It can't be a half-way job; it must be all the way, or your efforts may even backfire and result in more problems than you had at the start.

The magnitude of the nursery and cut flower industries here on the West Coast gives us an idea of the tremendous size of the over-all problem. In the nursery industry in California it is estimated that 350,000 cubic yards of soil are used each year — this is equivalent to the top foot of soil from 217 acres of land. This much soil fills many 1-gallon cans and flats, and the volume has been on the increase.

The presentations to follow approach these problems from a practical way, with practical procedures, to insure the nurseryman that he is doing a thorough job of maintaining a clean, sanitary production process.

The first speaker this afternoon will be Dr. Robert D. Raabe. Department of Plant Pathology, University of California at Berkeley. Dr. Raabe.

## THE DETERMINATION OF DISEASE-FREE PROPAGATING MATERIAL

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The title of this paper should probably be "The Determination of Pathogen-Free Propagating Material." The distinction between "pathogen-free" and "disease-free" is one which is technical and yet, it is important enough so that it should be mentioned here. Disease is a complex resulting from the interaction of a susceptible plant (called a suspect or a host), a causal agent (called a pathogen in infectious diseases) and an environment favorable for disease development. 'Disease-free' would mean the absence of disease as a result of the absence of any one or more of the three factors necessary for disease. Thus it would be possible to have plant material with a pathogen present but because of environmental conditions not favorable for disease development, there would be no disease. Later should favorable environmental conditions occur, disease would then result. If, however, the plant material is pathogen-free, disease would not result even though the plant might be placed in an environment favorable for disease development. This is not to say that once plant material is pathogen-free that it will remain so indefinitely. This aspect, however, is to be discussed by Dr. Wilhelm and Dr. McCain in the following papers.

Although the term 'propagation' includes both propagation by seed and by vegetative means, the number of seed-borne diseases is not extremely large. One of the advantages in propagating plants from seed is that many diseases are climinated this way. Because of this and the fact that the presence of seed-borne pathogens is determined by culturing technique similar to those used in determining the presence of pathogens in vegetative propagation material, the remarks here will be confined almost entirely to the vegetative reproduction of plants.

Inherent with the many advantages of asexual propagation is the disadvantage that if disease is present in any clone or selection, not only will there be a continuation of the disease, but there will be an increase in the amount of it. For this reason, there is a primary interest in obtaining pathogen-free plant material for this type of propagation. The purpose of this discussion is to focus attention on the methods used to determine if plant propagation material is pathogen-free. Actually, nearly all of the means used are for the determination of the *presence* of pathogens and it is by the absence of pathogens in such tests that pathogen-free material is found.

In order to discuss the determination of disease in plants, there should be an understanding of what disease is. Although there are many definitions of disease, one which might be used is "disease is an injurious disturbance in the form or function of a plant resulting from a continuous irritation." The last clause "continuous irritation" is put in so as to exclude certain types of injuries such as those resulting from wind, insect attack, etc. The symptoms of these are usually diagnostic so that the cause is obvious and the trouble can be eliminated.

In general, diseases are usually divided into two groups — non-infectious (non-parasitic), and infectious (parasitic). In the first group are found such diseases as those resulting from an unfavorable environment and include such troubles as nutrient excesses, nutrient deficiencies, air-pollution damage, etc. Since they are not infectious, they are not directly important in the selection of disease-free propagating material. They are indirectly important in that in selection of propagating material, healthy, vigorous plants will give the best results. They are also important in that the symptoms of such diseases might be similar to the symptoms of infectious diseases and when this is true, the actual cause of the trouble needs to be determined.

The first step in diagnosing a plant disease is usually the observation of symptoms. Symptoms may be defined as "the visible manifestation of the diseased plant." Symptoms are of many types and in clude the following: Chlorosis (yellowing) and other discolorations, necrosis (killing of tissue), wilting, and alternations in growth habit such as stunting, overgrowths, proliferations, etiolation and other growth abnormalities.

Obviously symptom expression plays an important part, not only in the diagnosis of plant disease, but also in the selection of plant propagating material, since plants obviously diseased as recognized by the symptoms, are usually avoided, and sometimes are rogued or destroyed, as they should be.

Symptoms alone, however, can not be relied upon as the only means of determining disease in plants for several reasons. One is that sometimes symptoms of different diseases might be similar and further tests are needed to determine the actual causes of the diseases. The other and more important reason is that many plants may be infected with a pathogen and the symptoms have not yet appeared. With some diseases, symptoms may not appear, especially in certain

varieties. This is particularly true with virus diseases of plants such as dahlia and chrysanthemum though certain fungus infections such as Verticillium wilt may be carried in varieties of some plants which act as 'Typhoid Marys.'

Since symptoms alone cannot usually be relied upon for diagnosis, additional procedures are followed. The first of these is the use of a hand lens or a microscope. The magnifications resulting from the use of these frequently make identification of the causal organism possible. Sometimes, however, further diagnosis is necessary. The symptoms frequently indicate the type of disease present and if so, the correct diagnosis procedures can be followed. For example, if a bacterial or fungus pathogen is suspected, an attempt is made to isolate the causal organism. This is usually done by surface sterilizing small pieces of infected tissue and incubating them on or in some type of culture media until the organism develops enough so that it can be identified. Diseases commonly detected in this way include Verticillium wilt, water-mold root rots, damping-off, the Fusarium wilts, bacterial wilt of carnation and other plants, and bacterial leaf and stem blight of pelargonium.

Since viruses have not been cultured, their presence is detected by trying to transmit them by means of juice inoculation, or grafting on budding to healthy plants, which will act as indicators. At present, much research is directed toward finding reliable indicator plants for many virus diseases. Such plants have been found for viruses which occur in carnations, stone fruits, chrysanthemums, roses, gladiolus, dahlias, and strawberries, to mention a few. One of the problems is that many of the virus diseases are complexes, i.e., they are the result of infection by more than one virus. This complicates the finding of an indicator plant. Frequently indicators can be found for certain components of a complex, and a series of indicators may be needed to show all the viruses present. This is also complicated by the presence of naturally-occurring inhibitors which prevent the transmission of some viruses.

Another means by which viruses may be detected is by the use of serology. This is done by injecting the purified sap of a virus-infected plant into an animal such as a chicken, rabbit or horse. After allowing sufficient time for the animal to produce antibodies specific for that virus, blood is taken from the animal and the blood serum extracted. In a laboratory test, a small amount of purified sap from a plant suspected of having the same virus is added to a small amount of the serum. If the virus is the same as that originally injected into the animal, there will be a positive reaction, usually a precipitate, giving proof of the presence of that particular virus in the suspected host. This technique, though used only experimentally in the United States, is used for large scale detection of the presence of viruses in commercial crops, such as flower bulbs and potatoes in European countries, particularly in Holland.

In conclusion, it should be stated that although no method will work for all diseases, by using a combination of the symptoms, culturing and/or transmission tests, a trained person can usually diagnose

most diseases. Such diseased plants should be discarded and only those free of disease should be used for propagation of plants.

MODERATOR MAIRE: Thank you, Dr. Raabe. We will now continue with our discussion of this subject with a talk by Dr. Stephen Wilhelm and Dr. Arthur McCain on how to produce clean propagating materials.

## PRACTICAL TECHNIQUES FOR THE PRODUCTION OF CLEAN PROPAGATING MATERIALS

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Practical techniques for the production of clean propagating materials involve three basic operations, and these lie at the heart of the subject matter of the fields of plant pathology and horticulture. The raising of superior plants through advances in horticultural science and the control of plant disease are our common objectives and no longer should anyone just assume that plant diseases are inevitable and crop losses to be expected. The three basic operations referred to above are: (1) getting rid of the pathogen at the source (2) geting rid of pathogen carry-over in the soil or from other growing or propagating media (3) getting rid of all sources of contamination by which the pathogen can be reintroduced into growing operations. The first operation — getting rid of the pathogen at the source —means obtaining pathogen-free planting stock, and the full meaning of "clean stock" as used in this talk is stock that is not carrying any known injurious organisms, fungi, bacteria, nematodes or viruses. The second operation — getting rid of pathogen carry-over in the growing media — involves methods of disinfecting, fumigating, or steaming, soils and other growing media, and for this subject matter area, I wish to direct your attention to University of California Manual 23, entitled the U. C. System for Producting Healthy Container-Grown Plants, Chapter 8-13 inclusive, edited by Dr. K. F. Baker. The third operation — getting rid of all sources of contamination — includes maintaining stocks pathogen-free by preventing the reintroduction of pathogens with tools, containers, tractors, water, worker, or insects, etc. This important subject matter area will be discussed by Dr. McCain, and in a practical way was illustrated by the high standards of hospital cleanliness depicted in the talk of Fred Real of the Four Winds Nurseries, San Jose.

Much of our past thinking in plant pathology, perhaps forced upon us by expediency and at the bottom, of our own wishes to serve agriculture, was to provide controls for plant diseases. This we have done, and recommendations involving plant sprays, dusts, drenches, with timing of application that coincides with vulnerable stages in the life cycles of the causal organisms, are readily available. Essential as these measures are to agriculture and horticulture, this approach to control by "fighting" the diseases never got us to the bottom of