Chapter A11

Reducing Virus Associated Crop Loss Through Resistance to Insect Vectors

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Introduction

The development of sustainable, environmentally-benign methods of crop protection is an important priority in agricultural research. A variety of insects attack crops, causing damage and reducing yields and crop quality. Insects cause crop loss directly through feeding on leaves, flowers, fruit or seed. A subset of insects damages crops indirectly, through transmission of plant viruses, resulting in reduced yield and crop quality. Breeding for disease resistance has been an important strategy for protection of crops against fungal, bacterial or viral diseases; however, resistances have not yet been identified or transferred for many major diseases. Although integrated pest management (IPM) strategies have been implemented with noted success, insect control has more often relied on the use of pesticides, leading

G. Loebenstein and J. P. Carr (eds.), Natural Resistance Mechanisms of Plants to Viruses, 241-260.

to the evolution of pesticide-resistant insects and to increasing health and environmental concerns. The development of pest resistant plants is an attractive alternative strategy for the control of insects and the direct damage they cause. For a target pest that is also the vector of a plant pathogenic virus, the question arises as to whether an effective insect resistance could also serve as a component in an integrated control strategy for insect vectored viruses.

Use of insect control to reduce losses due to viral disease is not a new concept. There are several instances in which crops are sprayed with pesticides for protection from vectors and the viruses they transmit. Similarly, systemic insecticides such as Imidocloprid are applied to the root zone with irrigation water to reduce vector populations. While effective in controlling insect populations, both methods have met with varying degrees of success in reducing viral infection (Perring et al. 1999). Another method used for vector control is the application of insecticide to non-crop plants that harbor virus and/or vectors, to reduce vector populations before they have an opportunity to transmit viruses to nearby crops. In California, insecticide sprays targeting weeds have been used since the mid-20th century to control *Beet curly top virus* (BCTV; Genus *Curtovirus*). The insecticide applications are directed at the overwintering breeding hosts (annual and perennial weeds) of the beet leafhopper (*Circulifer tennellus*) to decrease the spring populations of the vector (Cook, 1943). Growers pay over $1.25 million annually for spraying 80,000-200,000 acres of uncultivated land on the west side of the San Joaquin Valley with insecticide (Clark, 1995). Although it is somewhat difficult to measure the efficacy of the insecticide treatments, this control measure is thought to work well in certain years and locations, and be inadequate in others (Cook, 1943; Morrison, 1969). The use of insect resistant plants for reduction of losses due to viral disease would be a logical extension of these existing strategies. This chapter examines the biological features of the interactions among virus, vector and host that would determine the potential success of using insect resistance as a component of an integrated control strategy for insect transmitted viruses.

Goals of insect control vs. control of viral diseases

A review of the practical concerns for crop protection is necessary before considering how the interactions of virus, vector, and host plant could impact the efficacy of crop protection strategies. There are fundamental similarities and differences between protecting crops against economic loss caused by the direct attack of pests vs. that caused by viral disease. In either case, the concern is to

minimize the economic loss, not to achieve the absence of the pests or the virus in the field, although absence of the pest/virus could engender the least damage. The differences between losses based on direct pest damage vs. viral disease are based on the probable thresholds for economic losses in these two cases. It is possible to sustain some direct pest damage yet suffer little economic loss, provided that the damage does not seriously impact the yield or quality of the crop. For example, foliar pests such as leafminer can cause foliar damage in tomato, but economic damage could be minimal if the leafminer populations are low enough to avoid significant defoliation. Greater economic loss would result if the pest damaged the harvested portion of the plant, resulting in lower acceptable threshold levels for such pests. There is a greater opportunity for limiting direct damage after initial infestation with insects than indirect damage due to viral infection. If insect pest levels rise above acceptable thresholds during a growing season, it is possible to reduce pest levels by deployment of control strategies to prevent or limit economic loss. In contrast, a plant can become infected by a virus after it is visited by as few as one viruliferous vector. If the plant is not resistant to the virus, the virus spreads throughout the plant, causing damage ranging from minimal to complete economic loss. In addition, the presence of infected plants in a field increases the risk of infection and damage to other plants at that location, since the infected plants provide a local source of virus. Therefore, the tolerance for the presence of some level of the insect could be much higher if the goal is control of direct damage caused by the pest rather than the reduction of infection by a viral pathogen vectored by the insect.

Viruses transmitted by insect vectors and the nature of virus transmission

Most insect transmitted viruses are vectored by insects with piercing-sucking mouthparts such as aphids, whiteflies, leafhoppers, or thrips. We will chiefly focus on these four major vectors, although some plant viruses are also transmitted by beetles, mites or other types of chewing insects. One characteristic common to insects with piercing-sucking mouthparts is the use of stylets (hollow tube-like structures that can puncture cell walls) for feeding (Pollard, 1977; Backus, 1985; Hunter and Ullman, 1992). Vector transmitted viruses have a specific association with the vector insect that is required for efficient transmission. Some types of viruses associate with the stylet itself, while others associate with other areas of the insect digestive tract, including the foregut, midgut and hindgut. Others allow the virus to pass into the hemocoel (body cavity) where it circulates in the hemolymph (the equivalent

of blood in the insect) and can pass to other parts of the insect body (for reviews see Gray and Banerjee, 1999; Ng and Perry, 2004).

The specific relationship between vector and virus that determines transmission is a complex relationship involving not only the virus and vector, but also host plant and environmental influences. In addition, the nature of virus acquisition and association with the vector, other actions including landing and probing the food source, as well as feeding patterns may influence efficiency of virus transmission. Acquisition period refers to the time necessary for a vector to obtain virus from an infected plant during feeding. Transmission period refers to the length of time following acquisition during which a vector remains capable of transmitting the virus to a new host. Viruses transmitted by biological vectors are classified as nonpersistent, semipersistent, or persistent based on the nature of the transmission event. Nonpersistent viruses are acquired rapidly by vectors as the insects probe different plants with their stylets while seeking suitable food sources (known as test probing), or during the feeding process itself. Nonpersistent viruses remain associated with insect mouthparts, and can be transmitted for only a few minutes to a few hours (Gray and Banerjee, 1999; Pirone and Perry, 2002). In stark contrast, persistent viruses, once acquired by the vector, are usually retained for the life of the insect. Acquisition and transmission periods are much longer for persistent viruses, ranging from a few hours to several days, and often involve lengthy latent periods during which the virus cannot be transmitted. Between these two extremes are the semipersistent viruses. These viruses are also acquired quickly by vectors, but unlike nonpersistent viruses, semipersistent viruses are generally retained by the vector for periods of days to a few weeks.

Nonpersistent and semipersistent viruses have been shown to be specifically associated with the epicuticular lining of insect mouthparts, specifically the stylet or foregut. This lining is shed when the insect molts, and any virus associated with it is lost at that time (Ammar et al. 1994; Gray and Banerjee, 1999; Martin et al. 1997; Wang et al. 1996a). Nonpersistent and semipersistent viruses, which associate with insect mouthparts and do not cross membranes within the vector, are known collectively as noncirculative viruses.

Persistent viruses require virus particles to be fully ingested by the insect and transported to the insect hemocoel and ultimately into the salivary glands from which they can be transmitted to new plants during feeding (Gray and Banerjee, 1999). This type of transmission is referred to as circulative, because the virus must circulate through the body of the insect. Circulative transmission requires movement across cell membranes within the vector.

There are two types of circulative viruses; those that simply move through the body of an insect, and those that actually replicate inside the insect. Those that do not replicate in the insect vector are known as circulative nonpropagative. Those that replicate in the vector are known as circulative propagative viruses.

Some viruses are transmitted by chewing insects, such as beetles and eriophyid mites. Beetle-transmitted viruses are generally believed to be transmitted through regurgitant. Virus is acquired during insect feeding, and is transmitted to new plants through regurgitant produced by viruliferous beetles. Some beetle-transmitted viruses, like persistent circulative viruses transmitted by piercing-sucking insects, can be transmitted for very long periods of time. In some, but clearly not all cases, virus becomes circulative in the hemolymph of the vector. *Southern bean mosaic virus* (SBMV; genus *Sobemovirus*) is not circulative in the Mexican bean beetle, but is circulative in two other beetle species (Wang et al. 1992). Other viruses, such as *Bean pod mottle virus* (BPMV; genus *Comovirus*) have long transmission periods, however, BPMV is not detected in the hemolymph of the bean leaf beetle vector (Wang et al. 1992). This suggests that circulation of virus may not be critical for beetle transmission, at least for some beetle transmitted viruses.

Mite transmitted viruses include *Wheat streak mosaic virus* and numerous other viruses in the genera *Rymovirus* and *Tritimovirus* (family *Potyviridae*). While mechanisms of mite transmission are not well known, indications are that these viruses, like aphid transmitted potyviruses, can in some cases be acquired with very short feeding periods of a few hours (Thresh, 1971).

Dynamics of vector feeding and effect on transmission

The mechanics of virus transmission differ dramatically between circulative and noncirculative viruses, and within these, between nonpersistent and semipersistent (all noncirculative), and between persistent viruses (circulative-propagative and circulative-nonpropagative). Nonpersistent viruses are associated with the stylets of the vector and are retained for only a few hours. These stylet-borne viruses are acquired rapidly by their vectors, predominantly aphids, and are readily lost during feeding or probing. Interestingly, nonpersistent viruses are transmitted most efficiently when acquisition feeding periods are short. Transmission efficiency decreases with prolonged acquisition feeding, suggesting that bound virus may be easily dislodged during extended feeding, and cannot be reacquired immediately (Gray and Banerjee, 1999). Many insect vectors conduct test

probes on different tissues to identify desirable feeding sites. Test probing is likely the predominant means by which most nonpersistent viruses are transmitted. Although a number of differing theories exist on how transmission of nonpersistent viruses occurs, the process is clearly a specific relationship involving interactions between one or more virus proteins and proteins or other factors associated with the cuticular lining of the stylets (Pirone and Blanc, 1996). Some of the best-known examples of the nonpersistent viruses are the members of the *Potyviridae*, including *Potato virus Y*, *Tobacco etch virus, Turnip mosaic virus* and others.

Semipersistent viruses are generally associated with the insect foregut, rather than stylets. These viruses are usually retained for periods ranging from a few hours to several days (Perring et al. 1999). Efficiency of transmission increases with longer acquisition feeding periods. This suggests that unlike nonpersistent viruses, semipersistent viruses can continue to accumulate until all binding sites become saturated (Gray and Banerjee, 1999). Examples of semipersistent viruses are found in the *Caulimoviridae*, *Closteroviridae* and other virus families.

Transmission of persistent circulative viruses and circulative propagative viruses involves movement of virus across cell membranes within the digestive tract of the insect. Following ingestion, virus is actively taken up by epithelial cells of the midgut or hindgut of the insect, and is translocated across the gut membrane to the hemocoel. The virus moves through the hemocoel, and sometimes other tissues, ultimately reaching the salivary glands from which it is secreted with saliva and transmitted to new plants through probing or feeding (Gray and Banerjee, 1999). Circulative nonpropagative viruses are found in the *Luteoviridae* and *Geminiviridae*. During whitefly feeding these viruses are ingested by the vector and become circulative in the hemocoel of the whitefly vector prior to transmission. Once acquired, circulative nonpropagative viruses can be transmitted for extended periods ranging from weeks to the life of the insect (Gray and Banerjee, 1999).

Circulative propagative viruses are similar in many respects to circulative nonpropagative viruses, but differ in that propagative viruses can replicate inside the vector. Circulative propagative viruses are found in a number of families, but can be represented by *Tomato spotted wilt virus* (TSWV; genus *Tospovirus)*. TSWV is transmitted by both larval and adult thrips of numerous *Frankliniella* and *Thrips* species (Nagata and Peters, 2001), although plant-to-plant spread occurs by adult transmission. Acquisition of sufficient quantities of virus for transmission was as short as 5 minutes, with maximum efficiency by 21 hours, although the mean was 1 hour (Wijkamp et

al., 1996; Nagata and Peters, 2001). Similarly, inoculation access periods of 5 minutes resulted in 6% transmission to *Petunia hybrida*, and 17% to *Datura stramonium* (Nagata and Peters, 2001). Consequently, any method that would be effective in controlling transmission of TSWV or other circulative propagative viruses would need to essentially prevent feeding altogether.

Under what circumstances could vector control effectively reduce virus transmission?

It is clear that insect-transmitted viruses are extremely variable with regard to the many factors associated with transmission. Clearly many could not be controlled effectively by efforts at reducing vector feeding or vector numbers. This may not be universally true, however, and numerous examples exist to support this possibility. It is true that the best form of resistance is against the virus itself, since this will not only prevent damage to the crop exhibiting the resistance, but will also reduce the pool of available virus, thus reducing spread to additional crops. In many cases, however, resistance to virus infection is not available, or is not easily incorporated into commercial varieties. This can result from interspecific sexual barriers between the crop species and the wild relative that is the source of the resistance, the multigenic nature of the resistance trait, or association of the resistance trait or gene(s) with deleterious effects. Chemical control of vectors, while reducing populations, is becoming less desirable through efforts to use more environmentally friendly production methods. While virus control based on reducing vector population or feeding may not be a universal solution to all virus problems, it may be a valuable and effective tool for many. Review of the application, to date, of strategies to control the damage caused by plant viruses through genetic control of vectors has indicated a steady increase in interest for this type of control, ranging from as few as eight cases in 1976 (Kennedy, 1976) to over 20 in 1987 (Jones, 1987, 1998).

The factors that will determine efficacy of vector control for control of plant viruses are many and varied. Of paramount importance is the mode of transmission. Nonpersistent viruses are unlikely to be controlled through any type of vector management that allows significant levels of probing or feeding on the tissue. Additionally, controls that will ultimately kill the insect over a period of time will also be ineffective, as nonpersistent viruses

can be transmitted quickly by test probing in a matter of seconds (Perring et al., 1999).

Control of persistent circulative viruses through methods that reduce or prevent vector feeding may offer more potential, however, effectiveness will also be influenced by the nature of transmission. Circulative viruses, once acquired, move throughout the body of the insect. Consequently, ingestion will lead to uptake, and sequential ingestion will likely lead to more and more virus accumulation in the vector. The begomovirus, *Tomato yellow leaf curl virus* (TYLCV; family *Geminiviridae*) is transmitted by the silverleaf whitefly, *Bemisia tabaci* biotype B. The virus can be acquired by individual whiteflies with acquisition access periods and inoculation access periods as short as 5 minutes each (Atzmon et al. 1998; Czosnek et al. 2001), although efficiency of virus acquisition improves with longer feeding periods. Czosnek et al. (2001) also demonstrated that all individual whiteflies were able to transmit with inoculation access periods of 30 minutes. TYLCV can be acquired and transmitted with very short feeding periods on susceptible host plants, yet can be retained by the vector for long periods. Similar results are found with other members of the *Geminiviridae* as well (Duffus, 1987). Since the virus only needs to be ingested, it is simply a matter of sufficient virus being acquired for some of it to progress through the insect and reach the salivary glands in an infectious state.

One of the more promising virus genera for which vector based control may be effective is the genus *Crinivirus* (family *Closteroviridae*). These semipersistent viruses require longer feeding periods for efficient virus acquisition and transmission than many other plant viruses (Wisler and Duffus, 2001). In addition, efficient transmission of criniviruses usually requires several whiteflies feeding for extended periods. For example, *Beet pseudo yellows virus* (BPYV) can be transmitted with 10 percent efficiency by individual viruliferous greenhouse whiteflies (*Trialeurodes vaporariorum*), and *Cucurbit yellow stunting disorder virus* (CYSDV) can be transmitted with 3 percent efficiency by individual silverleaf whiteflies (*B. tabaci*, biotype B also known as *B. argentifolii*). These viruses can both be transmitted with approximately 85% percent efficiency when 40 and 60 vector whiteflies are used in single plant transmissions of BPYV and CYSDV, respectively. Consequently, limiting the amount of feeding by whitefly vectors can in some instances dramatically reduce the rate of plant infection by these criniviruses, although it is not known how universal this is among semipersistent viruses in general.

Studies by Wisler and Duffus (2001) compared numerous factors associated with vector acquisition and transmission among eight crinivirus

species and four vector species in two whitefly genera. Results were widely variable. Most criniviruses were transmitted by a single genus or in some cases a single species of whitefly (Wisler and Duffus, 2001). *Lettuce infectious yellows virus* is transmitted with high efficiency by the sweet potato whitefly (*Bemisia tabaci* biotype A), but with very low efficiency by the silverleaf whitefly. One crinivirus, *Tomato chlorosis virus* (ToCV), is the only known virus to be transmitted by 4 different species of whitefly in two different genera (Wisler et al. 1998). Interestingly, there were clear differences in ToCV transmission efficiency between each of the vector species. *B. tabaci* biotype B transmitted ToCV most efficiently, followed by *T. abutilonea*, *B. tabaci* biotype A, and *T. vaporariorum* in order of decreasing efficiency (Wisler and Duffus, 2001). This variability in transmission characteristics among virus species must be considered when evaluating the potential of vector-based reduction of virus infection.

Criniviruses can be vectored by whiteflies in both *Bemisia* and *Trialeurodes* genera (Wisler et al. 1998; Wintermantel, 2004). The specific relationship between virus and vector differs for each virus-vector combination with respect to acquisition period, transmission period and virus retention time in the vector. While ToCV was only retained by *B. tabaci* biotype B for 24 hours, *Cucurbit yellow stunting disorder virus* was retained for up to 9 days in the same vector (Wisler and Duffus, 2001). Most criniviruses also have extensive latent periods in their hosts ranging from three to five weeks after transmission before disease symptoms become apparent on plants. It is clear from comparisons even within the genus *Crinivirus* that a number of semipersistent viruses exhibit vastly different traits with regard to insect transmission. In spite of this, semipersistent viruses overall are probably better suited for vector-mediated control than many other types of viruses, simply by the nature of transmission.

Insect resistance mechanisms in plants

Host/insect interactions for plant protection were originally classified as being due to antibiosis, non-preference, or tolerance (Painter, 1958; Beck, 1965), although the term “antixenosis” was suggested as a more accurate term than non-preference (Kogan and Ortman, 1978). Under antibiosis a resistant plant exerts an adverse effect on the growth and survival of the insect. Antibiosis can be due to physical characteristics of the plant or due to secondary metabolites such as toxins. Under antixenosis (non-preference), a plant exerts influences on insect behavior, deterring the insect from using the plant as a host (Painter, 1958; Beck, 1965), hence the use of the term “deterrence” in some references. “Tolerance”

indicates that the pest is neither deterred from the host plant nor adversely affected by the host plant, but the damage resulting from the pest infestation is reduced compared to that suffered by susceptible varieties of the crop (Painter, 1958; Beck, 1965; Reese et al., 1994). These systems of insect resistance may not be mutually exclusive. It is possible that a resistance mechanism could have aspects of both antibiosis and deterrence.

Breeding for insect resistance has a long history, although insect resistance has been used less than disease resistance in most crops. The wheat variety "Underhill" was reported to have Hessian fly resistance in 1782. Despite resistance breakdown over the years in a number of Hessian fly resistance sources, many wheat varieties have been bred to include this trait (Panda and Khush, 1995; Everson and Gallun, 1980). Another historical example is grape phylloxera (*Daktulosphaira vitifoliae*), a North American aphid that was inadvertently transferred to France *ca.* 1860. Grape phylloxera feeds on grape roots, resulting in decreased productivity and vine death. Wild North American grape possessed natural resistance to the pest. This resistance was transferred to develop phylloxera resistant rootstocks that saved the French wine industry. Rootstocks with similar resistance are still in use (Granett et al., 2001).

There are too many examples of pest resistances and mechanisms to cover in this chapter but some examples can be cited to illustrate the differences in mechanisms and their potential utility. Some systems of natural insect resistance are based upon physical structures or characteristics. A resistance to potato leafhopper (*Empoasca fabae)* in bean (*Phaseolus vulgaris*) is due to a high density of hooked nonglandular trichomes. These trichomes act as physical barriers, entrapping nymphs as their hooks became imbedded in the nymphs' bodies (Pillemer and Tingey, 1976, 1978). The waxy surface of plants has also been implicated in reducing insect infestation. "Glossy" mutants, lacking the normal waxy layer or "bloom" of non-mutant plants, have been found in a number of crop species. Sadasivan and Thayumanavan (2003) list instances in *Brassica,* raspberry, castor, sorghum, wheat, sugarcane, and onion in which the glossy plants are more susceptible to a variety of insect pests than the normal waxy plants. This could be due to adverse effects of the waxy layer on the ability of insects to adhere, move, or feed on the plant. Differences in wax layer may also affect the choice of the plant as for feeding or oviposition. Consequently such waxy surfaces may confer either antibiosis or antixenosis depending on their mode of action against different pests.

A number of insect resistance systems are based upon secondary metabolites that are toxic or otherwise detrimental or noxious to pests. Secondary

metabolites are a very diverse array of compounds that are produced by plants but which are not considered essential for basic metabolic function or processes. There are too many secondary metabolites to describe in any detail here (see Hadacek, 2002; Singer et al. 2003; Sadasivan and Thayumanavan, 2003), but a few well-known examples are 2-tridecanone, cucurbitacins, and glycoalkaloids.

The 2-tridecanone, a methyl ketone, is a secondary metabolite in glandular trichomes that is the basis of insect resistance in *Lycopersicon hirsutum* var. *glabratum* (Williams et al. 1980; Fery and Kennedy, 1987). 2-tridecanone has been implicated in the resistance of *L. hirsutum* to tobacco hornworm (*Manduca sexta*), spider mite species *(Tetranychus spp.),* Colorado potato beetle (*Leptinotarsa decemlineata*), tomato pinworm (*Keiferia lycopersicella*) and beet armyworm (*Spodoptera exigua)* (Kennedy, 1976; Gonçalves et al. 1998; Farrar and Kennedy, 1991; Lin et al. 1987; Maluf et al. 1997). This compound is quite toxic, and also acts as an oviposition and/or feeding deterrent.

Some compounds provide resistance to one pest, but increase the damage caused by another pest. An example of this is found with the cucurbitacins. These tetracyclic triterpenoids confer resistance to spider mites in cucumbers through feeding deterrence (antixenosis). However, cucurbitacins are also feeding stimulants for cucumber beetles, thereby increasing damage caused by the latter pest (DaCosta and Jones, 1971). Problems also arise if the control compound is detrimental to humans. Foliar glycoalkaloids of potato are associated with Colorado potato beetle resistance due to the toxicity of the glycoalkaloids toward the pest (antibiosis). However, glycoalkaloids are also toxic to humans, and high foliar glycoalkaloid levels can be correlated with high glycoalkaloid levels in tubers. Consequently, this means of resistance must be used with care (Tingey, 1984).

An increasing number of crops are protected against various pests through expression of foreign genes in plants. Such plants are referred to here as genetically modified organisms (GMOs). A group of delta-endotoxins, known as Bt, derived from *Bacillus thuringiensis*, are used to protect an increasing number of crop plants from insect pests. This method has been so widely used in important crops that Bt GMO crops are the second most utilized GMO crops (James, 2003). GMO crops with a transgene other than Bt delta-endotoxins are also being tested for efficacy against target insects (reviewed in Ferry et al., 2004). The compounds included in this work include: biotin-binding proteins (Burgess et al., 2002, Kramer et al., 2000); chitinases (Wang et al., 1996a); spider venom peptides (Penaforte et al., 2000), enzyme inhibitors and lectins (Ceci et al., 2003, Rahbé et al., 2003); toxins from bacterial symbionts of entomopathogenic

nematodes (Kramer et al., 2001); enhancins from insects (Cao et al., 2002); and even plant hormones (Smigocki and Neal, 1998). Many of these transgenic pest resistance mechanisms are based on a toxin or other compound(s) that are detrimental to pest health and survival. For example, an insect feeds on a Bt GMO plant until it ingests sufficient toxin to be killed. Therefore, many of these GMO systems for insect resistance may be classified as examples of antibiosis.

Several of the natural and GMO systems of antibiosis for insect resistance control insect pests and their direct damage quite well. If the target pest were also a virus vector, would the resulting insect resistance also be expected to reduce crop loss due to insect transmitted viruses? In these resistance systems, insect feeding on the plant is usually required for acquisition of the toxin or to trigger either natural genes or transgenes involved in a response to herbivore activity. If this feeding is as long as or longer than the transmission period for a particular virus, the system would probably allow sufficient time and opportunity for virus transfer before the resistance mechanism against the insect effectively eliminated it as a vector. Therefore, the likelihood that this type of a pest resistance would significantly affect viral disease transmission is minimal. Similarly, natural pest resistance that is based upon antibiosis can reduce pest population growth and pest use of plants, thereby reducing crop loss caused directly by pests. However, in most of these virus-host systems the interaction of the insect with the host plant would be of sufficient length such that virus transmission would not likely be reduced by antibiosis.

Would resistance based upon antixenosis be any more likely to affect virus transmission or reduce economic loss due to insect vectored viruses than antibiosis? Insect resistance based on antixenosis could be of benefit if the deterrence were sufficiently strong and rapid that insect feeding was prevented or delayed enough to reduce or slow transmission rate, infection and symptom development. The first case of this may be the antixenosis found in some Solanaceous species due to the production of acylsugars.

Acylsugar mediated pest resistance and its possible effects on insect vectored viruses

One system of pest resistance that is largely due to deterrence is the resistance in various species in the *Solanaceae* that is based upon the production of acylsugars. Acylsugars are secondary metabolites that are produced by and exuded from type IV glandular trichomes. The wild tomato

L. pennellii has high densities of type IV trichomes on all aboveground green tissues of the plant (Lemke and Mutschler, 1984) and acylsugars comprise *ca*. 90% of the exudates of these trichomes (Burke et al. 1987; Fobes et al. 1985). Structurally, these acylsugars include 2, 3, 4-tri-*O*-acylglucoses, 3', 3, 4-tri-*O*-acylsucroses and 3', 3, 4, 6-tetra-*O*-acylsucroses, with a range of odd and even short- to medium-chain length fatty acid constituents (Burke et al., 1987; Fobes et al., 1985; Shapiro et al., 1994). The fatty acid constituents are present in different combinations and proportions on acylsugars across an array of *L. pennellii* accessions (Shapiro et al., 1994). These acylsugars mediate the resistance of *L. pennellii* to many pests of tomato including: fruitworm (*Helicoverpa,* formerly *Heliothis zea*); tomato pinworm (*Keiferia lycopersicella*); beet armyworm (*Spodoptera exigua)*; silverleaf whitefly (*B. tabaci* biotype B); leafminer (*Liriomyza* spp); potato aphid (*Macrosiphum euphorbiae*), and green peach aphid (*Myzus persicae*) (Goffreda et al., 1988; Rodriguez et al., 1993; Liedl et al., 1995; Hawthorne et al., 1992; Juvick et al., 1994.) Acylsugars also mediate pest resistance in other genera in the Solanaceae, including *Nicotiana, Solanum*, *Petunia*, *Datura,* as well as other *Lycopersicon* species (Gibson, 1976c; Gibson and Valencia, 1978; King et al., 1987, 1990; Severson et al., 1985; Holley et al., 1987; Neal et al., 1989, 1990; Kennedy et al., 1992; Cutler et al., 1986; Buta et al., 1993).

Experiments using acylsugars purified from *L. pennellii* LA716 demonstrated that acylsugar-mediated resistance is largely due to deterrence of the affected pests. Appropriate application of the pure acylsugars reduces feeding of aphids *Myzus persicae* and *Macrosiphum euphorbiae* (Rodriguez et al., 1993; Goffreda et al., 1988, 1989), and sharply reduces oviposition and feeding of leafminer *Liriomyza trifolii* (Hawthorne et al*.,* 1992) and whitefly *Bemisia tabaci* (Liedl et al*.,* 1995). In a study using pure acylsugars, neonate fruitworm (*Helicoverpa zea*) and beet armyworm (*Spodoptera exigua*) larvae, the resistance to these pests was expressed as reduction of larval feeding, which led to a decline in larval development and survival when alternative food supplies were not available (Juvik et al., 1994). This deterrence is very strong. In a potato aphid study that used electronic feeding monitoring (EFM), 35% of aphids placed on *L. pennellii* plants failed to probe over a 45 minute period, and the remaining pests showed a delay of over 20 minutes in the time to first probe, as well as highly significant reductions in the number of probes, and percentage of time spent probing over the test period. Similarly, 22% of aphids placed on the interspecific hybrid *L. esculentum* x *L. pennellii* failed to probe over a 45 minute period, and the remaining pests showed a delay of over 13 minutes in the time to first probe, as well as highly significant reductions in the number of probes and percentage of time spent

probing over the test period (Goffreda et al., 1988). A subsequent EFM study on green peach aphid produced essentially the same results. That is, highly significant reductions in percentage of insects that probed, significant delays in the time to first probe, as well as highly significant reductions in the number of probes and the percentage of time spent probing on the plants that produced acylsugars (Rodriguez et al., 1993). Considering the dynamics of insect-vectored virus transmission, this strong alteration in aphid behavior could have significant impact on the likelihood and efficacy of virus transmission by this vector.

Testing insects with pure acylsugars revealed several unique advantages of acylsugar-mediated pest control. First, the system mediates strong resistance to a broad spectrum of both chewing and sucking insects. In comparison, transgenic insect resistant plants have utilized the Bt toxin for control of chewing pests, and such toxins are generally not active against phloem-feeders (Gasser and Fraley, 1989; Gill et al., 1992; Meeusen and Warren, 1989). Second, the unusual mode of action associated with acylsugar-mediated resistance has advantageous consequences. Antibiosis-based systems producing toxins such as Bt impose strong selection pressure, that can favor the generation of resistant pest biotypes. Thus, it may be possible for an extremely strong deterrence-based mechanism to impose substantial pressure toward generation of resistant biotypes, as well. Pests known to be sensitive to acylsugars, however, are not limited to feeding on tomato. Indeed most of these pests have a wide range of acceptable host species. Consequently, the deterred insects are likely to find alternative hosts, thus reducing the selection of resistant pest biotypes. Another disadvantage of toxin-based antibiosis systems of resistance is the problem of tritrophic relationships, in which the presence of the toxin in the insect pest is detrimental to a beneficial predator of the pest (Kennedy, 2003), although this is rather unlikely in the case of Bt-mediated protection since the delta-endotoxin is specific to a relatively narrow range of insect species. A deterrence system, such as the acylsugar system, will not result in toxic pests, and so should not have this affect, although the reduction of pest populations in a field would probably also result in reduced levels of predator populations that can be supported in that location. Acylsugars do not affect bee visitation, since the acylsugars are not present on the petals or anthers within the flowers.

The goal for the development of acylsugar-producing tomato lines has been insect control, and data to date indicate that this goal should be attainable once the lines are brought to fully acceptable horticultural type. Field tests showed that the two acylsugar-accumulating breeding lines,

produced by transfer of the trait from *L. pennellii* to tomato after five backcrosses to tomato, substantially reduced *B. tabaci* eggs and nymphs (Mutschler et al. in prep). Considering that the acylsugar-mediated deterrence discourages insects from feeding on these plants, might acylsugars also provide protection against some insect transmitted viruses? This must be tested directly against different vector/virus combinations under a variety of typical field conditions and environments. As discussed above, crinivirus transmission may be reduced and development of disease symptoms delayed by external treatments that limit whitefly feeding periods on hosts to very short time periods, making this an attractive virus/vector/host combination to test. Preliminary tests indicated that tomato hybrids producing acylsugars significantly reduced the rate of *Tomato infectious chlorosis virus* symptom development on the plants over a season with heavy whitefly pressure. Plants that did not produce acylsugars developed virus symptoms up to a month earlier than those that produced acylsugars. In fact, many acylsugar-expressing lines never became infected, while most non-acylsugar expressing plants did. This illustrates the potential for this type of vector-based resistance in controlling semipersistent viruses affecting tomato (Wintermantel and Mutschler, unpublished data). The results of one season, with one virus/vector combination is encouraging but does not indicate the efficacy of the resistance across virus/pest combinations, with different levels of pest pressure, or in different environments. The recent production of new acylsugar tomato lines will facilitate the trials needed to assess the potential of acylsugar-mediated resistance for control of both insect vectors and the viruses they transmit, and how antixenosis can be used as part of an integrated strategy for the control of losses due to viral diseases.

Conclusions

Insect resistance has considerable value for control of pests and the direct damage they cause to crops. Although there is no practical example currently in use of indirect control of viral disease through plant resistance to insect vectors, we believe that there is real potential for such control with some combinations of virus/vector/crop/resistance mechanisms. The virus/vector combinations most likely to be controlled for a specific crop would be those that involve semipersistent viruses and/or viruses that require relatively long feeding periods for efficient virus acquisition and transmission. The most effective host plant resistance systems could be those that are rapid acting, perhaps constitutive, and thus have the potential for preventing or delaying vector feeding, rather than killing the vector after feeding. Use of pest

resistance to decrease losses due to viral diseases is unlikely to be the sole control utilized, but could be a valuable component of an integrated control strategy when coupled with other measures to decrease the exposure of the crop to viruliferous vectors. The combination of vector resistance with genetic resistance to viruses would be complementary, and perhaps help reduce the speed or likelihood of selecting virus strains that overcome sources of virus resistance. Cooperative work is needed to complete development and utilization of some of the more promising of these pest resistance systems. Efforts should focus on using this vector control material in coordinated field trials to determine its value against direct losses caused by insects, and on losses due to insect vectored viruses. These studies could determine the utility of vector control strategies, the conditions for their effective use, and the best means to deploy such resistances within a coordinated strategy of integrated pest management.

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