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State of Hawai'i
DEPARTMENT OF AGRICULTURE
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February 15, 2023

TO: Advisory Committee on Plants and Animals

FROM: Kimberly Johnson
Pioneer Hi-Bred International, Inc (Corteva Agriscience)

THROUGH: Wil Leon Guerrero, Microorganism Specialist
Plant Quarantine Branch
Hawaii Department of Agriculture

SUBJECT: Request to: (1) Allow the Importation Maize chlorotic mottle virus (MCMV), a virus on the List of Restricted Microorganisms Part A, by Permit, for Laboratory Work and Shade House Inoculations by Pioneer Hi-Bred International, Inc.; and (2) Establish Permit Conditions for the Importation of Maize chlorotic mottle virus (MCMV), a Virus on the List of Restricted Microorganisms Part A, for Laboratory Work and Shade House Inoculations by Pioneer Hi-Bred International, Inc.

I. Summary Description of the Request

PQB NOTES: *The Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicant from procedural information and advisory comments and evaluation presented by PQB. With the exception of PQB notes, hereafter "PQB NOTES," the text shown below in Section II from page 2 through 7 of the submittal was taken directly from Kimberley Johnson's application and subsequent written communications provided by the applicant, Ms. Kimberly Johnson. For instance, the statements on page 6 of the submittal regarding the effects on the environment are the applicant's statements, and not PQB's. This approach for PQB submittals aims for greater applicant participation in presenting import requests in order to move these requests to the Board of Agriculture (Board) more quickly, while distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including proposed Permit Conditions and Advisory Subcommittee Review are identified as sections III and IV of the submittal, which starts at pages 7 and 11 respectively.*

We have a request to review the following:

COMMODITY: Single shipment of three 50ml Falcon tubes containing leaves inoculated with Maize chlorotic mottle virus with appropriate escape proof packaging.

SHIPPER: [ ] **CBI**

IMPORTER: [ ] **CBI**

PQB NOTES: *The SHIPPER and IMPORTER are redacted in an effort to protect Corteva's trade secret information and preventing the disruption of biotechnology research and the safety of Corteva's research personnel and research location including fields. Throughout this submittal In instances where the applicant has made this claim, "CBI" will be indicated for the information between "[" and "]". See Appendix A for the permit application.*

CATEGORY: MCMV is a microorganism on the List of Restricted Microorganisms Part A. Microorganisms on this list are classified as high risk. Pursuant to Hawaii Administrative Rules (HAR), Chapter 4-71A, any microorganisms on the List of Restricted Microorganisms Part A, which includes MCMV, can only be introduced into the State under permit approved by the Board, subject to conditions established by the Board.

II. Information Provided by the Applicant in Support of the Application

PROJECT: The inoculations of maize chlorotic mottle virus (MCMV) in Hawaii are part of a Maize Lethal Necrosis (MLN) project done in collaboration with The International Maize and Wheat Improvement Center (CIMMYT) and the Bill and Melinda Gates Foundation (BMGF), (see Boddupalli P, et al. Virus Research 282 (2020) 197943). MLN is caused by simultaneous infections of maize by both MCMV and one of the *Potyviridae* viruses, causing severe loss of yield. This is prevalent in eastern Africa where maize yield losses from MLN can range from 23-100% of the crop.

The overall project includes many collaborators and multiple potential strategies to combat this disease. Corteva Agriscience's role in this project is to develop certain gene edited maize lines with potential resistance to MCMV. Both gene edited maize lines and non-edited

maize controls will be used and only the MCMV component of MLN infection will be addressed in this study.

PURPOSE: MCMV is a component of MLN infection. Testing the gene-edited maize lines for resistance to MCMV will provide insight into MLN resistance.

OBJECTIVE: The objectives are to test certain gene-edited maize lines against MCMV in an environment similar to Africa's. As MCMV is also a plant pest in Hawaii and other parts of the world, genetic resistance to MCMV would provide additional means of controlling this disease.

PROCEDURE:

- The MCMV isolate was originally collected in Waialua, Hawaii, and shipped to Johnston, Iowa for laboratory confirmation of purity, increased, and then shipped back to Hawaii for use in this project.
  - This helps ensure that only pure strains of MCMV will be utilized in the proposed field studies.

- The federal Interstate Movement and Release permit P526P-21-04098 for import and shade house release of MCMV isolates, was reviewed by Hawaii state officials and has been approved by USDA/APHIS.

- Packaging/Handling: All packages will minimally consist of both inner/primary and outer/secondary packages securely sealed so that both are designed to be effective barriers to escape or unauthorized dissemination of the listed materials/organisms. The inner/primary package(s) will contain all regulated materials/organisms and will be cushioned and sealed in such a way to remain sealed during shock, impact, and pressure changes that may occur. The outer/secondary shipping container will be rigid and strong enough to withstand typical shipping conditions (dropping, stacking, impact from other freight, etc.) without opening.

- Organisms will be shipped, or transported to the shadehouse, in packages designed to be leak-proof and escape-proof. Inoculum may be transported to the shadehouse via personal or project/company owned vehicles.

- Inoculations will be performed on approximately 100 maize plants, including controls, in a shadehouse in Honolulu County.

- MCMV inoculations will be performed per protocols in Boddupalli P, et al. Virus Research 282 (2020) 197943.

  Basic procedure and safeguards:

  - Infected leaves are ground with a carborundum abrasive in a buffer solution to make a sap solution (a viscous liquid containing mostly water and plant material).
  - Sap solution is kept in sealed Falcon tubes until use.
  - Sap solution is rubbed directly on leaves for inoculation. No spraying is involved.
  - Personnel will wear proper PPE (lab coat, safety glasses, nitrile gloves).
  - All un-used inoculum is chemically treated with a 10% bleach solution to kill all plant pests prior to disposal.
  - At the conclusion of the study, all plants will be incinerated and residue buried.

DISCUSSION:

1. **Person Responsible: [ ] CBI**

2. **Safeguard Facility and Practices:** [ ] **CBI**

   1. Corteva takes necessary precautions designed to prevent escape of regulated microorganisms received under permit. See additional information in #2 below.

   2. • All Corteva employees complete yearly biosafety training.

      • The samples will arrive in a 50ml Falcon tubes, double bagged in a sealed cooler inside a sealed cardboard box and stored in a similar container at our permitted facility. Inoculum will be prepared at the permitted facility and transported in sealed Falcon tubes, then double bagged. These will be devitalized after inoculations are complete.

      • All regulated microorganisms are stored in a secure facility with an orange "Restricted Access" sign(s) stating that regulated materials are stored inside.

  - All facilities are in a restricted access/gated area with security coded gates, only employees are able to access the facility.
  - The shadehouse will be locked so that only staff associated with the study can access it.

- In area that are common use, orange restricted access signs are posted on specific shelves, racks, equipment, etc. associated with the regulated material.
  - Only authorized personnel will have access and will be trained to handle permitted microorganisms.
  - All areas have placards indicating the area contains permitted microorganism.

- Regulated microorganisms are separated from non-regulated microorganisms to avoid inadvertent mixing.
  - Examples: different incubators or separate shelves within the incubator

- Regulated microorganisms are labeled to avoid inadvertent mixing with microorganisms not received under permit.
  - Any unlabeled microorganisms or microorganisms with an unknown status (i.e. regulated or non-regulated) will be handled as regulated.

- Necessary precautions are taken, which are designed to prevent escape of regulated microorganisms received under permit.

    - The samples will arrive in a 50ml Falcon tubes, double bagged in a sealed cooler inside a sealed cardboard box and stored in a similar container at our permitted facility. Inoculum will be prepared at the permitted facility and transported in sealed Falcon tubes, then double bagged. These will be devitalized after inoculations are complete.

- Regulated microorganisms will be maintained at the work address listed on the permit and internal protocols dictate that regulated articles may not be removed from designated storage or contained experimental area.

[Please see Page 7 for the map of Corteva's Parent Seed Station in Waialua.] **CBI**

3. Method of Disposition:

All un-used inoculum will be chemically treated by inactivation with 10% bleach solution to kill all plant pests prior to disposal. At the conclusion of the study, all plant material will be incinerated and residue buried.

4. Abstract of Organism:

Taxonomic Classification:

Domain: Virus
Group: "Positive sense ssRNA viruses" Group: "RNA viruses"
Family: *Tombusviridae*
Genus: *Machlomovirus*
Species: Maize chlorotic mottle virus

EPPO code: MCMV00

Additional Characteristics:

MCMV was first identified in Peru in 1971 and has since been found in parts of the central continental US, Hawaii, Mexico, South America, Asia and eastern Africa.

The Family *Poaceae* is the only natural host of MCMV, with optimal temperature ranges being any maize-growing region.

The virus is transmitted by 6 species of *Chrysomelid* beetles and thrips *Frankliniella williamsi* and is also seed transmitted but at a low level. Insect netting is used in the shadehouse as an additional barrier.

MCMV has a single-stranded positive sense RNA genome that is encased in a 30-nm icosahedral virion.

5. Effects on the Environment:

We have no reason to believe that this study would have any impact on the surrounding areas, economy, or society.

MCMV has been observed in Hawaii and the Family *Poaceae* is the only natural host, we have no reason to believe that an accidental release of MCMV would have an environmental, economic, or societal impact on other plants, animals, or humans.

It is well known that the virus is present in Kauai, Oahu, Molokai and Maui. Please see: Nelson S, Brewbaker J, Hu J. 2011. Maize chlorotic mottle. Honolulu (HI): University of Hawaii. 6 p. (Plant Disease; PD-79) (Attachment 1). Corteva is unaware of publications that document the prevalence or scope of infection to non-crop species.

Please note, the virus (inoculum) in use was isolated from the Waialua location and is endemic to Oahu. It has been kept in a BLS2 containment facility at Corteva in Johnston, Iowa where its purity was maintained and confirmed.

III. Proposed Permit Conditions:

1. The restricted article(s), *Maize Chlorotic Mottle Virus (MCMV)*, shall be used for laboratory work and plant inoculations in a shade house, purposes approved by the Board of Agriculture (Board), and shall not be sold, given away, and/or transferred in Hawaii, unless approved by the Board. Release of the restricted article(s) into the environment is prohibited.

2. The permittee, Pioneer Hi-Bred International, [address CBI], shall be responsible and accountable for all restricted article(s) imported, from the time of their arrival to their final disposition.

3. The restricted article(s) are subject to the pre-entry requirements of section 4-71A-8 and the inspection requirements of section 4-71A-9, Hawaii Administrative Rules (HAR).

4. The restricted article(s) shall be safeguarded at the Pioneer Hi-Bred International, [address CBI], a site inspected and approved by the Plant Quarantine Branch (PQB) prior to importation. Prior to the removal of the restricted article(s) to another site, a site inspection and prior approval by the PQB Chief is required.

5. The restricted article(s) shall be maintained by [CBI], as the responsible person, or by trained or certified personnel designated by the permittee.

6. The restricted article(s) shall be imported only through the port of Honolulu except as designated by the Board. Entry into Hawaii through another port is prohibited unless designated by the Board.

7. Each shipment shall be accompanied by a complete copy of the PQB permit for the restricted article(s) and an invoice, packing list, or other similar PQB approved document listing the scientific and common names of the restricted

article(s), the quantity of the restricted article(s), the shipper, and the permittee for the restricted article(s).

8. At least four sides of each parcel containing the restricted article(s) shall be clearly labeled with “Live Microorganisms” and “This Parcel May be Opened and Delayed for Agriculture Inspection” in 1/2-inch minimum sized font.

9. The permittee shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application, and as approved by the Board and the PQB Chief.

10. The approved site, restricted article(s), and records pertaining to the restricted article(s) under permit shall be subject to post-entry inspections pursuant to section 4-71A-16, HAR. The permittee shall make the site, restricted article(s), and records pertaining to the restricted article(s) available for inspection upon request by a PQB inspector.

11. The permittee shall immediately notify the PQB Chief verbally and in writing under the following circumstances:

    a. If any theft, accidental release, exposure, or disease outbreaks involving the restricted article(s) under this permit occurs.

    b. If any changes to the approved site, facility, and/or procedures regarding the restricted article(s) occur or are to be made, the permittee shall obtain written approval from the PQB Chief as soon as practicable (if unplanned) or prior to implementation (if planned). Also, the permittee shall submit a written report documenting the specific changes to the PQB Chief.

    c. If a shipment of the restricted article(s) is delivered to the permittee without a PQB “Passed” stamp, tag or label affixed to the article, container, or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State, then the permittee shall not open or tamper with the shipment and shall secure, as evidence, all restricted article(s), shipping container(s), shipping document(s) and packing material(s) for PQB inspection.

    d. If the permittee will no longer import or possess the restricted article(s) authorized under this permit.

12. The permittee shall submit an annual report of all the restricted article(s) imported for the calendar year by January 31st of the following year. The report

shall include the permit number, scientific name and quantity of each restricted article(s) imported, and status of use of the restricted article(s) imported and possessed.

13. Upon completion or termination of the study, the restricted article(s), media, and plants inoculated with the restricted article(s) shall be destroyed by autoclaving. In the event autoclaving is not possible, the permittee shall obtain written authorization from the PQB Chief for an appropriate alternate method of destruction prior to implementation.

14. The permittee shall submit a final report on the method of destruction of the restricted article(s) to the PQB chief within 30 days of completion or termination of the use of the restricted article(s).

15. The permittee shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspection(s), which identifies the practices and procedures to be adhered to by the permittee to minimize or eliminate the risk of theft, escape, or accidental release of the restricted article(s), including the risk of introduction and spread of diseases and pests associated with the restricted article(s) to the environment. The permittee shall adhere to all practices and procedures as stated in this biosecurity manual.

16. It is the responsibility of the permittee to comply with all applicable requirements of municipal, state, or federal law pertaining to the restricted article(s).

17. The permittee shall submit a copy of all valid licenses, permits, certificates or other similar documents required by other agencies for the restricted article(s) to the PQB Chief. The permittee shall immediately notify the PQB Chief in writing when any of the required documents are suspended, revoked, or terminated. This permit may be amended, suspended, or canceled by the PQB Chief upon suspension, revocation, or termination of any license, permit, certificate, or similar documents required for the restricted article(s).

18. Any violation of the permit conditions may result in citation, permit cancelation, and enforcement of any or all of the penalties set forth in HRS §150A-14.

19. The permittee is responsible for costs, charges, or expenses incident to the inspection, treatment, or destruction of the restricted article(s), as provided in Act 173, Session Laws of Hawaii 2010, Section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

20. A cancelled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancellation, any restricted article(s) imported under permit may be moved, seized, treated, quarantined, destroyed, or sent out of State at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee.

21. The permit conditions are subject to cancellation or amendment at any time due to changes in statute or administrative rules restricting or disallowing import of the restricted article(s) or due to Board action disallowing a previously permitted use of the restricted article(s).

22. These permit conditions are subject to amendment by the PQB Chief in the following circumstances:

    a. To require disease screening, quarantine measures, and/or to place restrictions on the intrastate movement of the restricted article(s), as appropriate, based on scientifically validated risks associated with the restricted article(s), as determined by the PQB Chief, to prevent the introduction or spread of disease(s) and/or pests associated with the restricted article(s).

    b. To conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

23. The permittee shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents, employees, and the Board of Agriculture members for any and all claims against the State of Hawaii, its officers, agents, employees, or Board of Agriculture members that may arise from or be attributable to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the state or federal employee is a permittee in the employee's official capacity.

24. For all laboratory use of the restricted article(s), the permittee shall comply with the Centers for Disease Control and Prevention (CDC) Biosafety Level 2 laboratory design, safety equipment and standard and special microbiological practices as found in the current edition of the CDC's handbook, "Biosafety in Microbiological and Biomedical Laboratories."

IV. Advisory Subcommittee Review

This request was submitted to the Advisory Subcommittee on Viruses for their review. Their recommendations and comments are as follows.

PQB NOTES: *Some questions were brought up regarding the exclusion of pests, particularly thrips from entering the shade house, sap solution and plant material disposal and standard operating procedures for handling MCMV materials. For the shade house, the applicant states that thrips screens will be used to provide a physical barrier to insects from entering. In addition, they have the ability to use pesticides if insect thresholds are reached so as to not compromise the experiment.*

As for the disposal of un-used inoculum, plant material, and contaminated material, a 10% sodium hypochlorite will be used. Treated waste will be double bagged prior to disposal. The Advisory Subcommittee on Viruses were satisfied with the applicant's responses to these questions.

1. I recommend approval ___ / ___ disapproval to allow the importation Maize chlorotic mottle virus (MCMV), a virus on the List of Restricted Microorganisms Part A, by permit, for laboratory work and shade house inoculations by Pioneer Hi-Bred International, Inc.

Dr. Edward Desmond: Recommends approval.

Dr. Michael Shintaku: Recommends approval.

Comments: "My recommendation leans heavily on their statement that the virus they wish to import was originated in Waialua."

Mr. David Clements: Recommends approval.

Comments: "Given the presence of the virus in Hawaii there is minimal risk and Hawaii provides an appropriate location to conduct the proposed test. Nevertheless, it is important that the appropriate precautions and procedures are in place. In the application a heavy emphasis is placed on the precautions and procedures on the application of sap solution to the test plants in the shade house, whereas there is minimal description placed on the preparation of the sap solution in the lab from the imported

infected leaf samples. The virus is in its most concentrated form at this stage and proper precautions and procedures are important at this stage. As stated above, "for laboratory and shade house inoculations.: Both areas of work need to be treated with equal emphasis. Also, there is no mention of BSL-2 handling in the application. My approval is conditional in the HDOA is satisfied that the importer has the proper documents and procedures in place to address the initial handling of the imported material."

Dr. Hongwei Li: Recommends approval.

Comments: "The MCMV isolate to be imported was originally collected in Waialua, Hawaii; and MCMV has also been found on other Hawaii islands. The proposed project to study the MCMV resistance of gene edited maize poses a minimal environment impact."

Dr John Hu: Recommends approval.

Dr. Raquel Wong: Recommends approval.

2. I recommend approval ___ / ___ disapproval to establish permit conditions for importation Maize chlorotic mottle virus (MCMV), a virus on the List of Restricted Microorganisms Part A, by permit, for laboratory work and shade house inoculations by Pioneer Hi-Bred i International, Inc.

Dr. Edward Desmond: Recommends approval.

Dr. Michael Shintaku: Recommends approval.

Mr. David Clements: Recommends approval.

Comments: "The permit conditions listed in the application are appropriate for the material to be imported and the work to be conducted. To emphasize the points in the above comments, these are directly addressed by permit conditions, these are covered by conditions 13, 15, and 24."

Dr. Hongwei Li: Recommends approval.

Dr John Hu: Recommends approval.

Dr. Raquel Wong: Recommends approval.

ADVISORY COMMITTEE REVIEW: May we request your recommendation and comments at the next meeting of the Advisory Committee on Plants and Animals.

CBI DELETED COPY

PQ-7 (01/04)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No._____

☐ Approve Permit No._____ Date:_____

☐ Disapprove ☐ Other_____

Processed by:_____ Date:_____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: August 25, 2021

In accordance with the provision of Chapter _____ , Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
3 Falcon tubes(50ml ea.)	Corn (maize) leaves inoculated with MCMV	Zea mays/Maize chlorotic mottle virus (MCMV)

RECEIVED
AUG 27 2021
PLANT QUARANTINE BRANCH

NO PAYMENT
Date: 8/27/21 Initial:

Name and address of shipper: [ ] CBI

(Mainland or Foreign address)

Approximate date of arrival: January 2022

Mode of Shipment: ☐ Mail ☑ Air Freight ☐ Boat

Type of Permit:
- --- Import
  - ☑ one time only ☐ multi-shipments
- --- Intrastate shipment
  - ☐ one time only ☐ multi-shipments
- ☐ Possession

Object of importation:
- ☐ Kept caged at all time
- ☐ Used for propagation
- ☐ Imported for exhibition
- ☑ Imported for liberation
- ☐ Other purposes - specify _____

Please type or print clearly.

Applicant's Name Kimberly Johnson

Company Name Pioneer Hi-Bred International, Inc
(if applicable)

Hawaii Mailing Address [ ] CBI

[ ] CBI

Telephone number [ ] CBI

Facsimile number [ ] CBI

Fee Amount Enclosed (cash, check or mail order) $_____

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).

   The MCMV isolate was originally collected in Waialua, Hawaii, and shipped to Johnston, Iowa for laboratory confirmation of purity, increased, and then shipped back to Hawaii for use in this project. The inoculations of maize chlorotic mottle virus (MCMV) in Hawaii are part of a Maize Lethal Necrosis (MLN) project done in collaboration with The International Maize and Wheat Improvement Center (CIMMYT) and the Bill and Melinda Gates Foundation (BMGF), (see Boddupalli P, et al. Virus Research 282 (2020) 197943).

2. Person responsible for the organism (include name, address and phone number).

   [

   ] CBI

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

   [

   ] CBI

4. Method of disposition.

   All un-used inoculum is sufficiently heat or chemically treated to kill all plant pests prior to disposal. Corteva protocol prohibits MCMV isolates from having direct contact with the environment outside of the shade house.

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

   MCMV (Family: Tombusviridae Genus: Machlomovirus) has been observed in Hawaii, all measures will be taken to ensure there is not an accidental release. Maize (Family: Poaceae) is the only natural host of MCMV, an accidental release would not have significant impact on other plants, animals or humans

****

I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature Kimberly Johnson **(Applicant)** **Date** Aug 25, 2021

College of Tropical Agriculture and Human Resources
University of Hawai'i at Mānoa

Plant Disease
December 2011
PD-79

Maize Chlorotic Mottle

Scot Nelson,[1] James Brewbaker,[2] and John Hu[1]
[1]Department of Plant and Environmental Protection Sciences
[2]Department of Tropical Plant and Soil Sciences

Maize (*Zea mays* L., corn) ranks as the second largest crop in the world, according to the Food and Agriculture Organization of the United Nations. The annual yield of maize in 2009 exceeded 800 million tons, and its value exceeded $51 billion (FAOSTAT 2009). Maize has become an integral part of modern society: It is a staple food for humans and livestock and has a variety of other uses. Corn has also become Hawai'i's most valuable crop.

Seed crops are Hawai'i's most valuable farm commodities. In 2008, seed corn made up 95% of the seed market and was valued at $213.6 million (Statistics of Hawaii Agriculture 2009). Eleven farms grew corn seed crops that year on over 6,300 acres. Farmers also grew 400 acres of sweet corn, valued at more than $1.23 million. With ever more acres being devoted to sweet corn, forage, feed grain, biofuel, and popcorn, maize has become essential to Hawai'i's modern agricultural economy.

Disease symptoms on a tasseling corn plant in Hawai'i infected with *Maize chlorotic mottle virus* (MCMV): pale green stripes or blotches running parallel to leaf veins, mosaic, and severe stunting (reduced distance between leaf internodes).

A range of important insects, weeds, and plant pathogens cause damage to corn in Hawai'i. Among the pathogens, fungi and viruses cause yield-limiting diseases to which temperate-climate field corn and sweet corn often lack resistance. Pathogens that cause fungal diseases include southern rust and a viral disease, maize mosaic, caused by *Maize mosaic virus* (MMV), both of which have been targeted for decades by the corn-breeding program at the University of Hawai'i's College of Tropical Agriculture and Human Resources (UH-CTAHR). Open-pollinated varieties such as Hawaiian Supersweet #9 (yellow) and Hawaiian Supersweet 'Silver' (white) are among the few sweet corn varieties available with a high level of resistance to MMV and southern rust. All sweet corn and field corn hybrids derived from these varieties and released by CTAHR are highly resistant (www.ctahr.hawaii.edu/hfs).

A newly emergent viral disease of corn, maize chlorotic mottle, appeared on Kaua'i in the early 1990s. *Maize chlorotic mottle virus* (MCMV) now poses a significant threat to temperate-zone seed corn crops and some of the locally developed tropical corn varieties. This disease was controlled for several years on Kaua'i by destroying infected crops, but it has since spread to other islands, including O'ahu and Maui. Most of the temperate-zone seed corn produced in Hawai'i is

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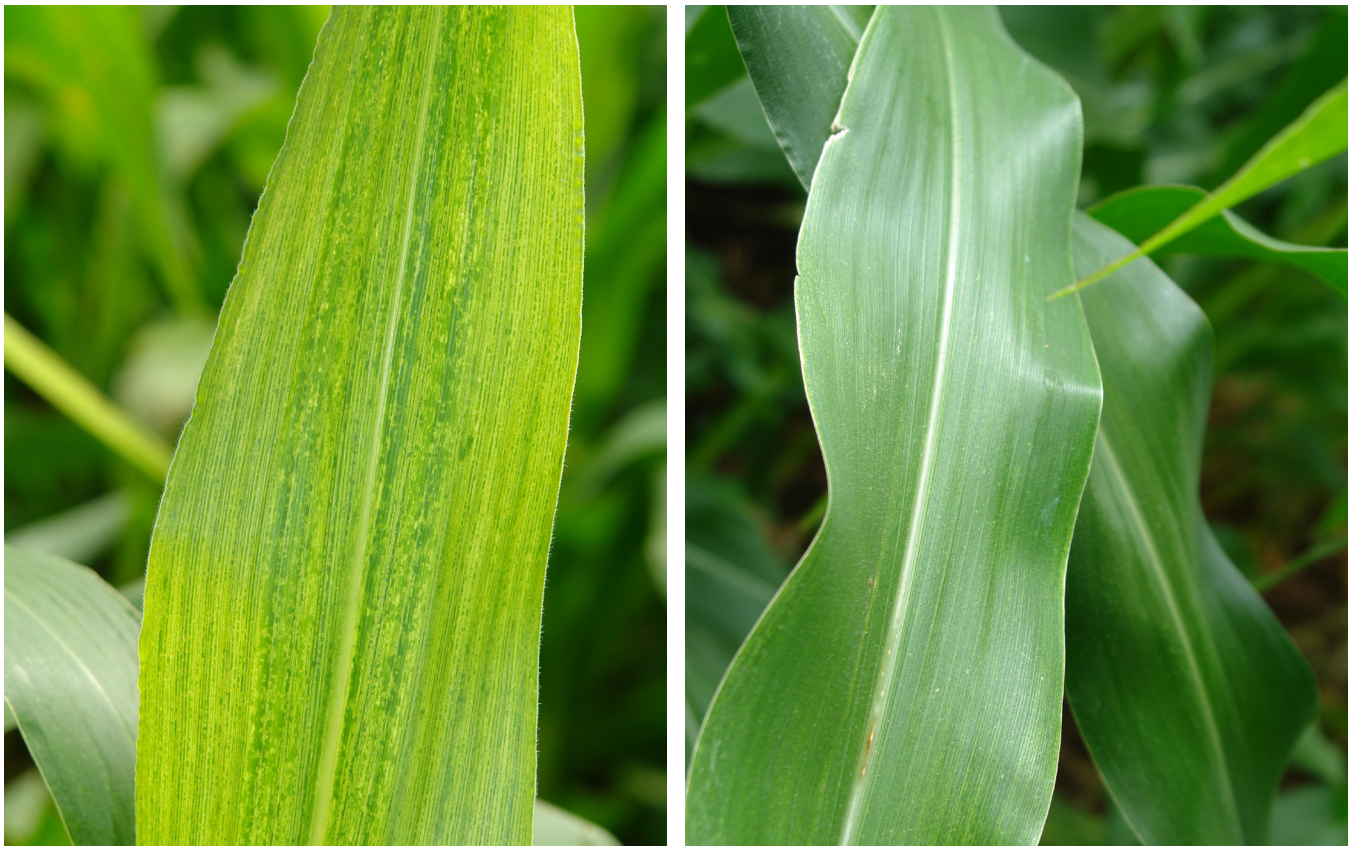

Typical mosaic symptoms on corn leaves (left) caused by MCMV in Hawai'i. Nearly asymptomatic leaves are shown at right.

highly susceptible to MCMV, and locally bred sweet corn varieties often show some genetic susceptibility. Producers of corn seed on all Hawaiian islands now spray regularly after planting to control the insects that spread of the virus.

In Hawai'i MMV, transmitted by the leafhopper, *Peregrinus maidis*, is more damaging than MCMV (Ming et al. 1997). However, the former disease can be effectively controlled by regular insecticide applications. MMV resistance in corn is monogenic and rare among temperate-zone corn varieties but common in all Hawai'i germplasm. MMV resistance in corn does not provide resistance to MCMV. When both viruses are present in a plant, in fact, the plants often age prematurely. The condition is called corn lethal necrosis (CLN).

The level of MCMV resistance varies widely among pure lines that have been tested in Hawai'i, so it is considered a quantitative trait. Almost all temperate climate inbreds and hybrids are highly susceptible to the virus. As a result, growers in Hawai'i's seed industry routinely spray insecticides on their predominately temperate-zone seed stock to control the thrips that are the vector of MCMV. In contrast, many tropical field corn lines and varieties are fully resistant. In CTAHR breeding nurseries at Waimanalo Research Station, the disease was first observed in early 2011. Maize chlorotic mottle is not widespread, but an epidemic occurred at a Kahuku sweet corn farm in 2010, causing severe yield loss. Preliminary inheritance studies on the inheritance of traits suggest a polygenic control of the disease, with resistance being partially dominant. This encourages the commercial production of hybrids only if both parents are resistant to the pathogen.

There are a number of important unanswered questions about MCMV and its vectors, as well as about maize chlorotic mottle. Here we discuss these ques-

tions, the pathogen, vectors and virus transmission, and the disease epidemiology and symptoms. We suggest integrated practices for its successful management and also identify areas of future research needed to gain a fuller understanding leading to better management of epidemics of maize chlorotic mottle.

Pathogen

Maize chlorotic mottle virus is the only species in the genus *Machlomovirus* (family *Tombusviridae*). The virions of this single-stranded RNA virus are isometric, and the single-component particles have a smooth spherical or hexagonal shape (Scheets 2010). The virus was first reported to infect *Z. mays* in Peru (Hebert and Castillo 1973). MCMV is not widespread in the United States, having been reported only in Nebraska, Kansas, and Hawai'i. Globally, the virus occurs in Argentina, Mexico, and Peru. At least two genetically and geographically distinct strains of MCMV have been reported, MCMV-P (Peru) and MCMV-K (Kansas) (Nyvall 1999).

Transmission

MCMV transmission occurs through insect vectors, mechanically, and by seed at very low rates (Jensen et al. 1991). MCMV is possibly also transmitted though infested soil, as the virus can survive in corn residue (Nyvall 1999). Continuous maize production in a field greatly increases the incidence of maize chlorotic mottle.

The following insect species can transmit MCMV (Nyvall 1999):

- Corn thrips (*Frankliniella williamsi*)
- Three species of corn rootworms (*Diabrotica*): the southern corn rootworm (*D. undecimpunctata*), the northern corn rootworm (*D. lonicornis*), and the western corn rootworm (*D. virgifera*)
- The corn flea beetle (*Chaetocnema pulicaria*)
- The flea beetle (*Systena frontalis*)
- The cereal leaf beetle (*Oulema melanopa*)

Corn thrips is the only widely distributed vector of MCMV in Hawai'i and is likely the primary vector. It transmits MCMV in a non-persistent manner. Although maize is the preferred host, corn thrips can survive on a number of plants, including cassava, beans, maize, sorghum, onions, various grasses, rice, peppers coriander, peas, and the weedy species *Bidens pilosa* and *Tithonia diversifolia* (Capinera 2008; ICPEI Thrips 2011; Frison and Feliu 1989; King and Saunders 1984).

Plant host range for MCMV

Maize is the only natural host reported for MCMV. Hosts that can be infected experimentally are limited to the grasses in the family *Poaceae* (Scheets 2004). Among these grasses, 73 plant species in 35 genera have been tested for susceptibility to virus strains MCMV-Kansas, MCMV-Peru, or both (Table 1).

Table 1. Plants tested for susceptibility to strains of MCMV (Scheets 2004).

Immune genera[1]	Susceptible genera	Genera with both immune and susceptible species
Axoponus	*Andropogon*	*Agropyron*
Chloris	*Avena*	*Bromus*
Elymus	*Bouteloua*	*Cenchrus*
Festuca	*Buchloe*	*Cynodon*
Lolium	*Calamovilfa*	*Dactylis*
Oryza	*Eleusine*	*Digitaria*
Paspalum	*Eragrostris*	*Echinochloa*
Poa	*Euchlaena*	*Panicum*
Saccharum	*Hordeum*	*Phalaris*
	Secale	*Setaria*
	Sorgastrum	*Zea*
	Sorghum	
	Spartina	
	Tripsacum	
	Triticum	

[1]Status of hosts listed in this table are a result of experimental inoculations, not natural field infection.

Hosts of MCMV found in Hawai'i include the following plant species (common names in parentheses) (Brunt et al. 2010):

- *Bromus mollis* (soft chess; soft brome)
- *Panicum dichotomiflorum* Michx. (fall panic grass; fall panicum)
- *Panicum maximum* Jacq. (guinea grass)
- *Panicum miliaceum* (proso; broomcorn millet)
- *Zea mays* (corn)

The host status (either natural or experimental) of other

Variation in host response to infection by MCMV among inbred corn lines growing at Waimanalo, Hawai'i. Symptoms range from severe mosaic and stunting (foreground) to virtually asymptomatic (background). These corn lines express varying degrees of resistance and susceptibility to the virus, suggesting that improved varieties can be developed through traditional breeding and selection.

Bromus spp. and *Panicum* spp. found in Hawai'i for the Hawaiian strain(s) of MCMV is unknown.

Disease symptoms

Disease symptoms vary in severity depending on plant age at the time of infection, environment, and maize variety or genotype (Scheets 2004). Symptoms of maize chlorotic mottle include the following:

- Leaf mosaic with fine, chlorotic, longitudinal yellow streaks parallel to leaf veins develops about 10 days after inoculation.
- Streaks may coalesce to create chlorotic mottling.
- Chlorotic mottling may be followed by leaf necrosis, stunting, and plant death.
- Ears are short, malformed, and partially filled, often with prematurely aged husks
- Yield reductions are possible with natural infections and range up to 60% with experimental infections (Scheets 2004).
- Male inflorescences (tassels) may be shortened.

Disease diagnosis and virus detection

In maize, MCMV has been detected in leaves, pollen, female and male inflorescences, ear husks, cotyledons, and seeds (pericarps, endosperm, cotyledons, and embryo) (Scheets 2004). The most reliable methods for detecting MCMV in host tissues include ELISA (enzyme-linked immunosorbent assay), Northern blots, and polymerase chain reactions (PCR) for detection of virus RNA. There are no reliable local lesion hosts. Maize chlorotic mottle may be difficult to diagnose based on symptoms alone, because some of its symptoms (stunting, chlorosis) may resemble those caused by nutrient deficiencies or maize mosaic. CTAHR can provide diagnostic inbreds with high susceptibility or high resistance for virus-resistance testing (www.ctahr.hawaii.edu/hfs).

Disease management

The most effective management of maize chlorotic mottle is through the integration of cultural practices with insecticides and host resistance. Superior resistance to MCMV is widely available in tropical corn seed stocks and provides the best control for this disease. Most temperate-climate varieties of field and sweet corn, however, are highly susceptible. Some locally bred sweet corn hybrids are intermediate in tolerance.

The following integrated practices are recommended to control maize chlorotic mottle:

- Plant maize lines resistant to MCMV.
- Apply insecticide sprays, weekly if necessary, to control thrips vectors.
- Control alternate weed hosts, especially grassy

weeds, to reduce populations of MCMV and corn thrips.
- Scout fields regularly and remove any symptomatic plants.
- Keep unnecessary people and machines out of the field to reduce mechanical transmission and spread of MCMV.

Deployment of host resistance

The most effective management strategy is to plant MCMV-resistant varieties. Many tropical inbreds and varieties were highly resistant to MCMV in 2011 trials. For example, 30 of 40 (75%) of UH-bred field-corn inbreds tested were resistant. Complete immunity, however, has not been observed. Sweet corn developed for the Hawai‘i market often shows some susceptibility, but the level of resistance is being increased through breeding. The severity of the disease depends on the cropping environment, or location. Therefore, the best approach is to employ the integrated management practices listed above, including the use of disease-resistant plants.

Future research needs

To manage maize chlorotic mottle disease more effectively in Hawai‘i, the following questions still need to be answered. What other grassy weeds are potential hosts of MCMV? Is more than one virus strain present in Hawai‘i, and how do strains differ? What genes are responsible for host resistance, and how can these genes be incorporated into commercial seed stock? How effectively can Hawaiian strains of MCMV be spread by seed? How does the environment in which corn is grown affect the severity of disease? Are thrips the only vector of MCMV in Hawai‘i? If so, can the disease be managed by controlling them? What is the best strategy for controlling thrips in Hawai‘i? Research is needed to address these questions in order to develop improved scientific understanding and better management of epidemics.

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Young corn plants infected with MCMV in Hawai‘i showing the typical symptoms of leaf mosaic.

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