การศึกษาโรคไวรัสของพืชชนิดที่มีแมลงเป็นพาหะ ณ มหาวิทยาลัยเกษตรศาสตร์

The Study of Insect Transmission of Plant Viruses at Kasetsart University, Bangkhen, Bangkok, Thailand

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There are more than 300 different kinds of plant viruses known today. Many cause extensive damage to crop plants. In fact in some regions of the world certain viruses are limiting factors to the growing of certain economic crops. In Thailand viruses are major pests of important crops such as tobacco, rice, sugar cane, cucurbits, and cabbage. The vast majority of the known plant viruses are transmitted by means of insects. Thus a control program for virus diseases would involve the study of the relationship between the plant viruses and the insect vectors. A project (No. 9) has been initiated under the auspices of the Kasetsart/Hawaii University Contract, USOM Thailand, to support research in this particular field of study which has not been undertaken previously in Thailand.

This paper is progress report on the project and it covers the facilities and equipment available and work which has been done.

FACILITIES AND EQUIPMENT

Facilities and equipment available are very satisfactory for research in the field of vector-virus relationships. Basic facilities consist of an insect-proof greenhouse (Lord and Burnham, U.S.A.) and a potting compound.

The greenhouse is 12.7 m. long and 6.5 m. wide. It is divided into two equal parts by a solid glass partition. One compartment is used for the growing of healthy test and colony plants, the other for diseased or treated plants. The sides of the greenhouse are of corrugated cement asbestos board at the base and 30×30 mesh stainless steel screen on the upper portion. The same kind of screen covers the vents at the ridge of the roof. The rest of the greenhouse is of high grade clear glass.

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In each compartment are two 14-inch wall fans attached to the asbestos board base. The openings of the fans are covered on the outside by the fine mesh screen. The fans are made to blow inward. Entrance to each compartment of the greenhouse is by doors located at each gable end. A concrete walkway leads from one door longitudinally to the other. The remaining floor of the greenhouse consists of a 4-inch layer of broken clay bricks. In front of each fan, on the inside of the greenhouse, there is a loose stack of bricks which are kept wet by means of a perforated water trough on the top. The fans blowing on the wet bricks produce a cooling effect which is adequate to keep the temperature inside of the greenhouse low enough to grow plants even during the hot season. The floor of broken clay bricks, which is almost always wet, also helps to maintain a low temperature. In each compartment there are eight pot benches, 2.2 m. x 0.9 m., each of which can accommodate as many as 200 three-inch pots.

The potting compound adjoins the greenhouse. It consists of three compost soil bins and a potting shed. The potting shed is 8.0 m long and 4.0 m wide. Inside are racks for the various sizes of clay pots, a bin for sterilized soil, a potting bench, and a storage room for tools and equipment.

The soil used in this research unit is used over and over. Periodically it is tested for pH and fertillizer is added when necessary. When a plant is to be discarded, it is dumped together with the soil into the compost bin. After a few months of composting the soil is screened, placed in the sterilized soil bin and fumigated with methyl bromide.

Essential equipment for research in vector-virus relationship is not extensive and is available in adequate quantities. Included are hand lenses, timers, brushes, cages, aspirators, and many kinds of glassware.

WHAT HAS BEEN DONE

During the initial stage of the project the amount of work accomplished was very limited due to the lack of an insect-proof greenhouse, which is a necessity in vector-virus research. At this stage work was confined mostly to surveying and observation of the important virus diseases of Thailand. Whenever possible diseased specimens were brought back to the laboratory, the juice extracted, froze and kept in that state for future use. Almost a year and a half passed between the placing of the greenhouse and its arrival in Thailand from the United States. In two months the greenhouse was erected and the potting compound constructed. With the completion of the facilities a period of few months of "breaking in" of the unit was necessary. During this period the method to cool the inside of the greenhouse was devised. Other necessary and useful information was obtained such as: the number of days required for such plants as cucumber, cabbage, and tobacco to germinate and to grow to test plant size; that shading was unnecessary; that two waterings a day, once in the morning and once

in midafternoon were adequate; that operation of the fans at night was unnecessary.

After the research unit was considered to be operational the next objective was to find a vector and virus suitable for the demonstration of various techniques and the methodology of research in this field of study. In the search for the virus the following characteristics were considered desirable: (1) non-persistent, thus sap-inoculable and aphid trans missible, (2)aphid transmissible at a relatively high rate, (3) ability to survive and manifest itself in the hot and humid greenhouse, (4) transmissible by a vector which has good laboratory behavior, (5) importance as a pest of an economically important crop of Thailand. The search took a long time. After testing viruses from more than 30 different sources, a mosaic virus on cabbage from Petchaboon was found which has all the desirable characteristics.

Concurrent with the search for a virus, a search was conducted for a suitable vector. Vector requirements were: (1) an aphid, (2) colonize well under insectary conditions on an easily grown colony plant, (3) perform well as a laboratory animal, i.e., begin feeding rapidly when placed on a plant, (4) large enough so that the proboscis can be seen easily with at the most a 20X hand lens. Aphids were collected from various sources in the field and brought into the laboratory. Non-viruliferous colonies were then derived from the field aphids through the usual time-consuming procedure employed for this purpose. The aphids were then tested for their suitability. Three different *Aphis* species and *Myzus persicae* (Sulzer) were considered. A pink strain of *M. persicae*, a major pest of tobacco in the Chiengmai area, proved to be a suitable vector. The identification of the vector was confirmed by specialists of the United States National Museum.

During the process of building up the vector-virus research unit to an operational status experience was gained in several aspects of this field of study. These included the transmission of virus by grafting, tranmission by means of sap inoculation, watched and timed transmission with one aphid per test plant, transmission with many aphids per test plant, host range studies, and the method of obtaining non-viruliferous aphids.

With the selection of a suitable virus and vector, a large experiment was initiated on the study of the change in trasmissibility of a mosaic virus of cabbage, with regard to time, using *M. persicae* as the vector. The experiment is as yet not completed. At weekly intervals for five consecutive weeks transmissibility is tested from virus source plants. There are three different treatments in this experiment. In one treatmnt the virus source is the youngest opened leaf of a plant selected at random each week from a "population" of ten diseased plants which were in turn selected from a larger "population" of diseased plants. In another treatment the youngest opened leaf is the virus source each week as in the previous treatment but in this case

the same plant is used each week. In the third treatment, at the first week of testing the youngest opened leaf is the virus source and for four consecutive weeks, thereafter the same leaf is tested. At least three series will be conducted per treatment, using 50 test plants per treatment per week. One series has already been completed and two others are now in progress. This experiment provides experience in standard technique, methodology, and analysis in a major phase of vector-virus relationship research.

The vector-virus relationship research unit at Kasetsart University is now operational. The facilities are excellent, the equipment adequate, and the personnel adequately trained and capable. The potential of the research unit is substantial and much work can be expected to emanate from it if financial support is consistent.

สรุป

ในประเทศไทยโรคไวรัสเป็นโรคที่พบและทำความเสียหายให้แก่พืชที่สำคัญ ๆ หลายชนิดมาก เช่น ยาสูบ ข้าว อ้อย ลำใย และพืชผักต่าง ๆ แต่โรคต่าง ๆ นั้นมีแมลงเป็นปัจจัยสำคัญต่อการแพร่เชื้อ ดังนั้นการศึกษาความสัมพันธ์ระหว่างแมลงพาหะกับเชื้อไวรัสที่เป็นสาเหตุของโรค จึงมีความสำคัญในทางที่จะหาวิธีป้องกันกำจัดโรค ด้วยเหตุนี้ ทางมหาวิทยาลัยเกษตรศาสตร์จึงได้ร่วมมือกับฝ่ายสัญญาความร่วมมือช่วยเหลือระหว่างมหาวิทยาลัยเกษตรศาสตร์กับมหาวิทยาลัยฮาไวอี เริ่มต้นดำเนินงานในด้านนี้ขึ้น โดยได้จัดสร้างโรงกระจกชนิดกันแมลงได้ (insect - proof greenhouse) จำนวน 1 หลัง นอกจากนี้ได้จัดหาและสร้างเครื่องมือ เครื่องใช้เบ็ดเตล็ดต่าง ๆ ที่จำเป็นอีกมาก ในด้านเจ้าหน้าที่ก็ได้เริ่มฝึกฝนให้รู้จักเทคนิคต่าง ๆ เป็นอย่างดี ในระหว่างเริ่มต้นของโครงการนี้ได้ดำเนินในด้านการสำรวจเสียเป็นส่วนมาก ทั้งนี้เพื่อหาโรคไวรัส และพาหะที่มีลักษณะเหมาะสมเพื่อใช้ในงานทดลองนี้ ซึ่งหลังจากได้ทดลองจากโรคของพืชชนิดต่าง ๆ กับแมลงต่าง ๆ กันมากมายแล้ว ปรากฏว่า เพลี้ยอ่อนของยาสูบ (*Myzus persicae*) จากเชียงใหม่ เป็นแมลงพาหะที่มีลักษณะเหมาะสมกับเชื้อไวรัสของโรคใบด่าง (mosaic) ของผักกาดที่ได้จากเพชรบูรณ์ได้ดี ซึ่งขณะนี้ได้เก็บไว้ใช้ในงานวิจัยและใช้สอนเกี่ยวกับการทดลองในด้านต่าง ๆ ที่กำลังดำเนินงานอยู่ต่อไปอีก