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QUARANTINE ISSUES AND RESEARCH DEVELOPMENTS

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ABSTRACT

The Plant Protection and Quarantine (PPQ) Division of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture, is the regulatory agency responsible for protecting the health and safety of domestic plant and other natural resources. PPQ safeguards these resources from the risks associated with the entry, establishment, or spread of plant pests and noxious weeds by the application of appropriate technologies for exclusion, detection, and response activities. The Center for Plant Health Science and Technology (CPHST) is the scientific support organization of PPQ and, as such, helps to ensure that the methods, protocols, and equipment used by PPQ and field personnel are effective and efficient science-based operations. Major issues facing CPHST include improved risk assessment technologies and processes; inspection, detection, identification and delimitation technologies; and alternatives to methyl bromide and other treatments. CPHST research and technology transfer activities stemming from these issues range from Asian longhorned beetle and anthrax to *Xanthomonas*, molecular diagnostics and trace element analysis to LucID keys and automated image analysis, electronic 'sniffers' to web crawlers, chemical fumigation to irradiation and radiofrequency, and from dry heat and steam to cold treatment and vacuum. These activities will be discussed in the context of the safeguarding mission of PPQ.

INTRODUCTION

Today's technology and marketing expertise make it possible for consumers to purchase foods that are only hours from their agricultural origins half a world away. Global trade is growing rapidly and, with it, comes the increased chance of an accidental introduction of an exotic pest that could have devastating consequences to the domestic agriculture and natural resources of an importing nation. Huge financial resources are expended annually by the world's trading partners in an effort to detect, mitigate, or otherwise deal with accidental and deliberate introductions of exotic pests (Fig. 1). When indirect costs such as loss of production and export markets are factored in, the economic impact can be staggering. For example, it is estimated that establishment of the Mediterranean fruit fly, Ceratitis capitata, alone in the continental U.S. would cause annual losses in excess of one billion dollars. These threats result in the establishment of sanitary and phytosanitary (SPS) measures intended to safeguard a nation's resources while allowing the movement of agricultural commodities among trading partners. Thus, quarantine and regulatory issues require a global perspective.

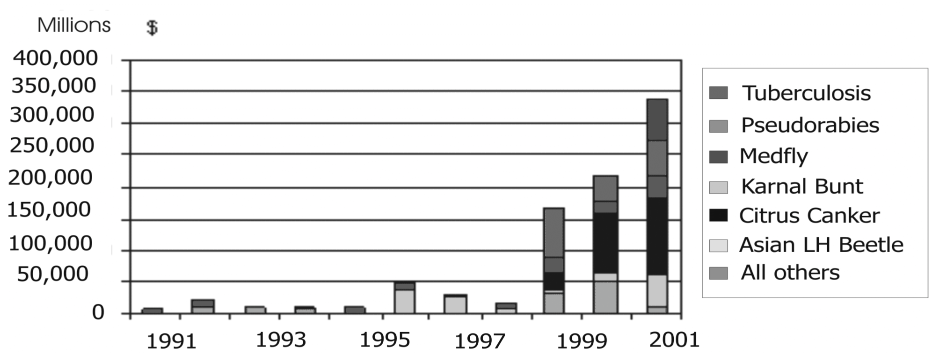

Figure 1. Trends in cost of conducting emergency programs for all exotics within APHIS USDA.

HARMONIZATION OF SPS MEASURES

The World Trade Organization (WTO) is the international organization that deals with the rules of trade between nations. Through the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), signatory nations to the WTO agree on certain basic concepts in setting their animal and plant health requirements. Working through the International Plant Protection Convention (IPPC), national plant protection organizations (NAPPOs) harmonize these SPS measures by formulating International Standards for Phytosanitary Measures (ISPMs). These measures form a common, effective and transparent safeguarding process among trading partners. To date, nineteen (19) ISPMs have been ratified and adopted (and others await endorsement and ratification) that cover a spectrum of international plant protection issues (FAO 2004).

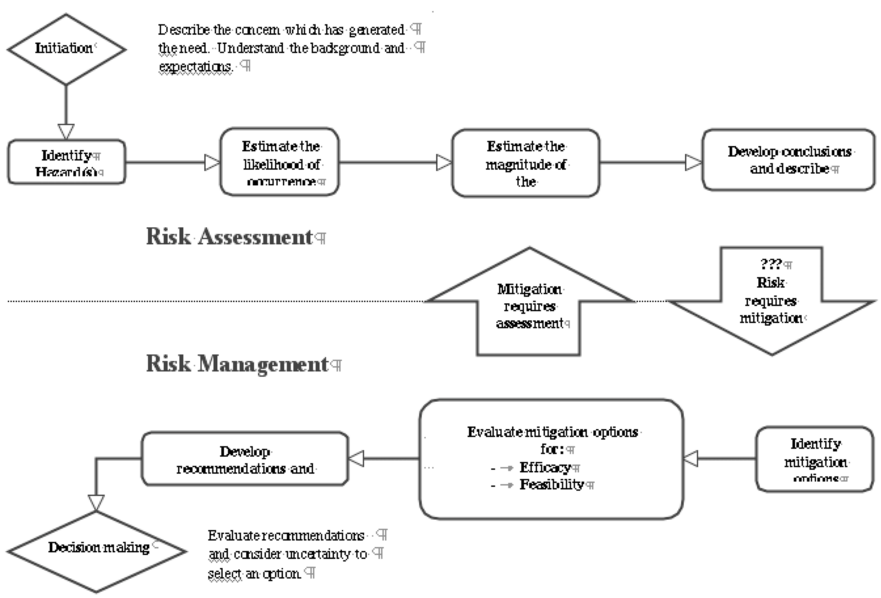

Figure 2. Risk and Pathway Analysis is composed of an initiation stage where the hazards are identified, a risk assessment stage where the probability of consequences of establishment is evaluated, and finally a risk management stage where mitigation options are evaluated and recommendations developed

REGULATORY ACTIVITIES IN THE UNITED STATES

The Plant Protection and Quarantine Division (PPQ) of the Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has the regulatory authority for safeguarding U.S. plant and other natural resources and, as such, is the official NAPPO representing the U.S. on the IPPC. In an effort to meet the challenges of the expanding global markets, PPQ recently underwent a stakeholder review of its plant regulatory activities (Anonymous 1999). The major issues pointed out in this review were the need 1) to strengthen our ability to conduct risk assessments, 2) to respond to increased smuggling interdiction activities based on consumer demand for exotic and specialty products, and 3) to strengthen the scientific and technological base of the agency and renew its ties to academia. In response to these recommendations, PPQ expanded the capabilities of its Pest Epidemiology and Risk Assessment Laboratory (PERAL), formed the Safeguarding, Intervention and Trade Compliance (SITC) program, and established the Center for Plant Health Science and Technology (CPHST).

CENTER FOR PLANT HEALTH SCIENCE AND TECHNOLOGY

CPHST is the scientific support organization for PPQ. Its mission is to identify introduction pathways used by exotic plant pests and weeds; assess the risks these exotics pose; develop, adapt and support technology to detect, identify and mitigate the impact of exotics; and ensure that the methods, protocols and equipment used by PPQ are effective and efficient. CPHST is composed of 250 scientists, analysts and researchers at ten (10) principle laboratories and eight (8) supporting units throughout the U. S. and Guatemala. Our stakeholders include land owners, universities, private industry and organizations, local, state and federal agencies, and foreign governments. CPHST provides services ranging from optimization of existing pest management practices and development of new technologies for pest exclusion, survey and management to providing technical support to stakeholders for treatments and facility certification. This work is embodied in about 200 work plan-based research and technology projects that are centered around five (5) program areas (www.cphst.org).

Risk and Pathway Analysis (RPA). SPS regulations are enacted primarily to foster and facilitate trade while safeguarding natural resources of the importing nation. However, depending on the amount of risk an importing country is willing to accept, SPS regulations can become barriers to trade. The nature of any SPS regulation will depend on the amount of risk associated with the introduction and potential establishment of the target pest. It is the responsibility of the importing country to establish its own regulatory standards. The process whereby that is accomplished must be based on science and the resulting standards should be applied only to the extent necessary to protect human, animal or plant life or health. Further, regulatory standards should not arbitrarily or unjustifiably discriminate between countries where identical or similar conditions prevail.

Because of our obligation under the SPS Agreement and the IPPC, signatories must make phytosanitary decisions using science-based RPAs. CPHST's PERAL laboratory conducts science-based risk analysis based on the international standard (FAO 1996) to establish the risk of introduction and establishment of alien pests to the U.S. In a regulatory framework, RPA is the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it (Sequeira 2002). Operationally, risk analysis is a multi-stage process (Fig. 2) that includes 1) initiation phase where pests are identified as a risk factor or hazard, 2) risk assessment stage where the pests are evaluated as to the likelihood of occurrence or establishment, and 3) risk management stage where mitigation measures for eliminating or reducing pest risk are evaluated (Devorshak and Griffin 2002). PERAL completes fifty RPAs.

Molecular Diagnostics and Biotechnology (MDB). The MDB program identifies, develops or adapts state-or-the-art technologies for molecular-based detection and identification of agents of interest to the agency and provides scientific support for the development of genetically modified insects for the control of crop pests. Active projects include development of rapid, molecular identification technologies for immature fruit fly and other invasive species intercepted at ports of entry and techniques to identify point of origin of fruit fly introductions. Other projects dealing with biological control and eradication programs include development of genetically modified organisms such as pink bollworm (*Pectinophora gossypiella* (Saunders)), Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)), and the Mexican fruit fly (*Anastrepha ludens* (Loew)) that possess markers and lethal genes.

Survey Detection and Identification (SDI). This program provides scientific basis for survey programs to rapidly detect and identify pest threats and develops and implements robust diagnostic tools and cutting edge spatial technologies and decision support systems. Active research projects include the development of automated image analysis for the purpose of rapidly identifying large numbers of insects collected in field trapping surveys, and the application of geographic information systems and spatial analysis for predictive modeling of pest populations and management strategies.

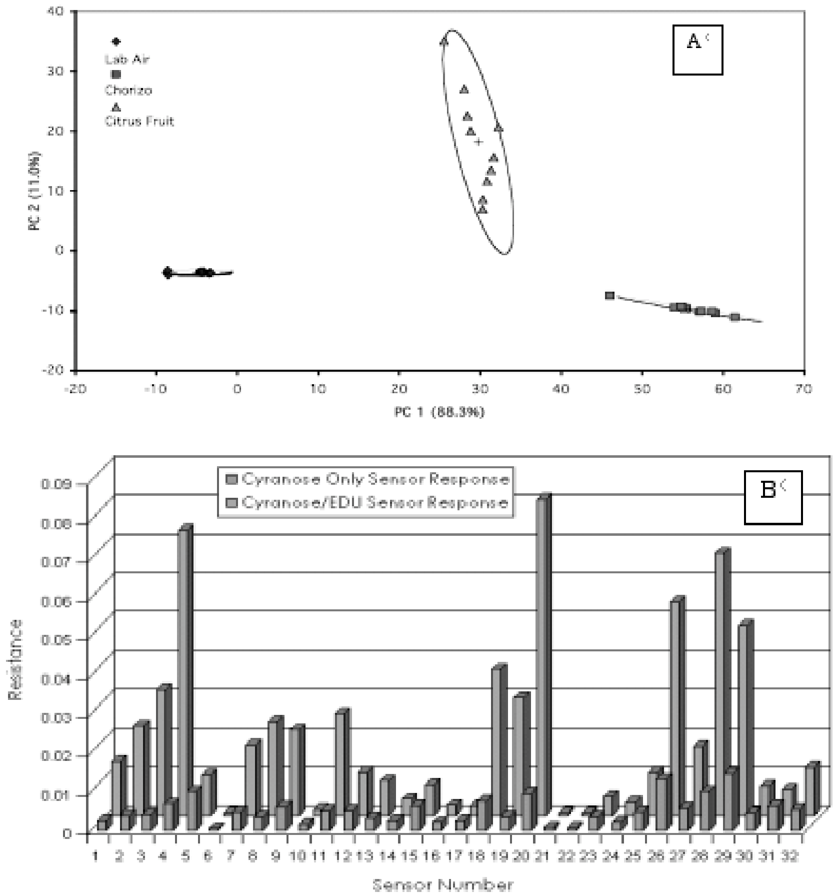

Figure 3. Evaluation of the Cyranose 320 electronic nose. Separation of principal components of lab air, chorizo and citrus fruit rind (A) and amplification of response to bananas when a sample concentrator (Airsense Electronic Detection Unit) is coupled to the unit (B).

Integrated Pest Management and Eradication (IPME). IPME deals with exotics that have become introduced and established domestically. The program provides scientific support to regulatory program managers and decision-makers engaged in strategic planning and deployment of emergency programs that are designed to safeguard and mitigate against the established quarantine pests. These efforts are focused on the response phase of an introduction by mobilizing technology and expertise for rapid detection and delimiting the spread of the pest. Activities involve providing science-based strategies for detecting, monitoring and mitigating recently-established populations of *Ralstonia solanacearum* race 3 biovar 2 and Sudden Oak Death, *Phythopthora ramorum.*

Agricultural Quarantine Inspection and Port Technology (AQI). This program develops, adapts and supports technology to detect, identify and mitigate the risk posed by quarantine pests in pre-clearance programs and ports of entry. Core activities in the AQI program include quarantine treatment development, treatment manual support for ports of entry, and methyl bromide use database maintenance. Although quarantine and preshipment (QPS) uses of methyl bromide are exempt from the provisions of the Montreal Protocol (EPA 2003), it is incumbent upon the agency to reduce or eliminate MB usage wherever possible. In 2003, PPQ monitored 7,553 QPS fumigations that used 175,100 kg of MB (Table 1). The major issues facing AQI are development of technologies to detect and identify exotic pests in preclearance and ports of entry inspections and development of commodity quarantine treatments, particularly those that can replace MB. A few selected examples of research and technology development programs within the AQI national program dealing with these issues will follow.

TABLE 1
Methyl bromide usage in the United States for year 2003 monitored by USDA APHIS PPQ during 7,553 fumigations.

Commodities (Top five)	Methyl Bromide (kg)
Grapes	71,340
Logs (Oak)	11,690
Ceramic tile	11,310
Logs	9,575
Chilean fruit (Grapes)	7,984
All other	63,230
Total	175,100

DETECTION AND IDENTIFICATION TECHNOLOGIES

Electronic nose. A major pathway for introduction of exotic pests and contraband is passenger baggage and mail. Presently, inspectors search randomly selected baggage and containers; sometimes dogs are used. A portable, sensitive electronic nose that can identify the contraband items would be significantly more efficient, accurate and reliable. A cooperative project with Argonne National Laboratory is developing such an instrument. Containing a series of polymer and metal oxide sensors, the hand-held portable unit can distinguish between dried meats and fruit (Fig. 3A). In order to improve sensitivity, a sample concentrator was incorporated into the prototype instrument (Fig. 3B). Theoretically, the sensors can be selected and 'trained' to detect a wide range of contraband materials, ranging from bioterror agents like anthrax to insects, drugs, and explosives.

Trace element analysis. In order to adequately monitor and inspect commodities for exotic pests, the origin of the commodity must be known. The geographical area from which a commodity originates determines the specific pests an inspector might encounter. For a variety of reasons, some commodities arrive at ports of entry from intermediate locations, their origins having been effectively disguised. CPHST's Analytical and Natural Products Laboratory is developing a method to determine the origin of agricultural commodities by analyzing the profile of trace elements found in the commodity. This method is based on the fact that plant genetics and the elemental composition of the soil contribute to unique trace element profiles. The method has worked well in discriminating orange juice blends and garlic samples by points of origin (Fig. 4). Critical to the validity of these tests is obtaining authentic reference samples from the agricultural production areas in question.

Agricultural internet monitoring (AIM) system. APHIS regulated organisms are being sold on the internet. Manual searches of URLs reveal that the potential number of implicated websites could number in the thousands. There is a need for advanced computer technology services to assist in locating and regulating the unwanted sale and movement of these organisms and commodities over the internet. The goal is creation of a secure intranet-based web application that semi-automates the process of webcrawling, evaluates sites for risk, sends information letters, and archives and retrieves information. CPHST is working in cooperation with The Southern Region Center for Integrated Pest Management (CIPM) in Raleigh, NC. CIPM has already developed the first phase of a demonstration project that provides tracking of internet sites selling illegal items. CIPM is currently in discussion with a number of agencies and organizations (U. S. National Plant Board, Western

Australia Ministry of Agriculture, wildlife organizations, etc.) for continued development and implementation of the software.

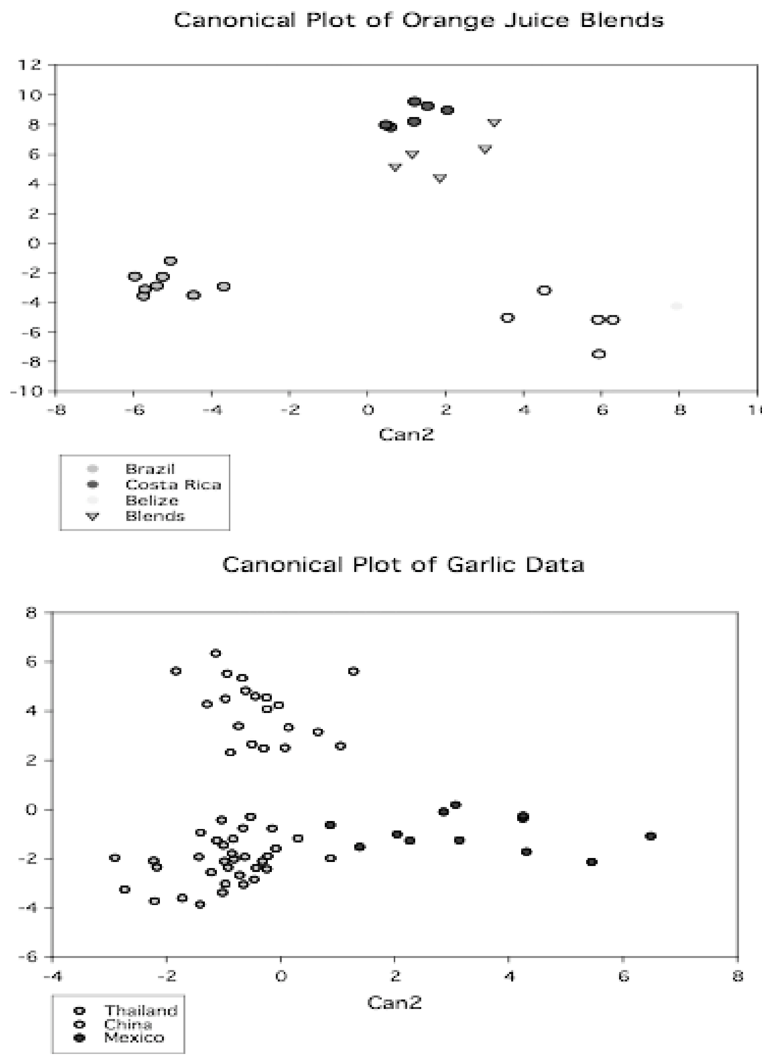

Figure 4. Separation of orange juice blends and garlic of different origins analyzed for composition of trace elements and then subjected to canonical analysis. The principle component scores for the first two canonical variables are plotted on the X and Y axes. (Samples furnished by U.S. Customs and Border Protection Laboratory, Savannah, GA).

LucID for pest identification. PPQ's safeguarding mission must be accomplished without impeding trade. However, identification of an exotic pest at a port of entry may require expertise located elsewhere, in which case the infested commodity must be rejected or held in quarantine pending identification of the pest. This is particularly critical for perishable commodities. Having technology available at ports of entry that will improve the identifier's skills is necessary to maintaining the flow of trade. One such technology is LucID. LucID is an expert system that uses interactive, multimedia identification software. Developed in 1994 at the University of Queensland, the software provides a rapid, reliable and user-friendly approach to pest identification, allowing for more accurate identification in a shorter period of time. CPHST has completed construction of about ten such keys of regulatory importance and another ten are under construction. These and other keys are available at www.lucidcentral.com.

DEVELOPMENT OF COMMODITY QUARANTINE TREATMENTS

Fumigants. CPHST is actively involved in developing mitigating treatments for agricultural commodities imported into the U.S., particularly those treatments that can replace MB or reduce its usage. Under the terms of the Montreal Protocol, the target date for phase out of MB in developed countries is only months away although QPS usage will remain exempt until suitable replacements are found (UNEP 1999). One area of research is the development of ozone-friendly fumigants to replace methyl bromide. In cooperation with CSIRO, Australia, and the Animal and Plant Quarantine Institute, The Peoples' Republic of China (PRC), CPHST is experimenting with carbonyl sulfide, sulfuryl fluoride and Cyanogen for low temperature fumigation of solid wood packing (SWP) material targeting the Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motchulsky) that overwinters in trees in northern China. Results of these experiments (Barak et al. 2003) showed: 1) carbonyl sulfide is not a viable alternative for MB with SWP in 4.4°C, 24-hour fumigations of green, solid wood naturally infested with ALB larvae; 2) cyanogen is not very effective against ALB in fresh, sawn 10- x 10-cm SWP, probably because of its high solubility in water (and excessively green wood) and may be more effective in drier wood, as is used in SWP in commerce; and 3) sulfuryl fluoride is not very practical for controlling ALB larvae in high moisture wood at relatively cold temperatures (i.e., ≤ 10°C), and tests are planned to evaluate the potential dose reductions of sulfuryl fluoride as a function of temperature.

TABLE 2
Methyl bromide doses applied in each container (6.1 m, $33m^3$) for fumigating solid wood packing material comparing APHIS-PPQ Treatment Schedule T-404-b-1-1, a revised schedule with intermediate doses that allows for reduced methyl bromide usage, and an optimal treatment schedule that can sustain minimum CT products when good fumigation practices are used.

Temperature (°C)	Treatment schedule doses applied		Intermediate schedule doses applied		Optimal schedule doses applied	
	kg	pounds	kg	pounds	kg	pounds
4.4	2.64	5.83	2.64	5.83	2.38	5.24
10.0	2.64	5.83	2.11	4.66	1.85	4.08
15.6	2.64	5.83	1.85	4.08	1.58	3.50
21.1	1.58	3.50	1.58	3.50	1.32	2.9
Total used	9.50	20.97	8.18	18.07	7.13	15.72
Total saved	---	---	1.32	2.91	2.37	5.25

From Barak et al. (2003)

Containers fumigated abroad may expose inspection personnel to unsafe levels of residual atmospheric fumigant due to inadequate aeration during inspection and unloading after arrival at U. S. ports of entry. Because containers fumigated at lower temperatures require higher doses, there exists a potential risk for unsafe residues due to increased sorption and slower degassing. An on-going project, in cooperation with the Animal and Plant Quarantine Institute, PRC, is aimed at refining the MB treatment protocol for fumigating SWP material at low temperatures. The APHIS Treatment Manual (USDA 2004) allows only two dosages of MB (48 g/m3 at 21.1°C or above and 80 g/m3 at 4.4 to 20.6°C) for this treatment. The cooperative tests were conducted in China and were predicated on the assumption that additional MB dosages at temperatures between those given in the treatment manual could reduce the amount of methyl bromide required for container fumigations and likewise reduce the levels of residual atmospheric fumigant present upon inspection at ports of entry. The operational-sized fumigation trials showed that adding two intermediate dosages to the treatment schedule can save significant amounts of MB in SWP fumigations (Table 2). Additionally, more efficient fumigation techniques for containers would allow doses lower than those in the proposed treatment schedule and still maintain adequate CT products. Good fumigation practices coupled with the lower doses could result in world-wide reductions of hundreds of tons of MB per million containers fumigated. However, in spite of the lower doses of MB used to fumigate containers for SWP at point of origin, 6% of the fumigated containers that arrived in the U.S. had atmospheric MB residues greater than 5 ppm.

Irradiation. The U.S. has approved irradiation as a phytosanitary treatment (USDA, 2003) and its application is governed by international standard (FAO 2003). Presently, this treatment targets fruit flies and a few other arthropods (Table 3). It is an excellent substitute to MB fumigation of fruits (Hallman 2003). Irradiation is unique among quarantine treatments in that it does not require mortality but rather prevention of reproduction. The U.S. Final Rule governing this treatment requires that there be reciprocity on the part of trading partners in that they must have similar legislation providing for the use of this treatment. To date, reciprocal agreements with international trading partners are not yet in place and the only irradiated U.S. imports allowed are interstate shipments and imports to the mainland from the Hawaiian Islands. Continuing research by CPHST, the Agricultural Research Service (ARS), USDA, and other research partners is broadening the scope of irradiation as a phytosanitary treatment to include more pest treatment options. Until specific treatment doses can be determined for additional pests, the generic dose concept (Hallman and Loaharanu 2002) is being considered for all tephritid fruit flies and 7 other arthropod pests.

*Radiofrequency***.** That part of the electromagnetic spectrum known as radiofrequency (Rf) spans roughly 3 kHz to 300 GHz. Within that range are the microwaves with frequencies of ≥ 1 GHz. It has been known for many years that the heat produced by Rf waves can kill insects (Headlee and Burdette 1929). This technology has potential application to dried fruits and nuts (Wang et. al. 2001), where the pests contain relatively higher moisture content than the host and are thusly selectively targeted, but thermal Rf technology has little or no application to fresh commodities that are susceptible to the heat produced by microwave Rf. A non-thermal application of Rf has recently been demonstrated that has potential as a quarantine treatment for a wide variety of pests on fresh agricultural commodities (Lagunas-Solar et al. 2003). The biocidal method is known as metabolic stress disinfestation and disinfection (MSDD) and relies on a combination of fluctuating pressure differentials with an oscillating or pulsed Rf field while in a modified atmosphere containing ≤ 0.01% oxygen. Preliminary studies suggest that a variety of target pests can be controlled in ≤ 12 hours using MSDD (Lagunas-Solar et al. 2003). CPHST is working in cooperation with ARS and the University of California at Davis to further evaluate the potential of MSDD as a quarantine treatment.

TABLE 3
Pests and doses for which irradiation is approved as a phytosanitary treatment for imported fruits and vegetables.

Pest Species	Common Name	Dose (Gy)
Bactrocera dorsalis	Oriental fruit fly	250
Ceratitis capitata	Mediterranean fruit fly	225
Bactrocera cucurbitae	Melon fly	210
Anastrepha fraterculus	South American fruit fly	150
Anastrepha suspensa	Caribbean fruit fly	150
Anastrepha ludens	Mexican fruit fly	150
Anastrepha obliqua	West Indian fruit fly	150
Anastrepha serpentina	Sapote fruit fly	150
Bactrocera tryoni	Queensland fruit fly	150
Bactrocera jarvisi	(no common name)	150
Bactrocera latifrons	Malaysian fruit fly	150
Sternochetus mangifera	Mango seed weevil	300
Euscepes postfasciatus	Sweetpotato scarabee	400
Omphisa anastomosalis	Sweetpotato stemborer	400

Miscellaneous treatments. Both dry and steam heat treatments are being targeted at a variety of pests and commodities. European snails hitchhiking on military containers coming from the Mediterranean, the Golden cyst nematode on potato farming equipment in New York state, and karnal bunt spores on wheat in Texas are a few examples. Based on the results of a recently-completed fluid dynamics computational model of a cold chamber, improvements will be made in the APHIS PPQ in-transit cold treatments for fruit fly control. Other projects in progress are development of controlled atmosphere treatments for stone fruits to meet quarantine restrictions, evaluation of the impact of organosilanes and hot water washing on surface pests on commercially processed pears, verification of hot water treatment of mangoes, and irradiation of passenger baggage for devitalizing bioterror agents.

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