VECTOR POSTERS

Biology of the recently introduced aster yellows phytoplasma and aster leafhopper (Hemiptera, Cicadellidae) in Hawaii

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Aster leafhopper (AL) (*Macrosteles* sp.) is the vector of Watercress Yellows Phytoplasma (WYP), a phloem-limited bacterium that causes disease in watercress in Hawaii. AL and WYP were both first found in Hawaii in 2001. Diseased watercress (*Nasturtium officinale*) has symptoms of reduced leaf size, leaf yellowing and crinkling, and occasionally witches' brooms. The threat of WYP causing disease in other susceptible crops is of concern to Hawaii's diversified agriculture industry. PCR-based tests using primers specific for phytoplasmas were positive for phytoplasma presence for all symptomatic plants, but not from healthy, non-symptomatic plants. Sequence analysis of cloned PCR products indicate that the watercress is infected with a phytoplasma nearly identical to the severe strain of western North American aster yellows (SAY) and to onion yellows (OY). Aster leafhopper adults collected from the field were also found to be positive for this bacterium. We studied this leafhopper's host range and its ability to vector WYP, using twenty-five different plant species, including crops of economic importance and native Hawaiian plants, as test plants for oviposition and adult survival experiments. We have found that this leafhopper breeds on watercress in commercial fields. We have also shown by confining field-collected insects on susceptible host plants that AL transmits WYP to watercress. Transmission experiments using *Plantago major* and *Lactuca sativa* as host plants also showed the capacity of this insect to vector WYP. The information on host plant range and WYP transmission will help to improve control strategies for this serious disease of watercress in Hawaii.

Use of immunofluorescence confocal laser scanning microscopy to study the distribution of plant viruses and pathogenic bacteria in vector insects and in host plants

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The distribution of maize mosaic virus (MMV, *Rhabdoviridae*) and of corn stunt spiroplasma (CSS, *Spiroplasma kunkelii,* Prokaryotes, Mollicutes) in their insect vectors was studied by immunofluorescence confocal laser scanning microscopy (iCLSM). MMV is transmitted in a propagative manner by the planthopper *Peregrinus maidis* (Hemiptera, Delphacidae), whereas CSS is similarly transmitted mainly by the leafhopper *Dalbulus maidis* (Hemiptera, Cicadellidae). Three preparation methods were used in conjunction with iCLSM on these vectors: dissected whole-mount organs, thick (6-8 µm) paraffin sections, and hemocyte smears. These preparations were incubated with primary antibodies (antisera to MMV or CSS), followed by the secondary fluorescent antibody Alexa Fluor 488 (green fluorescence), then treated with the nuclear stain propidium iodide (Ammar and Hogenhout, 2005; Ammar et al. 2005). The latter stained the nuclei red, which helped in the identification of the tissues observed and in the subcellular localization of the above pathogens.

Using iCLSM to localize MMV in its planthopper vector *P. maidis*, 2-4 wk post-exposure to MMV-diseased plants for 1 wk, MMV antigens/accumulations were found associated with the nuclei in almost all the vector tissues examined including the midgut, anterior diverticulum, Malpighian tubules, hemocytes, epidermis, muscles and fat tissues. Additionally, extensive perinuclear accumulations of MMV were found in tracheal cells and in cells of the brain and other nerve ganglia of this vector. In the principle salivary glands MMV accumulations were associated with the cell periphery in addition to the nuclei. Earlier studies by transmission electron microscopy (TEM) indicated that in maize leaf cells and in most of the vector tissues examined, virions of MMV mainly assemble by budding through nuclear membranes then accumulate in perinuclear space (Ammar and Nault, 1985; McDaniel et al., 1985). However, in secretory cells of the salivary glands, MMV virions were found to bud through the plasma membrane accumulating in intercellular and extracellular spaces, apparently facilitating release of virions into the salivary ductules and ducts, which is necessary for virus transmission by the vector (Ammar and Nault, 1985).

The three preparation methods mentioned above for iCLSM were also successful in studying the distribution of the bacterial mollicute CSS in its leafhopper vector *D. maidis*. CSS antigens/accumulations were detected in the midgut, filter chamber, Malpighian tubules, hindgut, fat and muscle tissues, hemocytes, tracheae and in several lobes of the salivary glands. However, CSS was not detected in nerve cells of the brain or other nerve ganglia of *D. maidis*. The percentage of leafhoppers with detected CSS in various organs/tissues 2-3 wk post-exposure to CSS-infected plants for 1 wk, was up to 95%, and 75% of these leafhoppers inoculated CSS into maize test seedlings prior to iCLSM processing.

To localize MMV or CSS in the plant host by iCLSM, paraffin sections as well as free-hand sections of fixed un-embedded leaves from infected maize were used. CSS accumulations were detected mainly in phloem tissue, whereas MMV accumulations were detected in epidermal, mesophyll, and phloem tissues of infected maize leaves, which is consistent with previous TEM studies of these two pathogens in maize plants (McDaniel at al., 1985; Nault and Bradfute, 1979).

Although TEM provides higher resolution for the localization of viruses, mollicutes or other pathogens in host plants and in insect vectors/hosts at the cellular and subcellular levels, iCLSM has the following advantages compared to TEM: a. both processing and examination of specimens are considerably faster; b. much larger and more numerous samples can be processed and examined; and c. the distribution of pathogens can be studied at the tissue and organ levels, or even in the entire insect through thick sectioning. Additionally, compared to epifluorescence microscopy, iCLSM provides three-dimensional images of the studied organs indicating their spatial relationships, which can be valuable for studying the routes of pathogens in their vectors.

Because of these advantages, iCLSM is being used at present to study the temporal movement of MMV and of CSS in tissues and organs of their insect vectors at various times post acquisition, which is necessary to determine the routes and transmission barriers for these pathogens in their vectors (Ammar, 1994; Hogenhout et al., 1993). Additionally, iCLSM can be used to study the localization of various pathogen associated/induced proteins in insect vectors or host plants, which may provide important clues to elucidate various aspects of pathogen-vector/host relationships.

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