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Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus

Dhana Raj Boina[a] and Jeffrey R Bloomquist[b*]

Abstract

By 2014, huanglongbing (HLB), the most destructive disease of citrus, and its insect vector, the Asian citrus psyllid (ACP), *Diaphorina citri* (Kuwayama), became established in all major citrus-growing regions of the world, including the United States, with the exception of California. At present, application of insecticides is the most widely followed option for reducing ACP populations, while application of antibiotics for suppressing HLB disease/symptoms is being practiced in some citrus-growing regions. Application of insecticides during the dormant winter season, along with cultivation of HLB-free seedlings and early detection and removal of symptomatic and asymptomatic trees, has been very effective in managing ACP. Area-wide management of ACP by application of insecticides at low volume in large areas of citrus cultivation has been shown to be effective in managing HLB and reducing management costs. As insecticide resistance is a major problem in sustainable management of ACP, rotation/alternation of insecticides with different chemistries and modes of action needs to be followed. Besides control of the insect vector, use of antibiotics has temporarily suppressed the symptoms of HLB in diseased trees. Recent efforts to discover and screen existing as well as new compounds for their antibiotic and antimicrobial activities have identified some promising molecules for HLB control. There is an urgent need to find a sustainable solution to the HLB menace through chemical control of ACP populations and within HLB-infected trees through the judicious use of labeled insecticides (existing and novel chemistries) and antibiotics in area-wide management programs with due consideration to the insecticide resistance problem.

Keywords: *Diaphorina citri*; citrus greening; insecticide; insecticide resistance; antibiotic

1 INTRODUCTION

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is an important insect pest of citrus crops worldwide.[1] Both ACP and the causal organism of huanglongbing (HLB) it transmits originated in Asia and spread to other citrus-growing parts of the world, including the Americas.[1–8] In the Americas, ACP was first reported in Brazil in the 1940s, and in the United States it was observed in Florida in 1998[9,10] and later found in all citrus-cultivating states, including Alabama, Arizona, California, Georgia, Hawaii, Louisiana, Mississippi, South Carolina and Texas.[11–13] In Australia, ACP was first reported in 1915, but it was eventually eradicated within 10 years.[14] Although ACP has been present in Brazil for >60 years, the first report of HLB disease occurred in 2004,[15,16] and a year later HLB was reported in the United States in Florida,[5,17] followed by all citrus-growing states except Alabama, Arizona, Mississippi and South Carolina.[12,13] A recent report documented the presence of HLB within California in a residential lime citrus plant.[13,18,19]

ACP causes both direct and indirect damage to citrus trees. Direct damage is caused by heavy ingestion of phloem sap by adults and nymphs and injection of toxins through saliva.[20] Indirect damage results from transmission of three species of phloem-harboring bacteria belonging to the genus *Candidatus* Liberibacter, which are thought to be associated with HLB, or citrus greening disease.[5] Both nymphs (fourth and fifth instars) and adults are able to acquire and transmit the bacterium, but adults, because of their flying ability, are significant contributors to the spread of HLB.[21–24]

ACP is an efficient insect vector of HLB causal pathogen, *Candidatus* Liberibacter asiaticus (*Ca*Las), in Asia and the Americas, and high ACP populations in HLB-resident areas lead to high incidence and spread of HLB. The establishment of ACP in a region makes its complete eradication highly unlikely; therefore, ACP populations should be kept to as low as possible to minimize HLB spread. In the field, build-up of ACP populations depends on temperature and new flush. Female adults must feed on new flush to mature eggs and lay eggs on tender shoots and new sprouts, which ensures continuous supply of appropriate habitat for nymph development in subsequent days.[25–29] Temperatures between 24 and 30 °C are most favorable for both adult survival and reproduction, as adults survived 30–50 days and females laid 500 to 800 eggs at these temperatures.[30–33] The production of new flush, on the other hand, depends on tree age, weather conditions and citrus variety.[25,34]

All known cultivars of citrus are susceptible to HLB, and it has wiped out citrus production in some Asian and African

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* *Correspondence to: Jeffrey R Bloomquist, Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA. E-mail: jbquist@epi.ufl.edu*

[a] *National Institute of Plant Health Management, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, Rajendranagar, Hyderabad, Telangana, India*

[b] *Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA*

countries.[1,5,13] HLB causes both qualitative and quantitative loss to citrus production. Infected trees exhibit tree decline and become unproductive (30–100% reduction in yield),[19] with a variety of symptoms that intensify as the disease progresses. Trees die in 5–8 years, as there is no chemical or other control method currently available that can completely cure the disease.[5] Juice from diseased tree fruits tastes bitter, with high acid content and off-flavor,[5,35,36] thus reducing the market for both fresh and processed fruits. The destruction of nearly 100 million citrus trees in 40 countries worldwide reaffirms the severity of the disease.[8,19,35,37–40] In Florida, about 10.8 million out of 60 million orange trees may have been infected with HLB.[19,41] About 10 million orange trees have been removed in Brazil alone, as the usual practice to minimize HLB spread is removal of the entire orchard when the infection rate reaches 28%.[19,41] Therefore, the sustainability of the world citrus industry is under threat if HLB and its spread by the vector are not controlled.[42]

Given the high reproductive potential of ACP under favorable conditions[30,43] and strong dispersal ability of adults,[44] it is difficult to control the disease once it is established in an area. The suppression of vector populations with insecticides and reduction or elimination of disease symptoms with antibiotics are some of the viable options for managing this disease system. Interest and funding for research on this pest disease complex and on chemical control options for ACP and HLB management has resulted in a large body of relevant literature. Recent reviews on ACP and HLB dealt extensively with the biology and distribution of ACP,[1,6,40,45] as well as the distribution, transmission, epidemiology and symptomatology of HLB,[1,5,40,46,47] with less focus on specific chemical control methods for the vector or disease.

The principal aim of this review is to bring the salient findings from original scientific studies on chemical control of ACP and HLB into a single fold, so that the reader will be able to find, at ease, the required information on managing ACP and HLB through chemical means. At the same time, we minimize repeating information already available on the biology and distribution of ACP, and management of ACP and HLB by non-chemical means. Although a significant amount of literature in this review comes from studies conducted in the United States, we tried to include information from important citrus-growing countries around the world as well. The first part of the review deals with chemical control of ACP, including foliar-applied broad-spectrum and reduced-risk insecticides, soil- and trunk-applied systemic insecticides, the role of insecticides in reducing HLB transmission, factors affecting the susceptibility to insecticides, insecticide resistance and its management and compatibility of insecticides with biocontrol agents in the citrus crop ecosystem. The second part of the article deals with chemical control of HLB itself, mainly using various antibiotics and other antimicrobials.

2 CHEMICAL CONTROL OF ACP

With the worldwide establishment of HLB, there is a significant increase in both the portfolio of insecticides and insecticide applications made per year (1–21) to fight the ACP-HLB threat.[28,29,38,48–57] The per hectare annual cost of ACP management in citrus could range from $US 240 to > $US 1000, depending on the type of insecticide used, application frequency and method of application.[38] The chemical control options currently available for managing ACP in the field are foliar applications of broad-spectrum insecticides and reduced-risk insecticides, as well as soil or trunk applications of systemic insecticides. The majority of these insecticides act on the insect nervous system (Table 1). Monitoring the onset and duration of flush periods and infestation of flush by immatures using visual inspection and adult populations using various trapping methods facilitates timely intervention with insecticide applications and reduction of ACP populations. Information on insecticides labeled for ACP management is given in Table 2.

2.1 Foliar-applied insecticides

Among the various types of insecticide, foliar-applied broad-spectrum insecticides are the most widely available and commonly used for controlling both adults and immatures of ACP. These insecticides rapidly kill the insect vector upon contact/feeding owing to their quick mode of action and sensitivity of the insect nervous system to poisoning by these compounds (Tables 1 and 3). They have a broad spectrum of activity and act by contact and/or systemic action(s). They are usually applied as a spray at high volume using conventional ground air-blast applicators (tractor driven) or as a mist at low volume using specially designed and truck-mounted applicators with fine-orifice nozzles.[58]

Young trees need continuous protection from ACP feeding and pathogen transmission owing to their frequent flushing pattern and ACP colonization. Soil application of systemic insecticides offers continuous protection and is a widely followed practice. However, these soil applications are generally supplemented with foliar applications of broad-spectrum insecticides.[59] In this review, trees of ≤4 years age are considered young,[29] and those of ≥5 years age are considered mature trees; when there is no mention of tree age in a field study, we assumed mature trees.

2.1.1 High-volume spray applications

Studies conducted in the United States and other countries on young and mature trees documented that application of broad-spectrum organophosphates (OPs) (e.g. chlorpyrifos, dimethoate and phosmet), synthetic pyrethroids (SPs) (e.g. fenpropathrin and zeta-cypermethrin), an avermectin (abamectin) mixed with 435 horticultural mineral oil (HMO) and neonicotinoid and sulfoximine (e.g. imidacloprid and sulfoxaflor + 435 HMO) at field labeled rates and an experimental use permit butenolide compound (flupyradifurone + 435 HMO) provided protection from ACP.[29,59–61] The protection lasted anywhere between 1.5 and 6 weeks, depending on the insecticide, rate used, application method, tree age (canopy size) and citrus variety.[45,54,55,57,62–65] Field studies conducted on young King mandarin trees in Vietnam and mature (five-year-old) Valencia orange trees in the United States, however, found that foliar application of neonicotinoids (clothianidin, imidacloprid and thiamethoxam) and an anthranilic diamide (cyantraniliprole) gave the longest-lasting protection (8–9 weeks), and among the three neonicotinoids, imidacloprid provided maximum control of adults (50–90%).[66,67] In general, broad-spectrum insecticides, such as OPs, carbamates and SPs, exhibited more rapid killing of both adults and nymphs of ACP than systemic neonicotinoids, but neonicotinoids showed longer-lasting residual activity.[49,68] As a result, broad-spectrum insecticides need more frequent applications than neonicotinoids.

Unlike young trees, chemical control options for ACP on mature trees in Florida are limited to foliar applications of insecticides, because the only available insecticide for soil applications, aldicarb (banned in Florida), had reduced efficacy in controlling ACP on mature trees.[59] Furthermore, soil application of neonicotinoids,

Table 1. Chemistry, mode of action and IRAC classification of insecticides used in citrus for controlling the ACP[a]

Chemistry group	Mode(s) of action	IRAC classification[b]	Insecticide(s)
Cyclodiene organochlorines	Antagonist of γ-aminobutyric-acid-gated chloride channels	2A	Endosulfan
Organophosphate	Acetylcholineesterase inhibition	1A	Aldicarb, fenobucarb, methomyl and oxamyl
Synthetic pyrethroid	Sodium channel modulation	3A	α-Cyhalothrin, cypermethrin, fenpropathrin, λ-cyhalothrin and ζ-cypermethrin
Avermectin	Chloride channel activation	6	Abamectin
Neonicotinoids and sulfoximines	Agonist of nicotinic acetylcholine (nACh) receptors	4A	Acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam
		4C	Sulfoxaflor
Butenolides	Agonist of nACh receptors	4D	Flupyradifurone
Spinosyns	nACh receptor allosteric modulators and antagonists of GABA-gated chloride channels	5	Spinosad and spinetoram
Insect growth regulators	Juvenile hormone mimic	7C	Pyriproxyfen
	Chitin biosynthesis inhibition (benzoylphenyl ureas)	15 type 0	Diflubenzuron, flufenoxuron, lufenuron, novaluron and teflubenzuron
		16 type 1	Buprofezin
Selective homopteran feeding blockers	Paralysis of cibarium or mouthparts used for ingesting plant sap	9B	Pymetrozine
METI insecticides	Mitochondrial complex I electron transport inhibition	21A	Pyridaben, fenpyroximate
Tetronic and tetramic acid derivatives	Acetyl CoA carboxylase inhibition	23	Spirodiclofen and spirotetramat
Anthranilic diamides	Ryanodine receptor modulation	28	Chlorantraniliprole, cyantraniliprole
Compounds of unknown or uncertain mode of action	Chitin synthesis inhibition, feeding and oviposition deterrence, suffocation and alterations in cuticle composition	UN	Azadirachtin, sucrose octanoate, Silwet L-77, Kinetic, petroleum spray oil, horticultural spray oil, *n*C24 horticultural mineral oil and oil

[a] Includes insecticides that are under experimental use, as well as insecticides that were labeled for use on citrus but are now banned.
[b] Based on the mode of action classification of the Insecticide Resistance Action Committee (IRAC), version 7.3, February 2014 (www.irac-online.org).

especially imidacloprid, is not recommended for mature trees, as application at full labeled rate did not result in concentrations that killed feeding ACP.[59] Mature trees produce a major flush in late winter and early spring, followed by minor flushes in early summer, late summer and fall, with ACP populations reaching peak numbers during these flushing periods.[33,69] Therefore, foliar applications with broad-spectrum insecticides during late winter to mature trees, when few overwintering adults are the only survivors, exhibited a profound effect on ACP populations for a longer period.[53] For example, a single foliar application of chlorpyrifos, oxamyl or fenpropathrin to mature orange trees in January significantly suppressed the ACP populations (both adults and nymphs) for up to 6–7 months, including major and minor flush periods.[53] Dormant winter-season foliar application not only helped in immediate suppression of overwintering ACP adults but also maintained natural enemies, which might have helped in keeping the ACP number to low levels in the following months.[53]

Application of insecticides effectively managed ACP, which in turn resulted in HLB reduction.[70,71] Insecticide applications (foliar or soil applied) in newly planted or well-established groves significantly delayed or lowered the incidence and spread of HLB compared with untreated groves. Similarly, reviving citrus production in an area of China where severe outbreak of HLB occurred[48] also emphasized the importance of insecticides in HLB management through suppression of ACP populations (at least 10–13 insecticide sprays during flushing periods). A mathematical model was developed to predict the transmission of HLB pathogen among the flush of a diseased tree.[72] The model considered various factors, including ACP stage, insecticidal intervention, removal of symptomatic flush, etc., and suggested that insecticidal applications made for ACP control reduced the spread of disease among the flush of a diseased tree.[72] Moreover, a significant reduction in disease spread was expected when the applications were made with an effective insecticide (high efficacy against ACP) at a frequency of ≥2 applications per year soon after the incidence of disease.[72]

An additional example is available from studies in Malaysia, where a significant reduction in ACP life stages on new flush, and in turn a significant reduction in incidence and spread of HLB within the grove, was made possible by application of imidacloprid alone or triazophos alternated with cypermethrin/chlorpyrifos at biweekly intervals.[28] Imidacloprid caused significant reduction in incidence and spread of HLB within the grove compared with other treatments: 9.2% imidacloprid and 22.7% triazophos/cypermethrin/chlorpyrifos versus 42.2% in control.

2.1.2 *Low-volume mist applications*

Multiple foliar insecticide applications using conventional high-volume air-blast applicators increased the cost of citrus production. The search for a technology that could reduce burgeoning ACP management costs resulted in adoption of low-volume mist applications originally developed for mosquito

Table 2. Insecticide products labeled for use in citrus for controlling the ACP in India and the United States

Insecticide	Labeled rate $acre^{-1}$	Method of application/comments	State,[a] country
Chlorpyrifos 4 E	1893–2839 mL	Foliar (during bloom, apply 1 h after sunset to 2 h before sunrise)	CA, USA
	2365.88 mL	Foliar (apply during non-bloom)	FL, USA
Dimethoate 400	473.2–946.4 mL	Foliar (limit to two applications on mature fruit)	CA, USA
Dimethoate 4 E	473.2 mL	Foliar (applying during non-bloom, not more than two applications per season)	FL, USA
Oxydemeton-methyl 25 EC	480–640 mL		India
Carbaryl XLR Plus	2839–4732 mL	Foliar (during bloom, apply 1 h after sunset to 2 h before sunrise)	CA, USA
β-Cyfluthrin XL	94.64–189.3 mL	Foliar	CA, USA
Cyfluthrin	189.27 mL	Foliar	CA, USA
Fenpropathrin 2.4 EC	473.2 mL	Foliar (apply citrus trees of 3 years age or older)	CA, USA
	473.2 mL	Foliar (apply during non-bloom)	FL, USA
Pyrethrin 5 EC II (organic insecticide)	502.75 mL	Foliar	CA, USA
Pyrethrin 1.4 EC	1893.70 mL	Foliar	CA, USA
ζ-Cypermethrin	127.3 mL		CA, USA
ζ-Cypermethrin	121.90 mL	Foliar [limit to four applications (90.71 g AI) per acre per season, do not apply during bloom]	FL, USA
Abamectin SC	66.54–125.68 mL	Foliar [limit to 251.37 mL (25.40 g AI) abamectin per acre per season]	CA, USA
Diflubenzuron 80 WGS	177.18 g	Foliar	CA, USA
Diflubenzuron 80 WGS +	177.18 g + 2% v/v	Foliar (limit to three applications per season, apply during bloom, do not apply when temperature exceeds 34.4 °C)	FL, USA
Fenpyroximate	946.34–1892.70 mL	Foliar	CA, USA
Fenpyroximate	946.35–1892.70 mL	Foliar [limit to two applications per season (1892.70 g AI), apply during bloom, allow 14 days between applications]	FL, USA
Clothianidin 50 WDG	90.71–181.43 g	Soil drench [for non-bearing trees only, apply during non-bloom, should not be applied within 1 year of fruit harvest, not to apply more than 362.87 g (181.43 g AI) per acre per year]	FL, USA
Imidacloprid	207.01–414.02 mL	Soil drench (limit to 226.79 g AI per acre per season, use the highest recommended rate for mature trees, do not apply during bloom)	CA, USA
Imidacloprid 4 F	236.58–473.17 mL	Soil drench (limit to 226.79 g AI per acre per season, use the highest recommended rate for mature trees, do not apply during bloom)	CA, USA
Imidacloprid 1.6 F	295.73–591.47 mL	Foliar [apply during non-bloom, limit to 226.79 g AI per acre per growing season regardless of application type (soil and/or foliar)]	FL, USA
Imidacloprid 4.6 F	207.01–414.02 mL	Foliar [apply during non-bloom, limit to 226.79 g AI per acre per growing season regardless of application type (soil and/or foliar)]	FL, USA
Imidacloprid 4.6 F	207.01–414.02 mL	Soil drench [limit to 828.05 mL (453.59 g AI) per acre per year, but do not exceed 414.02 mL per application]	FL, USA
Imidacloprid 2 F	473.17–946.35 mL	Soil drench [limit to 226.79 g AI per acre per growing season regardless of application type (soil and/or foliar) for 180 cm tall trees]	FL, USA
Imidacloprid 17.8 SL	20 mL	Foliar	India
Imidacloprid + β-cyfluthrin	94.63–189.27 mL	Foliar [limit to 189.27 mL (4.53 g AI β-cyfluthrin per acre and 45.15 g AI imidacloprid per acre per season), limit to 22.67 g AI β-cyfluthrin per season or 45.35 g AI cyfluthrin per season or 45.35 g AI cyfluthrin and β-cyfluthrin in all forms per acre]	CA, USA
Thiamethoxam	113.39–155.92 g	Foliar [limit to 311.84 g (78.01 g AI) per acre per season]	CA, USA
Thiamethoxam 25 WG	113.39–155.92 g	Foliar [limit to 311.84 g (78.01 g AI) per acre per season, do not apply during prebloom and bloom]	FL, USA
Thiamethoxam 75 SG	51.87–104.04 g	Soil drench [limit to 104.04 g (78.01 g AI) per acre per season, do not apply during prebloom and bloom]	FL, CA, USA
Thiamethoxam 25 WG	40 g		India
Thiamethoxam + abamectin	162.65–251.37 mL	Foliar (limit to 251.37 mL per acre per season or 21.31 g AI abamectin or 78.01 g AI thiamethoxam per acre per season)	CA, USA
Thiamethoxam + chlorantraniliprole	141.74–198.44 g	Foliar (limit to 396.89 g per acre per season or 78.01 g AI or 90.71 g AI per acre per season)	CA, USA
Thiamethoxam + chlorantraniliprole	198.44 g	Foliar (limit to 396.89 g per acre per season or 78.01 g AI thiamethoxam per season, do not apply during prebloom and bloom)	FL, USA
Thiamethoxam + abamectin + petroleum oil 97 + %	251.37 mL + 2% v/v	Foliar (limit to 502.75 mL per acre or three applications per season or limit to 78.01 g AI thiamethoxam per acre per season or limit to 21.31 g AI abamectin per acre per season, must be mixed with 0.2% oil, do not apply during prebloom and bloom)	FL, USA
Phosmet 70 W	453.59 g	Foliar (apply during non-bloom, not more than two applications per season)	FL, USA

Table 2. Continued

Insecticide	Labeled rate acre^{-1}	Method of application/comments	State,[a] country
Spirotetramat	295.73 mL	Foliar	CA, USA
Spirotetramat 250 SC + petroleum oil 97 + %	295.73 mL + 3% v/v	Foliar [limit to 591.47 mL (9.07 g AI) per acre per season, do not apply 10 days prior to bloom and during bloom]	FL, USA
Spirotetramat MPC + petroleum oil 97 + %	473.17 mL + 3% v/v	Foliar [limit to 591.47 mL (9.07 g AI) per acre per season, do not apply 10 days prior to bloom and during bloom]	FL, USA
Sulfoxaflor SC + petroleum oil 97 + %	118.29–147.86 mL + 2% v/v	Foliar [limit to 502.75 mL (7.54 g AI) per acre per season, only one application allowed between 3 days before bloom and until after petal fall per year]	FL, USA
Chlorantraniliprole	127.57 g	Foliar [limit to 255.14 g chlorantraniliprole (90.71 g AI) per acre per season]	CA, USA
Spinetoram DG	113.39–170.09 g	Foliar	CA, USA
Spinetoram WG + petroleum oil 97 + %	113.39 g + 2% v/v	Foliar [apply during non-bloom, limit to 340.19 g (85.27 g AI) per acre per season, do not make more than three applications per year)	FL, USA
Spinosad (organic insecticide)	295.73 mL	Foliar	CA, USA

[a] CA – California; FL – Florida (Rogers *et al.*[29] and Refs [125] and [126]).

control, with some modifications.[58,73–76] In Florida, abamectin, carbaryl, *zeta*-cypermethrin, diflubenzuron, dimethoate, fenpropathrin, malathion and spirotetramat have modified labels to apply them at low volumes for ACP control [Special Local Needs label 24(c)].[58,76] As per the 24(c) label, when these products are applied at low volume, a minimum spray volume of 7570 mL acre^{-1} or 2 gal acre^{-1} and a spray droplet volume median diameter (VMD) of $\geq$90 μm should be maintained.[58] Studies conducted under Florida conditions have shown that ACP control obtained at low-volume ground applications is as good as or better than high-volume conventional ground applications.[73]

There are several advantages of using low-volume applications compared with high-volume conventional applications, the major one being a significant reduction in cost (5–6 times).[74–76] Other benefits of applying at low volumes are that the sprayer and spray tank are truck mounted and can move at a speed of 5–16.9 kilometers per hour (kph) [10 miles per hour (mph)], facilitating coverage of large areas in a short time [as high as 97.16 ha (240 acres) per night under good weather conditions] and eliminating the task of loading and unloading the spray equipment each time applications are made.[73,76] It is estimated that the use of low-volume applications could save Florida growers on an average $US 40–72 million per year in ACP management costs.[74,75] Major drawbacks of this technology are limited choice of insecticides and insecticide drift. To avoid drift, it is recommended to make applications when the wind speed is <16.9 kph (10 mph), and nights are the best time for low wind speeds.[74,75]

2.2 Soil- or trunk-applied systemic insecticides

Young trees need continuous protection from ACP feeding and pathogen transmission owing to their frequent flushing pattern and ACP colonization. Soil application of systemic insecticides offers continuous protection, and is a widely followed practice (Table 4). Although soil applications are supplemented with foliar treatments of broad-spectrum insecticides,[59] soil-applied systemic insecticides do not exhibit the quick knockdown effect on ACP exerted by foliar applied broad-spectrum insecticides. Systemic insecticides can be applied to soil by soil drench, chemigation (through drip irrigation) or spot application at bed/swale side or to tree trunk (trunk application, trunk paint and trunk injection).[49,50,64,66,69–71,77–79] Soil application methods are widely followed in the United States, while tree trunk application methods are usually followed in other citrus-growing countries of the world (India and Vietnam). There are only a few classes of chemistry available for soil application. Insecticides used for these applications belong to OP (dimethoate, methamidophos, methidathion and monocrotophos),[40,55] carbamate (aldicarb and oxamyl)[69] and neonicotinoid (acetamiprid, clothianidin, imidacloprid, nitenpyram and thiamethoxam) chemistries (Table 4).[64,66,70,71,77,79]

Although foliar applications of broad-spectrum insecticides are highly effective against adults, achieving the same level of control of immatures by this method is less likely[53] owing to the cryptic nature of immatures and the possibility of feeding on newly expanded shoots not covered by insecticide spray. Supplementing foliar applications with soil applications for young trees which need continuous protection from ACP for at least 4 years (or $\leq$180 cm tall)[29,59] reduces the chances of infestation of young trees. It is generally recommended that young trees need at least one soil application per growing season to protect them from ACP.[29,80] In Florida, young non-bearing trees are protected by soil application of neonicotinoids such as imidacloprid, thiamethoxam and clothianidin, once in the fall and once in the spring. Because of use restrictions, total insecticide used should not cross the maximum quantity allocated per season (Table 2).[29,59]

Based on a 3 year study, Powell *et al.*[77] recommended soil drench with imidacloprid at 24 weeks (6 months) to control ACP adults and reduce the percentage tree and shoot infestation in young sweet orange trees. Other studies reported that imidacloprid and other soil-applied insecticides such as aldicarb (banned in Florida), oxamyl and thiamethoxam offered shorter durations of protection (3–12 weeks) from ACP for young trees.[50,64,77] Among different insecticides, imidacloprid provided the longest residual activity and ACP suppression in young trees.[81] Use of imidacloprid on young trees with continuous production of flush has other benefits as well. Owing to its antifeedant and deterrent effects on adults, fewer adults were attracted to new flush, resulting in fewer eggs being laid on treated plants both in laboratory and field conditions.[79,81]

Similarly to young trees, seedlings and resets in newly established groves need continuous protection from ACP infestation, and both imidacloprid and thiamethoxam, owing to their effective killing action and longer-lasting residual activity, were highly

Table 3. Insecticides used for foliar applications in citrus for controlling the ACP[a]

Insecticide	Trade name[b]	Rate	Reference	Country
Endosulfan	Thiodan 35 EC	200.00 mL tree^{-1}	Farmanullah and Gul[65]	Pakistan
Chlorpyrifos	Lorsban 4 E	1133.60 g AI acre^{-1}	Qureshi and Stansly[53]	USA
	Lorsban 4 E	1182.92 g acre^{-1}	Pena *et al.*[62]	USA
	Lorsban 4 E	2365.85 g acre^{-1}	Rogers *et al.*[29]	USA
	Lorsban 4 E	2365.85 g acre^{-1}	Childers and Rogers[64]	USA
	Lorsban 4 EC	1892.68 g acre^{-1}	Childers and Rogers[64]	USA
	Lorsban, Clorpirifos, Nufos	1.00 mL L^{-1}	Belasque *et al.*[38]	Brazil
	–	0.0125%	Leong *et al.*[28]	Malaysia
Malathion	Malatol 1000 CE	0.50 mL L^{-1}	Belasque *et al.*[38]	Brazil
Dimethoate	Dimethoate 4	473.17 g acre^{-1}	Rogers *et al.*[29]	USA
	–	300.00 mL acre^{-1}	Ahmed *et al.*[63]	Pakistan
	–	0.40 and 0.80 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
	Dimethoate 30 EC	0.55%	Rao and Shivankar[56]	India
	Dimethoate 40 EC	0.001%	Abbaszadeh *et al.*[54]	Iran
	Many CPs	0.5–0.8 mL L^{-1}	Belasque *et al.*[38]	Brazil
Metasystox-R	MSR 2 E	1419.51 g acre^{-1}	Qureshi and Stansly[50]	USA
Methamidophos	–	500.00 mL acre^{-1}	Ahmed *et al.*[63]	Pakistan
Methidathion	Supracide 40 EC	150.00 mL tree^{-1}	Farmanullah and Gul[65]	Pakistan
	Supracid	0.50 mL L^{-1}	Belasque *et al.*[38]	Brazil
	–	0.40 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
Phosmet	Imidan 70 W	454.00 g acre^{-1}	Rogers *et al.*[29]	USA
	Imidan	0.50 g L^{-1}	Belasque *et al.*[38]	Brazil
Triazophos	Hostathion 40 EC	0.03%	Leong *et al.*[28]	Malaysia
Fuzalon + teflubenzuron	Darton 21.7 EC	0.0025%	Abbaszadeh *et al.*[54]	Iran
Fenobucarb	–	101.21 g AI acre^{-1}	Gatineau *et al.*[70]	Vietnam
	–	0.50 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
Methomyl	–	0.005 g AI L^{-1} (2 L tree^{-1})	Khan *et al.*[57]	Pakistan
Oxamyl	Vydate 2 L	226.72 and 453.44 g AI acre^{--1}	Qureshi and Stansly[53]	USA
Carbosulfan	Marshal	0.25 mL L^{-1}	Belasque *et al.*[38]	Brazil
Etofenprox	Trebon	0.25 mL L^{-1}	Belasque *et al.*[38]	Brazil
α-Cyhalothrin	Karate 2.5 EC	50.00 mL tree^{-1}	Farmanullah and Gul[65]	Pakistan
Cypermethrin	–	0.125%	Leong *et al.*[28]	Malaysia
Fenpropathrin	Danitol 2.4 EC	137.65 g AI acre^{-1}	Qureshi and Stansly[53]	USA
	Danitol 2.4 EC	473.17 mL acre^{-1}	Rogers *et al.*[29]	USA
	Danitol 2.4 EC	473.12 mL acre^{-1}	Childers and Rogers[64]	USA
	Danitol 2.4 EC	68.42 and 102.42 g AI acre^{-1}	Boina *et al.*[96]	USA
	Daniben	0.50 mL L^{-1}	Belasque *et al.*[38]	Brazil
Formetanate	Dicarzol	0.50 g L^{-1}	Belasque *et al.*[38]	Brazil
λ-Cyhalothrin	–	0.0125 g AI L^{-1} (2 L tree^{-1})	Khan *et al.*[57]	Pakistan
	Karate 50 SC	0.10–0.20 mL L^{-1}	Belasque *et al.*[38]	Brazil
	Karate 50 SC	80 mL acre^{-1}	Belasque *et al.*[38]	Brazil
γ-Cyhalothrin	Nexide	0.025 mL L^{-1}	Belasque *et al.*[38]	Brazil
ζ-Cypermethrin	Mustang	121.86 g acre^{-1}	Rogers *et al.*[29]	USA
Bifenthrin	Talstar	0.15–0.2 mL L^{-1}	Belasque *et al.*[38]	Brazil
Deltamethrin	Decis 100 Ultra	0.075 mL L^{-1}	Belasque *et al.*[38]	Brazil
Abamectin[c]	Agri-Mek 0.15 EC	283.40 g acre^{-1}	Childers and Rogers[64]	USA
	Abamectin 1.9 EC	0.38 mL L^{-1}	Rao and Shivankar[56]	India
	Vertimec, Kraft, Abamex, Abamectin, Nortox	0.2 mL L^{-1}	Belasque *et al.*[38]	Brazil
Acetamiprid	Assail 70 WP	64.89 acre^{-1}	Childers and Rogers[64]	USA
	Assail 30 SC	59.42 g AI acre^{-1}	Qureshi and Stansly[50]	USA
	Mospilan 20 SP	0.00025%	Abbaszadeh *et al.*[54]	Iran
Dinotefuran	–	0.20 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
Imidacloprid	Imicon 2.5 WP	0.0063 g AI L^{-1} (2 L tree^{-1})	Khan *et al.*[57]	Pakistan
	Couraze 1.6 F	295.70–591.40 mL acre^{-1}	Rogers *et al.*[29]	USA
	Nuprid 1.6 F	295.70–591.40 mL acre^{-1}	Rogers *et al.*[29]	USA
	Pasada 1.6 F	295.70–591.40 mL acre^{-1}	Rogers *et al.*[29]	USA
	Provado 1.6 F	295.70–591.40 mL acre^{-1}	Rogers *et al.*[29]	USA
	Provado 1.6 F	295.70 mL acre^{-1}	Childers and Rogers[64]	USA
	Provado 1.6 F	147.85 mL acre^{-1}	Childers and Rogers[64]	USA
	Provado 1.6 F	56.69 g AI acre^{--1}	Qureshi and Stansly[50]	USA

Table 3. Continued

Insecticide	Trade name[b]	Rate	Reference	Country
	Provado 1.6 F	443.55 mL acre^{-1}	Qureshi and Stansly[50]	USA
	Confidor 200 EC	0.01%	Leong *et al.*[28]	Malaysia
	–	250.00 g acre^{-1}	Ahmed *et al.*[63]	Pakistan
	–	0.20 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
	Provado 200 SC, Kohinor	0.20 mL L^{-1}	Belasque *et al.*[38]	Brazil
	Provado 200 SC	160 mL acre^{-1}	Belasque *et al.*[38]	Brazil
Thiamethoxam	Actara 25 WG	113.36–155.87 g acre^{-1}	Rogers *et al.*[29]	USA
	Actara 25 WG	113.36 g acre^{-1}	Childers and Rogers[64]	USA
	Actara 25 WG	10.00 g tree^{-1}	Farmanullah and Gul[65]	Pakistan
	Actara 25 WG	0.0003%	Abbaszadeh *et al.*[54]	Iran
	Actara 250 WG	0.10 g L^{-1}	Belasque *et al.*[38]	Brazil
Flupyradifurone	Sivanto 200 SL	297.66, 340.19, 396.89 g acre^{-1}	Qureshi *et al.*[61]	USA
Thiamethoxam + chlorantraniliprole	VoliumFlexi	198.38 g acre^{-1}	Rogers *et al.*[29]	USA
Thiamethoxam + abamectin[c]	Agri-Flex	251.34 mL acre^{-1}	Rogers *et al.*[29]	USA
Cyantraniliprole	HGW 10 SE	600 mL acre^{-1}	Tiwari and Stelinski[67]	USA
Sulfoxaflor + citrus oil	Sulfoxaflor 20 SC	80.79–161.59 g acre^{-1} + 2% v/v	Stansly *et al.*[60]	USA
Azadirachtin	Neemix 4.5	180.00 mg L^{-1}	Weathersbee III and McKenzie[93]	USA
	Azadirachtin 1000 PPM	3.65 mL L^{-1}	Rao and Shivankar[56]	India
Neem oil	–	1.00%	Khan *et al.*[57]	Pakistan
	–	6.76 mL L^{-1}	Rao and Shivankar[56]	India
Neem seed water extract	–	3.00%	Khan *et al.*[57]	Pakistan
Buprofezin	Applaud 70 WP	240.00 mg L^{-1}	Tiwari *et al.*[89]	USA
Diflubenzuron	Micromite 80 WDG	184.00 mg L^{-1}	Tiwari *et al.*[89]	USA
Diflubenzuron[c]	Micromite 80 WDG	177.12 g acre^{-1}	Qureshi and Stansly[50]	USA
Flufenoxuron	Cascade 10 DC	150.00 mL tree^{-1}	Farmanullah and Gul[65]	Pakistan
	Cascade 5 DC	0.0005%	Abbaszadeh *et al.*[54]	Iran
Lufenuron	Match 050 EC	60.00 mL tree^{-1}	Farmanullah and Gul[65]	Pakistan
Novaluron	Rimon 10 EC	0.55 mL L^{-1}	Rao and Shivankar[56]	India
Pyriproxyfen	Knack 0.86 EC	64.00 mg L^{-1}	Boina *et al.*[88]	USA
	Admiral 10 EC	0.0007%	Abbaszadeh *et al.*[54]	Iran
Spinosad	Spintor 2 SC	177.42 mL acre^{-1}	Childers and Rogers[64]	USA
	Spinosad 45 SC	0.15 mL L^{-1}	Rao and Shivankar[56]	India
Spinetoram[c]	Delegate WG	113.36 g acre^{-1}	Rogers *et al.*[29]	USA
Spirotetramat[c]	Movento 240 SC	295.70 mL acre^{-1}	Rogers *et al.*[29]	USA
	Movento MPC	473.12 mL acre^{-1}	Rogers *et al.*[29]	USA
	Spirotetramat 150 OD	354.84 and 473.12 mL acre^{-1}	Qureshi and Stansly[50]	USA
Spirodiclofen	Envidor 2 SC	591.40 mL acre^{-1}	Qureshi and Stansly[50]	USA
Pyridaben	GWN-1715 75 WP	300.40 g acre^{-1}	Qureshi and Stansly[50]	USA
Pymetrozine	Fulfill 50 WG	52.00 mg L^{-1}	Boina *et al.*[90]	USA
	–	0.50 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
Sucrose octanoate	–	1600.00–8000.00 mg L^{-1}	McKenzie and Puterka[92]	USA
Silwet L-77	–	0.05%	Cocco and Hoy[94]	USA
Kinetic	–	0.05%	Cocco and Hoy[94]	USA
Silwet l-77 + imidacloprid	–	0.05% + 1/10, 1.4 or 1/2 of the lowest labeled rate	Srinivasan *et al.*[52]	USA
Silwet L-77 + abamectin	–	0.05% + 1/4 or 1/2 of the lowest labeled rate	Srinivasan *et al.*[52]	USA
Kinetic + imidacloprid	–	0.05% + 1/10, 1.4 or 1.2 of the lowest labeled rate	Srinivasan *et al.*[52]	USA
Kinetic + abamectin	–	0.05% + 1/4 or 1/2 of the lowest labeled rate	Srinivasan *et al.*[52]	USA
Petroleum spray oil	–	5.90 mL L^{-1}	Rao and Shivankar[56]	India
Horticultural spray oil	F1-435-66	3785.00 mL acre^{-1}	Qureshi and Stansly[50]	USA
*n*C24 horticultural mineral oil	–	0.03% v/v	Leong *et al.*[28]	Malaysia
Oil	Volk 80O	0.0015%	Abbaszadeh *et al.*[54]	Iran

[a] Includes insecticides that are under experimental use, as well as insecticides that were labeled for use on citrus but are now not recommended/banned.

[b] — denotes that no information is available on the trade name.

[c] Application with petroleum oil 97 + % is recommended for effective control of ACP (Rogers *et al.*[29]).

[d] For aerial application.

Table 4. Insecticides used for soil/trunk applications in citrus for controlling the ACP[a]

Insecticide	Trade name[b]	Method	Rate	Reference	Country
Metasystox-R	–	Trunk drench	0.62 mL L^{-1}	Powell *et al.*[77]	USA
Methidathion	–	Soil drench	0.40 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
Aldicarb	Temik 15 G	Soil incorporation	8.50 g AI tree^{-1}	Powell *et al.*[77]	USA
	Temik 15 G	Soil incorporation (bed side)	1133.60, 2267.20 and 3744.93 g AI acre^{-1}	Qureshi and Stansly[69]	USA
	Temik 15 G	Soil injection	56.68 g tree^{-1}	Childers and Rogers[64]	USA
	Temik 150 G	Soil application	25–75 g tree^{-1}	Belasque *et al.*[38]	Brazil
Oxamyl	Vydate L	Soil drench	1892 and 3785 mL acre^{-1}	Qureshi and Stansly[50]	USA
Clothianidin	Belay 50 WDG	Soil drench	90.68–181.37 g acre^{-1}	Rogers *et al.*[29]	USA
	Dantotsu	Soil drench	0.19 g tree^{-1}	Ichinose *et al.*[55,71]	Vietnam
	–	Trunk injection	1.97 g tree^{-1}	Ichinose *et al.*[71]	Vietnam
Imidacloprid	Admire Pro 4.6 F	Soil drench	414.57 mL acre^{-1}	Setamou *et al.*[79]	USA
	Admire Pro 4.6 F	Soil drench	206.99–413.98 mL acre^{-1}	Rogers *et al.*[29]	USA
	Admire Pro 4.6 F	Soil drench	266.13 mL acre^{-1}	Qureshi and Stansly[50]	USA
	Admire Pro 4.6 F	Soil drench	77.11 g AI acre^{-1}	Qureshi and Stansly[50]	USA
	–	Soil drench	1.92 g AI tree^{-1}	Powell *et al.*[77]	USA
	Admire 2 F	Soil drench	473.12–946.24 mL acre^{-1}	Rogers *et al.*[29]	USA
	Admire 2 F	Soil drench	7.39 mL tree^{-1}	Childers and Rogers[64]	USA
	Alias 2 F	Soil drench	473.12–946.24 mL acre^{-1}	Rogers *et al.*[29]	USA
	Couraze 2 F	Soil drench	473.12–946.24 mL acre^{-1}	Rogers *et al.*[29]	USA
	Nuprid 2 F	Soil drench	473.12–946.24 mL acre^{-1}	Rogers *et al.*[29]	USA
	–	Soil drench	0.20 g tree^{-1}	Ichinose *et al.*[55,71]	Vietnam
	–	Trunk injection	2.00 g tree^{-1}	Ichinose *et al.*[71]	Vietnam
	Confidor	Trunk application	0.15 g AI tree^{-1}	Gatineau *et al.*[70]	Vietnam
	Provado	Soil drench	3.5–15 mL tree^{-1}	Belasque *et al.*[38]	Brazil
Thiamethoxam	Platinum 75 SG	Soil drench	54.11–108.52 mL acre^{-1}	Rogers *et al.*[29]	USA
	Platinum 2 SC	Soil drench	72.57 g AI acre^{-1}	Qureshi and Stansly[50]	USA
	Actara 25 WG	Soil drench	0.17 g tree^{-1}	Ichinose *et al.*[71]	Vietnam
	Actara 25 WG	Soil drench	0.17 g tree^{-1}	Ichinose *et al.*[55]	Vietnam
	Actara 25 WG	Trunk injection	1.67 g tree^{-1}	Ichinose *et al.*[71]	Vietnam
	Actara 250 WG	Soil drench	1.25–3.75 g tree^{-1}	Belasque *et al.*[38]	Brazil
Cyantraniliprole	HGW 20 SC	Soil drench	440–880 mL acre^{-1}	Tiwari and Stelinski[67]	USA

[a] Includes insecticides that are under experimental use, as well as insecticides that were labeled for use on citrus but are now not recommended/banned.
[b] — denotes that no information is available on this trade name in the literature.

effective in controlling ACP and reducing/preventing HLB incidence in the field.[55,70,71,78] For example, in Vietnam, newly planted seedlings in the field were protected by following one of two recommended practices. The first was use of the neonicotinoids imidacloprid (0.2 g plant^{-1}), thiamethoxam (0.17 g plant^{-1}) or clothianidin (0.19 g plant^{-1}) 5–10 days before planting in the grove by drenching of potting soil and making subsequent applications at 60 days interval in the field.[55,71] The second was treating with a reduced rate of imidacloprid (0.15 g plant^{-1}), but applying more frequently (30 days interval) to trunks of newly planted seedlings, which significantly delayed the incidence and spread of HLB.[70] However, overreliance and practice of repeated application of a single insecticide (e.g. imidacloprid) or insecticides of the same or different chemistries with the same mode of action (e.g. neonicotinoids) lead to heavy selection pressure, resulting in insecticide resistance and shortening the effective use period of an insecticide or a group of insecticides. Therefore, developing and implementing proper IRM tactics may prevent or delay insecticide resistance evolution in field populations.

Once the soil-applied insecticides are taken up by plants, they are metabolized to intermediate compounds in due course of time, reducing the lethal concentrations and leading to sublethal effects on target pests. For instance, analysis of imidacloprid titer in leaf tissue samples collected from plants after soil application suggested that a significant control of ACP nymphs can only be expected when imidacloprid titers exceed 0.2 mg kg^{-1} plant tissue weight or 200 ppb.[79] Adverse effects of a sublethal concentration (0.1 mg L^{-1}) of imidacloprid on feeding, biology and reproduction of ACP supported this hypothesis.[81] The degree of manifestation of these adverse effects, however, depended upon imidacloprid concentrations in the treated plants and duration of ACP feeding.[83] Similarly to the negative effects of imidacloprid on pathogen transmission by insect vectors,[82–86] the antifeedant nature of imidacloprid at sublethal concentrations has implications for the spread of HLB, as it interferes with the successful transmission of the pathogen by disrupting or reducing ACP feeding activities at phloem sieve elements.[82,83] A similar reduction in phloem feeding activities of zebra chip disease vector, potato psyllid *Bactericera cockerelli* (Sulc), was observed with imidacloprid.[86]

2.3 Integrated control considerations for insecticides

All these findings suggest that neonicotinoids, being systemic in nature and with long-lasting residual activity, can protect the newly planted seedlings and young trees from ACP infestation and subsequent HLB transmission.[55,70,71,82,83] However, the above conclusion needs to be interpreted cautiously as the outcome of such practice may depend on several aspects, such as ACP population, presence or absence of HLB and severity if present, financial status of grower and prevailing local conditions. Use of systemic insecticides helps in preserving natural enemies, and biological control is considered as a major component of ACP management programs by reducing the number of foliar applications made. Continuous exposure of all life stages of ACP to neonicotinoids with the same mode of action may lead to strong selection pressure for individuals with resistant alleles and subsequent control failures. Therefore, care should be taken not to overuse neonicotioids by alternating them with soil application of insecticides having different chemistries and modes of action, and supplementing with intermittent foliar applications of broad-spectrum insecticides. Overall, a management tactic that consists of combining one foliar application of broad-spectrum insecticide during late winter for suppressing overwintering adults, followed by soil application of labeled systemic insecticides such as aldicarb (banned in Florida), oxamyl and methidathion for reducing immature populations on new flush, is more effective than any other practice.[76]

2.4 Reduced-risk insecticides

By discovering, developing and incorporating pest-insect-specific insecticides, targeted application technology, maximal control of the target species and no or minimal kill of beneficial insects and other natural enemies can be achieved.[87] Reduced-risk insecticides with novel chemistries and modes of action fulfill this requirement. Furthermore, application of reduced-risk insecticides in rotation with broad-spectrum insecticides may reduce the selection pressure for resistant alleles in target pest populations that is imposed by the latter compounds.

The insect growth regulators (IGRs) are known to be highly effective in killing immatures, especially nymphs, of several sucking insect pests, including ACP. Although nymphs are not involved in spread of HLB (nymphs confine themselves to a single tree during their development), controlling them is as important as controlling adults, because pathogen transmission efficiency of infected adults was found to be greater when they acquire the pathogen as nymphs rather than as adults.[24] The majority of the data on efficacy of IGRs against ACP come from laboratory studies,[88,89] while few studies evaluated their efficacy in the field.[50,54,56] Three IGRs, pyriproxyfen (juvenile hormone mimic), buprofezin and diflubenzuron (both are chitin synthesis inhibitors), showed promising ovicidal and nymphicidal activities against ACP, as well as adverse effects on reproduction (both fecundity and egg viability) and morphology of adults emerging from treated older nymphs.[88,89] Under field conditions, the protection offered by IGRs, (diflubenzuron, flufenoxuron, lufenuron, novaluron and pyriproxyfen) ranged from 3 days (diflubenzuron) to 4–6 weeks (lufenuron and flufenoxuron).[50,54,56,65] Given the potential of IGRs to reduce adult fecundity and control immatures, IGRs are an important and promising rotational tool in insecticide resistance management (IRM) programs for ACP.

Greenhouse and field studies have shown that the spread of plant pathogens by insect vectors continues even at low density levels.[51,55,70,71,90] Application of insecticides with antifeedant activity or rotating broad-spectrum insecticides with antifeedants such as pymetrozine and imidacloprid[83,90] may further reduce HLB transmission by low numbers of ACP present in the field after insecticidal intervention. The continuous feeding required by ACP adults for disease transmission (30 min–7 h)[21,22,91] could be disrupted with antifeedants. In support of this idea, pymetrozine applied at the labeled rate significantly reduced the feeding activities of adults on treated plants or directly treated adults, as measured by electrical penetration graphs (EPGs).[90] This effect resulted in reduced disease transmission by ACP adults feeding on treated plants compared with controls.[90]

Other groups of reduced-risk insecticides that have potential for ACP control are oils, botanical insecticides such as neem-based formulations, sucrose octanoate (α-D-glucopyranoside, β-D-fructofuranosyl octanoate) and organosilicone spray adjuvants.[28,52,92] All these are effective against nymphs, while oils have the additional effect of repelling adults from new flush, thereby reducing oviposition. Neem-based pesticides exhibited toxicity to both nymphs and adults in laboratory studies, but the protection offered by neem seems to be short lived (<5 days) in the field.[56,93] Foliar sprays with sucrose octanoate, a synthetic analog of natural sugar esters produced in foliar trichomes of wild tobacco, *Nicotiana gossei* Domin, offered effective protection from both nymphs (4 weeks) and adults (1.5 weeks) of ACP in mandarin orange (Ortanique tangor).[92]

HMOs, petroleum oils and other oils have been used successfully to provide protection from ACP in the field that was comparable or even better than conventional insecticides.[28,54,56,94] For instance, in Malaysia, foliar application of nC_{24} HMOs (0.35% v/v) at weekly intervals to mature honey mandarin trees caused significant reductions in the incidence and spread of HLB, which was even better than broad-spectrum insecticide applications (only 11.2% diseased plants versus 22.7% in triozophos/cypermethrin/chlorpyrifos and 42.2% in untreated). The significant effect of HMOs on HLB containment was the result of significant reductions in the percentage of new flush infested with adults, and reduced extent of oviposition and number of adults per new flush, compared with controls.[28] Attempts were also made to use Silwet L-77™ and Kinetic™ alone, two spray adjuvants usually mixed with conventional insecticides for ACP control. These compounds exhibited significant nymphicidal activity at the labeled rate (500 mg L^{-1}) under laboratory, greenhouse and field conditions.[52] However, non-registration of these spray adjuvants as pesticides precludes their sole use for ACP control in the field.[52]

2.5 Role of insecticides in reducing HLB transmission

The main objective behind insecticide applications is to reduce the numbers of ACP that can transmit the HLB pathogen. Several studies have documented that application of insecticides, mainly with systemic action, significantly reduced the transmission of plant viruses by vectors.[84,85] This has been proved with ACP as well.[28,70,71] ACP, like any other phloem feeder, reaches these tissues by inserting its mouth parts into the plant and releasing saliva (salivation), followed by ingestion of phloem sap. HLB pathogens grow and multiply in phloem tissues of the plant.[95] During the processes of salivation and sap ingestion at phloem tissues, inoculation and acquisition of HLB bacteria, respectively, occur, depending on whether the plant is infected or not. Thus, for successful reduction in HLB spread by ACP, insecticide applications must serve at least one of two purposes. It should kill adults quickly upon contact or ingestion of insecticide before initiation of feeding activities or it must interfere with the salivation and ingestion processes taking place at phloem tissues and significantly reduce them so that acquisition or inoculation of pathogens is prevented or minimized.

Using an EPG technique, Serikawa *et al.*[82,83] determined the role of three insecticides belonging to different chemistries and modes of action in preventing transmission of HLB pathogen to healthy plants by infected ACP. Of these, fenpropathrin with its quick knockdown effect was found to be the most promising in preventing disease transmission by ACP. It killed all the infected adults within 8 min after contacting the treated plant and well before adults made any feeding attempts. Imidacloprid, on the other hand, did not exhibit such quick kill, but all adults feeding on treated plants died (in 4 h) well before the end of the observation period (12 h).[83] Imidacloprid significantly reduced the salivation and sap ingestion periods compared with those on untreated plants (controls), suggesting its potential for preventing HLB pathogen transmission.[83] In contrast to the findings with fenpropathrin and imidacloprid, adults feeding on aldicarb-treated plants were still alive at the completion of the 12 h observation period. Moreover, phloem feeding activities of adults on aldicarb-treated plants were comparable with those of adults on untreated plants, indicating poor performance of aldicarb in preventing HLB pathogen transmission.[82]

Insecticides that exhibit greater scope for preventing pathogen transmission by ACP [which kill ACP adults within minutes or hours (4 h), before they are able to acquire and/or transmit the HLB bacteria], such as fenpropathrin and imidacloprid, are highly useful in HLB management. Nevertheless, even if vectors acquire pathogens, the latent period and nature of pathogen status in a vector, i.e. persistent/propagative (once the vector acquires the pathogen it can transmit the pathogen throughout its life) versus non-persistent/non-propagative (transmission of pathogen by the vector is transitory or lasts for a shorter period), can impact upon the role of insecticides in reducing transmission by vectors. For instance, infected ACP may be killed following exposure to insecticides before the completion of the latent period (7–25 day 'waiting' period during which transmission does not occur). Similarly, *Ca*Las being propagative in ACP (infected ACP has the potential to transmit pathogen throughout its lifespan),[24,95] insecticides that kill the infected ACP contribute to transmission reduction. Also, by spraying diseased trees with insecticides having quick knockdown action (e.g. fenpropathrin) prior to removal,[29] both inoculum source and number of infected ACP that could potentially transmit the pathogen are reduced.

2.6 Factors affecting the susceptibility of ACP to insecticides

Apart from several abiotic and biotic factors mentioned earlier in this review, factors such as spray droplet size, discharge rate (mg AI min^{-1}), insect life stage, pathogen infection, resistance status (activity levels of detoxifying enzymes and composition of target site proteins) and environmental conditions such as temperature may influence the susceptibility of ACP to insecticides and in turn the efficacy of insecticide applications in the field.

2.6.1 *Spray droplet size and discharge rate*

Studies conducted under laboratory conditions demonstrated that foliar application of fenpropathrin at smaller droplet sizes (40.0–50.0 μm diameter) provided better control of all life stages of ACP than at larger droplet sizes (174.0–265.0 μm diameter).[96] However, there are problems of potential spray drift at smaller droplet sizes and spray run-off at larger droplet sizes. Therefore, application of insecticides in general, and fenpropathrin specifically, at high volume using air-blast applicators at 100–150 μm droplet sizes may result in effective control of ACP in the field.[96]

2.6.2 *Temperature*

Environmental conditions, such as prevailing temperature in the field, play an important role in the efficacy of insecticides and in turn effective management of ACP, because insecticide toxicity to a target insect pest varies with post-exposure temperature.[97,98] For example, in a laboratory study, the toxicity of 11 insecticides belonging to different chemistries and modes of action against ACP adults varied with post-exposure temperature.[98] Based on these findings, it can be suggested that application of insecticides belonging to the SP class, such as *zeta*-cypermethrin, fenpropathrin and lambda-cyhalothrin, in winter months, when the temperatures are low, maximizes insecticidal activity and may result in effective management of ACP. On the other hand, application of insecticides belonging to the OP and carbamate classes, such as chlorpyrifos, dimethoate and carbaryl, in summer months, when the temperatures are high, should provide greater control of ACP.[98]

2.6.3 *Insect life stage and body colour*

Insecticide susceptibility is known to vary among different life stages and among different body (abdomen) color morphs of the same life stage of ACP and cotton whitefly, *Bemisia tabaci* (Gennadius), owing to differences in feeding behavior, total protein content and activity levels of detoxifying enzymes.[99–101] Although experiments with nymphs and adults are not directly comparable owing to differences in endpoint observation, in general, ACP nymphs were more susceptible to pymetrozine than adults on potted plants sprayed with pymetrozine at the labeled rate (52 mg L^{-1}).[90] Eggs of ACP were more susceptible to buprofezin and diflubenzuron than nymphs,[89] while fourth-instar nymphs were less susceptible to carbaryl, chlorpyrifos, fenpropathrin and spinetoram than adults.[102] The higher activity levels of three detoxifying enzyme families, general esterases (GEs), glutathione *S*-transferases (GSTs) and cytochrome P450 monoxygenases [mixed-function oxidases (MFOs)], in fourth instars compared with adults was a possible reason for the reduced susceptibility.[102] However, the different bioassay conditions of adults to insecticide compared with nymphs in these studies may have accounted for some of the differences observed between these life stages. Adults with orange/yellow abdomen color were more susceptible to chlorpyrifos, fenpropathrin and imidacloprid than adults with blue/green and grey/brown color, probably owing to the lower expression of five *CYP4* family genes in the former color morph than in the latter color morphs.[101]

2.6.4 *Pathogen infection status*

Biotic factors, such as microbial infection (bacterial, fungal, viral and rickettsial) tend to change insecticide susceptibility of insects.[103–106] Physiological changes taking place after microbial infection that compromise the host fitness are thought to be the reason.[105] In a laboratory study, it was shown that infection of ACP adults with *Ca*Las increased their insecticide susceptibility by 1.2–3.1-fold compared with uninfected adults.[103] This increase in susceptibility was attributed to significant reductions in both total protein content and activity level of GEs compared with uninfected adults.[103] Significant reductions in activity levels of two other insecticide-detoxifying enzymes, GSTs and MFOs, in *Ca*Las-infected adults compared with uninfected adults further supported the above conclusion.[107] A subsequent study on gene expression profiles of MFOs belonging to the *CYP4* family in *Ca*Las-infected and uninfected sexes suggested that

*Ca*Las-infected males were major contributors to this increased susceptibility, with significantly lower expression of four of five *CYP* genes studied than their uninfected counterparts.[104]

2.7 Insecticide resistance and its management

Establishment of baseline susceptibility data for field populations of ACP to insecticides is an important initial step for monitoring the evolution of insecticide resistance. Monitoring the onset and progress of resistance evolution in field populations is also essential for implementing IRM tactics. Comparison of insecticide susceptibility data (LD_{50} values) between a laboratory susceptible colony and field populations of ACP collected from Florida in 2009 and 2010 suggested varying levels of decreased susceptibility of adults and fourth-instar nymphs to 14 and five synthetic insecticides respectively, belonging to OP, carbamate, SP, neonicotinoid and macrocyclic lactone (avermectin and spinosyns) classes.[102] In general, field populations of adults and nymphs showed a modest level of decrease (typically less than fivefold) in susceptibility to most of the insecticides tested during the first year of resistance monitoring, with greatest resistance in adults observed to chlorpyrifos (12-fold at Lake Alfred, Florida) and imidacloprid (15-fold at Groveland, Florida). Studies conducted in subsequent years (2010 and 2011) at various diagnostic doses (LD_{95}) of a laboratory susceptible colony suggested a further significant decrease in susceptibility of some field populations to chlorpyrifos, carbaryl, bifenthrin, fenpropathrin, imidacloprid, thiamethoxam and spinetoram.[108]

Elevated levels in one or more of the three main detoxifying enzymes in nymphs and adults from field-collected populations may be responsible for decreased susceptibility to several of the tested insecticides currently used in the field.[107,108] For instance, a 48 h exposure to imidacloprid (0.2–1.0 mg L^{-1}) induced the expression of five *CYP* genes in uninfected mixed-sex adults, and the degree of induction varied with exposure concentration and *CYP* gene involved.[104] In addition, reduced activity of acetylcholinesterase (AChE) enzyme, as well as reduced sensitivity of AChE to inhibition (2–4-fold) by selected OP and carbamate insecticides in adults from field-collected populations also supported the observed resistance in field populations to OP and carbamate insecticides.[109]

These findings indicate that field populations in Florida are being subjected to intense selection pressure and evolving resistance to some insecticides. By implementing proper IRM tactics, evolution of insecticide resistance in field populations can be slowed or delayed, which is essential for sustained use of insecticides for ACP management. Some simple IRM tactics (specific examples presented in previous sections) that can be implemented for this purpose are (1) developing and evaluating citrus flush-based insecticide rotation modules with low-volume spray technology that includes insecticides from different classes of chemistry and modes of action, and (2) optimizing the use of binary insecticide mixtures (either tank mix or commercial formulation) having different target sites and/or modes of action using low-volume spray technology. Foliar spray modules designed with the above tactics in mind are more sustainable, and mixtures are generally considered a short-term IRM strategy, with rotations preferred.[110] Some studies recommended need-based applications of foliar insecticides (peak populations corresponding to flush periods)[45,48,53] while others recommended calendar-based applications at regular intervals.[28,55] However, the best choice for protecting trees from HLB transmission by ACP is to select insecticides or mixtures of different chemistries and modes of action that show higher efficacy and long-lasting residual activity, and to rotate/alternate them for maximizing the efficiency of insecticide applications and reducing the potential for insecticide resistance. Clearly, there is an urgent need for developing more effective and sophisticated IRM strategies for tackling the problem of resistance development in ACP control. Along these lines, an IRM program for ACP was recently announced by the Insecticide Resistance Action Committee (IRAC)[110] with integrated recommendations for rotations of modes of action, taking into account the different life stages of the ACP, time of the growing season, etc.

2.8 Compatibility of insecticides with biological control agents of ACP

Although both biological and chemical control strategies are important components of an IPM program for ACP, once the HLB disease incidence occurs and/or becomes widespread in an area, the chemical control option has advantages over the biological control option and should be adopted as the main vector and disease management option.[1,25] Synthetic insecticides, owing to their quick mode of action, rapidly kill the target insect upon contact/feeding and very quickly reduce ACP populations capable of transmitting HLB, whereas biocontrol agents, owing to their slow action, may not be effective in preventing HLB spread. In addition, reduced-risk insecticides with systemic action (applied to the soil) help in preserving the diversity of biological control agents and maintain ecological stability.[69,70]

In a laboratory study, various foliar-applied broad-spectrum insecticides exhibited mixed toxicity effects to an important biological control agent of ACP, *Tamarixia radiata* (Waterston).[111] Chlorpyrifos, carbaryl and fenpropathrin exhibited significant acute and residual toxicity to adults of *T. radiata*, even at rates lower than the labeled rates. Although abamectin, fenpyroximate and spirotetramat exhibited significant acute toxicity to *T. radiata* adults, they showed no residual toxicity to *T. radiata*. Imidacloprid was acutely toxic, but its residual toxicity depended on environmental conditions; i.e. high rainfall and cooler temperatures reduced the toxicity to *T. radiata* adults.[111] Diflubenzuron, sucrose octanoate, Silwet L-77 and petroleum oil 435 are safe to *T. radiata* adults and the predator complex of citrus insect pests, making them a favorable alternative to broad-spectrum insecticides as well as an important component of IPM for ACP.[87,94,111]

3 CHEMICAL CONTROL OF HLB BACTERIA

HLB is often associated with three species of gram-negative bacteria belonging to the α-proteobacteria class (*Candidatus* Liberibacter spp.), which harbor and multiply in plant phloem tissues, leading to plugging of the phloem sieve tubes.[5,112] Of the three species of *Candidatus* Liberibacter, *Ca*Las has greater geographical presence, ranging from the Asian continent to the Americas.[5]

3.1 Trunk-applied/injected antibiotics and budwood/root soaking in antibiotic solution

Among the few management options available for temporary cure of the HLB disease (controlling the disease and/or reducing the severity of symptoms), the use of antibiotics is receiving renewed interest. Antibiotics are administered either by trunk injection[113–115] or by dipping/soaking of budwood or plant roots in antibiotic solution.[112,116–119] Budwood immersed in tetracycline (1000.0 mg L^{-1} for 2 h or 500.0 mg L^{-1} for 3 h) solution yielded good results.[118] Tetracycline hydrochloride is the most commercially applied therapeutic treatment for HLB control. This antibiotic is not bactericidal but bacteriostatic, necessitating frequent applications at regular intervals (annual basis) for continuous suppression of HLB pathogen.[113] The frequent application of tetracycline makes it less cost effective and increases the chances of the pathogen becoming antibiotic resistant.[113] In addition, repeated trunk injections of tetracycline led to phytoxicity in injected citrus trees.[113,115]

Although both methods of treatment (trunk injections and budwood soaking) with tetracycline resulted in temporary relief of HLB symptoms (1.0–1.5 years),[113,115,118,119] trunk injections provided the best results in Taiwan, China, Reunion, the Philippines, South Africa and India.[113,115] One report claimed budwood treated by dipping in 500.0 mg L^{-1} of ledermycin/penicillin combined with 500.0 mg L^{-1} of carbendazim for 1 h resulted in complete control of HLB disease.[119] When tested in different seasons, tree trunks injected with antibiotics during spring season provided the best results.[115]

Recent attempts to evaluate the potential of several new antibiotic compounds in suppressing HLB bacterial titers and symptoms yielded some promising findings.[112,116,117] Among various antibiotics evaluated, penicillin G sodium salt (50.0 mg L^{-1}), oxytetracycline (100.0 mg L^{-1}) and a combination product, penicillin G potassium salt + streptomycin (P + S) (100.0 mg L^{-1} + 10.0 mg L^{-1}), were found to be highly effective in suppressing or eliminating HLB bacterial titers in various test plant systems, and the disease suppression lasted for 3–14 months.[112,116,117] Soaking *Ca*Las-infected periwinkle stem cuttings in the above solutions for 4 h and subsequent application of the same solution to potting soil by drenching at 7 and 14 days post-transplanting completely suppressed HLB bacterial titers in the rooted and regenerated cuttings (below detectable level by qPCR, i.e <500 cells g^{-1} plant tissue).[117] The P + S combination treatment also completely eliminated the HLB bacterial titers in lime budwood by overnight soaking, in two-year-old grape fruit plants by root soaking for 4 h and in six-year-old citrus plants by trunk injection. Compared with periwinkle stems, however, higher concentrations of antibiotics were required for suppressing HLB symptoms in budwood and citrus tress, which ranged from ten- to 5000-fold, indicating that the higher the citrus plant age, the higher the antibiotic concentration required.[112,116,117] Another advantage of P + S combination is that disease suppression can be obtained by spray application. For instance, three foliar applications made at 1 week intervals were sufficient to suppress the disease in infected periwinkle plants completely.[117] The antibiotics kasugamycin (an aminoglycoside antibiotic at 1000 mg L^{-1}) and metronidazole (a nitroimidazole antibiotic at 100 mg L^{-1}) were not effective in suppressing HLB in citrus budwood, while 2,2-dibromo-3-nitrilopropionamide (DBNPA) (20% solution) (a fast-acting and broad-spectrum biocide at 100 or 200 $\mu L\ L^{-1}$) was effective in suppressing the disease, but not completely in any of the test plant systems.[112,116,117]

In addition to suppressing HLB symptoms, there are additional advantages of using antibiotics. Antibiotics significantly increased the percentage of regeneration and biomass of regenerated cuttings, which are highly useful when rescuing and conserving citrus germplasm, as *Ca*Las infection severely affects the regeneration of plant material.[112,117] Thus, it is possible to rescue *Ca*Las-infected rare germplasm and valuable breeding materials using these antibiotics. For example, treating infected budwood from four citrus germplasms (Campbell Valencia, Kona, Cleopatra Mandarin and Sugiyama) with the P + S combination resulted in complete suppression/elimination of HLB symptoms in survived budwood.[116] Although these compounds exhibited significant anti-*Ca*Las activity, as of this writing, their use as HLB control agents has not been approved by the United States Environmental Protection Agency. Approval for use of these compounds for HLB management may be expedited considering the severity of HLB in the state of Florida and elsewhere. When approved for use, care must be taken to use them in such a way that provides maximum suppression of HLB symptoms in plants with no phytotoxicity.

Recently, attempts were made to discover *Ca*Las antimicrobial compounds that target a novel site, the SecA protein, and inhibit its ATPase activity. The SecA protein is involved in preprotein translocation from cytosol into or across the cytoplasmic membrane of bacteria.[120] In one study, out of >5000 compounds screened, 17 exhibited a significant inhibition of *Ca*Las SecA ATPase activity ($>50\%$) at 20.0 μM.[120] In another study, five small molecules out of 20 000 screened exhibited $>50\%$ ATPase activity inhibition at nM concentrations.[121] As these findings represent the initial stages of development of these compounds, it might take considerable time before they are available for use in the field. Overall, rescreening some of the existing antibiotics used for curing other plant diseases may shed light on promising antibacterial compounds for treating infected budwood and young trees.

4 CONCLUSIONS AND FUTURE DIRECTIONS

The HLB and its insect vector, ACP, are well established in almost all citrus-growing regions of the world. As there is no permanent cure for HLB, application of insecticides for keeping the ACP populations at low levels is one of the most promising management options for minimizing incidence and spread of HLB. This tactic, when combined with other practices (planting disease-free material and removal of symptomatic trees), greatly reduces the incidence and spread of HLB. Depending upon the pest status and plant age, intervention with insecticide applications could be need or calendar based.

Reduced susceptibility in field populations of ACP to a few insecticides in Florida signals an impending problem of insecticide resistance in ACP. The immediate need is to develop and implement effective IRM strategies, as recently suggested by the IRAC.[110] Monitoring ACP field populations for insecticide resistance should be continued in the future for determining the impact of control strategies on resistance evolution. For this, we need to develop simple and accurate methods for detecting resistance and its mechanisms (conferred by target-site insensitivity and detoxifying enzymes) in individual insects from representative populations. Future studies on resistance should be focused on (1) molecular-based methods for detecting alterations at gene level, i.e. point mutations, gene amplifications, gene duplications, etc., and (2) the genetics of resistance and its evolution in field populations. In addition, resistant colonies should be developed to determine the stability of resistance, patterns of cross-resistance, genetic nature and mode of inheritance and fitness costs.

An ACP management approach that is gaining momentum among Florida and Texas citrus growers is area-wide management of ACP by establishing Citrus Health Management Areas (CHMAs) as per the recommendation made by National Research Council (NRC) Committee on strategic planning for the Florida citrus industry,[8] wherein all the citrus growers in an area (10 000–50 000 acres) take up spray applications in a coordinated way (at least within a week).[74,75,122] By following such a coordinated approach, recolonization of ACP in treated groves within few days of application from neighboring untreated groves can be minimized, leading to a reduced number of insecticide applications made in a year.[122] Anecdotal reports suggest that such an area-wide management approach significantly reduced the ACP numbers in the entire sprayed area.[123] Furthermore, this approach reduced the movement of ACP from abandoned and less-managed groves into well-managed groves, which is otherwise thought to be very high.[123] Maintaining CHMAs by practicing area-wide management of ACP with low-volume ground/aerial mist applications should be exploited to the fullest, as they have a significant impact on overall management of ACP and HLB and result in a significant reduction in management costs. Additional studies are needed for quantifying the reduction in incidence and spread of HLB following an insecticide or a series of insecticide applications, in order to establish how insecticide applications can be optimized for managing this pathogen–host system.

The only major chemical tool currently available for direct HLB pathogen management is the use of antibiotics. The recent interest in evaluating new antibiotics for suppressing/eliminating HLB symptoms by reducing the bacterial titers yielded some promising candidate compounds. As some of the antibiotics exhibited phytotoxicity after a single or multiple uses, further research needs to be conducted to determine whether repeated applications of these antibiotics result in phytotoxicity. Studies should determine the effective dose of antibiotics required for curing HLB infection in trees of different age and size, if possible. It will be interesting to see whether suppression of HLB symptoms is reversible or irreversible, and if they are reversible then the frequency of applications for effective cure needs to be determined. Also, more studies need to be conducted for discovering and screening novel compounds with antibiotic and antimicrobial activity that will have potential impact on HLB.

A management strategy that includes generating transgenic trees resistant to feeding from ACP and infection of HLB pathogen (NRC recommendation, please see NRC 2010[8] for complete list of recommendations for ACP and HLB management), as well as application of insecticides for ACP population and antibiotics for HLB symptom suppression, is a viable and sustainable solution for the HLB crisis in citrus. Demonstrating progress in this direction, scientists from Texas A&M University have inserted two genes coding for defensin proteins from spinach into citrus plants in order to confer resistance to HLB.[124] Currently, field trials are under way to see how these transgenic plants perform to the challenge posed by HLB under field conditions. Other potential strategies proposed by the NRC Committee for ACP control are the sterile male technique (or transgenic ACP incapable of transmitting *Ca*Las) and RNA-interference-based methods. As all the above-proposed strategies are in the initial stages of development and may take considerable time before they can be used, chemical control with insecticides and antibiotics combined with other practices of reducing HLB inoculum is the only major viable option that continues to play a vital role in managing ACP and HLB, now and in the near future.

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