PROEXAG
NON-TRADITIONAL AGRICULTURAL EXPORT SUPPORT PROJECT

CARDAMOM MOSAIC VIRUS IN HONDURAS: OCCURRENCE AND RECOMMENDATIONS FOR ITS CONTROL

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EXECUTIVE SUMMARY

Cardamom is a widely grown spice crop in India, Guatemala and, to a lesser extent, Honduras. In the 1970s, a viral disease of Cardamom, Cardamom Mosaic, was observed in Guatemala where it has since spread throughout most of the country and is a major factor in limiting optimal production of this crop. The virus associated with the disease is a member of the potyvirus group and is transmitted by the banana aphid (Pentalonia nigronovosa) in a nonpersistent manner. Recent observations have indicated that the virus (Cardamom mosaic virus, or CarMV) may be present in certain cardamom plantations of Honduras, thus posing a threat to cardamom production in that country.

The scope of this assignment involved five areas: (1) to conduct field surveys in cardamom plantations suspected of having the cardamom mosaic virus in order to verify the presence of that virus, assess its effect on crop production and develop recommendations for control; (2) to teach local technicians laboratory methods for serologically detecting the virus; and (3) to discuss with cardamom growers and technicians the subjects of cardamom, cardamom mosaic virus and methods for its control. Previous surveys indicate that the virus is found in only two locations: near Lake Yojoa and near the Guatemalan border. My survey was conducted on two farms (Rocas and Sabillon) located near Lake Yojoa. Cardamom plants showing typical cardamom mosaic symptoms were observed on approximately 30 manzanas of Rocas farm. Apparently, the CarMV was brought into the plantation through infected rhizomes. In the adjacent farm, Sabillon, only two plants with virus symptoms were observed, although about 150 plants with symptoms had been pulled out in March 1987. Infected plants showed chlorosis on the leaves with green streaks interspersed, creating a mosaic pattern. These symptoms are those observed in infected cardamom plants growing in Guatemala. Thus, visual symptoms confirmed that CarMV was present in Honduras.

A lecture on the properties of CarMV, its occurrence in Guatemala and the methods for controlling the virus was given to a number of people interested in cardamom. Subsequent discussions centered on CarMV, its potential danger to the cardamom industry of Honduras and possible ways to control the virus. The ELISA (enzyme-linked immunosorbent assay) technique was taught to technicians using the facilities at FHIA (Fundacion Hondurena de Investigacion Agricola). ELISA is a rapid and specific serological method that is useful for detecting CarMV in cardamom and other possible hosts. It should be valuable for use in subsequent CarMV control programs initiated in Honduras.

Since CarMV is not yet widespread in Honduras, it is extremely important that a strong effort be made to prevent CarMV

from spreading to farms that do not have the virus. Immediate control measures should be centered around this goal. It is recommended that (1) in order to prevent further spread of the virus through propagation materials, movement of plants from farms with the virus (e.g., Rocas, Sabillon, and a farm near the Guatemalan border) should be strictly forbidden; (2) a buffer zone free of cardamom plants must be maintained around the infected farms and, specifically, the establishment of cardamom plantations within 5 km of infected farms should be prohibited; (3) importation of cardamom plants into Honduras must be prohibited, unless they are checked and found to be free of CarMV; (4) all nurseries must be required to maintain and propagate virus-free plants; (5) more surveys should be conducted to determine if CarMV is in other parts of the country; (6) educational programs and meetings aimed at making people more aware of CarMV and its potential danger should be continued; (7) a centralized laboratory should be used for doing ELISA tests (the laboratory at FHIA is well equipped and seems to be the logical place to do ELISA tests for CarMV); (8) once the necessary equipment for ELISA is obtained, the people who participated in the ELISA training course should do further tests to gain experience (the advisor has left a supply of antisera and chemical solutions at FHIA. He will also maintain contact with the participants through Juan Moya, to give advice and further information); and (9) ELISA tests should be used judiciously for the detection of CarMV in cardamom samples. The cardamom program of FEPROEXAAH does not have sufficient manpower to use ELISA for routine detection of CarMV. Visual diagnosis is sufficient for this purpose.

TECHNICAL REPORT

A. Scope of Assignment

The scope of this assignment covered three areas as stated below:

1. Conduct field surveys in cardamom plantations suspected of having the cardamom mosaic virus in order to verify the presence of the virus, assess its effect on crop production and develop recommendations for control.

2. Teach local technicians the laboratory methods for serologically detecting the virus.

3. Discuss with cardamom growers and technicians the subjects of cardamom, cardamom mosaic virus and methods for its control.

B. Introduction and Background

Cardamom is a highly priced spice that is widely used in India as a cooking additive and in Middle Eastern countries as an ingredient for coffee. Central American countries have an ideal mountainous climate for the growing of cardamom. In fact, Guatemala is one of the world's largest cardamom producers. Cardamom production in Honduras is small compared to that of Guatemala, but it does have good conditions for growing the crop.

Although viral diseases have had significant effects on cardamom production in India, such diseases were not observed in cardamom plantations of Central America until the 1970s (1). The viral disease was first observed in Guatemala. The associated virus, Cardamom Mosaic Virus (CarMV), has spread through the entire southern region of Guatemala and to areas of the north, and it is a major factor in reducing the production of cardamom in Guatemala. The presence of CarMV in plantations of Honduras has not been thoroughly documented, although observations by local personnel (such as Juan Moya) indicate that the disease is present in at least two farms and possibly a third.

CarMV is a potyvirus that is easily spread through the use of infected propagation materials and by the banana aphid (Pentalonia nigronovosa) in a nonpersistent manner. The virus from Guatemala was recently characterized and antiserum prepared to the isolated virus particles (1). These flexuous rod-shaped particles are about 720 nm long. Leaves of infected plants show chlorosis and numerous green streaks causing a distinct mosaic pattern. Subsequent leaves show mosaic patterns, the plants show weaker growth resulting in smaller crop production and also

poorer quality fruit. Over a span of a few years, the production of the infected plant falls significantly below that of comparable healthy plants. The effect of the virus on the plant's productivity is also dependent on the age at which the plant is infected. Plantations started with infected rhizomes show very poor crop yields while those infected after the plants are several years old would have significantly more yield. Symptoms of CarMV resemble the viral disease described in India as "katte" disease of cardamom.

Recent surveys (Moya, personal communication) indicate that CarMV is present in Honduras. Thus, the cardamom industry of Honduras is faced with a potentially severe threat of CarMV. Efforts must be made to control this disease.

C. Occurrence of CarMV in Honduras

The establishment of CarMV in Guatemala and its subsequent effect on crop production is well documented. However, the situation in Honduras was not completely clear, as far as this advisor was concerned. Thus, an important objective was to determine if the virus was indeed present in Honduras and, if so, how widespread it was.

Previous surveys by Juan Moya and colleagues indicated that CarMV was present in two adjacent farms near Lake Yojoa. Finca Rocas was reported to be severely infected (about 30 manzanas) while only about 150 infected plants were in the adjacent farm (finca Sabillon) only 1 km away. According to accounts, plants with mosaic symptoms were detected in finca Rocas since 1985.

The advisor, Juan Moya and other persons visited fincas Rocas and Sabillon. The accompanying persons included eight members of IHCAFE, two foremen from the farms and two from the Plant Health Department of the Ministry of Natural Resources. The virus is widespread in a large sector of finca Rocas. Apparently CarMV was brought to the farm through infected rhizomes that were used to establish the initial plantings in 1982. Infected plants showed symptoms identical to those observed in Guatemala. From the symptomatology alone, the Advisor concluded that this was indeed the same disease that was observed in Guatemala. The sector with the disease was not growing well and the foreman indicated that production in that sector had decreased by more than 50 percent.

The disease was also observed in a nearby area of slightly higher elevation in finca Rocas. However, the disease was located only in the section that had been previously planted with rhizomes from the lower area of the farm in which the virus was prevalent. The disease was not observed in the other sections (although only a brief survey was made) that had been started from seedlings, and thus should have been free of the virus at

the time of planting. Apparently, viral movement via aphids is very slow in this farm.

The relatively slow movement of the virus by aphids was also indicated by observations on finca Sabillon, the farm adjacent to finca Rocas. Sabillon farm was planted in 1984. In March 1987, about 150 plants with CarMV symptoms were found in two locations. Apparently, the virus had been introduced by aphids from finca Rocas. The infected plants were removed in March and we observed only two new plants with symptoms in that same area. This apparently slow movement shows the effectiveness of distance and geography (mountains, hills, valleys) in slowing the spread of CarMV.

According to Mr. Moya, data from surveys done in other parts of Honduras indicate that they are free of CarMV, except for a farm near the Guatemalan border. Only one plant with the virus was observed and the plant and those adjacent to it were removed. The advisor did not have sufficient time to look at other cardamom plantations.

Based on symptomatology, the advisor believes that the disease caused by CarMV is present in Honduras. Furthermore, the virus particles that we observed in infected tissues under the electron microscope (done at the advisor's laboratory using leaf tissue from finca Sabillon) were similar to those previously observed with CarMV infected tissues from Guatemala (1).

Previous observations coupled with those of the advisor strongly indicate that CarMV is not widespread in Honduras. If these observations are correct, the virus situation in Honduras is very different from that in Guatemala. In the latter country, CarMV is widespread, while in Honduras the virus is confined to one or possibly two locations: near Lake Yojoa and near the Guatemalan border. Thus, it is extremely important to keep the virus localized in the specific farms and try to eradicate it if possible. At the minimum, the virus should not be spread to other farms through propagation of infected rhizomes. The importance of this step cannot be overemphasized. Otherwise, Honduras would quickly fall into the same situation as Guatemala.

D. Detection of CarMV by Serological Methods

Identification of the causal agent or the agent associated with the disease is a crucial initial step towards the development of control measures. CarMV symptoms in cardamom are quite characteristic and, thus, visual diagnosis is reliable. However, confirmation of the specific cause can be done serologically since antiserum to CarMV was previously produced by the advisor (1). Furthermore, serological tests are extremely valuable in detecting the virus in other plants that may serve as alternate hosts, and in detecting the virus in cardamom plants or

parts of the plant that do not show symptoms and thus may serve as sources for spread of the virus. ELISA (enzyme-linked immunosorbent assay) is a rapid, relatively simple, and specific serological method that has been used to detect CarMV in infected plants found in Guatemala. Therefore, the advisor sought to train local technicians in the use of ELISA and to use CarMV antiserum (prepared to CarMV from Guatemala) in ELISA tests for detecting the virus in leaf samples taken from symptomatic plants from Honduras.

Three days were devoted to training technicians on the use of ELISA. Gloria Molina, Leslie Rios, Roberto Jillegas Panchami from FHIA (Fundacion Hondurena de Investigacion Agricola), Nestor M. Tranconi from IHCAFE (Instituto Hondureno del CAFE) and Juan Moya from FEPROEXAAH (Federation of Association of Producers and Exporters of Agricultural and Agroindustrial of Honduras) took part in the training course. It was held at the laboratory of FHIA at La Lima. Facilities were provided by FHIA and the advisor provided CarMV antisera, conjugates and the necessary buffers. Participants were divided into two groups with each group doing the entire ELISA test to detect CarMV. Test samples were cardamom leaves showing typical CarMV symptoms and corresponding healthy leaves. Theoretical aspects of ELISA were discussed, as well as the specific steps.

The laboratory was well equipped except that it lacked micropipets to accurately dispense solutions into test wells. This was the main hindrance to doing the test properly. Otherwise, the training course went well and the participants were eager and very willing to learn.

The results were not clear. Although the virus-infected samples produced more reaction than the healthy ones, the advisor had experienced far better results in Guatemala and at Cornell University. Unfortunately, the tight time schedule did not permit a repetition of the test. Judging from leaf symptoms and preliminary ELISA results, the advisor was quite certain that the leaf samples with symptoms were infected with CarMV. As a further test to identify the disease, symptomatic leaf samples were taken to Cornell University and examined with the electron microscope. Virus particles showing similar shape to those of CarMV (as those observed in Guatemala) were detected from tissues with symptoms and not in tissues from healthy plants. However, more tests will have to be conducted to document the use of ELISA for detecting CarMV under Honduran conditions.

The facilities needed to perform ELISA tests on a routine basis are quite minimal. According to Mrs. Gloria C. Molina, FHIA has ordered the necessary pieces of equipment for setting up ELISA to be used primarily for detection of citrus tristeza virus in conjunction with a planned citrus virus certification program of FHIA. Ideally, the facilities of FHIA could be used for ELISA

tests to detect CarMV in cardamom and other plants. However, the specifics of this arrangement would have to be worked out with FHIA. The advisor would be willing to help in establishing the facilities if the personnel want such advice or help.

Although laboratory facilities are available for doing ELISA tests for CarMV in Honduras, the ELISA test should be used judiciously. FEPROEXAAH does not have the manpower to run thousands of samples, nor would it be wise even if the manpower was available. The ELISA test should be used to verify visual observations that CarMV does occur in various locations in Honduras. Once this has been established, then there is no routine need to use the ELISA. A very important use of the ELISA would be to determine whether alternate hosts (plants other than cardamom that can be infected by CarMV) play an important role in the epidemiology of the disease in Honduras. Then, the ELISA should be used to test prospective stock plants that growers may want to use to establish CarMV-free nurseries. Lastly, ELISA could be used to test samples of nursery plants to monitor the presence of CarMV in established nurseries before these plants are distributed to farms.

While the technique of ELISA is quite simple, the technicians should also be well grounded in the theory and various practical aspects of ELISA and other serological methods that could be used to detect CarMV. The initial training that the advisor gave to the technicians was a good beginning, but the technicians should be exposed to more training and hands-on experience. Such training will help to minimize misinterpretation of data and also will help in the correct utilization of the technique(s) in the cardamom program.

The advisor's laboratory has produced antiserum to CarMV from Guatemala. So far, this is the only antiserum available for CarMV. The advisor will continue to cooperate with the cardamom program in Honduras and supply the antiserum as he sees fit. Furthermore, he would try to clarify any questions of serology or other matters that personnel in Honduras have on cardamom.

E. Dissemination of Information on CarMV

Another objective of this assignment was to educate personnel on the properties of CarMV, its effects on cardamom based on the advisor's experience in Guatemala and to discuss possible control programs that could be used in Honduras. This phase of the assignment was important because the success of any CarMV control program started in Honduras will require the cooperation of technicians, growers, nurserymen and government officials. Thus, all of these people must be made to understand, as much as possible, the nature of CarMV, its effect on crop production and the options available for trying to control this virus in Honduras.

A seminar and discussion session was held at FEPROEXAAH headquarters throughout the morning of September 30. Thirty people, including extension agents, technicians from IHCAFE, cardamom growers and agriculture bankers, were present. The advisor gave a lecture, complete with slides, on the properties of CarMV, its detection, its prevalence in Guatemala and on possible approaches to control the disease in Honduras. Following the lecture, questions were fielded from the audience. Many questions were asked, the discussions were lively and the audience participated fully. The advisor felt that the audience became convinced of the potential detrimental impact CarMV on cardamom production in Honduras and the importance of preventing its spread to other farms.

As mentioned earlier, the advisor discussed various aspects of CarMV and cardamom culture with people who accompanied him on the field trip to fincas Rocas and Sabillon. These contacts were especially important because several of the attendants are IHCAFE extension agents in charge of cardamom in different parts of the country. The discussions made them more aware of what to look for and of the importance of preventing the spread of the virus.

It is important that Mr. Moya and colleagues continue, as they have been doing, to hold meetings with growers and other interested persons. Such meetings should focus on informing them on the nature of CarMV and its potential seriousness. Furthermore, they should relay specific instructions and details on control programs as these develop.

F. Possible Control Programs for Honduras

[Note: The advisor did not have sufficient time in this initial assignment to determine the personnel or agencies to be most suited for carrying out the CarMV control programs. Thus, the discussions below deal only with possible control programs and not with which agencies should carry them out.]

Control programs for plant viruses generally proceed in the following order: prevention, eradication, stopping further spread and learning to "live" with the virus disease once it is widespread. Naturally, the ideal control would be to prevent the virus from coming into the country or area of concern. In this case, it would be Honduras.

The virus situations in Guatemala and Honduras appear to be quite different at the present time. In Guatemala, CarMV is widespread in the south and parts of the north. Thus, in most of southern Guatemala, farmers must learn to "live" with the virus because it is virtually impossible to eradicate it. On the other hand, Honduras apparently has CarMV in only two locations, near Lake Yojoa and near the Guatemalan border. Furthermore, the

viral infection near the Guatemalan border was confined to only one plant (which was subsequently destroyed), according to observations by local personnel. Thus, although CarMV was not prevented from entering Honduras, the second and third modes of control (eradication and stopping of further spread of the disease) still can be applied in Honduras.

The first and immediate control measure to be taken is the prevention of further spread of the virus, especially in the Lake Yojoa region where large sections of a farm are infected. Fortunately, the two farms near Lake Yojoa have the virus are isolated from other cardamom farms by forests and by natural mountainous barriers. This would help to minimize the chances of the virus being spread to other infected farms by aphids. It is absolutely essential that the virus be prevented from spreading from these farms through infected rhizomes. That is, no planting material should be taken out of these farms and used to establish other plantings. Furthermore, isolation of these farms should be maintained by not allowing the establishment of cardamom plantations within a reasonable distance (for example, 5 Km, although the minimum distance cannot be determined for sure) of the infected farms. This would reduce the probability of aphid transmission of CarMV to neighboring farms, the subsequent transmission to nearby farms and so on. The key is to immediately isolate the infected farms to prevent further spread.

Ideally, efforts should be made to eradicate the virus from the infected farms. Although this is technically possible by destroying all infected plants of the farms, it may not be economically and politically feasible for obvious reasons. Unfortunately, the infected area of finca Rocas is large (about 30 manzanas).

It is also important that CarMV be prevented from coming into Honduras from other countries. All cardamom plants entering Honduras should be required to be free of the virus. Furthermore, cardamom nurseries should also be checked to ensure that their plants are free of the virus.

Fortunately, it appears that the cardamom industry of Honduras does not have to "live" with the virus at the present time. That is, cardamom growers (except those from the two farms mentioned above) do not have to produce cardamom in the presence of the viral infection. It is essential that efforts be made to maintain this condition.

Although CarMV appears to be presently confined to only a farm near the Guatemalan border and to two farms near Lake Yojoa, efforts must be made to monitor all cardamom producing areas for the virus. This can be done by routine surveys and by making growers aware of the disease and the visual symptoms of infected plants.

Recommendations:

1. In order to prevent further spread of the virus through propagation materials, movement of plants from farms (e.g., Rocas, Sabillon, farm near Guatemalan border) with the virus should be strictly forbidden.

2. Maintain a buffer zone free of cardamom around the infected farms. Specifically, prohibit the establishment of cardamom plantations within 5 km of infected farms.

3. Prohibit the importation of cardamom plants into Honduras unless they are checked and found to be free of CarMV.

4. Require that all nurseries maintain and propagate virus-free plants.

5. Conduct more surveys to determine if CarMV is in other parts of the country.

6. Continue to conduct educational programs and meetings aimed at making people more aware of CarMV and its potential danger.

7. Further tests should be conducted to establish unequivocally by serology that the CarMV in Honduras is related to that in Guatemala. The advisor is willing to help with this aspect by doing some work at Cornell.

8. Use a centralized laboratory for doing ELISA tests. The laboratory at FHIA is well equipped and seems to be the logical place to do ELISA tests for CarMV.

9. Once the necessary equipment for ELISA is obtained, the people who participated in the ELISA training course should do further tests to gain experience. The advisor has left a supply of antisera and chemical solutions at FHIA. He will also keep in contact with the participants, through Juan Moya, to give advice and further information.

10. ELISA tests should be used judiciously for detecting CarMV in cardamom samples. The cardamom program of FEPROEXAAH does not have sufficient manpower to use ELISA for routine detection of CarMV. Visual diagnosis is sufficient for this purpose.

Reference

Gonsalves, D., E. Trujillo, and H. C. Hoch. 1986. Purification and some properties of a virus associated with cardamom mosaic, a new member of the potyvirus group. Plant Disease 70: 65-69.