The Plant Pathogen Containment Facility at Frederick, Maryland

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Microorganisms that cause disease in man or in the plants and animals essential to man's well-being are potentially dangerous when used in experimental research. There is no acceptable alternative to doing the research because these diseases must be controlled, and the knowledge required to devise control strategies comes primarily from experimental research. As a result, special procedures and facilities have been developed that permit the necessary experimentation while ensuring the safety of the public, the researchers, and the environment (12,13). Some aspects of the needed research can be done in areas where the pathogens are endemic (5).

During the 1940s and early 1950s, the U.S. Department of the Army designed and built special research facilities for safe experimentation with plant (6), animal, and human diseases at Fort Detrick, Frederick, Maryland, and for animal diseases at Plum Island (off Long Island), New York. The facility at Plum Island was transferred in 1953 to the U.S. Department of Agriculture (USDA) and has since functioned as the national center for research on contagious foreign diseases of animals. In April 1971, the plant pathogen containment facility, staff, and other facilities of the Plant Pathology Division at Fort Detrick were taken over by the USDA. This acquisition became the Plant Disease Research Laboratory (PDRL). In 1972, some of the Fort Detrick facilities designed for research on human diseases were transferred to the U.S. Department of Health, Education and Welfare, to form the National Cancer Institute's Frederick Cancer Research Facility.

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Research Within PDRL's Containment Facility

A facility in which necessary investigations can be conducted safely with plant pathogens of any type from any area at any time is a valuable national asset. The ability to simultaneously compare collections of pathogens from different regions of the world is a unique research advantage of containment study.

Equipment and techniques are available within the containment facility to culture both obligate and facultative microorganisms; to produce, collect, and store inoculum; to apply inoculum to test plants under controlled, monitored conditions; to quantitate disease development, increase, and spread parameters; and to quantitate the effect of disease on crop yields.

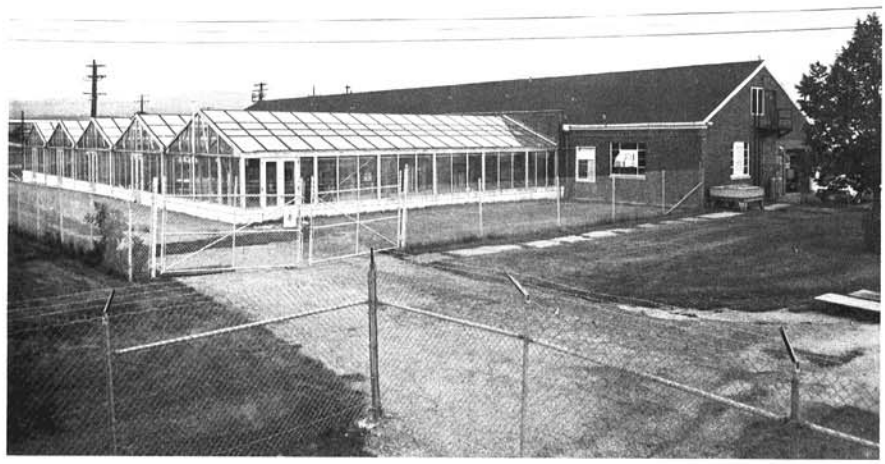
Comparisons have been or are being made with pathogens causing downy mildew of corn and sorghum, rusts of corn, maize streak, rust of soybean, soybean dwarf, and rust of skeleton weed and with pathogens potentially useful as agents for the biological control of noxious weeds (5). For example, isolates of fungi causing downy mildew, each identified by its contributor as *Peronosclerospora sorghi*, were received from India, Thailand, and Texas. These were compared under the same conditions for virulence, host range, and other biological characteristics. The culture from Thailand differed markedly from the others and probably should be designated as a species other than *P. sorghi* (M. R. Bonde, *unpublished*). Comparative studies with isolates of the nonendemic *Phakopsora pachyrhizi*, the cause of soybean rust, have demonstrated pathogenic races of the fungus and quantitative differences among isolates for factors affecting aggressiveness (1,10).

The containment facility has been used to provide information on citrus canker, caused by *Xanthomonas campestris* pv. *citri*, and on sugarcane smut, caused by *Ustilago scitaminea*. This research, planned by USDA scientists not assigned to the laboratory, was implemented and pursued by them in cooperation with staff at PDRL. Scientists from Brazil, the Philippines, and South Africa have worked in the containment facility on cooperative projects related to soybean rust or southern corn leaf blight.

Research on the potential use of plant pathogens as biological control agents for noxious weeds in the United States has been conducted in the facility since 1975. This involves testing many types of pathogens from areas of the world where the target weeds are indigenous. The biology, host range, and potential effectiveness of these pathogens are evaluated, and the data obtained contribute to the knowledge required by state and federal authorities for making decisions regarding production and release of organisms as weed control agents (4).

Description of the Facility

The plant pathogen containment facility is a brick-faced concrete block building 41 × 176 ft with five attached glasshouses (Fig. 1). Each glasshouse is 25 × 60 ft and is glazed with large Thermopane panels supported by a steel superstructure. All exterior windows in the masonry structure are double-glazed, with glass on the interior and Plexiglas on the exterior side. The masonry structure is divided into four functional areas: 1) a room containing the main exhaust and supply fans and filter systems, preheat equipment for supply air, and electrical transformers; 2) a room containing two 300-ton air-conditioning compressors (one is always on standby status), heat exchangers and associated pumps for the main chilled water system, a smaller compressor system supplying chilled brine to remote dew chambers, and a fuse panel for the 4,160 VAC power



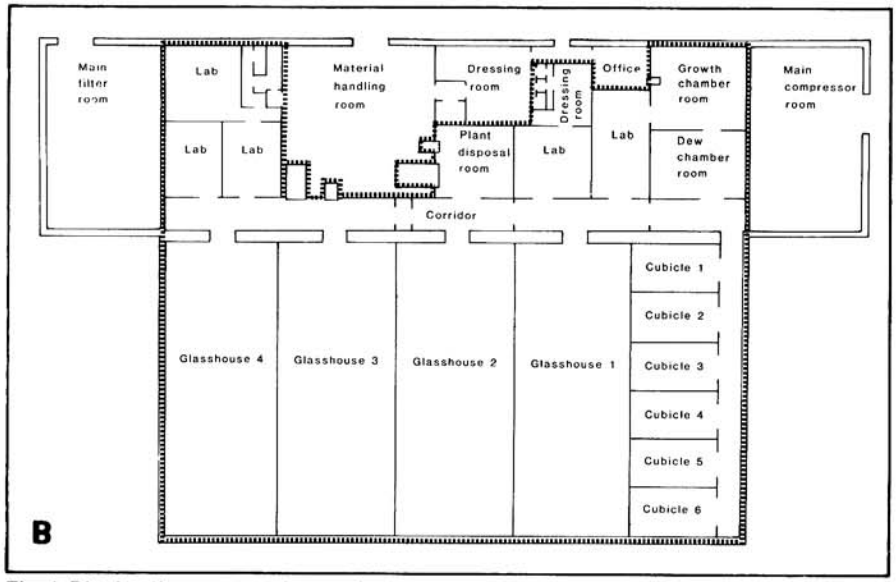


Fig. 1. Plant pathogen containment facility. (A) Five glasshouses are attached to masonry building housing laboratories and ancillary equipment. (B) Floor plan, with delineation of the contaminated "hot" area where pathogens are present as aerosols during research.

supply; 3) an area containing an office, locker room, toilet facilities, and plant handling room (this area is accessible by two doors to the outside and provides entrance to the interior of the building); and 4) the interior area containing two air locks with showers and change rooms for entry and exit of personnel, laboratories, growth and dew chamber rooms, and a central corridor providing access to the glasshouses.

The interior area can be divided into two separate complexes, each isolated from the other and from the outside and each with independent air supplies and sewage systems. When operated in this manner, one complex has access to two of the glasshouses and the other to three. Four glasshouses are not divided. The fifth is divided into six 10×20 ft cubicles and an access corridor (Fig. 2).

The attic of the masonry building houses electric, gas, water, steam, and chilled water lines and supporting equipment such as water still, air handlers, ductwork, and secondary filter systems. A steel-reinforced 4-in.-thick concrete slab separates the attic from the building below. The floor of the entire complex is a 6-in. slab of reinforced concrete.

Filters in the air supply and exhaust ducts to the individual areas have permanent leakproof fluid channel seals. Duct joints, seams, and filter frame holders are soldered. Fittings are sealed into the ductwork to permit steam decontamination when necessary. All joints in walls and all penetrations of pipe, ductwork, etc., are sealed inside and out with silicone caulking.

Air control. Outside air is preheated as necessary and passed through dust-stop roughing filters into the main supply duct. From this supply, branch ducts, each with its individual fan and tandem double-filter unit, deliver fresh makeup air to each cubicle, greenhouse, and laboratory area.

Exhaust air from all regions within the area where pathogens are present is drawn through tandem double-filter units that are 99.9% efficient in the trapping of airborne particles of a mean diameter of 0.5 μm or greater. The air is then carried by the main exhaust duct and filtered again through deep-bed filters before discharge to the outside atmosphere. Thus, all air leaving contaminated areas passes through three successive filter systems, each capable of removing airborne bacteria or fungus spores. Containment of viruses as disease-causing propagules is accomplished by containing their hosts and/or vectors.

The tandem arrangement of the filters in the supply and exhaust air systems permits replacement of the upstream filter while containing pathogen aerosols in the containment areas, since the second (downstream) filter remains in place. Also, supply and exhaust air-handling units can be repaired without decontamination, because the supply side never is exposed to pathogens and the exhaust side is totally within the containment area.

Within each laboratory and glasshouse area, individual air-handling units condition and recirculate the air internally over steam and chilled water coils and through household-type dust-stop filters. The volume of air recirculated can be varied by two-speed motors and pulley selections. In the large glasshouses, the air volume recirculated varies from 10,000 to 12,500 cfm and in the cubicles, from 1,350 to 1,500 cfm (1 cfm = 57 L/sec). Air moves over experimental plants at an average velocity of 0.5 m/sec or less. As with the exhaust system, the recirculation system is physically within the containment area, and any repairs can be made without danger of pathogens escaping from the facility.

Fresh air is supplied to each area at a rate of 5–10% that of the recirculated volume. Simultaneously, air is removed through the exhaust filters at a rate exceeding the supply air rate, thus causing each area to operate at an air pressure negative to the outside atmosphere. Dampers within the supply and exhaust ducts can be adjusted so that certain areas within the containment area operate at an air pressure negative to others even though all are negative to the outside atmosphere.

The main corridor is positive to four of the five glasshouses and all cubicles; the fifth glasshouse is used to propagate experimental plants and is positive to the corridor to prevent entry of aerosols of unwanted plant disease organisms. In the shower and contaminated dressing room areas, air pressures are strongly negative to the clean-side dressing areas, providing a constant inflow of air that effectively prevents aerosols from reaching the clean side. Although strongly positive to the shower rooms, the air pressure in the clean areas is still negative to the outside atmosphere.

As a fail-safe provision, the controls on supply and exhaust air systems are electrically interlocked. The supply fans cannot be operated unless the exhaust fans are also running. If the exhaust fans should stop unexpectedly, the supply system is automatically cut off, preventing

the development of positive pressure within the containment area.

Control of environment. All cubicles and glasshouses are equipped with programmable cam controllers and recorders for wet and dry bulb temperatures. Dry bulb temperature can be controlled from 15 C and higher. Relative humidity can be controlled above about 50% by intermittent injection of steam. Proper selection of the wet and dry bulb cam controls can provide a film of water simulating dew on plants throughout an entire glasshouse.

There are eight reach-in environmental growth chambers for temperature and light studies with smaller plants.

A dew chamber room contains nine small (about 0.75 × 0.75 × 1.5 m high, inside dimensions) and two large (0.75 × 1.25 × 1.5 m high) dew chambers (Fig. 3). Simulated dew at a range of intensities can be formed on plants in these chambers at air temperatures of 10–36 C. A 12-point potentiometer continuously records air temperature within each chamber.

Sewage. All liquid and solid waste from within containment areas drains into welded wrought-iron pipe sewage lines enclosed within poured concrete protective casings. The effluent drains by gravity into six holding tanks (50,000 gal each). The effluent is then pumped through a series of heat exchangers where heat is picked up from the outgoing treated sewage. During passage of the effluent through the heat exchangers, temperatures rise from ambient to about 113 C. Steam is injected to bring the temperature to about 135 C, and the effluent then flows through some 1,500 ft of insulated pipe that exposes the effluent to 135 C for about 14 minutes. The heat-treated effluent then passes back through the heat exchangers, where it gives up its heat to the incoming raw sewage. The treated sewage is sampled through a rubber diaphragm type of sampling adapter and tested for sterility. The sterile effluent is carried by standard sewage lines to a conventional sewage treatment plant.

Protocol

Most plant pathogens do not cause disease in humans, although some cause allergenic reactions and some produce products harmful to man. The organisms used in the plant pathogen containment facility at Frederick require only normal handling to safeguard the health of working personnel; for example, concentrated aerosols of fungus spores are similar to nontoxic industrial dust in that conventional precautions and equipment to prevent inhalation or excessive exposure provide adequate protection. Major emphasis in the standard operating procedures has been to preclude escape of microorganisms while minimizing restrictions that hamper experiments.

Exterior doors of the containment facility are kept locked at all times, as are the two doors providing access to the containment areas through the air locks with showers. Authorized laboratory personnel have keys, and others requiring entrance (visitors, workmen, etc.) are escorted by members of the laboratory. One professional and one support scientist coordinate operation of the facility as part of their overall research responsibilities.

Before entering the containment area, personnel must remove all clothing and jewelry and put on laboratory clothing (head covering, socks, shoes, and coveralls) available in the clean-side dressing rooms. They may then proceed through the air lock and shower area into the "hot" side dressing room and on into the research area. Normally, showering during the entry process is not required.

When leaving the containment area, personnel again remove all clothing and leave it in the inside dressing room. They then shower and shampoo thoroughly and exit through the air lock into the clean-side dressing room. Eyeglasses worn into the containment area are also washed thoroughly during the showering process.

Material normally enters the containment area through one of five double-ended autoclaves equipped for steam, dry heat, or ethylene oxide gas sterilization (Fig. 4). Incoming material usually does

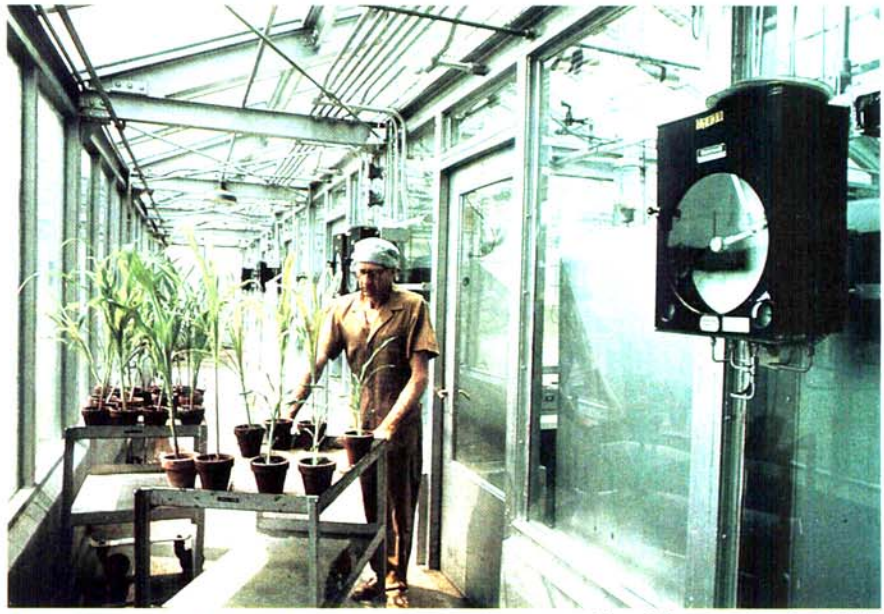


Fig. 2. Moving experimental plants through glasshouse corridor with access to six cubicle growing areas.

Fig. 3. Harvesting conidia of a downy mildew pathogen from source plants previously placed in large dew chamber to provide conditions promoting sporulation.

not require sterilization. Living plant material, other than seeds or pathogen cultures, is brought in only in special instances, and then extra effort is taken to prevent the introduction of unwanted disease organisms and insects.

All material leaving the containment area is processed through the autoclaves. Discarded plant material, soil, pots, and most items that can tolerate the treatment are steam-sterilized; those that cannot, such as microscopes, cameras, and film, are sterilized by the cold gas treatment (10% ethylene oxide with 90% CO_2 to reduce flammability).

A security fence encloses the area surrounding the glasshouses, maintaining a perimeter zone free from traffic of any kind and reducing the probability of accidental or intentional damage to the glass. The condition of the building and the support equipment is monitored at intervals throughout the day and night, 7 days a week, by trained maintenance and security personnel. An intrusion alarm system is installed in the containment complex and if activated, sends a signal to the security control panel of the guard force indicating an unauthorized attempt to enter the containment laboratory. Sensors also monitor the operation of key equipment and conditions in glasshouses and cubicles, transmitting a signal to a remote, constantly manned control panel should a malfunction occur.

Frederick, Maryland, is in a relatively low hazard area in regard to snow, hail, tornadoes, and earthquakes. No structural damage or glass breakage has been caused by these phenomena in the containment facility during its 28 years of operation. The most severe storm of record (1975) with winds attaining 100 mph did not damage the structure, but conventional glasshouses suffered extensive glass breakage.

Other possible emergency situations have been identified and specific procedures formulated to respond to them without compromising the containment integrity. Such emergencies include fire, serious illness or injury to a worker requiring immediate medical aid, glass breakage from vandalism, and loss of electric line power. Such events present serious problems in any facility, but within a containment area where any responsive actions must be taken in a manner that also prevents loss of containment capability, the problems become more complex.

No containment facility, regardless of sound engineering, sophisticated hardware, and detailed operational procedures, will assure the integrity of the complex if the personnel working within it are inadequately informed, inept, or lacking in dedication. All PDRL workers whose duties require them to enter the containment area are thoroughly trained in operational procedures and made aware of all ongoing research within the facility. They know and understand the reasons behind the rules and regulations. It is their willing dedication and

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K. R. Bromfield

Dr. Bromfield, a research plant pathologist at USDA's Plant Disease Research Laboratory in Frederick, Maryland, received Air Force training in meteorology at New York University, a B.S. in forestry from Pennsylvania State University in 1949, and a Ph.D. in plant pathology from the University of Minnesota in 1957. Since 1950, he has investigated and led research on the etiology and epidemiology of rusts, especially stem rust of wheat, peanut rust, and, since 1971, soybean rust.

C. H. Kingsolver

Dr. Kingsolver served as director of USDA's Plant Disease Research Laboratory from its inception in 1971 until his retirement in 1979. He received advanced degrees in plant pathology from Iowa State University in 1941 and 1943. Following service in the U.S. Navy, he was assistant professor of plant pathology at the University of Missouri from 1946 to 1951. Except for 5 years (1957–1962) as an agricultural administrator in the Agricultural Marketing Service, he was associated with the U.S. Army Biological Laboratories at Fort Detrick from 1951 to 1971, heading their Plant Pathology Division from 1965 to 1971. He has held appointment as adjunct professor at Pennsylvania State University since 1971 and is an independent consultant.

conscientious adherence to an effective protocol within the specially designed and operated facility that ensure the essential condition of pathogen containment.

Special Equipment and Techniques

Assessment of the epidemic potential of exotic pathogens is the primary thrust of research within the containment facility. This requires the acquisition, development, or adaptation of special equipment and techniques (in addition to equipment normally used in a well-equipped plant pathology laboratory) to utilize the facility's capability to fullest advantage.

Cyclone spore collectors (3,11) are used in sizes and configurations ranging from "microcyclones" for collecting from single rust pustules to those for collecting from large populations of mature plants. These facilitate rapid, convenient collection of required quantities of "dry" propagules, such as uredospores of rust fungi. Other types of spores are collected in water, Freon, or other liquids, as appropriate, and concentrated by filtration or centrifugation for use as inoculum. The method of choice for maintaining cultures of the rust fungi under investigation and, more recently, of many other pathogens is storage of their propagules in liquid nitrogen (2,8).

Quantitative inoculations with propagules of foliar pathogens that infect and spread in nature by airborne spores are made in turntable settling towers specially developed for this purpose (9). Modifications to the settling towers also permit quantitative inoculation with spores suspended in various liquids. When less stringent control of inoculum deposition is acceptable, plants may be placed in dew chambers and immediately atomized with spores suspended in water, Freon, or other liquid or dusted with spores diluted with "talc."

A temperature gradient plate (Fig. 5) provides precise control of temperature from below to above the physiological response range of the pathogen being studied and facilitates determining the effect of temperature on spore germination, germ tube growth, and early infection in detached plant tissues. Built-in dew chambers (7) provide controlled dew conditions for studies involving relatively small plants (1 m tall or less) in pots. Up to 11 conditions of dew period air temperature, dew duration, dew intensity—or combinations of these—can be compared simultaneously.

Temperature and light effects on small potted plants are studied in eight reach-in environmental growth chambers of conventional design and operation. Six Plexiglas chambers ($0.8 \times 1.8 \times 1$ m high, inside dimensions) equipped with gloveports and individual temperature control capability are available in the glasshouse areas. These chambers exclude and contain aerosol particles with a mean diameter of 0.5 μm or greater and permit simultaneous work with several pure isolates of a pathogen.

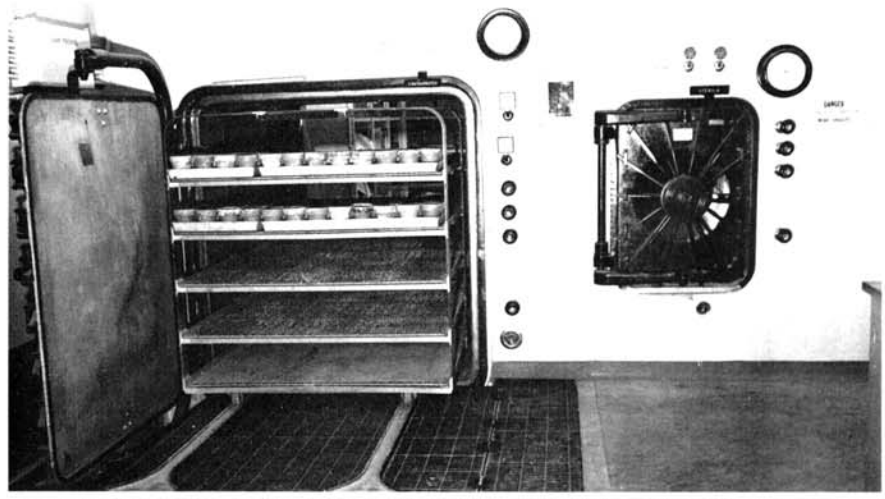

Fig. 4. Double-ended autoclaves through which materials enter and leave contaminated research area.

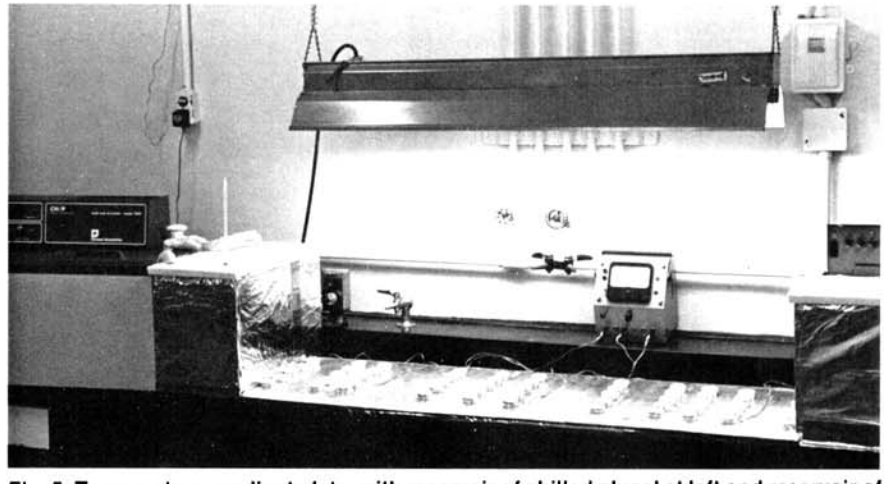

Fig. 5. Temperature gradient plate, with reservoir of chilled glycol at left and reservoir of warm water at right. Plastic petri dishes containing media for spore germination are arrayed on the aluminum plate at positions providing desired temperatures.

To study diseases throughout the growth of the suscept plants and to determine the effects of disease on yield, large plantings can be made in glasshouse soil beds, each $2.8 \times 17 \times 0.5$ m deep (Fig. 6), or in large metal cans containing 0.03 m^3 of soil. Plants are spaced within and between rows in accordance with accepted agronomic practice for the particular crop. Because no fully satisfactory artificial light source has been found for growing high-light crops (eg, maize), such plantings are made at the time of year normal for field plantings (early May to early June). Glass is kept clean to obtain the maximum possible insolation within the growing areas; this is feasible during the summer season without excessive heat buildup because of the large capacity of the air-conditioning system.

Initial inoculation of the plantings is made in a manner and at a time appropriate for the purpose of the particular study, such as pathogen spread from a known infection focus or effect of crop age at first infection on disease and yield. Misting or steam-injection systems, in combination with temperature control, can be programmed to provide leaf wetness (simulating dew or rain) on all plants throughout the glasshouse (Fig. 7). Wetness periods of controlled duration can be programmed throughout the study. Crops with low light requirements can be studied out of their normal season. Light sources adequate for day length extension and some photosynthetic effect are available.

A portable television camera and auxiliary equipment within the containment area permit closed-circuit color transmission to monitors on the clean side of the facility. In addition to recording visual observations on videotape for subsequent analysis, the system can be used to provide maintenance personnel with needed visual information. Also, large groups of visitors may be shown areas of interest and experiments in progress without having to change clothing and enter the containment area.

Fig. 6. Large planting bed containing maturing soybeans.

Fig. 7. Misting soybean plants to provide leaf wetness for germination of *Phakopsora pachyrhizi* in a study of soybean rust.

Discussion

During the last decade, interest has been increasing in techniques and facilities for the study of potentially dangerous organisms. The recent controversy in regard to recombinant DNA research was widely publicized, and while the guidelines originally developed have since been made less stringent, the scientific community and the general public were made aware as never before that different levels of risk must be identified and specific safeguards for dealing with each risk level must be developed.

The containment facility we have described represents the highest level of plant pathogen containment. It differs from the highest level of containment for human pathogens only in the absence of gloveport cabinets designed to protect the research operator. Plant pathogens, however, pose little or no danger to research operators. The containment facility for plant pathogens has the same integrity as those for human or animal pathogens in terms of preventing escape of pathogens from its interior.

PDRL operates three other containment units used for research activities posing lesser risks. These units are equipped with filtration systems and air locks and operate at negative air pressure. Before entering them, personnel put on head coverings, laboratory coats, and shoe coverings. These garments are removed when personnel leave and are retained within the facility for future use or until sterilized through a double-ended autoclave. Pathogens within these areas are usually handled in laminar flow hoods, and no mass aerosols of pathogens are generated. Small environmental chambers permit some inoculation studies with potted plants, but no large numbers of diseased hosts are produced. Facilities operated in this manner are adequate for some studies.

As interest and activity in the biological control of noxious weeds, destructive insects, and other harmful organisms increase, there will be more and more demand for importation of exotic organisms. Much of this research will be on a state or regional basis, directed toward resolution of local problems. This will intensify the need to define risk levels, develop research procedures, and construct additional facilities providing adequate protection.

Less than a decade ago, the list of projects requiring a capability for plant pathogen containment was short, indeed. Today, the number of proposals for such research exceeds the capacity of available facilities. Although the construction of containment complexes is being discussed and proposed at various locations, to our knowledge no construction is currently under way. The technology and operational philosophy to design, build, and safely operate such facilities are available, and their construction should be encouraged.

During the coming years, research in containment facilities will contribute vital knowledge needed to assess crop vulnerability to foreign pathogens, to identify and evaluate genes conferring resistance to potentially destructive foreign diseases, to resolve identification and taxonomic problems with certain exotic pathogens, and to implement biocontrol programs.

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