantine personnel. The general rule is to rear natural enemies for at least one generation to prevent release of obligate hyperparasites and to discover if there is a problem with pathogens. Weed control agents, especially Lepidoptera, occasionally have disease problems when reared in quarantine. Goodwin (1984) discussed diseases commonly encountered during insect rearing. At the FBCL we are fortunate to have strong taxonomic support locally from Division of Plant Industry and University of Florida taxonomists and pathologists and from ARS/USDA insect pathologists.

Diseased specimens of biocontrol interest may be sent to quarantine facilities by insect pathologists conducting foreign exploration or by other foreign explorers. Dead specimens are released to pathologists. Until recently diseased material was usually sent directly to pathologists, but that appears to be changing. Regulations concerning handling of insect pathogens are currently being formulated.

Document Shipments

Often while the foreign explorer is packaging a shipment to send to quarantine and while quarantine personnel are processing an incoming shipment, documentation of the shipment seems unimportant compared to the need to keep the insects alive. Surprisingly, documentation may be equally or even more important. Many shipments arrive in quarantine with all material dead; many attempts to colonize species in or out of quarantine fail and many species are not established in the field. Without adequate quarantine documentation there would be no record of those projects. The information on the importation forms and the voucher specimens are the only tangible results of the project. Researchers often have difficulty recognizing the importance of documentation and are wary of providing information they think might detract from their future publications. However, rarely are there conflicts with publications and every quarantine facility should document projects as carefully as possible. Fisher (1964) illustrated a card that was used to document shipments at University of California facilities. Coulson (1987) illustrated and discussed record forms (941 & 942) used by federal and some state quarantine facilities, including the FBCL, to document shipments into and out of quarantine, respectively. Both the California and federal forms document the collecting locations, collectors, dates, species shipped and emerged, packaging, transport method, numbers shipped, condition received, disposition of material and other information.

Many quarantine facilities prepare an annual report of importations and exportations that is distributed to other facilities and interested persons. It can usually be obtained by writing the quarantine officer. These reports are valuable for communication but are especially valuable as an historical record. They are usually produced from computer data bases similar to that described by Dysart (1981). Coulson et al. (1988) published a national summary of insects released in the United States during 1981 based heavily upon the quarantine shipment forms. Publication of updates is planned.

Documentation of projects through a voucher insect collection is an important responsibility of quarantine facility personnel. The voucher collection, including specimens of both the hosts and the natural enemies, can be maintained at the facility or in a museum, although collections at both locations would be best.

Conduct Research

Many introductions into quarantine are relatively easy to process and to move out of quarantine. They may be pure laboratory cultures from well known institutions; they may be a natural enemy group, for example *Aphytis* (parasites of armored scales) with a proven safety record; they may be diseased insects that can be transferred to the pathologist when they die. Other introductions are not so easy to process. Research of both short and long duration may be needed before they can be cleared for release. New species of parasites or species with unknown biologies in genera or families that include hyperparasites must be reared and their behavior carefully observed. Fisher (1964) suggested techniques to determine if a species is a hyperparasite. Predators and occasionally parasites may require studies of their host ranges if they are not well known. This is especially true today with the concern that society has for non-target native organisms. Carabid beetles that eat mole cricket (*Scapteriscus*) eggs have been studied in the FBCL for five years in an attempt to evaluate their threat to native Orthoptera or other groups.

Biology studies may be conducted while the researcher is waiting for a long-lived natural enemy to die to obtain a specimen for identification or while awaiting an identification of specimens that have been sent from one taxonomist to another. During a project at the FBCL on fall armyworm (*Spodoptera frugiperda* (Abott & Smith)) parasites from South America, a researcher conducting laboratory cross-mating studies with armyworms obtained permits to receive the pest moths from quarantine before we obtained an identification and permits to release the parasites. Some projects involve research that is considered safer if conducted in quarantine. During a project on fire ant (*Solenopsis invicta* Buren) natural enemies, a suspected parasitic ant associated with fire ant queens was studied in quarantine.

Weed control research requires long term studies in quarantine especially if there has been little foreign research. Coulson & Soper (1989) discussed protocol for these types of studies. The researcher must demonstrate through host range tests that the control agent will not endanger crops or native plant populations. A federal inter-agency Technical Advisory Group advises APHIS officials on the safety of the release. A similar state committee advises Florida officials who must agree to the release before forwarding the application to APHIS. Agents tested at the FBCL during a project to control the submersed aquatic weed *Hydrilla verticillata* (L.F.) Royle, were cleared for release in as little as one year and as long as five years.

Various types of research in addition to biocontrol research may be conducted at quarantine facilities. Extensive cross-mating studies to produce sterile hybrids of *Heliothis* moths were conducted at the Stoneville quarantine facility. A multi-year project was conducted at the FBCL to develop rearing techniques for species of mosquitoes from California. The FBCL was chosen for the project because of local expertise in mosquito rearing. Exotic cockroaches, orchid bees, and phengodid beetle larvae have been confined to the FBCL quarantine in support of local research projects. Quarantine is also used for rearing larvae of newly discovered immigrant insects to obtain adults for identification.

Conclusions

Countries conducting classical biocontrol projects should have a quarantine facility to process the imported material or should have use of a regional facility. Formerly, a quarantine room at the Commonwealth Institute of Biological Control in Trinidad functioned like a regional facility for portions of the Caribbean basin because of the many projects it supported. Perhaps a similar facility could be developed again. Because a newly invading pest usually threatens many neighboring countries, a concerted control effort with a system of several regional quarantines should be more efficient than quarantines in every country. The quarantine facility at the CCBCA, El Zamorano, Honduras, is planned as a regional quarantine for Central America.

As discussed previously, quarantine facilities need not be large. A renovated laboratory room could be sufficient for many small projects. I anticipate an increase in quarantine facilities of all sizes in the United States as weed control projects increase and as requirements for host range testing of parasites and predators increase. Quarantine facilities are safe as currently operated and help allay public fears about importation of exotic insects. I know of no reports of field establishments of pests that have escaped from a quarantine facility in the United States.

If new quarantine facilities are developed throughout the Caribbean, it is imperative that a documentation system be instituted and that voucher specimens be kept and carefully maintained. It would be helpful if emphasis were placed on energy efficient design of the structure compatible with the quarantine function. This might help avoid the large maintenance costs associated with quarantine facilities, especially as they become older.

As pest control moves from the chemical age to the biological age, personnel at quarantine facilities will help lead the way.

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