PLANT VIRUS DISEASES IN DENMARK—THEIR INCIDENCE AND CONTROL

H. RØNDE KRISTENSEN

FAO Consultant, Forest Research Laboratory, Bangalore

IT would be useful to list the important plant virus diseases which have received attention in Denmark during the last 15 years. Amongst agricultural plants, sugar-beet yellow virus, potato leafroll virus, potato virus Y and potato virus X are the most important. In horticultural crops some of those attacking *fruit trees* and causing diseases known as flat limb, rubbery wood, apple mosaic, green crinkle, stony pit, plum lime pattern, cherry ringspot and many others are conspicuous, while in fruit bushes, virus diseases are specially to be found *in black currant (reversion), in* raspberry (various mosaic diseases) and in gooseberry (yellow veinbanding).

Amongst the vegetables, tomatoes specially suffer from widespread virus attacks—in most *cases due to infection of tobacco mosaic virus*. And as to the ornamental plants, it may be truly said that few species have completely escaped virus infection,—the most important virus diseases being those attacking chrysanthe-*mums, carnations and dahlias*.

Amongst forest plants the mountain ash, *Sorbus aucupaira*, is often infected with a virus causing a ringspot—mosaic pattern on the leaves. Also various species of poplar are frequently found to be virus infected.

Most of the research work and investigations concerning plant virus diseases in Denmark are carried out at or are being conducted from the State Plant Pathology Institute, which is situated in Lyngby, a suburb of Copenhagen.

This Institute has several departments devoted to fungus diseases, virus diseases and physiological diseases as well as pests. The Institute has also an advisory department which is working in very close co-operation with a large team of agricultural and horticultural advisers covering the whole country. As there is a very close co-operation between the advisory department and all the other departments of the institute, any new or apparently new virus diseases found, will immediately be handed over to the specialists in the field for further investigation.

The virus specialists try as far as possible to visit all the places, from where attacks of new virus diseases have been reported in order to get a first-hand impression right on the spot. With such elaborate organisation the virus workers have a good chance to come to know of any new virus diseases in so far these diseases manifest themselves with external symptoms.

WORK ON PLANT VIRUSES

The work done on plant viruses can be broadly grouped under the following heads: A. Diagnostic Work; B. Spread and Sources of Infection; C. Control Measures.

A. DIAGNOSTIC WORK

Control measures are naturally effective only with early and reliable diagnosis. By experience the virus worker may in some cases be able to identify some virus diseases on basis of visible symptoms, but as these symptoms are very much influenced by environmental factors and also, as disorders other than viral may produce virus-like symptoms, it is often very difficult to carry out a virus diagnosis based on symptoms only. This is of course especially evident when the infected plants show no symptoms at all (as in the case of latent infection). In many cases the virus workers therefore are compelled to have recourse to use special identification methods for diagnosis. The methods which have so far been adopted in a very extensive way are infection experiments, (indexing to indicator plants) and serological investigations.

1. INFECTION EXPERIMENTS

To find out whether a suspected plant is virus infected or not and if possible to identify the virus in question inoculum from the plant to be tested is transferred to one or more species of indicator plants (test plants), which are likely to react in a special way in case of virus infection.

The methods used for transferring the infectious agent from the source of infection to the test plants, depend on the kind of virus, suspected to be involved, but they are mostly mechanical sap transmission, insect transmission and transmission by grafting. Incidentally, the *mode of virus transmission* can also give some indication of the nature of the virus. Infection experiments are now being used on a very great scale in Denmark—not only for pure academic purposes, but also in order to select completely virus-free mother plants of several economically important crops (see under control measure).

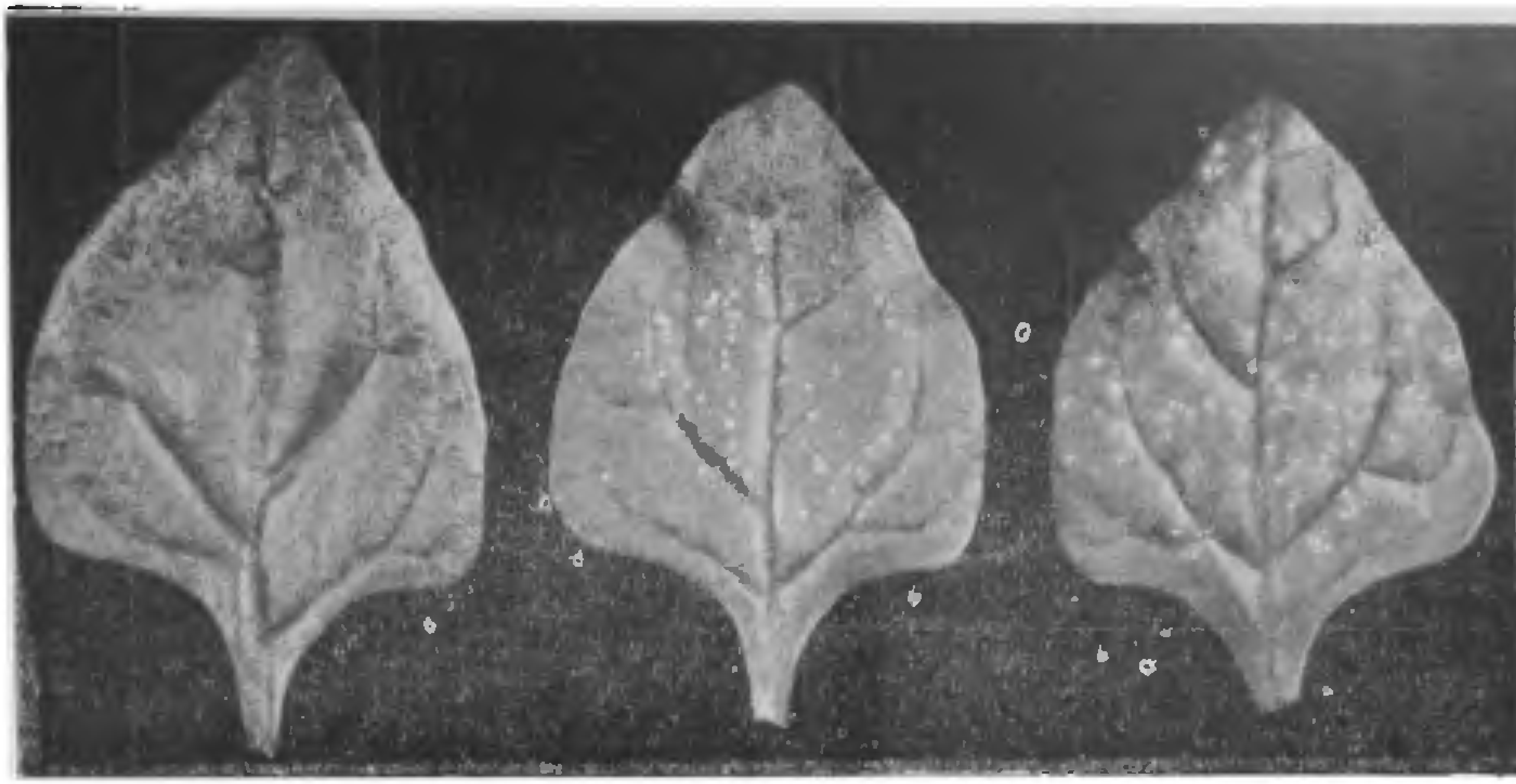

FIG. 1. Local lesions in *Tetragonia expansa* caused by Tobacco necrosis virus sap inoculation. Extreme left: uninoculated leaf.

2. Serological Investigations

During the last 8 years, serological methods have assumed growing importance in diagnosing plant viruses in Denmark, and antiserum against a number of different viruses have been produced. This has proved of special value to the potato farmers, as it is now possible to carry out routine testing for potato virus X and S on a very large scale with efficiency and accuracy.

The practical aspect of this work is so important that statutory provisions of the Ministry of Agriculture govern the supply of Danish seeds potato, Bintje, certified to be free of virus infection by serological tests. This practical application of a laboratory technique is a good example of Danish organisation wherein:

(1) The virologist produces the antiserum and devises the methods for its use; (2) The agricultural field research workers are trained in regular courses of instructions how to use the antiserum; (3) The actual tests are carried out at agricultural research stations. Check tests of samples are performed by the Government Plant Protection Service; (4) The growers of certified seed potatoes (Bintje) are using the tested material for further propagation under suitable conditions.

B. Spread and Sources of Infection

When trying to work out any control measures against plant viruses, it is important to know about their modes of spread as well as about the sources of infection. With these objectives, numerous transmission experiments have been (and still are) carried out in Denmark. Besides the experimental transmissions which are directly performed by the virus workers, a number of trial plots have been laid out at various agricultural and horticultural research stations all over the country to investigate the spontaneous spread of the more important virus diseases such as potato leafroll virus, potato virus Y, sugar-beet yellow virus, raspberry mosaic virus, etc.

Counts of the most important virus vector in Denmark—the green peach aphid, *Myzus persicae*—have also been performed for a number of years in order to gain information about the population of aphids occurring in various years, various periods of the year and in different parts of the country.

Sources of infection are of course the infected plants, not only those showing distinct virus symptoms but also plants with latent infection. To tackle any virus disease it is important to know which plants can act as virus sources, in other words, which plants are susceptible to the virus in question.

Rather large-scale experiments have therefore been performed to find out the resistance-susceptibility of a comprehensive range of likely host plants for some important viruses. Large-scale experiments are comparatively easy to perform, when working on sap transmissible viruses—the major items of cost being insect-proof greenhouses, where the plants can be grown under controlled conditions.

When working on viruses which can only be transmitted by insects or by grafting, matters become somewhat more difficult. In earlier days all the plants used in the insect transmission tests, had to be placed in cages, that often caused a poor growth of the plants and further these cages were rather uneasy to handle and fairly expensive and space consuming. This has now been improved.

Instead of placing the plants in cages, small cages are placed wherever wanted on the plant (see Fig. 2). By using this type of cages, it is possible to carry out aphid transmission tests in great numbers and to perform hostplant investigations with aphid-borne viruses in an easy way.

FIG. 2. Insect cage for virus transmission. Transmission by aphids of sugar-beet yellow.

Turning now to the viruses, which are—as far as we know only transmissible by grafting, rather reliable experiments may be performed outdoors, but naturally insect-proof greenhouses would also be preferable.

C. Control Measures

(I) *Sugar-beet yellow virus,* which is one of the most economically important viruses in Denmark, is transmitted by the aphid, *Myzus persicæ,* which in mild winters is able to survive in the sugar-beet clamps. From these clamps infective aphids during May-June migrate to the young sugar-beet plants in the field with the consequence that these plants in turn will be infected (early infection can cause up to 50% reduction in yield).

Extensive insecticide-trials have shown, that use of systemic insecticides, when the first aphids appear in the field reduces and delays the infection very much. It is however of the greatest importance to perform these sprayings at the right moments, and by collaboration between all interested parties, a spraying warning service has been established under the leadership of the Plant Pathology Institute. Based on very frequent reports from advisory officers all over the country the Institute issues the spray warning through papers and radio.

(II) *Potato viruses* are also of great importance, the most common viruses as mentioned earlier being potato leafroll virus, potato virus Y and potato virus X.

The first two are in most cases easily recognisable in the field and can therefore be removed by the inspections, which are carried out in the fields, where seed potatoes are grown. Virus X on the other hand is carried by many potato varieties without any external symptom and has to be recognised by serological tests. A very large number of such tests are carried out and it has thus been possible to select large quantities of virus-free seed potatoes (see under serological investigation).

3. Virus Diseases of Horticultural Plants

It is only in very recent years that the horticulturist has recognised that the technique by which many of his plants are propagated (vegetative propagation) is specially vulnerable to virus transmission. Indeed, it is rare to find virus-free stock of some important varieties of apple, raspberry, gooseberry, dahlia, chrysanthemum, carnation and narcissus.

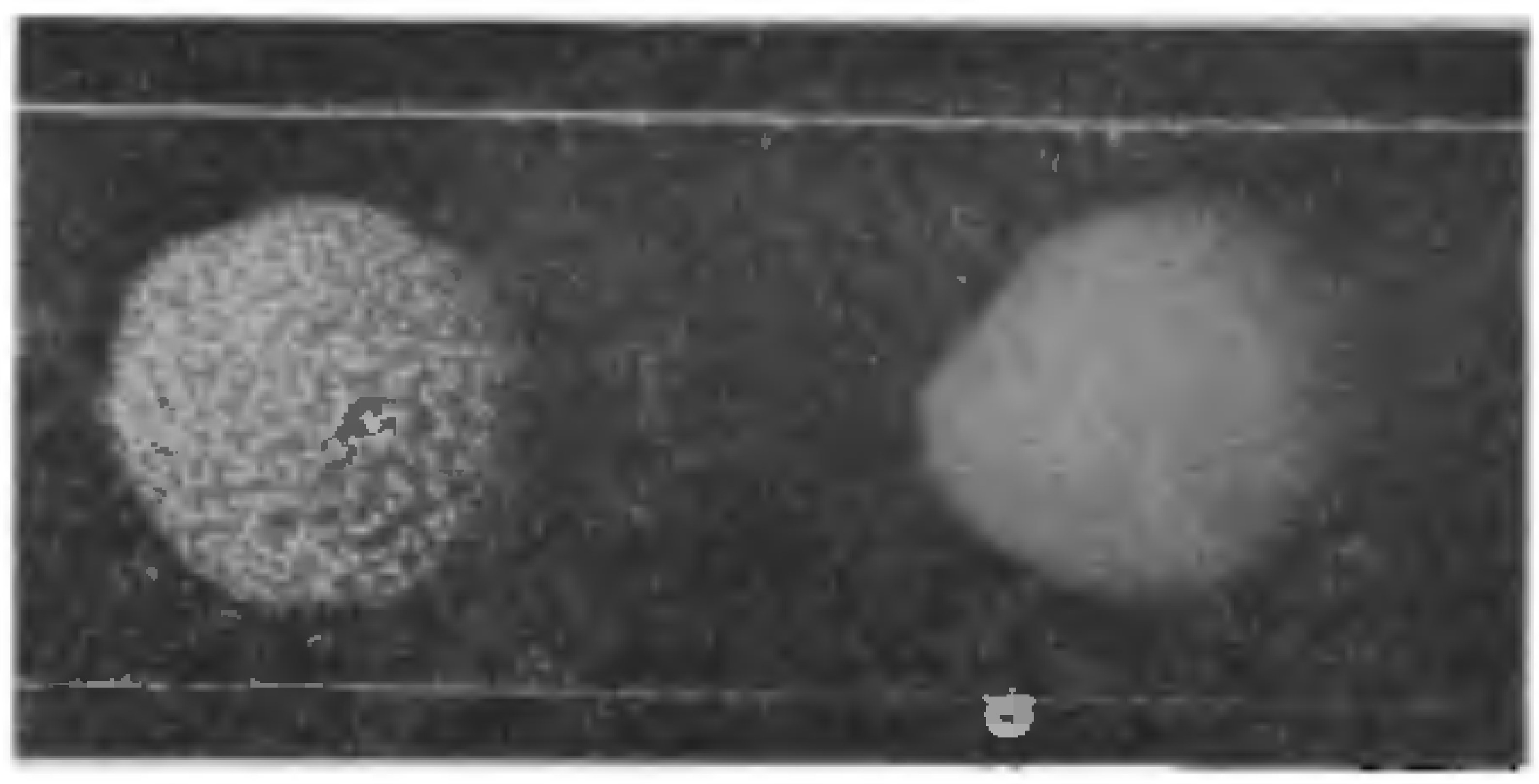

FIG. 3. Slide agglutination test. On left sap from virus X-infected potato leaf mixed with virus X antisera. On right, sap from healthy leaf similarly treated.

Realising what the consequences of many of these virus diseases may well be, several countries have now taken steps to prevent further spread. In the following I shall try to give a brief account of the measures we have taken in Denmark.

In 1948 "The National Committee for the Propagation and Sanitary Inspection of Horticultural Plants" was established. This Committee collaborating closely with the State Institute of Plant Pathology and the Horticultural Research Stations, has now taken over the majority of all field inspections of horticultural plants in Denmark.

By carrying out inspection and roguing in nurseries (which operation is now compulsory in Denmark) it has been possible to eliminate a large proportion of plants severely affected by virus diseases, and consequently the general standard of health among plants offered for sale in Denmark has considerably improved.

However, as these field inspections are carried out on the basis of visible symptoms only, the risk of approving plants with latent virus infection is quite obvious, and therefore the National Committee is keen on selection and multiplication of virus-free material from many different plant species.

To insure that the selected motherplants are totally free from any known virus, it is necessary to carry out thorough investigations. These investigations can in certain cases—as earlier described—be performed by serological methods, but where fruit trees, fruit bushes and strawberries are concerned, serological methods up to now, have not proved effective. In testing these plants, indexing is used, mostly through grafting and budding on many different test-plants.

In cases where it is impossible to find virus-free plants of certain valuable varieties it might be feasible to use heat treatments as a cure. As a rule a temperature of about 35° C. to 37° C. is used over varying lengths of time depending on the virus, the host plant and the time of year. In this way, it has been possible to inactivate Aspermy virus in chrysanthemum, mosaic virus in shallots and mild mosaic in raspberry. Attempts have also been made to combine heat treatment with tissue culture, but this work is still in the preliminary stage.

Apart from their scientific interest, plant viruses have a direct bearing on a very important world problem today, *viz.*, how to provide sufficient food for the rapidly growing populations. Increased food production can be achieved in many ways. One of the most obvious must be to reduce the heavy toll taken by pests and diseases among which the virus diseases take a high place. Therefore, every effort should be made to investigate these diseases as thoroughly as possible and apply the knowledge thus gained to step up our food production. This work calls both for national effort and international co-operation ; Denmark is trying to play its part. A measure of this is to be found in the next European conference on tree viruses meeting at Copenhagen in July 1960.

MEASUREMENT OF SOIL HUMIDITY BY RADIOACTIVE METHODS

γ-radiation and neutron emission from radioactive nuclei afford methods of measuring the humidity of soils. The proportionate reduction in the intensity of a beam of γ-rays in traversing a material is a function of the thickness and the nature of the material traversed. By comparing the attenuation produced when γ-rays pass samples of dry and humid soils it is possible to calculate the water contents of the soil.

In the actual experiment a long hollow tube, which carries at its one end a Co-60 γ-ray source kept in a small lead container provided with a window, is driven obliquely into the soil. At the surface of the ground, directly above the Co-60 source, is placed a radiation counter which indicates the intensity of the γ-ray after it has traversed the soil thickness from source to ground level. This method allows measurement of soil humidity up to a depth of 50 cm.

The second method is based on neutron density measurements. It is known that fast moving neutrons, from a neutron source, passing through a moderator, such as water, collide with the hydrogen nuclei (in water), lose their high speed, come into thermal equilibrium with the ambient atoms, diffuse according to the laws of gaseous diffusion, and finally, are captured by nuclei of surrounding atoms.

The rate of fall of neutron density in the surrounding moderator will be a measure of the hydrogen (water) content. In the actual experiment a pellet of a mixture of polonium and beryllium is used as the neutron source. The source and the neutron counter are embedded in the soil to be studied. By a run of preliminary experiments on soil at different stages of dryness, a curve is drawn giving the relation between neutron density and soil humidity. This curve is used in further experiments on samples of soil.

Although the method is time-consuming, it has the advantage that it is independent of the soil composition, density, temperature, etc.—*WMO Bulletin January* 1960.