PLANT VIRUSES AND INSECTS

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The principal families of insect vectors which cause the most damage to agricultural crops through the spread of plant diseases are in the order Hemiptera, and include the aphids, leafhoppers, delphacid planthoppers and whiteflies. Another important group of insect vectors found worldwide is the order Thysanoptera, the thrips. Other insects also spread plant diseases; however, aphids alone are responsible for spreading the majority of known plant viral diseases, followed closely by the leafhoppers, whiteflies, and thrips. The known number of plant disease vectors within these taxa is large, including Cicadellidae (leafhoppers, containing 49 known vector species), Aphididae (aphids, with the majority of 192 vector species), Aleyrodidae (whiteflies, with 3 vector species) and Thripidae (thrips, with 8 known vector species). Of course, these numbers change every year with the description and discovery of new viral diseases and new insect vectors. Furthermore, a group with only a few insect vectors still may be able to carry and spread a huge number of viral diseases to many different host plants, as occurs in the whiteflies and thrips.

In 1997, more than 380 viruses were known to be transmitted by these insect vectors; however, in the last five years the number has increased greatly (about 600 in 2001) and is increasing every year. This dramatic increase has been due in part to our ability to detect and characterize viral diseases more accurately, and also due to increased travel and trade between countries, which often result in the accidental introduction of either diseases or insects from one country into another.

Components involved in disease epidemiology

The epidemiology of plant diseases caused by insect-carried plant pathogens involves four main components: the pathogen, the insects, the plant and the environment. Thus, the transmission of a plant pathogen by an insect to a plant appears relatively simple. However, this situation is highly complex when one examines all the possible elements that can influence these interactions. The availability of the pathogen is affected by its quantity, location, and the strain within the plant. The insect's biology is influenced by population size, number of generations, longevity, dispersal patterns, feeding behavior and interactions with the pathogen. The plant's performance can be influenced by its level of susceptibility to the pathogen, multiple infections of different strains, and/or different pathogens, susceptibility to the insect, the location and stage of growth when exposed to the pathogen and insect. Environmental factors add another level of complexity as temperature, moisture, air currents, and cultural practices come into play. The discovery of a new plant pathogen that is carried and spread by an insect is usually the beginning of a long and difficult task toward understanding its epidemiology (all the elements that influence the development and spread of a plant disease).

Insect feeding mechanisms

The traits of morphology that contributes to the ability of these insects to transmit plant diseases so efficiently is their piercing-sucking feeding style. Insects in the order Hemiptera (aphids, leafhoppers, whiteflies), and Thysanoptera (thrips) have similar basic morphologies of the head and body. In the accompanying scanning electron micrograph you can see the compound eyes, and the proboscis of the insect. In Thrips this proboscis is referred to as a mouthcone due to the thick, short nature of the structure. The proboscis helps support the stylets as the insect works its stylets into the plant cells. The stylets are under muscular control so that they can be extended into the plant tissues. The stylets are each curved and are held against each other so that they go straight, one pushing and sliding against the other. However, when one stylet moves in front of the other the curve pushes the stylet in a lateral, sideways direction. Thus, the insect controls the direction in which it moves the stylets. Some leafhoppers feed in a manner whereby they will pierce into the plant tissues and then proceed to feed in a clockwise or counter-clockwise procession, emptying cells as they go, thus creating an emptied out ‘spot’ in the plant leaf, called a stiple. Others feed directly from the vascular tissues of plants, the phloem or xylem (Fig. 1).

All these insects have piercing-sucking mouthparts that allow them to feed on plants while causing minimal damage. This is important for virus transmission, as viruses require a living cell to reproduce. The insects use paired maxillary stylets to form a suction tube that is inserted into plant cells, similar to a flexible syringe needle. In the Hemiptera, these stylets form two canals, the food canal, and a smaller salivary canal where the saliva of the insect comes out during feeding. The Thysanoptera are unique in that thrips stylets form a single canal used for both sucking up plant fluids and to secrete saliva. The insect salivary secretions have several functions. There are at least two types of saliva, one is liquid and aids in the digestion of plant cells and cell debris so that they can be ingested, sucked up through the food canal. Another solidifies or hardens during feeding which functions to form a salivary sheath to help prevent leakage around the inserted stylets, and to hold the stylets firmly in place during feeding. The saliva also is thought to prevent or hinder the plant’s response to repair its damaged cells so the insect can continue feeding once it finds the desired location inside the plant (i.e., the phloem or xylem) (Fig. 2).

There are also mandibular stylets. These are paired, thicker stylets on the outside of the maxillary stylets. The aphids, leafhoppers and whiteflies all have symmetrical, paired, matching mandibular stylets which function to pierce the hard epidermis of plants and to assist attaching the insect firmly to the plant surface. Only the thrips have an asymmetrical morphology with one of the mandibular stylets being reduced or absent, and the remaining stylet being closed at the end, forming a needle-like structure, closed at the end. The thrips use the single, mandibular stylet to pierce a hole into the epidermis of the plant surface so that the slender, paired maxillary stylets can be inserted to feed in a piercing-sucking manner from plant cells that are deeper (Fig. 3).

By being able to feed in such a precise and direct manner, insects that feed in a piercing-sucking manner can avoid many of the plant's natural defenses. These insects also can deposit viruses directly into specific tissues from which they feed, such as into the vascular tissues of a plant. Once a virus has been introduced into the vascular tissues, it can spread rapidly throughout the plant to cause disease. Furthermore, piercing-sucking feeders cause less damage to the plant than a chewing insect, so plant cells that are infected with a virus often survive the feeding and support virus survival within the plant.

Inside the head there are several valves whereby the insects can stop the procession of food into their midgut. The plant sap is drawn up the food canal of the maxillary stylets. The food is then held in place by the precibarial valve where it is tasted by gustatory sensilla. The food is then drawn into the cibarium, the pumping chamber of the mouth. The cibarium also has gustatory sensilla for tasting and evaluating the quality of the food as the insect sucks up the plant sap. The food then passes the esophageal valve and enters the esophagus and passes through to the midgut which is the area of the alimentary tract where most nutrients are absorbed. In most plant sap-feeding insects, there is a region of the midgut where the hindgut coils around and is attached to it. This is the filter-chamber region of the insect's alimentary canal. Plant phloem and xylem, the liquids within plants' vascular tissues, are full of water; insects which feed on these as a primary food source have adapted over time the ability to shunt or direct excess water directly into the hindgut. This allows the insect to concentrate food and nutrients in the midgut for maximum absorption and to release excess water without having to process it through the midgut.

Tools to understand feeding (electronic monitoring of insect feeding)

Scientist have many methods to study insect/virus interactions. One such method is the invention of an electronic feeding monitor system, EMS, that allows someone to measure aspects of feeding as they occur. This is very important in studies where the amount of time an insect spends feeding needs to be measured. The EMS allows the scientist to know how many times an insect inserts its stylets into a plant, how long the insect fed, and if the insect was feeding from the phloem or xylem tissues within the plant. Being able to examine insect feeding so closely enables the early selection of crop varieties that may have resistance to insect feeding before they are planted in the field. Plant varieties which can disrupt insect feeding may be useful to prevent the transmission of some virus diseases. The EMS works by running a low voltage of electricity through a plant, usually by placing a copper electrode into the moist soil of a potted plant. The insect then has a fine gold wire glued onto its back, using electrical-conductive paint, so that the electricity will pass up through the plant, and then through the insect when it inserts its stylets into the plant. When the insect either salivates out, or ingests plant fluids up the food canal, the electricity passes through the insect, which acts as a variable resistor, and goes back into the EMS, which then amplifies the signal so that it can be recorded.

Insect vector-plant pathogen interactions

There have been two systems of terminology established to describe the association and transmission of plant diseases by insect vectors which feed in a piercing-sucking manner. One is based on how long the virus persists in the insect vector, and the second is based on the route of virus movement through the insect vector. They can be combined as follows: 1) the non-persistently transmitted, stylet-borne viruses; 2) the semi-persistently transmitted, foregut-borne viruses; 3) the persistently transmitted, circulative viruses; and 4) the persistently transmitted, propagative viruses. Using this terminology, virus ‘transmission’ is referred to as ‘non-persistent,’ ‘semi-persistent,’ or ‘persistent’ (Fig. 4).

The way a virus moves through the insect vector then is described by the terms: ‘circulative’ or ‘propagative.’ Circulative viruses pass into the insect hemolymph and circulate through the insects before being salivated back out during feeding. This involves the ability of the virus to pass several barriers within the insect, passing through the midgut membranes, and then the salivary gland membranes, to be able to be released back out with the saliva. These types of viruses do not replicate inside their insect vectors but merely pass through the insect. Viruses that reproduce inside the insect are considered propagative. Propagative viruses are able to enter the insect hemolymph but they also replicate once they infect an insect. As one would expect, a virus that is circulative is retained in the insect for a longer period of time than a virus that is non-circulative and merely stuck to the insects’ stylets (stylet-borne) or foregut (foregut-borne virus). Viruses that are propagative (replicating in the insect) are retained for the life of the insect.

Non-persistently transmitted, stylet-borne viruses are transmitted into the plant during short durations of feeding. Virus acquisition (the ingestion of a virus that results in the insect’s ability to transmit the virus to a plant), is brief, often just a few seconds of feeding. There is no latent period (the time that passes between when the virus is acquired and when it can be transmitted to a plant). Since these types of viruses usually are binding to the insect’s stylets for only a brief period of time, the insect does not retain the ability to transmit the virus for long periods. Usually, virus transmissibility is lost after a few minutes of feeding on a non-infected plant. Aphids transmit the majority of non-persistently transmitted viruses. The ability of viruses to bind to the insect’s stylets is aided by a helper component (a virus encoded, non-structural protein produced only in infected plants). During subsequent periods of feeding the virus is released, or washed from the stylets, thus depositing virus into the plant tissues.

Semi-persistently transmitted, foregut-born viruses are transmitted into the plant during longer durations of feeding (minutes). Virus acquisition increases with increased time spent feeding (minutes to hours), and the virus stays in association with the insect for several hours, being able to be transmitted into other plants. The virus is thought to be binding in the anterior areas of the alimentary tract, along the stylets to the foregut, and a few virus particles are released during each act of feeding. There is no latent period, the virus does not replicate, and the insect will lose the ability to transmit the virus during its life.

Persistently transmitted, circulative viruses do not replicate in the insect vector. These types of viruses are acquired and transmitted during long periods of feeding (minutes to hours), and there is a latent period of hours to days before the virus can be transmitted to another plant. This makes sense as the virus must move through the insect body and get into the salivary glands to be salivated back out before transmission can occur. Virus retention is long, but is dependent upon the amount of virus acquired into the insect body, and may last for the life of the insect, usually around 30 days.

Persistently transmitted, propagative viruses do replicate inside the insect. Virus acquisition time takes hours to days of feeding. The latent period can take weeks before an insect can transmit virus. The virus is retained for the life of the insect and often the virus is passed to the eggs (transovarial transmission).

Some insect-transmitted viral plant diseases

Aphids - Of all known aphids, about 250 are considered serious pests. They are pests because of their feeding, which reduces the vitality of the crops they feed on, but primarily due to the transmission of viral plant diseases. Perhaps the most important aphid pest is *Myzus persicae*, often referred to as the green peach aphid. *M. persicae* is a green or slightly reddish aphid which has peach as its primary host and a wide range of secondary hosts, including many brassicas. *M. persicae* is cosmopolitan in temperate climates occurring in the U.S.A., and a fair portion of Europe including the United Kingdom. Though it seldom occurs in numbers large enough to cause direct damage from feeding pressure, it is capable of transmitting and spreading over 100 viruses including the potato leaf roll, potato virus Y, yellow net and yellows viruses of sugar beet, cauliflower mosaic, plum pox, cucumber mosaic, lettuce mosaic, and turnip mosaic virus.

The pea aphid, *Acyrthosiphum pisum*, is a large green aphid with long antennae and legs. The pea aphid is found on many leguminous plants and transmits Lucerne mosaic virus, pea leaf-roll virus, pea enation mosaic virus and pea mosaic virus in the United Kingdom, and pea enation mosaic virus in the U.S.A. The cabbage aphid, *Brevicoryne brassicae*, is a serious pest of the major cabbage crops, cabbages, cauliflowers and Brussels sprouts. The main cause of its pest status is the transmission of cauliflower mosaic and turnip mosaic virus. The brown citrus aphid, *Toxoptera citricida*, is a dark, black, aphid that is the main vector of citrus tristeza virus in the subtropics and tropics. The melon aphid or cotton aphid, *Aphis gossypii*, also is an important aphid vector of viral diseases in citrus and on many other agricultural crops.

The control of aphid pests currently still involves large amounts of pesticides in most countries, but other more ecologically friendly methods have been used in other places for some time. These generally involve biological control, mostly the use of Hymenopteran parasitoids. These are small wasps that lay their eggs inside the aphids. Other methods include plant improvement, and monitoring aphid dispersal to predict when a pre-emptive spraying in smaller amounts might be effective. The most important element of insect pest control for all of us is education. Farmers as well as the general public need to become better informed as to the alternatives to, and proper uses of, insecticides.

Leafhoppers - A large group of plant viruses, the plant rhabdovirus group, consists of more than 70 members. They are transmitted by aphids, leafhoppers, planthoppers, lacebugs, and mites. These viruses infect and replicate in the insect cells, but each virus is specific to its insect vector. Some of them also can be transmitted mechanically, through artificial means using plant sap from infected plants. Another important leafhopper-transmitted virus is maize chlorotic dwarf virus (MCDV). This virus is a semi-persistently transmitted, foregut-borne virus, and is restricted to the phloem of maize. Transmission of this virus requires a protein that is produced by virus-infected plants. This protein, called the helper component (HC), is suspected to bind to receptor-like structures in the food canal of leafhoppers, thereby forming a matrix to which virus particles attach. Viruses are then slowly released from this matrix during feeding and, consequently, are transmitted to other plants when leafhoppers fly to neighboring plants and then feed. The insect vector of maize chlorotic dwarf virus is the leafhopper, *Graminella nigrifons*.

The beet leafhopper (*Circulifer tenellus*) is one of the most important insect pests of sugarbeets in the western United States because it is the vector of beet curly top virus, BCTV. Curly top virus is a severely devastating plant virus that affects more than 300 broad-leaved plants. Tomato, bean, squash, cucumber, melon, spinach, table beet, pepper, and some flowering plants are the most common cultivated plants affected in the western United States. Leafhopper populations survive on weeds and cultivated plants infected with curly top virus which serve as reservoirs for both the insect and virus. Leafhoppers are able to acquire the virus during very short feeding times. The leafhopper retains the ability to transmit BCTV for a month or more after acquisition.

Whiteflies - In the past decade, whiteflies as pests and vectors of plant viruses have become one of the most serious crop protection problems in the tropics and subtropics. Yearly losses are estimated in the hundreds of millions of dollars. Several species of whitefly cause crop losses through direct feeding, while others are important in virus transmission. *Bemisia tabaci*, for example, is the vector of African cassava mosaic, bean golden mosaic, bean dwarf mosaic, bean calico mosaic, tomato yellow leaf-curl, tomato mottle, and other *Begomoviruses* in the family *Geminiviridae*, affecting crops worldwide.

With the spread of an especially aggressive biotype of *B. tabaci* into the New World tropics (*B. argentifolii*), crop losses likely will continue to increase, resulting in higher pesticide use on tomatoes, beans, cassava, cotton, cucurbits, potatoes, sweet potatoes and other crops. There is an urgent need to develop integrated pest management systems aimed at reducing insecticide use and which will help re-establish the ecological equilibrium of predators, parasitoids, and microbial controls. Needed are crop varieties with resistance to the whiteflies, and/or to the whitefly-transmitted viral diseases.

This problem is manifested in the fact that whiteflies and the viruses they carry can potentially infect many different host plants, including agricultural crops and weeds. A pest problem on one crop, such as beans, cannot be tackled as a single problem, as neighboring crops or weeds also may be affecting the disease spread. The different viruses and forms of the whitefly also are difficult to identify, and/or separate on the basis of symptoms or morphology. Determining where the problems in food crops are coming from becomes almost impossible. Proper diagnosis of the problem depends on using sophisticated molecular techniques to characterize the viruses and whitefly vectors, followed by epidemiological work, usually based on dynamic modeling, to understand the incidence of disease spread.

Thrips - Thrips species in the genus *Frankliniella* are commonly referred to as flower thrips. The western flower thrips, *F. occidentalis*, has a worldwide distribution and is considered the primary vector of tospoviruses. Thrips feed on over 600 different plants and crops, especially on flowering plants where they also feed on pollen. Many thrips are pests of commercial crops due to their damage to flowers. Also, their feeding causes stunting, deformed and unmarketable fruits and vegetables.

Thrips in the genera *Frankliniella* sp. and *Thrips* sp. also spread plant diseases through the transmission of viruses such as tospoviruses. Tomato spotted wilt virus is the type member of the genus *Tospovirus* in the family *Bunyaviridae*. These enveloped viruses are considered among the most damaging of emerging plant pathogens around the world. Virus members also include the impatiens necrotic spot viruses, which infect many ornamental plants. Tospoviruses can kill plants or reduce yields of marketable fruits and vegetables (i.e., lettuce, tomato, peanut, watermelon and ornamental crops). To transmit tospoviruses, thrips must acquire the virus during the larval stage. Most thrips species over-winter as either adults or as pupae. A typical flower thrips generation time varies from between 7 and 22 days depending on the temperature. The eggs are about 0.2 mm long and reniform (kidney shaped); they take on average three days to hatch. Thrips have two larval stages, then go through a prepupal and a pupal stage. Adults take between one and four days to reach sexual maturity. The females of the suborder Terebrantia are equipped with an ovipositor which they use to cut slits into plant tissue into which they insert their eggs one per slit, while females of the suborder Tubulifera, which lack an ovipositor, lay their eggs on the outside surface of plants, either singly or in small groups.

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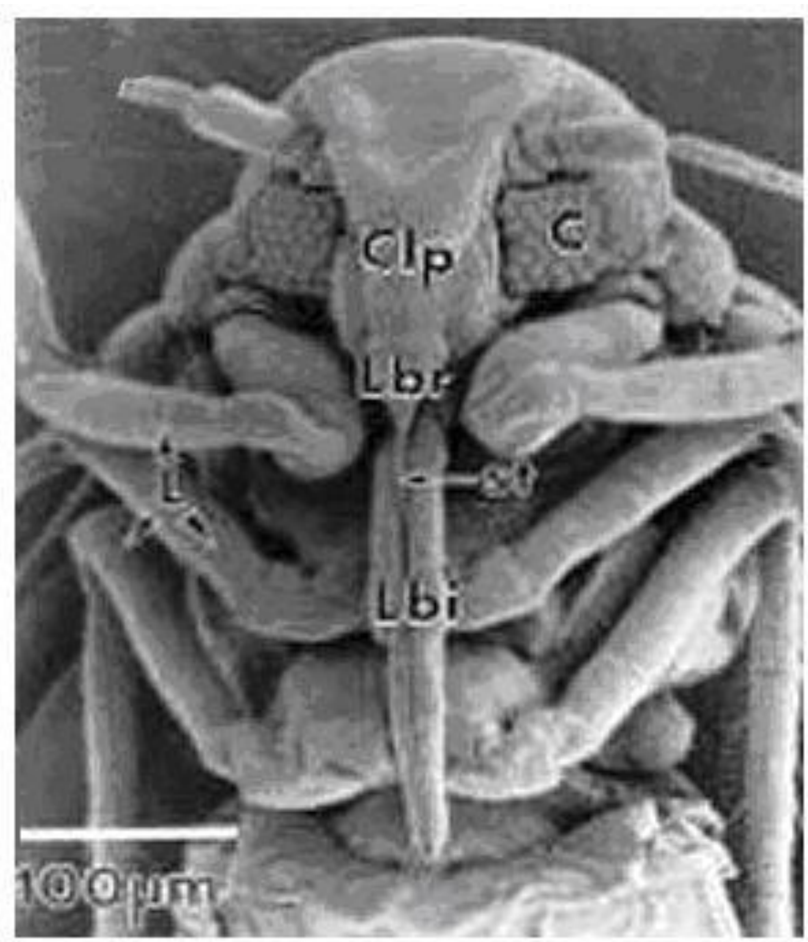

Figure 1. Scanning electron micrograph (SEM) of the whitefly, *Bemisia tabaci*, showing the ventral surface. Bod parts are as follows: C, compound eye; Clp, clypeus; Lbi, labium; Lbr, labrum; L, legs; St, stylet bundle.

Figure 2. Cross section showing stylets of a whitefly, with separate food and salivary canals: Dc, dendritic canal; Fc, food canal; Md, mandibular stylet; Mx, maxillary stylet; Sc, salivary canal.

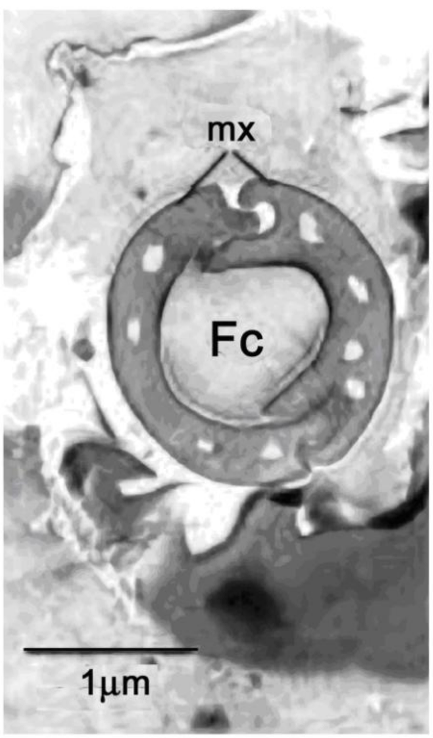

Figure 3. Cross section showing stylets of a thrips, with single canal used for salivation and food intake: Fc, food canal; Mx, maxillary stylet.

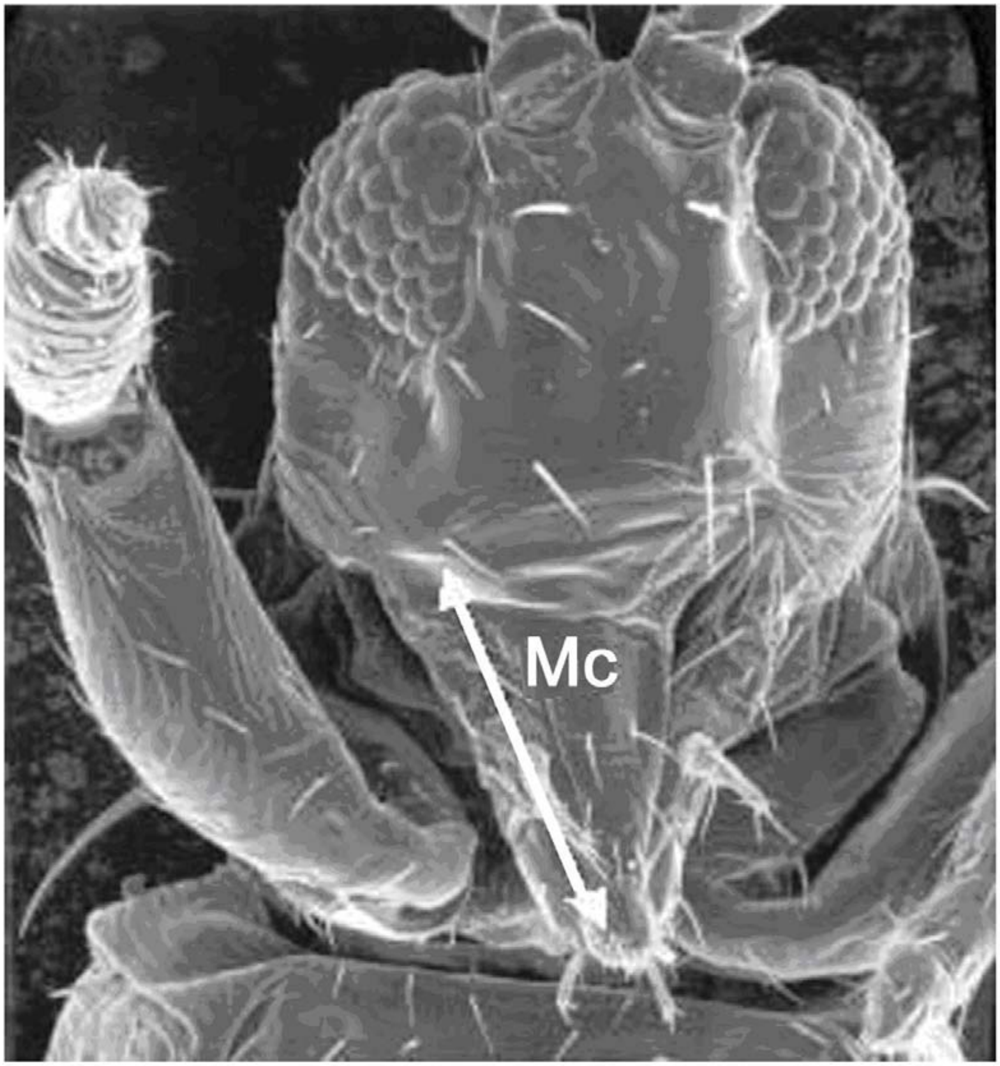

Figure 4. Scanning electron micrograph of a thrips showing the face and mouthparts: Mc, mouthcone.