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ALLERGY ARISING FROM EXPOSURE TO AIRBORNE CONTAMINANTS IN AN INSECT REARING FACILITY:

HEALTH EFFECTS AND EXPOSURE CONTROL

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Allergy Arising From Exposure to Airborne Contaminants in an Insect Rearing Facility:

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INTRODUCTION

In agricultural crop improvement, yield under various stress conditions and limiting factors is assessed experimentally. Of the stresses on plants which affect yield are those due to insects. *Ostrinia nubilalis,* the European corn borer (corn borer) is a major pest in sweet and field corn in the U.S. There are many ways to fight crop pests such as the corn borer, including (1) application of chemical insecticides, (2) application of natural predators and, (3) improving crop resistance through plant genetics programs.

Randomized field trials are used to determine the effectiveness of pest management programs. These trials frequently consist of randomly selected crop plots to which well-defined input regimes are instituted. For example, corn borers might be released onto crop plots in several densities at various stages of crop development, then sprayed with different levels of pesticide. These experiments are duplicated across regions and, in some cases across the country, to determine, in this instance for example, the best pesticide application rate for a given pest density and crop development stage. In order to release these pests onto crop plots, one must have an adequate supply of the insect pest. In winter months studies are carried out in the laboratory to examine chemical and natural pesticide effectiveness, as well as such things as the role of pheromones in moth behavior. The advantage in field trials is that yield data can be garnered directly.

In this country, insects are raised for crop research primarily through the U.S. Department of Agriculture, in cooperation with public Land Grant Universities and, by the private sector agricultural concerns--seed companies and others. To fulfill the Master of Public Health thesis requirement, it was agreed that a study would be carried out to quantify the airborne allergen exposure of persons working in a Land Grant University entomology lab where allergy to European corn borer was suspected.

The original investigational plan involved making personal and area size-selective aerosol concentration measurements in several lab areas and during various lab operations in which the corn borers were handled. Next, these samples were to be examined for the two predominant potential allergens known to be in the lab: mold spores and moth parts. Several mold genera were identified by the university environmental health department (UEHD) staff, an essential step in identifying potential

allergens and an aid for the treating allergy physician. Of the hundreds of mold genera an individual might react to, those found in the occupational setting are of primary interest here, although mold and other allergens in one's residential environment could easily compound occupational allergic disease. The abundance of fungi in this lab is due to the high relative humidity needed in moth rearing environments.

The *original* study strategy was to grow out the spores from personal and area sample aerosol material (collected to estimate allergen concentration), and to count the number of moth wing scales (to estimate moth airborne allergen levels).

As will be set out in this paper, significant problems with spore viability during the aerosol collection process and, the lack of a clear relationship between viable airborne spore concentrations and mold allergen air concentrations, led to the abandonment of this approach in attempts to quantify fungal airborne allergen concentrations.

The original investigational plan also included the goal of quantifying corn borer allergen exposure. The concept was to count moth scales under a light microscope in the same manner in which asbestos fibers are counted, taking appropriate measures to arrive at moth scale airborne concentrations. Two major shortcomings led to the abandonment of this strategy as well. The first problem is that the wing scales are fragile and break apart due to factors in the rearing facility and likely during sampling, such that wing scale counts become unreliable due to the many wing scale fragments which are created, and which are often too small to discern with light microscopes. The second factor is that virtually all of the insect parts from all life stages (egg, larva, pupa and adult moth) are understood to be allergens in susceptible human populations. Thus moth wing scale aerosol concentrations will not necessarily correlate to moth allergen aerosol.

A literature review revealed that the technology required to quantify laboratory personnel allergen aerosol exposure was available. The aerosol samples obtained in the lab could be sent out for analysis. This type of allergen analysis is often referred to as radioallergosorbent (RAST) testing. RAST tests make use of human antibody obtained from blood serum to quantify the allergen concentration in a sample which can then be extrapolated to allergen aerosol concentrations, often expressed in ng or ug allergenic protein per cubic meter of air. Although available, RAST testing is expensive to initiate and requires specific purified allergenic material to create standards against which

unknown allergen concentrations can be measured. Pure cat, dust mite, etc. allergens are available for common garden-variety allergens, but are less easily obtained for allergens of lesser stature. Allergens required in making standards for the corn borer would have to be purified from the insects from the rearing facility. Overall, it was decided that the technical and fiscal obstacles to RAST analysis were too great to be tackled in this project.

Under the original plan, allergen aerosol exposures could have been estimated, and control measures evaluated, important steps in creating an economically justifiable worker protection plan. However, given the lack of occupational allergen exposure standards, the question of what constitutes acceptable exposure levels and satisfactory control measures would remain unresolved.

Anecdotal evidence from lab personnel indicated a serious, ongoing allergy problem in the moth rearing facility, in spite of new exposure control strategies implemented on the advice of the UEHD. A powered purified air respirator was purchased for use in many moth handling operations, gloves and dedicated lab coats were required in certain areas and for certain procedures, and the entire lab area was thoroughly cleaned to remove an accumulation of adult moths from walls, ceiling light fixtures and vents, etc., and a large free-standing HEPA filtration unit was installed in the lab.

Given the ongoing nature of the problem, it was decided that an investigation without RAST analysis could yield important allergen sampling and exposure control strategies; at a minimum, it was thought such an investigation could bring to light the suspected inadequacies of the then employed sampling procedures and engineering control strategies used in the entomology lab. At this juncture it was decided that an investigation and paper, (one without airborne allergen exposure quantitation) would benefit the ongoing investigation by the UEHD team, which in turn would be of value to the entomology department. The paper which follows provides: (1) An introduction to the allergy problem at this particular entomology lab, (2) Details of the entomology lab facilities, procedures, allergen exposure controls, etc., (3) Background information on allergy in general, as related to the entomology lab, and (4) Allergen aerosol sampling (exposure assessment) and, (5) Allergen control strategies suited to the entomology lab.

SECTION 1 ALLERGY IN AN INSECTARY

Mary (pseudonym), a worker in a European corn borer moth rearing facility, in an university entomology department was experiencing symptoms of breathing irregularity, runny nose, itchy eyes and skin, suggestive of asthma, rhinitis, conjunctivitis and contact dermatitis. Her physician determined she was suffering from allergic disease which was "clearly associated with her work environment". Her symptoms were mild in 1989 when she began work in her current capacity as lab technician (with a B.S. in biology and several years experience in entomology research). Her condition worsened to the point that she will no longer work at this lab by the end of 1993.

Indications are that this is not an isolated case of occupational allergy to arthropods. Several surveys of occupational allergy to arthropods have been conducted. In a survey by Robert Wirtz[1], mailed to 136 institutions chosen from mailing lists of the Arthropod Rearing Society Newsletter and university Entomology departments, 84 (61.8%) of the institutions responded. Fifty (59.5%) reported at least one individual with an allergy coinciding with occupational exposure to an arthropod, host animal, or arthropod diet. Anecdotal reports offered in conversation with entomologists, agricultural engineers, and technicians during the research phase of this paper suggest that prolonged exposure to moth rearing environments may result in severe allergic symptoms, and that these individuals recognize arthropod rearing environments as serious threats to the health and careers of a portion of exposed persons.

SECTION 2 CORN BORER FACILITY AND PROCEDURES

2.1 Facility Layout

The floor plan of the entomology lab is given in Figure 1. The moth rearing chambers are located in room 241C. Most of the handling procedures are now carried out in 241C, although some operations, including cage cleaning and disinfection, are carried out in room 211. This is significant because room 241C is now under separate ventilation, including negative pressure relative to adjacent rooms and is exhausted directly to the roof. Additionally, a free-standing HEPA filter has been put into service in 241C (and in the main moth rearing chamber). All other rooms, including 211, are under general exhaust ventilation (GEV).

The rearing chambers (see photo 11 and cross-hatched area in figure 1) are similar in construction to walk-in coolers one might find in a restaurant or at a corner grocer. They open into the working area of 241C. The doorway out of 241C leading to the general lab area is draped with 1-1/2" plastic stripping to reduce moth migration out of 241C, as moths regularly escape from the rearing chambers. It is not clear when this barrier was installed.

Mary, the affected individual, had office space for over two years in room 241A, in very close proximity to 241C. Her current office is in room 232.

2.2 Personnel

Mary, the lab technician, is virtually the only individual who performed moth rearing tasks on a day-to-day basis year-round. Two other individuals, both student workers, worked closely with corn borers for periods of about 18 months. One of these persons did experience allergic response to her occupational environment (the allergen type suspected of inducing allerigic response is unknown). In the summer months two to eight undergraduate student workers were hired to rear corn borers. Currently two student workers are rearing corn borers to handle the tasks normally performed by the lab technician at this time of year; they are using personal protective equipment and have experienced no corn borer allergy.

2.3 Corn Borer Rearing Procedures

Corn borer moths are collected in fields, checked for disease and if free of disease are housed in cages in rearing chambers. Egg masses are deposited on waxed paper by the adult moths. These masses are punched with a air-powered punch (photo 1). The paper disks with egg masses attached are collected and pinned in small plastic containers (diet dishes) to which food and habitat material have been added (photos 2 and 3). This environment is covered with paper towels (tightly secrued) and set on racks for 21 days (room 211). The containers are not opened such that there is no exposure during this period to corn borer allergen. The eggs are allergenic and some exposure is likely during the punching operation, although personal protective equipment (PPE) is now required, including gloves, respirator (positive purified air) and lab coat. Note lack of PPE at time photos were taken.

A food source is placed in the cages which will feed the corn borers as they mature into moths (photo 4). This food source must be replenished, an operation carried out via a circular access port at the end of each cage (photo 11). The diet dishes with larvae (photo 5) are placed in the rearing cages. This media contains the frass (saliva, silk, excrement) of the larvae from the 21 day period discussed above. This allergenic material is loose and light and is a significant potential allergen exposure source. PPE is now required for this operation (photo 5), and at all times in the rearing chambers and room 241C..

The cage frames are wooden, 15" h x 15" w x 20" l. The sides are covered with fine mesh through which the moths cannot pass. The top of the cage is covered with 1/4" hardware cloth which the moths may pass through (photo 6). This is to allow the moths to lay eggs on sheets of waxed paper which are placed over the hardware cloth. The moths will only lay eggs on smooth surfaces, similar to the undersides of corn leaves. Rubber mats are then placed over the paper to secure it and minimize moth escape.

The waxed paper upon which eggs are deposited is replaced daily (photo 7). This is a delicate operation, one requiring PPE under current lab protocol. The waxed paper with egg masses has been in direct contact with adult moths and likely will have insect

frass and wing scales associated with it. These paper sheets are carried out of room 241C to 211, creating the potential for dispersion of allergen to the portion of the lab under GEV.

After drying for a day or so, the eggs are removed from the waxed paper. The first step of the removal process involves pulling the paper, egg masses down, under a metal bar to loosen them (photo 9). Next, the paper is forcefully and rapidly pulled taught to "pop" the eggs loose (photo not available). These two operations (now carried out in room 241C) have resulted in eggs and moth parts landing in the worker's hair, on clothes, etc. and is thought to be a major allergen exposure operation. PPE is currently required. The eggs are then collected with a camel hair brush (photo 10).

In this manner, the insects are propagated and multiply, requiring 70-120 cages during peak production, with up to 500 adult moths per cage. (photo 11).

2.4 Allergen Exposures

Given the lab layout, personnel and procedures, what are the likely sources of allergen aerosol exposure?

2.4.1 Rearing Chambers

The adult moths flutter about in their cages creating allergen aerosol from frass, wing scales and body parts. Thus the rearing chambers in which the insects are housed are a primary potential allergen exposure source, especially during the operation in which waxed paper is exchanged.

The cages are disinfected before each use and the rearing chambers are routinely cleaned and disinfected as well to minimize moth exposure to pathogens. This attention to good moth environment hygiene has the secondary benefit of keeping mold growth down. Immunoassay would be required to determine whether mold or moth aerosol allergen is higher in concentration.

A free-standing HEPA filtration unit has been installed in the main moth rearing chamber.

2.4.2 "Bench" Operations

The bench operations, egg punching, popping, etc. are carried out in room 241C with PPE, in association with a free-standing HEPA filter. Current precautions have reduced allergen exposures, but no efficient aerosol collection system, such as a vented, hooded workstation exists at this time. During a visit to the lab in December 1993, several moths were observed flying about in 241C, indicating a lack of stringent allergen exposure control.

2.4.3 Non-241C Lab Areas

The larval production carried out in the sealed diet dishes in room 211 presents virtually no exposure hazard. The procedure of carrying egg masses on waxed paper to 211 to dry does pose an exposure hazard, not only for the technician, but for any other personnel in the general lab environment. This includes clerical staff and others due to the potential for allergen aerosol to enter the GEV system from 211 and adjacent areas (room 211 is open to the other lab areas).

Due to the negative pressure of room 241C in relation to adjacent areas, allergen aerosol should be contained fairly well, with the exception of live moths escaping through the plastic strip curtain, and the removal of contaminated articles such as PPE worn in 241C (currently stored just outside 241C) and "dirty" moth cages (currently HEPA vacuumed in 241C, washed with soap and water in 211). A black light moth trap is operated adjacent to room 241C in an attempt to capture moths having escaped 241C.

At this point it is appropriate to step back from the immediate situation described in the entomology lab above in order to set the stage for the development of an allergen aerosol sampling and control strategy. Allergic disease can have multiple causal agents and may have multiple manifestations. It is therefore imperative that the industrial hygienist understand the broader scope of allergic disease in order to design allergen aerosol sampling and control strategies that will result in meaningful data and effective control measures.

SECTION 3 ALLERGIC DISEASE BACKGROUND

In order to assess and control the allergens causing the allergic reaction in the entomology lab, one must identify potential occupational and non-occupational allergens and then employ a physician to test the suspect etiologic allergens on the individuals at risk. This work had been completed by the time I began work on this project. UEHD staff had identified several mold genera in the lab and the treating physician had completed an evaluation of the patient including an history of potential allergen exposure. The physician confirmed the suspected occupational allergy to corn borer. To put this work in perspective, a list of potential allergenic agents is presented below, followed by a discussion of the human immune response to allergens, known as the immunoglobulin E (IgE) response.

3.1 Potential Etiologic Agents

The allergy symptoms Mary experienced were attributed to corn borer allergen. However, asthma, one of her conditions, is a poorly understood condition thought to have several causal agents. Allergen exposure and resulting IgE response in susceptible individuals is a major factor in development of asthma, but other agents could be responsible for the disease as well. Potential allergenic and non-allergenic agents are discussed below.

3.1.1 Allergens

Allergens are generally assigned to one of two classes. Animal and vegetable proteins make up the high molecular weight allergens. Chemicals such as anhydrides, isocyanates, etc.., are generally referred to as low molecular weight allergens or haptens. Haptens are strictly not allergens until they bind covalently with proteins forming a molecule which may be recognized by IgE.

The following is a non-exhaustive listing of allergens one would like to identify or exclude as contributors to atopic disease in an occupational setting such as the moth insectary of concern here.

3.1.1.1 High Molecular Weight Allergens

The term high molecular weight allergen refers to plant and animal protein which has the potential to bind to human IgE, based on chemical composition and molecule configuration. It has been shown that the allergens of greatest potency are proteins frequently associated with digestion. In cats, saliva is a potent allergen, as is urine of rats, feces of mites and frass of moths. Conversely, some insect parts have virtually no allergenicity. One investigator used whole body moth extract in aeroallergen research because the moth wing scale extracts did not contain allergen[2]. This is an interesting observation in light of the fact that many entomologists and arthropod allergy investigators speak of moth wing scales as the allergen of concern in disease development. However, allergens could easily reside upon moth wing scales in the insectary environment, and thus contribute to allergy indirectly. Whether or not moth wings scales are found definitively to be non-allergenic is not the critical issue; it is, rather, to realize that other moth parts, secretions and products of elimination are (also) allergenic.

3.1.1.1.1 Arthropod

Arthropods include insects, crustaceans, arachnids, and myriapods (e.g. centipedes) which are characterized by a chitinous exoskeleton and a segmented body. Of these, the moth (insect) and to a lesser extent, mite (arachnid) proteins are major potential allergen sources.

3.1.1.1.1.1 Moth

Moth hairs, setae (stiff hairs which may contain urticating chemicals), scales, exuvia, fragmented body parts, feces, venoms, and silk are known or potential allergens.

Many entomologists and other researchers speak of moth wing scales as *the* allergen of interest where moth related allergy is suspected or known. As previously stated, feces, hairs, silks and other allergen sources are likely more of a concern as potential allergens.

3.1.1.1.1.2 Mite

Mites have been shown to be potent allergens[3]. While typical moth rearing facilities guard against organisms (including mites) potentially detrimental to the health of the moth stock, mites should not be overlooked as an allergen source in moth rearing facilities due to an environment favoring their development (abundance of food and high humidity) found in moth rearing chambers.

3.1.1.1.2 Fungi

Due to the high relative humidity (often 70-80%) required in moth rearing chambers, the potential for fungi (mold) growth and spore production is great. Interestingly, fungal spores, caps and mycelia (vegetative structure) have been shown to contain common and unique allergen,[4,5] such that spore counts alone may not yield accurate estimates of aerosolized fungal allergen. Similarly, *viable* spore counts have limited utility since allergens need only bind to IgE to induce allergic response; viability is not requisite for such binding. In the unique case of individuals at risk for developing allergic bronchopulmonary aspergillosis, typically found in persons with depressed immune function, viable spore exposure is a significant factor in disease prevention and management. In the moth insectary of concern several mold genera were identified.

3.1.1.2 Non-Occupational Allergen Sources

An obvious yet important confounder in occupational allergy cases is that of the potential for non-occupational allergen exposure. Non-seasonal or weakly seasonal allergens as from pets, mold, and mite sources, for example, may contribute significantly to allergic disease, such that reductions in occupational allergen aerosol may not correlate well with allergic symptom responses. Skin prick tests and immunoassays can be of great value in identifying allergens to which the patient may be sensitized, allowing the physician to suspect, but not prove, potential allergen exposures.

3.1.2 Non-allergenic / Not Known To Be Allergenic Agents

Hyperresponsive airway disorders may be triggered by nonallergenic agents. Irritants are one such agent type. In relation to asthma, psychological factors, once considered etiologic agents, are now thought of as triggers but not causative agents. These two disease "agents" are addressed here.

3.1.2.1 Irritants

Asthma and other hyperresponsive airway disorders may have nonallergenic triggers. Examples include fresh paint, cooking odors, perfumes, cologne, insecticides, and household cleaning agents.[6] In addition, SO_2, cigarette smoke, and air pollution, both indoor and outdoor, can trigger asthma symptoms. With some types of chemical exposure, hyper-reactive airway disorders may persist for short periods or may be permanent. Lung function tests may detect a reduction in lung function, but would not offer insight into the disease etiology. A complete patient history and routine allergy testing are two of the diagnostic tools useful in diagnosing allergic asthma vs hyperresponsive airway disorders. Although important when they occur, non-IgE mediated hyperresponsive airway disorders are unlikely to be disease agents in aerosol allergen occupational environments such as insectaries and as such are not discussed at length.

3.1.2.2 Psychological Factors

Paul Greenberger, in *Allergic Diseases: Diagnosis and Management* describes the role of psychology in asthma succinctly when he states "Asthma has evolved from a disorder considered to be psychological to one recognized as extremely complex and of unknown origin." Greenberger goes on to say that stress clearly may influence asthmatic disease, much as it does in many aspects of our lives, but that a dose-response relationship of emotional stress in asthma must be considered as speculative. Additionally, specific behavior patterns, including disease denial, indolence, compulsive and manipulative behavior, and quackery have been observed in asthmatic patients, however, no evidence was presented to suggest any link between these observed behaviors and asthma per se.

With discussions of allergic disease types and potential etiologic agents with regard to occupational allergy in the moth rearing facility now set forth, the stage is set for a brief review of the IgE immune response.

3.2 IgE Mediated Immune Response

The human immune response to allergens is complex and not fully understood. The topics which follow are discussed briefly in relation to the moth insectary in an attempt to familiarize the reader with the basic concepts of IgE immune response in order to appreciate not only the complex nature of the human allergic response, but also to point out some of the important facets of the role for the occupational hygienist.

The first issue is that of the site of exposure/response - How does the site of allergen deposition affect the nature and site of the immune response and development of allergic disease? Secondly, what types of allergy tests are available and how reliable are they? Finally, if we are to set allergen aerosol exposure limits, what are the levels of exposure which lead to sensitization and to disease?

3.2.1 Sites of Response

Where does the exposure occur which leads to allergy? The answer is complex but seems twofold. Apparently, sensitization may occur in any one of several sites of exposure, while allergy symptoms appear to occur primarily at the site of exposure. For example, in moth allergen inhalation, the primary responses are runny nose, itchy eyes, etc., but virtually the whole body is sensitized, as is seen in the diagnostic use of the skin prick test by treating physicians to determine potential allergens.

It is important to state that not all individuals are thought to have the potential to develop allergy. Significantly, 50% to 60% of the population is not susceptible and will not develop IgE antibodies to inhalants regardless of the levels of exposure.[13] These individuals, as one would infer, will not be susceptible to allergic disease.

3.2.1.1 Allergen Exposure Leading To Sensitization

The simplified picture of sensitization has two primary components. When an allergen is recognized by IgE antibody and other serum constituents, there is a local response, but there is also an amplification and distribution of IgE and mediators throughout the circulatory system.

Duff and Platts-Mills[7] state that "The evidence establishing a causal relationship between inhalant allergy and asthma is currently extensive. Specific sensitization occurring in genetically susceptible individuals and leading to signs of allergic disease has been demonstrated with many different indoor and outdoor allergens. Sensitization has been demonstrated by skin testing, serum IgE antibodies, and bronchoprovocation challenges." Pollart, et al[8] demonstrated that in adults who visited the emergency room with acute wheezing, the prevalence of measurable IgE antibodies to one or more inhalants was four times that of nonwheezing controls (69% vs 15%). Among children with asthma, sensitization is unusual under the age of 2 years but becomes progressively more common up to about 8 years of age. Multiple factors, including status of the immune system, dosage of inhalant exposure, and length of exposure, all appear to play a role in the rate of sensitization.

How does the role of sensitization affect decisions made in the entomology lab? The significance lies in the fact that persons who do not have symptoms may not follow PPE protocol as well as those with symptoms, yet these less cautious individuals may become sensitized. Once sensitization occurs, the threshold allergen aerosol concentrations leading to symptoms are greatly reduced. The concept of sensitization also has portent for screening new hires: susceptible individuals may not test positive for corn borer allergy, be hired, yet go on to become diseased.

3.2.1.2 Allergen Exposure Leading To Disease

It is clear from the discussion above that allergen exposure in one site may sensitize virtually all body tissues through which blood flows. As discussed earlier, there is also an important role of the lymphatic system in allergy, but this is thought to be non-IgE mediated. It is primarily the mucosal surfaces, which by design are defensive tissue, that are often the site of allergic response and disease. In food allergy the intestinal

mucosa become irritated often leading to stomach upset. In hayfever, where pollen size is typically too large to reach lower airways, the response is seen in the mucosal surfaces of the eyes and nose. Where asthma is present the smaller particles capable of penetrating to the bronchi are also able to affect the eyes and nose, and this is typically seen.

The implication for the entomology lab is that particle size and aerodynamic diameter will impact the type of allergic response in susceptible individuals. This in turn has implications for the exposure control strategy one might employ: in order to remove the fine allergen fraction which one would expect to cause eye irritation, efficient aerosol capturing schemes are required--unlike the inefficient free-standing HEPA filter currently employed in room 241C of the entomology lab.

3.2.2 Diagnostic Tests for Allergy

There are several commonly employed tests used to determine the presence of IgE antibodies in an individual. The skin prick test, inhalation test, and blood serum immunoassay are the frequently used tests to determine sensitivity in atopic individuals.

Due to the variability of the various allergy testing methods, physicians rely heavily on patient history. In cases where the harmful allergen source is difficult to identify from the plethora of allergens to which a typical patient may be exposed, a detailed individual diary may be of great value in determining which environmental allergen exposures lead to disease symptoms.

Confounding common to each of these diagnostic tools exist. One problem is that of allergen purity / potency. Many commercial insect allergen preparations are made from whole body extracts which may vary in specific allergen content. In the area of fungal allergen, studies have clearly documented the inadequacy of commercially available mold extracts with regard to skin test and RAST.[9] Elution and dilution procedures, among others, may lead to test variability.

3.2.2.1 Skin Prick Test

The first test method commonly used to assay for allergy was the skin prick test. Suspect allergens diluted to levels considered safe, given known patient responses, are administered. The allergens to be tested, along with appropriate controls are placed on the skin and the skin is pricked. The irritation, known as wheal and flare, are recorded.

This test is not quantitative, but is easy to administer. A liability of the skin prick test is apparent low specificity. In a study of food allergy, the sensitivity of skin tests ranged from 73 to 100 percent. The sensitivity for five of the six foods ranged from 97 to 100 percent, with the sixth food at 82 percent[10]. Another source of confounding is the allergen source. The rating of wheal and flare response is also subject to variation.

3.2.2.2 Inhalation Tests

In vivo inhalation (nasal or bronchial) challenges represent another approach in allergy diagnosis where asthma is suspected or confirmed. One challenge employs an atomized spray of allergen inhaled by the patient. Another test involves inhalation, in the manner described above, of an agent such as histamine, which is generally thought to produce symptoms of bronchial hyperreactivity virtually indistinguishable from allergen inhalation challenges (in individuals with atopic disease). Early and late phase responses are detected primarily via lung function tests, typically a series of FEV_1 measurements.

3.2.2.3 Immunoassays

There is a wide variety of immunoassays available for the detection and quantitation of allergens. In *Allergosorbent tests,* such as the popular radioallergosorbent test (RAST), the allergen of interest is bound to a solid support, forming the allergosorbent. The allergosorbent is exposed to the patient's serum. If the serum contains IgE, which is immunologically specific for the allergen, the IgE binds to the allergen. The IgE that does not bind to the allergosorbent is washed away from the allergosorbent along with the rest of the serum. The allergosorbent is then reacted with a labeled antihuman IgE antibody (e.g. a mouse antibody which has the capacity to bind human IgE antibody).

The amount of the anti-IgE binding to the allergosorbent is proportional to the amount of anti-IgE bound to the sorbent; thus by quantitation of the amount of anti-IgE bound, the amount of specific IgE in the serum can be estimated.

As might be expected, test repeatability is a concern. One investigator remarked that "Even in a carefully controlled clinical laboratory, fairly large variations may occur randomly in assays for specific IgE."[13] However, when laboratory methods are carefully controlled, it has been demonstrated, for example, in paired trials, that results may be obtained which are not significantly different.[11] Or more simply put, there is evidence to suggest that results from different investigators should be compared only with caution, but that with proper controls and analysis, intra-investigation results may be compared with confidence.

What this all means in terms of the entomology lab is that the science of allergy diagnosis is imperfect, and results must be viewed with caution. The impact of non-occupational allergy will be virtually impossible to discern from occupational allergy. This may result in worker's compensation claims which may be difficult to win for individuals claiming harm due to occupational allergy.

3.2.3 Allergen Aerosol Required to Sensitize / Induce Clinical Disease

The level of allergen exposure which results in some level of disease varies greatly between individuals and may change dramatically as a given individual becomes sensitized to an allergen. Just as exposure to a disease agent, such as a virus, initiates a cascade of events including the amplification of antibody production and antigen"memory", a non-sensitized, susceptible, atopic individual may become exposed to an allergen, resulting in the same type of amplification, in this case of IgE, and with "memory" of the IgE-allergenic agent.

The variation in human response to allergen due to sensitized vs non-sensitized individuals combined with other confounding factors such as non-occupational allergen exposure precludes the setting of standards for individual, or personal occupational exposures. As with all occupational exposure standards, contaminant levels could possibly be set for allergen aerosol which are thought to protect most individuals, but

given the variability in human populations these standards would not guarantee protection for the most sensitive individuals.

In a paper outlining methodology for occupational allergen exposure standard setting, Dr. Charles Reed discusses his work with diisocyanates, a spray-wash air conditioner allergen and laboratory mice allergen. Where the exposed population is large enough to allow statistical analysis and where the offending allergen or hapten can be identified, "safe" occupational exposure levels have be defined.[12]

The procedure in each case involves 1) identification of hapten or allergen which has been determined to cause allergy in the workforce; 2) hapten or allergen aerosol measurement by means of personal and/or area samplers which have been appropriately calibrated and otherwise set up, both before and after engineering controls are initiated; 3) the quantitation of hapten or allergen via sensitive immunoassay; and 4) the statistical analysis of detailed symptom questionnaire results taken before and after implementation of engineering controls. Using these principles, levels of allergen or hapten aerosol levels thought protective of human health have been set. Little work has been done to date in this area of occupational allergen standard development. Similar attempts have been made in non-occupational settings to ascertain levels indicative of both sensitization and of allergic disease.

Epidemiological studies in homes have been carried out in an effort to determine allergen exposure levels which correspond to disease incidence. In a study of airborne mite allergen in homes of asthmatic children, there was a strong association between sensitivity (positive skin prick tests) to house dust mite and the presence of mite allergen in the air of the homes, using a Casella personal sampler.[13] The authors conclude by stating that "Until a prospective study has been done it will be impossible to predict a threshold level below which the frequency of allergy is reduced." Thus while it is possible to determine airborne allergen levels which induce sensitization and disease, to the knowledge of this writer, no such data yet exist for exposure to European corn borer allergen or that of other moths.

The above has very important implications for allergen exposure control measures in the entomology lab. Some of the most important considerations are:

1. Currently, there is no data suggesting minimum allergen aerosol concentrations to avoid sensitization, and to avoid disease in sensitized individuals.

2. Without standards for allergen aerosol concentration minimums, allergic individuals have little recourse to achieve air standards which mitigate their symptoms.

3. The only way to arrive at meaningful standards for moth allergen aerosol is to carry out a large epidemiological study of entomologists rearing moths to estimate safe allergen aerosol levels. Dose-response data and analysis on a scale as small as that in the entomology lab would be unreliable.

While discussions of allergic disease are interesting and insightful, to the industrial hygienist they are the springboard from which occupational allergen aerosol exposure schemes can be designed and carried out, leading finally to exposure control measures.

SECTION 4 EXPOSURE ASSESSMENT

In order to derive meaningful results from airborne allergen exposure measurements, one must understand the particle aerosol properties, the allergenic aerosol properties as contrasted to the non-allergenic aerosols sampled, and one must be confident that the sampling strategy - placement of samplers, type of sampler, etc. - is meaningful within the context of the questions under investigation. The discussion begins with the important subject of allergen aerosol properties.

4.1 Properties of Allergen Aerosols

The two allergen aerosol properties of primary interest to the industrial hygienist and other concerned professionals are aerodynamic diameter, (which is the property determining such matters as settling speed, where the particle will deposit when inhaled, etc.) and, biological function or composition, (which determines the potential for IgE recognition and binding, potentially leading to sensitization and allergic disease).

4.1.1 Particle Size

The range of inhalable airborne particle sizes, allergen or not, is over a hundred-fold. The lower range has a practical limit of about 0.1um due to the fact that particles of this size typically constitute only a small portion to the total aerosol mass. The upper limit is determined by rapid settling of large particles, few of which manage to be captured either by human airways or sampling devices. The mass contribution of these few particles will, however, contribute significantly to total mass in many environments. The European corn borer moth wing scale (about 100um by 5um) will have a much smaller aerodynamic diameter d_{ae} than physical diameter, and should be estimated using size-selective samplers. Airborne pollens are in the range of 20 to 60um d_{ae} (another source listed 12 to 70um d_{ae}) in diameter; mold spores usually vary between 3 and 30um d_{ae}; dust particles are often defined as having a size range of 1 to 10um, and by some authors, 1um to over 100um d_{ae}. Due to the fact that a percentage of fungal spores and pollen grains break apart (during insect activity, and possibly during aerosol sampling), the significance of geometric diameter and d_{ae} of these *whole* reproductive

structures becomes clouded; the allergenicity of fractured spores and pollen grains remains.[16]

4.1.2 Aerodynamic Size Distribution

Particle d_{ae} is the property which determines where in the airway allergens will deposit and as described earlier, determines to a great extent, the type of allergic disease experienced by the exposed atopic individual: allergic conjunctivitis, rhinitis, asthma and to a lesser extent, contact dermatitis. It also greatly influences the air monitoring strategy and the efficacy of specific engineering controls.

Studies have shown that there may or may not be differences in the allergen mass collected on a given stage of a particle impactor (a type of air sampling device in which particles break out of the air streamline as they airstream bends sharply impacting on a solid surface) in disturbed and quiet environments. In a study of rat airborne allergen using a cascade impactor, the highest percentage of allergen by mass was found in the size range of 2 to 15um d_{ae} in quiet and in disturbed environments.[14] Conversely, differences were found in the percent mass of cat allergen found on stages of an impinger in quiet and disturbed environments. In quiet air researchers found 62% of the allergen mass <2.5um d_{ae}; when the same environment was disturbed, the percent allergen mass <2.5um d_{ae} dropped to 41%.[14] Factors such as humidity, relative air movement between quiet and disturbed environments, allergen type and, sampling technique will influence these measurements. In a study of outdoor moth allergen in southern Minnesota, Wynn et al[15] found the highest percent allergen mass for true army worm and wheat-head army worm (moths) in two ranges: 0.8um to 1.4um and >4um d_{ae}. No data on European corn borer rearing chamber allergen d_{ae} data were found in a review of pertinent literature. In fact, in an insect allergy bibliography by Bellas[16] only 3 of 625 references concern the European corn borer.

In both exposure assessment and exposure control, aerosol aerodynamic diameter is a key factor which should not be overlooked, and which will be addressed again in later sections of this text. The third property of allergen aerosols, after size and aerodynamic diameter to be discussed in the context of this paper is that of (IgE-recognizable) allergen composition.

4.1.3 Properties of IgE Recognizable Allergen

The properties that allow human IgE to bind with allergen and lead to potential sensitization and disease are important in determining appropriate assay methods once aerosol collection methods have been determined. The high molecular weight allergens of concern in insect and animal rearing facilities are proteins. Water and lipid solubility play a role in exposure in both site and rate of uptake. The water-soluble portion of ragweed pollen, for example, affects the respiratory and conjunctival mucosae, and the lipid-soluble allergens of ragweed pollen may cause a typical contact dermatitis on exposed skin. In addition, there is evidence that allergen solubility increases at higher pH levels and that higher nasal mucosa pH in turn corresponds to increased prevalence of rhinitis. Not only does allergen composition influence allergic disease outcomes, it is the primary factor in determining the suitability and reliability of allergen quantitative assays, discussed under *immunoassays*, below.

Now that the properties of allergen aerosols have been outlined, the means by which airborne allergens are sampled and assayed are addressed.

4.2 Airborne Allergen Concentration Determination

Over the last 50 years or so airborne allergen collection methods have improved dramatically. Initially, little attempt was made to sample using aerosol collection rates, such that extrapolation to concentration values was impossible. Later, attempts at estimating airborne allergen concentrations were made, but came with serious limitations. The 1967 discovery of immunoglobulin E by Ishizaka and Ishizaka revolutionized the manner in which allergy could be studied, including the development of accurate, quantitative, aerosol allergen assays. The two primary components of aerosol allergen concentration determination are aerosol collection and allergen content determination.

4.2.1 Collection Methods

All impactors and a few liquid impingers rely on accelerating particles in a volume of air, subsequently subjecting that airstream to a sharp bend to cause impaction. These airstreams and impactions may break apart the allergen particle but will not reduce the overall allergenicity of the particle. The high molecular weight proteins which comprise the allergens found in insectaries and animal laboratories, including protein in mold spores could be denatured or otherwise altered during the analysis phase of allergen aerosol determination leading to changes in allergenicity. Protein physical integrity, however, must not be confused with the biological recognition by human IgE antibody. An area where misconception on this point may occur is in mold spore sampling. Many investigators of allergy have sought to quantify viable mold spores, that is, to determine viable, airborne, mold spore concentrations. While viable mold spore numbers may indicate the general level of mold growth and reproduction, other components, such as the mycelia (vegetative mold body) as described earlier, share common allergens with spores. Thus mycelia alone could become a source of allergen aerosol. Typical allergen handling and storage (often at 20 degrees C, for periods of several months) techniques currently employed by investigators using immunoassay suggest that mold spore viability is not an issue in allergenicity any more than that of allergen protein from insect parts or animal sources. Factors affecting protein solubility in polar and non-polar solvents can increase or decrease the allergen fraction reaching sites of human IgE, but this situation is not likely to be encountered in typical sampling situations. For these reasons, viable mold assay techniques will not be described. Attention is now turned to the advantages and shortcomings of specific sampling techniques.

4.2.1.1 Settling

In 1946 the American Academy of Allergy adopted a gravitational pollen collection method. Greased slides were exposed to the environment of interest for 24 hours, after which pollen grains were counted using a light microscope. One serious limitation was that the collection rate was unknown thus aerosol concentration estimates were not possible. It was understood that particles with greater aerodynamic diameter would settle onto the open slides more rapidly, such that these aerosols would be overestimated. As is now known, pollen fragments not readily identified, would not be

counted, further underestimating airborne pollen concentrations. Even if all pollen or fungal spores and pieces could be identified, the quantitation of allergenic material on a visual basis is not possible, for reasons mentioned previously such as allergen potency variation and cross-reactivity.

4.2.1.2 Rotating Arm Impactors

Narrow cylinders and filaments intercept particles from moving airstreams with high efficiency, providing the impetus for the design of rotating arm impactors. In common use, it is typical to whirl a narrow, adhesive-coated surface through the air at a high constant speed (i.e. motor driven) and to count impacted particles. Collection efficiency curves have been described for various particle sizes and wind speeds. Other characteristics of this type device have been discussed in detail which will not be developed here due to serious limitations of this technique. One limitation is that as particles are captured on the prepared sticky impaction surface, particle bounce will increase and capture rates will diminish. For low volumes required in visual inspection of pollen and mold spores this is not an overwhelming obstacle, but as previously described, visual quantitation of allergen is not possible. When immunoassay is desired, the amount of allergen material required is much greater. Collection efficiencies drop significantly before detectable allergen quantities can be reached, making this technique incompatible with immunoassay techniques.

4.2.1.3 Filter Cassettes

Filter cassettes, used in conjunction with small, battery operated, calibrated pumps, and often referred to as personal samplers, or with area samplers, have become the workhorse of the industrial hygienist's aerosol exposure assessment kit. There are many types of filter cassettes currently on the market. When choosing a filter cassette the concept likely to be of greatest importance in interpreting collection data is that of sampler collection efficiency. The sampler collection efficiency should match that of a recognized standard-setting body such as the American Conference of Governmental Industrial Hygienists (ACGIH), so that potential health effects can be estimated.

Additionally, the significance of the efficiency must be interpreted with caution. "Total" aerosol efficiency curves do not represent the size particles or mass which

actually reach human airways, and samplers based on this concept are not the choice of the informed aerosol investigator.

4.2.1.4 Cyclones

Cyclone samplers were developed for estimating exposure to respirable silica. While the collection efficiency curve has been well established for inorganic dusts, curves for organic dusts differ from those of inorganic dusts. These differences are well described in the literature. Cyclones share the limitation of the filter cassettes described above in that allergen mass which could be expected to deposit in the nasopharyngeal, bronchial, and alveolar regions is not provided.

4.2.1.5 Impingers

Impingers are of two major types: size-selective and non-size-selective. The principles of operation differ somewhat. With size-selective impingers air is drawn into the sampler where sharp turns allow some particles to cross the airstream and impact on a liquid surface. The glass construction material and liquid-filled chambers tend to restrict its use to laboratory settings. Impingers which are not size-selective rely on aerosol contact with a liquid as the airstream is drawn up through the bottom of the sampler. Other limitations and use considerations of the non-size-selective impingers are not addressed because these instruments are not size-selective, which restricts their usefulness.

4.2.1.6 Cascade Impactors

Cascade impactors can separate an aerosol into a particle aerodynamic size distribution that allows the investigator to characterize the mass of the primary size ranges according to their aerodynamic diameters. Cascade impactors are available for personal and area sampling. From the size distribution data, estimates of aerosol mass of the inhalable, thoracic and respirable fractions can be made. Typical problems associated with impactors include particles lost to bounce, reentrainment due to overloading, inaccurate calibration, and lack of sharp cutoff for each stage.[11,17] The potential sources of error described can be greatly minimized through proper, careful use of these

instruments and many investigators rely on them for allergen aerosol studies. The Anderson area sampler is the most widely used area sampler for bioaerosols.

The use of personal cascade impactors in allergen aerosol studies may be complicated in that the low amount of material collected on each impactor stage may near the detection limits of the immunoassay used to determine allergen content. However, the use of high volume samplers appears to have complications as well. Price et al[18] in a study of mite allergen in homes of asthmatic children, found that allergen concentrations using a Casella *personal sampler* (with a low flowrate of 2 l/min) were higher than those using the Sartorius MD8 area sampler (high flowrate of 42 l/m), yielding concentration ranges of 0-63ng/m^3 and 0-11ng/m^3 , respectively. The authors suggested the Sartorius, with a high flowrate, may actually clean the air leading to reduced aerosol concentration estimates. Given the constraints discussed above, some investigators have resorted to using personal cascade impactors as area samplers, running them, in one instance, for 12 hours in order to collect detectable allergen mass. While this type of data will not provide detailed information on allergen aerosol exposure of the individual, it can be used as a method to prioritize control efforts and subsequently it can be an important tool in evaluating the effectiveness of engineering controls.

4.2.1.7 Light-Scattering Monitors

Light-scattering aerosol monitors (aerosol photometers) draw air into a sampling chamber at a constant flowrate. Light is directed at the airstream where reflected and refracted light is measured to determine the particle size (geometric, not aerodynamic) and count. The operating principles of these devices have been discussed at length in several sources, including a review by Ness.[19] Ease of operation, and particle count data within each of several particle size ranges are two of the advantages of light-scattering monitors. The obvious and important limitation of these monitors is the inability to identify chemical and biological properties of the aerosol being sampled. Additionally, highly contaminated environments may preclude the use of these instruments due to inherent design parameters. The UEHD staff found that in highly contaminated entomology labs areas, the light-scattering monitor became over-loaded and failed to count particles in the air. Given what is known about the hazards of corn

borer allergen, air contamination should be kept at levels at least as low as that required to operate this type of air monitor.

While light-scattering monitors allow one to say little about allergenicity of an aerosol, in isolated environments or in environments in which the airborne contaminants have been well characterized, these instruments can provide important data on the efficacy of engineering controls. Wolf[19] in sampling aerosol in a pink bollworm rearing facility used a light-scattering monitor to document particle counts before and after engineering controls were implemented.

4.2.2 Sample Assays

Many assay techniques from viable spore counts to immunoassay have been discussed in previous sections. In a given context, virtually any of the assay techniques mentioned, and some that were not, may be quite suitable in a given context. For example, to determine the efficacy of particular engineering control measures, viable spore counts, pollen counts, etc. will provide control trend data. If the goal of the sampling program is to quantify IgE-binding allergen, a technique such as inhibition RAST will be required. Even with sophisticated techniques such as RAST, variability must be examined in order to arrive at meaningful conclusions about exposure levels. Even where reliable allergen quantitation is possible, the paucity of dose-response data makes the harmfulness of a given exposure tough to estimate. Given these uncertainties, it is still possible to state that for susceptible individuals, exposure to reduced allergen concentrations is desired. With the goal of reducing aerosol allergen exposures, the development of an exposure assessment strategy is quite useful.

4.3 Strategy for Exposure Assessment

Given the difficulty in determining threshold allergen exposure levels (levels which may lead to sensitization and disease), especially in small groups or for individuals where statistical inference cannot be applied efficaciously, and without exposure standards as a reference for exposure limits, the role of traditional 8-hour averaged personal aerosol exposure sampling is diminished. This in no way reduces the importance of personal sampling; in controlled prospective epidemiological studies

personal sampling would be the method of choice to determine levels at which sensitivity becomes manifest, and such studies are certainly needed for specific environments, such as insectaries. Until these studies are complete, and exposure guidelines or standards for allergen aerosol exposures are published, area samples may provide more pertinent data.

Area samples in an insectary, for example, can provide data which may indicate which laboratory procedures, engineering controls, etc. develop the least allergen aerosol in laboratory micro-environments. Once effective engineering controls and personal protection schemes are in place, personal sampling once again becomes a dominant sampling strategy, used to determine which moth rearing procedures present the greatest allergen exposure. The discussion which follows will focus on allergen exposure assessment as a means to compare reductions in ambient allergen aerosol levels, given various engineering controls and laboratory procedures, in different rooms of the laboratory.

4.3.1 Allergen Identification

The first step in performing an assessment of allergen aerosol concentration is to identify the potential allergens. In home or outdoor environments this can be a daunting task due to the vast range of potential exposures. However, in highly controlled environments such as that of insectaries, the potential allergen pool is greatly reduced, as outlined previously. In a moth insectary, the two primary allergen sources of concern will likely involve the insect being reared and several mold genera, owing to the high humidity of moth rearing chambers. Skin prick testing of laboratory personnel carried out at an allergy clinic will yield data on the most likely allergens encountered. This data will be used to help determine an exposure assessment strategy.

4.3.2 "Disturbed" vs. "Quiet" Environments

Some investigators have compared allergen aerosol concentrations in still room air with that of agitated, moving air at timed intervals. This data can provide evidence of allergen settling rates and has been used to argue the advantage of air sampling vs settled dust allergen sampling methods in, for example, studies of house dust mite allergen. These arguments seem somewhat esoteric: from the perspective of analyzing

laboratory procedures and engineering controls, sampling carried out in the typical work environment would best characterize true differences in allergen exposure for various controls and procedures.

4.3.3 Sampler Type

As described in collection methods above, particle size-selective aerosol sampling yields aerosol concentration data for several aerodynamic particle size ranges which can be related to the major human airway particle deposition sites and as such is quite suitable for the task at hand. If immunoassay of the collected aerosol is not available or is prohibitively expense, etc., a light-scattering aerosol monitor, of the type mentioned used by Wolf, could be employed to determine aerosol concentrations as a rough indicator of changes in airborne allergen concentrations for use in determining suitable aerosol engineering controls and laboratory procedures.

4.3.4 Sampling Location

The placement of sampling devices can dramatically influence the aerosol concentration data one obtains. Not only will concentrations vary within an environment, but wind direction and velocity often influence sampling outcomes. Some area samplers have rotating heads to minimize the influence of air currents. An understanding of the existing ventilation scheme is essential to appreciate potential worker exposures and in turn develop appropriate sampler placement schemes. Whatever placement scheme is decided upon, it should include some redundancy in case of equipment failure, especially where 'before and after' sampling is to be carried out.

4.3.5 Sampling Duration

Aerosol sampling times will largely be determined by airborne contaminant loads, sampler type and assay type. With a low volume size-selective sampler, the sampling period required could reach 12 hours or longer, when immunoassay is to be performed. With a light-scattering monitor the sampling duration may be invariable (some monitors have fixed, one-minute sampling periods), but shorter sampling times, if

optional, would reduce the likelihood of overloading in highly contaminated environments; the option of taking many short duration samples also exists.

Having discussed allergic disease and allergen exposure assessment strategies, the stage is set for an examination of aerosol exposure control.

SECTION 5 ALLERGEN AEROSOL EXPOSURE CONTROL

The National Institute for Occupational Safety and Health (NIOSH), other U.S. government agencies and private organizations have developed what has become known generally as the hierarchy of contaminant control. In this scheme, engineering controls are placed at the top of the hierarchy, administrative controls are next, and personal protection is considered the control measure of last resort. The underlying philosophy embraces the goal that the workplace should be safe and free from hazards, precluding the need for personal protective measures. Only where hazards are deemed particularly intractable are personal protective devices recommended. With this as a framework, a strategy for allergen aerosol exposure control is set out.

5.1 Engineering Controls

It may be useful to put the concept of allergen aerosol control in a overall control context. Allergen aerosols can pragmatically be reduced only as all aerosols are reduced in a given environment. Thus in many respects, the problem of allergen aerosol control can be visualized in terms of an industrial 'clean room', that is, one can ignore the fraction allergen in an aerosol if as is usual, that fraction is small, and the overall aerosol concentrations are very low. Investigators of laboratory rat allergen have reported allergen concentrations of nanograms per cubic meter. If, for example, the rat allergen as a percent of aerosol for particles 0.1 to 10um was found to be 10%, reducing the overall allergen in that size range by 99% would lead to a decrease of rat allergen of about 99%. These figures certainly could be calculated in a given facility and used as a reference to avoid the continual need for immunoassay.

A basic tenet of ventilation contaminant control is that the concentration of any substance in the air is a function of the rate of generation and the rate of removal. To lessen moth allergen generation the number of moths raised could be reduced; where this is not satisfactory, air changes and filtration capacity may be increased in order to lower allergen aerosol concentrations.

Given what is known about lung particulate deposition, aerosols greater than about 0.3um should be removed from the environment to a specified degree, where allergy

from allergen aerosol is a hazard or disease agent. High Efficiency Particulate Air (HEPA) filters are often rated as removing a percent of particles 0.3um or larger (99.5%). Obviously, the greater the efficiency, the cleaner (potentially) the air may become. More important than the filter collection efficiency is an understanding of basic air ventilation and filtration concepts such as contaminant control, filter loading, and energy requirements of various systems. The following concepts come from animal models which could be adapted to moth rearing facilities well and vice versa. The discussion centers around three primary allergen control measures: cage modifications, humidifier modifications, and room ventilation considerations.

5.1.1 Cage Modifications

There are no doubt as many ways to control allergen aerosols as there are people exposed to them. This said, the discussion which follows is a consideration of just two approaches to achieving the goal of protecting workers from harmful allergen aerosols: HEPA filtered cage enclosures and a system by Wolf which can be designed by local engineers and constructed on site.

5.1.1.1 HEPA filtered cage enclosures

HEPA filtered animal cage enclosures have been in use for some time as a means to protect animal stock from exposure to pathogens. These units are operated in what are commonly referred to as negative and positive pressure modes. In the negative mode, room air is drawn into the unit, passes over the animal cages, and is then exhausted into the room through the filters. In the positive, or animal protective mode, air passes through the filters, traverses the animals, and then is discharged into the room. Ziemann, et al[20] found that 70% of the airborne rat allergen was associated with respirable particles during positive pressure operating modes and that the corresponding negative pressure level fell to 36%, demonstrating that negative pressure operation of the cleaning unit reduces the dispersion of dust containing allergen, as well as the exposure of room occupants to airborne allergen of respirable aerodynamic diameter. A similar filtered enclosure is currently in use at a private moth rearing facility with apparent high user satisfaction.[21]

The appeal of these units aside from allergen control at the generation site is that they can be wheeled into the moth rearing or other facility, plugged in, and put to use immediately. Moth (or other lab insect or animal) pathogen exposure can be minimized, an important feature of the successful programs such as is found in this entomology lab. Some filtering units have double filtering to protect both the insect or animal from pathogens and to protect workers from airborne allergen.

For single-filtered units which are run in the negative pressure mode for worker safety, pathogen exposure of the reared species can be minimized. If the rearing room or chamber is sealed, positive HEPA filtered air may be fed into the room or chamber such that when entry doors are opened, 'clean' air escapes outward reducing pathogen entry from outside air.

Cost data for these units were not available, but through personal communication with one entomologist, it was found that cost could be prohibitive, thus the text which follows describes a 'home-made' allergen aerosol control system, presumably installed at lower cost.

5.1.1.2 Wolf Allergen Aerosol Engineering Control Strategy

Wayne Wolf[28], agricultural engineer for the U.S. Department of Agriculture, devised a method to control airborne allergen in a moth rearing facility. Several species of moth were being reared at the U.S. Agricultural Research Service's Western Cotton Research Laboratory, Phoenix, AZ. There are two major aspects to Mr. Wolf's control strategy: keeping moth aerosol from freely escaping rearing vessels, and including provisions for controlled, confined exhaust, and multistage air filtration of the insect cage.

5.1.1.2.1 Cage Design

Moths are reared in a variety of vessels and cages depending on species and stage of development (photos 3,11). In any case, air in these containers must be carefully controlled. Where solid-walled vessels are used, the vessels can be placed directly on rectangular ventilation duct for bottom exhaust. A mesh screen small enough to control

the insect is placed over the exhaust port. A filter top is employed to allow airflow through the vessel and into the ventilation duct (Figure 2).

Another approach to containing allergen aerosol is to place the cage or rearing vessel in a specially-built cabinet. A cabinet with a filtered top or side wall can be built to allow airflow from top to bottom or side to side (Figure 3). Screening the exhaust port would be optional since cages in the cabinet would be designed to contain the insects, and insects drawn into the ventilation duct would be carried toward the filtration system.

At the European corn borer rearing facility described in the opening remarks, large wire mesh cages are used for a portion of the rearing cycle. These moths do not lay eggs on rough surfaces, so wire mesh walls result in egg laying on waxed paper which is laid over the cage top, made of 1/4" hardware cloth (photo 6). This type of cage could be set into a smooth-walled, filtered-top box with an exhaust port leading to ventilation ducting.

The current cage design is unsatisfactory in limiting moth and mold allergen aerosol contamination to the ambient environment. The deficiencies concerning moth allergen are discussed first. The paper (egg-mass) changing procedure currently allows the escape of moths from the cages. As the paper is removed, moths may easily escape though the 1/4" hardware cloth cage top. The 1/4" mesh is required so that the moths can reach the smooth waxed paper upon which they prefer to deposit their eggs. This design is not satisfactory. One possible solution would be to build cages with slots in one end similar to bread board slots in kitchen cabinetry or writing trays in desks. The paper would be stapled or taped to the "bread board" and slid into the cage. Obviously, this type of cage design requires close cooperation of the entomology staff, and the example given here is not meant as a final solution to moth escape; refinements are required.

The other cage deficiency concerns mold allergen. The cage frames are wooden, and as such are prime habitat for mold growth in the warm, humid rearing chambers. The cage frames should be fabricated from plastic or metal which can be easily cleaned and does not absorb water.

5.1.1.2.2 Air Filtration

A wide variety of air filtration devices are available and are well described in *Industrial Ventilation: A Manual of Recommended Practice,* Ed. 21, 1992, American Conference of Governmental Industrial Hygienists. Due to the concern of insect pathogen exposure and the frequent need for constant temperatures and high humidity in moth rearing chambers, economics will likely dictate that filtered air be recirculated, at least in colder climates such as that of the entomology lab in question. The concept developed by Wolf and others of import is that of successive stage air filtration. To minimize worker exposure and maintenance costs, a succession of filters, from coarse to fine, inefficient to efficient, is recommended. Wolf used a cyclone filter followed by a "dry" (not defined in paper) filter, then a 95% efficient HEPA filter. In this manner the coarse filter can be changed relatively frequently, extending the life of the more expensive HEPA filters.

Proper filter system maintenance is critical. As HEPA filters become saturated with dust the pressure required to draw air through them increases and consequently the volume of air recirculated may drop, as well as the aerosol capture velocity. Capture velocity is the velocity required to draw a suspended particle into the airstream of an exhaust hood or duct. Airflow rates within the duct, to a large degree, determine whether captured particles will remain suspended in the airstream or settle out in the ductwork. Airflow rates at maximum filter loading should be adequate to keep captured particles suspended. Automatic warning systems are available that indicate maximum recommended pressure drops have been reached, and that filter replacement is required.

Air filter replacement necessarily increases the potential for allergen exposure. Personal protection including gloves, respirators, and protective clothing should be used whenever used filters are handled.

The allergen other than that of the insect being reared is that of mold, or more aptly, fungi. As such humidification systems are briefly addressed.

5.1.2 Humidifier Modifications

Recirculating water humidifiers can be a prime habitat for mold and endotoxin growth and should be avoided. Direct steam or ultrasonic humidification is commonly used in insectaries and is strongly recommended. Where recirculating humidifiers are operated, the use of fungicides is essential to minimize mold growth and spore production. The entomology lab moth rearing chambers are fitted with ultrasonic humidifiers, which break water into an aerosol. This type of humidifier minimizes mold spore production, although UEHD staff did find mold spores in the humidifier outlet port.

5.1.3 Workstations

In the European corn borer facility, an operation termed 'egg popping' is performed (photo 9). Paper strips upon which eggs have been laid are pulled taught across a metal blade to remove the eggs. The paper is then "popped" by quickly snapping the paper taught using two hands. This and operations like it have the potential to expose workers to high levels of airborne allergen. Properly designed ventilated workstations can greatly reduce the level of aerosol exposure during these operations.

Wirtz[22] states that "Hood selection is critical; some commercial laminar flow models are designed for product protection only and the air flow is directed into the face of the operator after generating airborne particulate matter." Such a system is clearly unacceptable in insectaries. A suitable bench hood configuration will have solid side walls and a slot hood in the rear with ample velocity to capture particles with aerodynamic diameters of at least 10um. This arrangement allows clean air to be drawn first past the worker, then around the contaminant source and into the hood. In order to more efficiently capture larger particles, one bench design called for a perforated bench top with air drawn down then ducted to a series of filters. This arrangement would likely require some type of hopper to collect the largest particles not carried away in the duct airstream. Such a hopper should be fitted with a disposable liner to minimize allergen exposure during disposal operations.

Whatever bench and exhaust hood system is employed, some large particles will fail to be captured and filtered. This material will tend to settle quickly and should be either wet-wiped or vacuumed with a HEPA filtered vacuum by a protected worker.

This type of hooded bench filtration system should be installed in room 241C of the entomology lab in a manner compatible with all of the "bench" operations currently performed in every stage of corn borer development.

5.1.4 Room Ventilation Considerations

The finer aerosol fraction generated in the moth rearing facility (primarily in room 241C) will remain airborne for periods from hours to days. This fraction could be carried into the general ventilation system, a situation which should be precluded through proper ventilation design. As mentioned previously, some positive pressure via HEPA filtered air should be constantly supplied to the primary rearing chamber to protect the species being raised from pathogens. An adjacent workroom, storage, changing or cleaning room should be provided under negative pressure (as is the case currently for room 241C). Air drawn into this adjacent area will minimize the escape of allergen into the general laboratory environment as doors are opened and closed and traffic moves through the area. This room should be supplied with exhaust and air filtration separate from the general ventilation system; whether this air is exhausted to the outdoor environment or filtered and recirculated will depend on local climate and environmental regulatory factors. Due to complicated wind patterns near building surfaces, unfiltered exhaust to the outdoors, especially where building air intakes are nearby, may lead to the reintroduction of allergen aerosol to the general ventilation system.

Currently, the waxed paper sheets with eggs masses (and moth frass and body parts) are wheeled out of the moth rearing chambers through 241C to room 211, where they are hung for several hours to several days (photo 8). Ideally, the egg masses should be kept in 241C to avoid contamination of the general lab environment. If the egg masses must be taken to room 211, they should be transported in plastic bags; dry cleaning bags would be quite suitable to the task. Additionally, the cages are HEPA vacuumed in 241C, then carried to room 211 where they are scrubbed with soap and water and disinfected. Both of these operations allow moth allergen to become airborne in the general lab environment which is ventilated by GEV. The potential for building-wide aerosol contamination from 211 cannot be overlooked. Ideally, rooms 241C and 211 should be contiguous space with ventilation separate from the balance of laboratory.

5.2 Administrative Controls

The concept of administrative controls for occupational hazards arose from situations in the workplace in which exposures exceeded recommended levels even after engineering control measures were instituted. The fact that exposure standards to insect allergens do not exist tends to undermine the administrative control concept; if the level of exposure which induces sensitization or causes disease is unknown it is difficult to determine when measures, such as worker rotation should be implemented. Many laboratories have small staff requirements; in college and university settings there may be only one technician and a handful of students at a facility making job rotation impractical. Until allergen aerosol standards are in place, the value of administrative controls for allergy in insectaries will remain questionable. In an attempt to avoid occupational allergy problems some employers have resorted to preemployment screening as a an administrative control measure, discussed below.

Screening tests typically have sensitivity and specificity of less than 100%, such that for a given test, a percentage of non-allergenic job applicants who test positive for allergy would be excluded from a potential employment opportunity; conversely, some job applicants who test negative for allergy will likely go on to become sensitized and possibly diseased. It is important that an employer understand the value of each specific screening test and to ensure that the screening program, if adopted, actually meets the goals of the program. In a study of preemployment screening practices for allergy to laboratory animals Newill, et al[23] states "Results indicate that, currently, the use of these screening criteria as determinants for hiring persons to work with laboratory animals is unwarranted." Even if a useful allergy screening program were developed, legal, moral and practical considerations remain. The legal and moral arguments surrounding this issue are probably best left to attorneys and ethicists, however, the practical considerations deserve attention.

In an existing laboratory there will be a pool of exposed workers who are affected by occupational allergy or who are at risk for occupational allergy. The health of these individuals requires that allergen exposure be controlled. At a minimum, employers should inform prospective employees of the recognized allergy hazards associated with the position and supply the job applicant with relevant information about occupational allergy and personal protection regimes.

5.3 Personal Protection

As with most airborne contaminants, allergen aerosols pose the greatest exposure threat to workers via the airways such that respiratory protection should be the focus of personal protection programs for airborne allergens. Exposures to skin, eyes, and oral exposures should be considered as well.

5.3.1 Respiratory Protection

A range of respiratory protection devices from simple dust masks to self-contained breathing apparatus (SCBA) similar to that worn by firefighters exists for occupational use. Problems with the less expensive devices include poor fit (ineffectiveness), labored breathing, and discomfort. SCBA equipment is both expensive and bulky. While there is no single best respirator, several entomologists have expressed their satisfaction with powered air-purifying respirators and this type of respirator is currently used in the entomology lab.

These devices are powered by an electrically operated blower with a rechargeable battery pack. The motor driven fan is attached to filters (HEPA should be specified) and the entire unit can be belt-mounted and worn on the waist. Ambient air is drawn in though the filters. The filtered air then moves through a breathing tube and into the headpiece. Excess air is provided to the headpiece, assuring that leakage is outward, and as a result, the user is constantly breathing filtered purified air. The helmet/face mask combination has the added benefit of protecting the eyes from aerosol exposure. This arrangement is quite suitable for the allergen aerosol found in many insectaries.

5.3.2 Skin Protection

Gloves and lab coats should be worn routinely in the primary insect rearing and processing areas. These items should remain in contaminated areas to avoid contamination of the general office areas of the laboratory. Currently, lab coats, etc., are removed and stored just outside room 241C, in the general lab environment. For operations such as cage cleaning or insect disposal in which high allergen concentrations exist, disposable coveralls may be appropriate.

5.3.3 Personal Hygiene

Sensitized individuals have reported severe reactions when insect handling is followed by touching or rubbing the eye. All laboratory staff working with insects should be (and currently are, in the entomology lab) directed to wear gloves to protect the skin (providing a psychological deterrent to contacting ocular, nasal, and oral body surfaces as well). Clothes changing rooms should be provided with laundry services to avoid prolonged allergen exposure, to prevent general lab environment contamination and, to prevent allergen exposure to family members, who potentially could become sensitized to moth allergen from clothing worn at work. Mary, the lab technician, strongly urges student workers to wear clothes to work only once before laundering, and recommends daily showering including hair washing. Shower facilities at work would be ideal.

SECTION 6 CONCLUSIONS

It is clear from surveys of facilities rearing or handling arthropods that inhalant allergy is likely to be a health problem for one or more laboratory personnel. The financial costs to the facility in lost work hours, redirected duty, medical, and potentially, legal, expenditures make occupational allergy and its prevention an important issue. From the standpoint of the individual, one's education, training and livelihood may be threatened by occupational allergy. In order to properly protect workers and prevent sensitization and disease, employers and employees must be aware of the prevalence and seriousness of occupational allergy, as well as sound allergen exposure control measures. This paper has outlined the major allergic diseases, described human immune response to allergens in a limited manner, and discussed allergen aerosol properties and control measures, yet important questions remain. The following topics are presented as two categories: 1) important features of the entomology allergy investigation and 2) areas in which further research and understanding would be most useful in reducing allergic disease in arthropod rearing facilities in general.

6.1 Highlights of the Entomology Allergy Investigation

When Mary began working in the entomology lab in 1989 there were virtually no personal protection or engineering controls in place. As her symptoms worsened, steps were taken piecemeal to improve both the PPE and engineering controls in the lab. Mary began to wear a dust mask. Next, a full PPE regime was instituted, including gloves, lab coat and a powered purified air supply hood-type respirator with face shield. A free-standing HEPA filter was put into operation in room 241C. The entire lab was vacuumed thoroughly. Mary changes and launders her clothes every day, and showers daily to reduce her allergen exposure. Still, Mary has become so highly sensitized that the current control measures are inadequate to protect her health. More importantly, the fact that she still has symptoms suggests that occupational allergen levels in the lab may not be sufficient to preclude sensitization in new workers.

Further action to reduce moth allergen exposure should include:

1. Verification that room 241C is under negative pressure relative to adjacent lab areas and that the exhaust from 241C is not likely to be vented back into the GEV through rooftop intake vents as is claimed by the lab technician. If rooftop re-entry into the building is found, HEPA filtration of room 241C exhaust should be implemented.

2. A HEPA filtered workstation should be built in room 241C to minimize worker exposure to allergen aerosol during procedures such as egg "popping" and cage vacuuming.

3. Moth rearing cages should be redesigned to reduce mold growth on wood frames and to minimize moth escape. Cages should be enclosed and HEPA filtered such that allergen is not released into the rearing chamber atmosphere.

4. Transport of cages, lab coats, egg-mass paper, etc. should be confined to room 241C. If this is not practical, room 211 should be isolated by both solid walls and proper, separate, ventilation in a manner that precludes moth allergen escape to the general laboratory.

5. The two primary sampling strategies discussed rely on 1.) RAST immunoassay and, 2.) size-selective particle counting by light-scattering monitor. If RAST immunoassay is available, area and personal size-selective sampling should be carried out on a "before and after" basis, that is, before and after changes are made in lab procedures, PPE, or engineering controls.

If RAST immunoassay is not available, estimates of allergen reduction, given by various work procedure and engineering control modifications, can still be had. Before and after size-selective particle counts can be made with a light-scattering monitor. The reductions observed in aerosol particle counts for each size range will likely be in the same proportion as the reductions in allergen particles in each size range. Obviously, no data on actual allergen aerosol concentrations can be derived by this method.

6. The PPE protocol established should be re-evaluated to ensure proper precautions are taken. This protocol should be written out, in part to fulfill OSHA requirements to

have written respiratory protection plans where respirators are used occupationally, and as a means to ensure continuity of PPE plan adherence as personnel changes occur.

6.2 Laboratory Allergy - Topics For Further Study

The precautions mentioned above will go a long way to reduced allergy in laboratory environments, yet important questions remain such as, how much protection is enough? How important is lab allergy in general? Can principles found effective in rat housing areas be applied to moth insectaries? Clearly more data is required than is currently available. Here are some areas where more data is needed:

Incidence and Prevalence

While surveys on allergy to arthropods have helped characterize the extent of arthropod allergy, response rates have been low. Future surveys should be funded sufficiently to maximize response rates. Due to a lack of understanding of allergy by some respondents, questionnaires must be written carefully and interpreted with caution, using appropriate epidemiological and statistical tools.

Standards

Currently sound data concerning the level of allergen exposure resulting in sensitization and/or disease is lacking. Without this information the development of exposure standards is impossible. While the number of workers exposed to arthropods nationally may preclude extensive investigation due to economic factors, the same could not be said about laboratory allergic disease in general. One author estimated that 90,000 workers are at risk of occupational allergy due to exposure to laboratory animals. Exposure and disease models developed in this area could be used to propose recommended employer practices, if not occupational health standards, for occupational exposure to arthropods. Currently NIOSH is conducting an extensive study of allergy in moth rearing facilities across the nation in an attempt to quantify some of the parameters of occupational allergic disease experienced in these occupational settings, but it is not clear that exposure standards will be developed as a consequence of this research.

Exposure Control Measures

Whether or not government or industry recommended employer practices and standards are developed soon, concerned employers and workers could benefit significantly from quantitative data developed for engineering controls, personal protection, and laboratory procedural changes. This work is being carried out with much greater frequency for laboratory animals than arthropod facilities. The *Federal Guide For The Care And Use Of Laboratory Animals*[24] calls for the development of allergy prevention measures; many of these could be adapted for use in arthropod facilities, but the effectiveness of these sister technologies must be evaluated on a case-by-case basis.

Finally, as with any endeavor where change is sought, dissemination of information is critical. Trade groups and professional organizations such as the Entomological Society of America (ESA) have been and continue to be instrumental in reaching arthropod facility managers, technicians, and others, who all benefit from availability of new occupational allergy research information.

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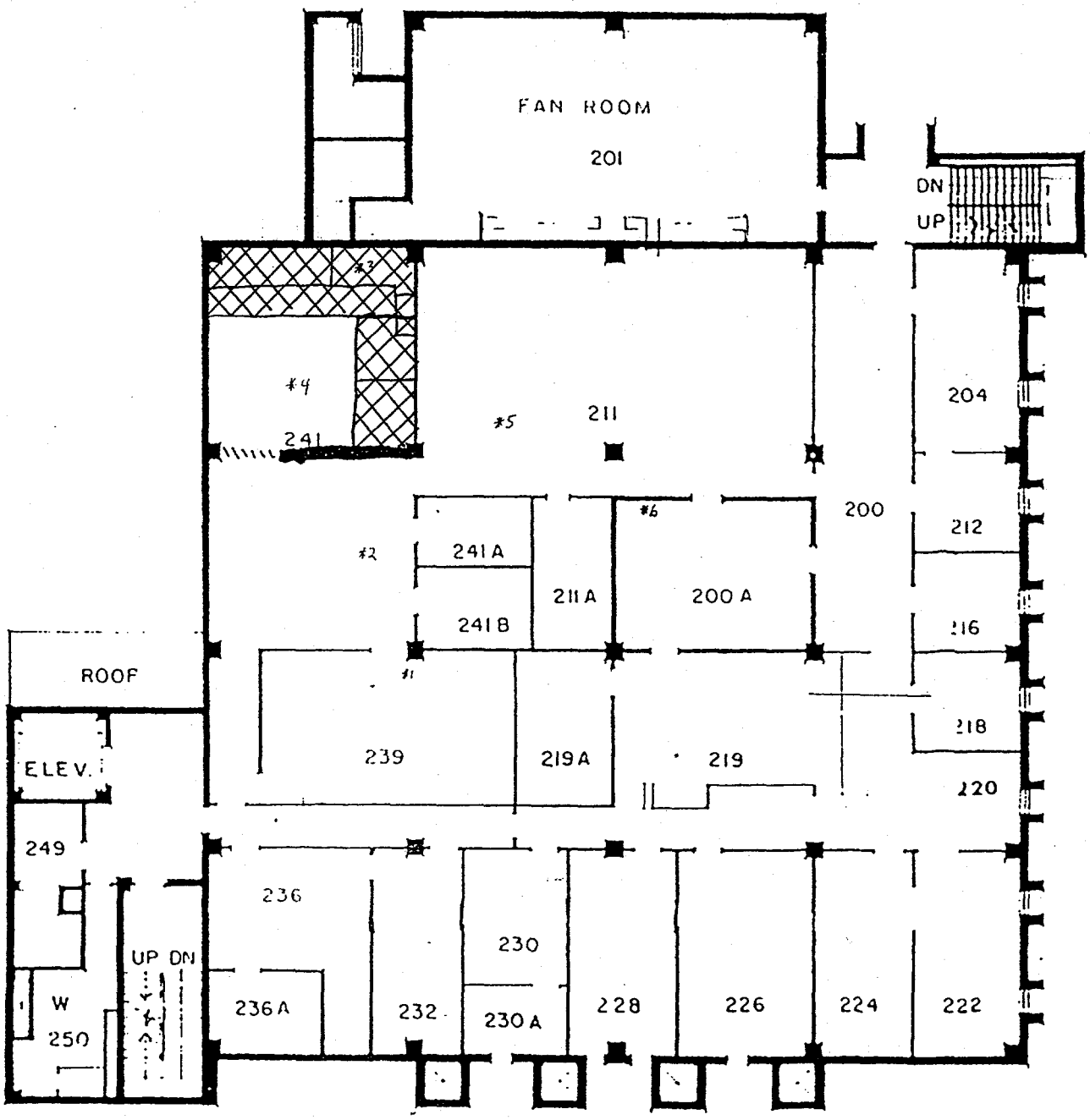
Figure 1
EntomologyLab Floor Plan



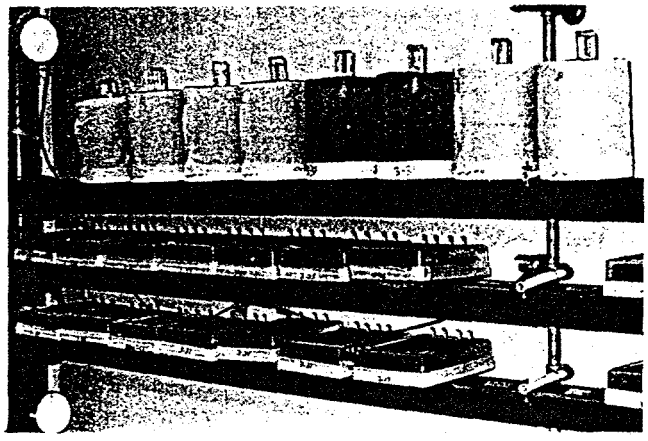


Figure 2.—Insect cages mounted on exhaust ducts to control moth scales. Air passes through cages and is filtered by cyclone and dry filters shown in figure 4.

(Figure 2)

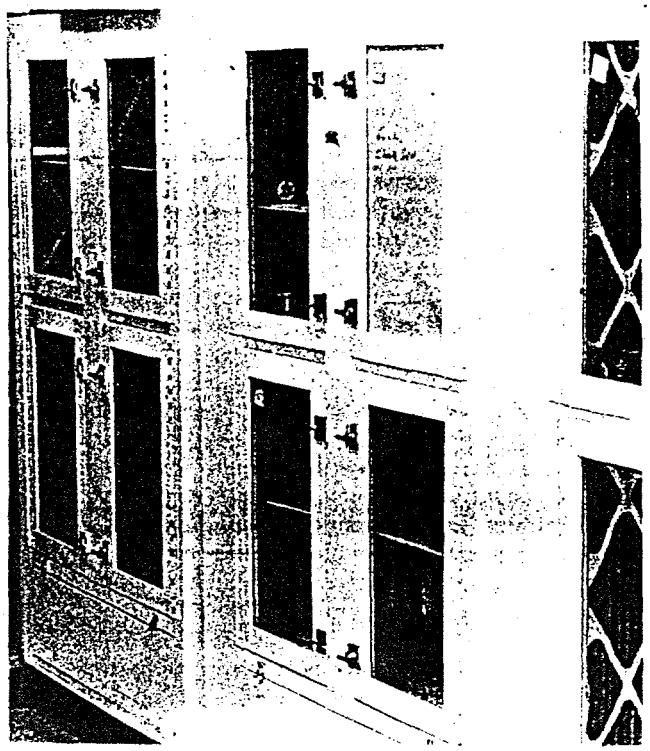


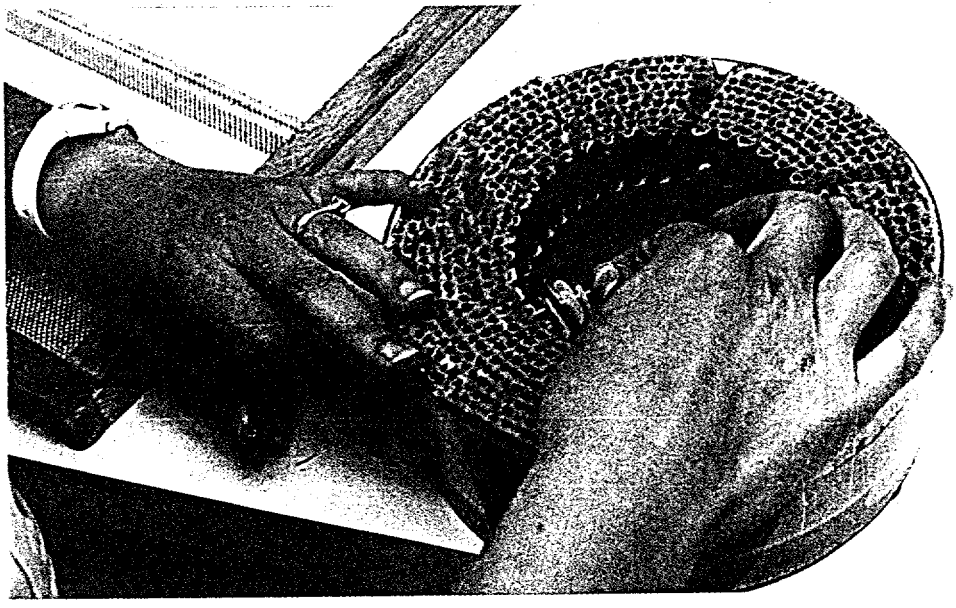
Figure 3.—Cabinets for separating egg-laying cages of different strains of insects. Each cabinet has air circulation from right to left across each shelf and has 35%-efficiency filters to collect scales.

(Figure 3)

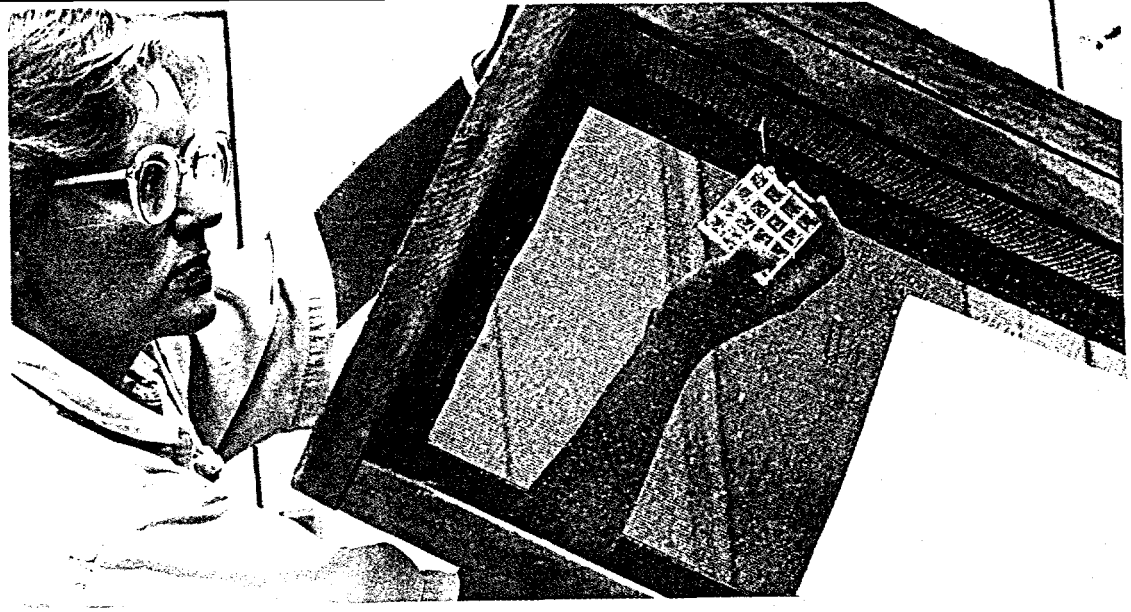
Laboratory Procedures and Equipment Photographs

1. Egg Punching Operation

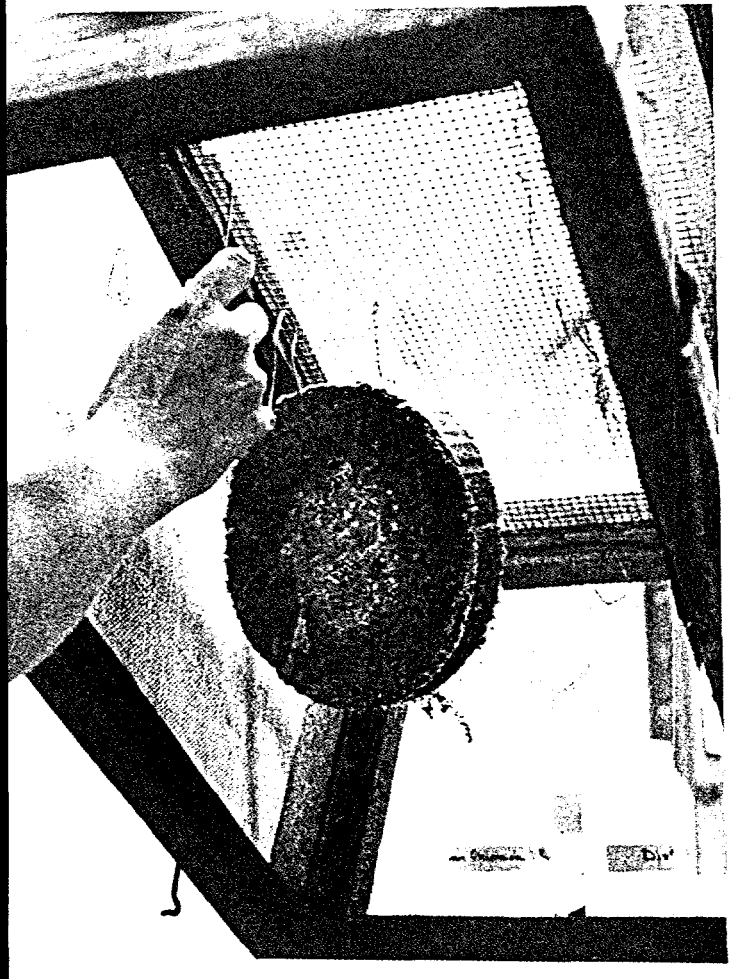
3. Diet Dish With Paper Towel Cover



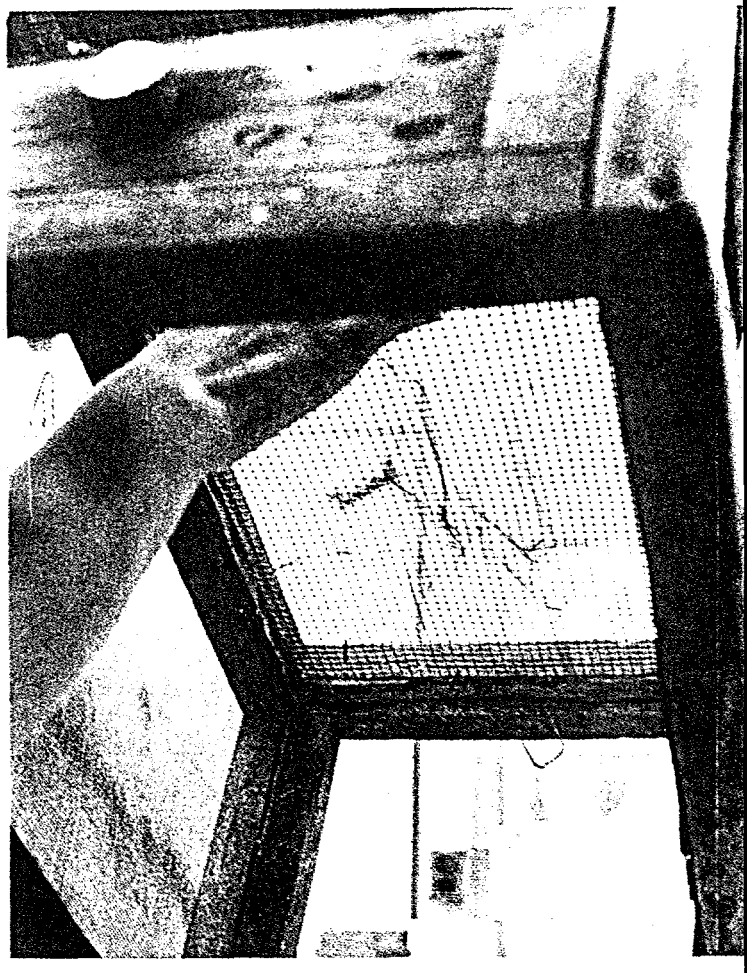
2. Preparation of Diet Dish



4. Adult Corn Borer Food Source in Cage

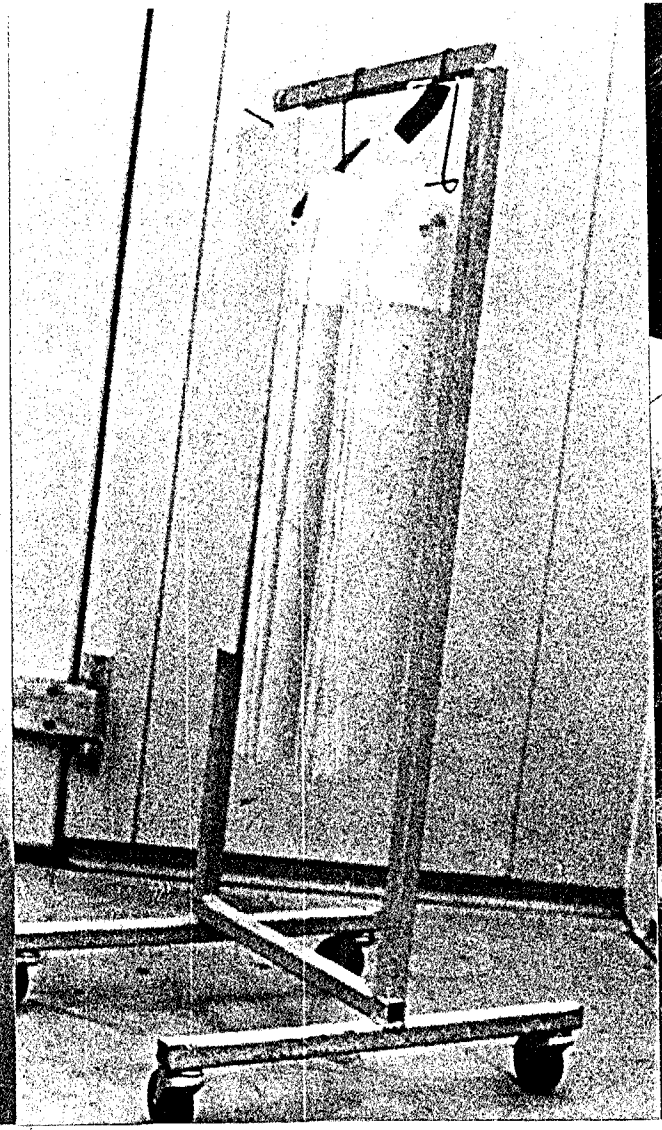


Corn Borer Larvae Placed in Cage

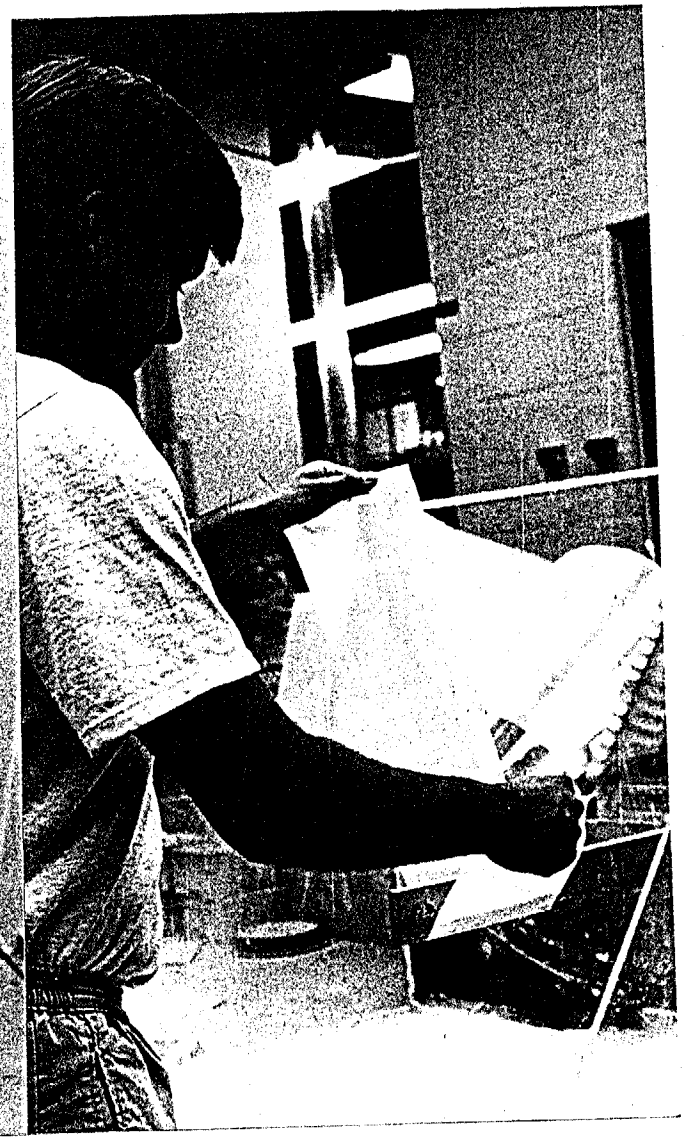


6. View of 1/4" Mesh Cage Top

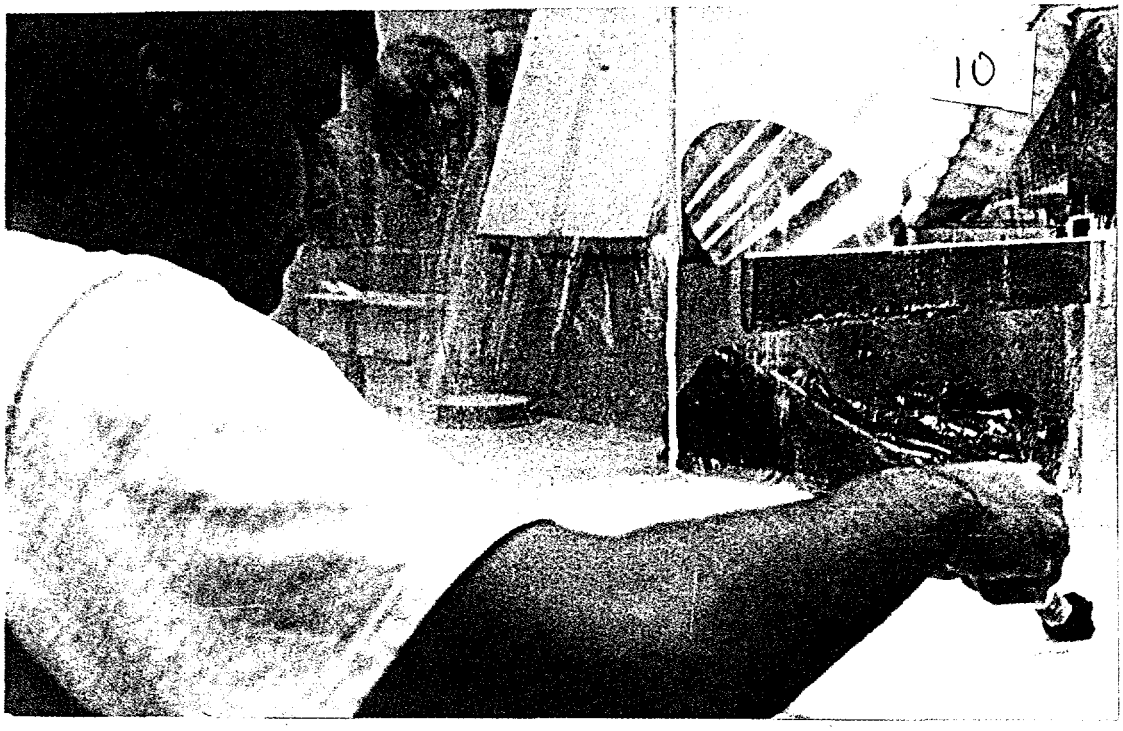
7. Waxed Paper Strips Being Replaced



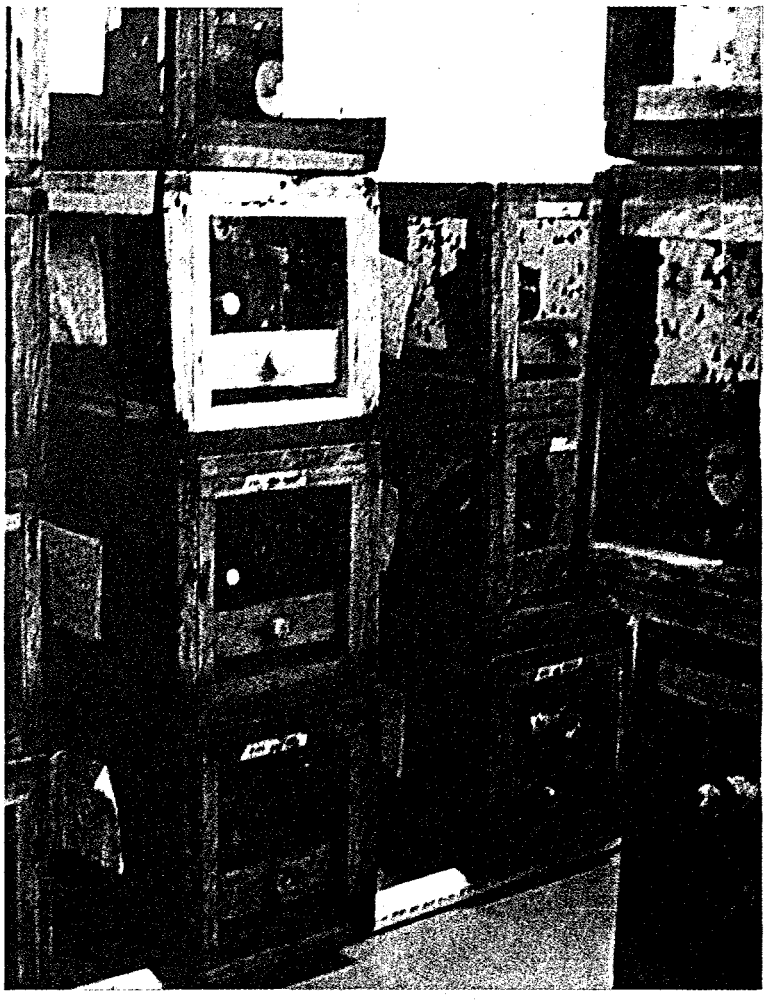
8. Paper with Egg Masses



9. Loosening Eggs Prior to "Popping"



10. Collecting "Popped" Eggs



11. Corn Borer Moths in Cages