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REVIEW ARTICLE

Status of acaricide resistance in major phytophagous mites infesting horticultural crops - mechanisms and management: A review

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Abstract

The persistent presence of mite pests poses an ongoing challenge to the sustainable cultivation of numerous economically important crops on a global scale. Acaricide application stands as a key element in management practices to date. Unfortunately, the relentless use of acaricides in the field has led to the development of resistance among mite populations to several acaricidal compounds. The advancement and application of reverse genetic tools, such as RNAi, have provided valuable insights into the molecular genetic mechanisms underlying resistance, particularly in model species like *Tetranychus urticae*, although such understanding remains limited in many agriculturally important phytophagous mite pests. This review emphasizes the status of acaricide resistance of major phytophagous mites, shedding light on the physiological and intricate molecular mechanisms underlying this phenomenon, while RNAi represents a promising research tool for studying gene function and resistance mechanisms in mites, its practical application in overcoming acaricide resistance remains in the experimental phase due to delivery challenges and species-specific variability in response.

Keywords: acaricides; phytophagous mites; resistance mechanisms and RNAi

Introduction

Mites, classified under the class *Arachnida*, subphylum *Chelicerata* and phylum *Arthropoda*, represent the second-largest arthropod group, following insects. As one of the earliest branches of arthropods, chelicerates include a diverse range of organisms such as horseshoe crabs, scorpions, ticks, spiders and mites (1, 2). Currently, around 40000 mite species have been documented, yet estimates suggest that an additional 0.5 to 1 million species remain undiscovered (3). Mites demonstrate remarkable adaptability, enabling them to occupy various terrestrial habitats; however, their ecological distribution and niche diversity are more limited compared to the extensive habitat range and specialization observed in insects (4). In agriculture and horticulture, mites are among the most damaging pests, posing a serious threat to crop yields and stored products.

Among the various mite families, Eriophyidae, Tenuipalpidae, Tarsonemidae and Tetranychidae are notorious for their phytophagous nature, feeding on plant tissues and causing significant economic losses (5). The family Eriophyidae consists of gall-forming and vagrant mites, which either induce abnormal plant growth or freely move across plant surfaces (6). Several species, such as *Aculus cornutus* and *Aceria mangiferae*, cause considerable damage in orchards and vineyards, while *Aculops lycopersici* is a major concern in greenhouse cultivation (5, 7). Tenuipalpidae, predominantly found in tropical and subtropical regions, includes approximately 1,100 species distributed across 38 genera (8). Among them, *Tenuipalpus* and *Brevipalpus* are the most agriculturally significant, with species like *Brevipalpus phoenicis, B. californicus and B. obovatus* infesting a wide range of host plants: 928 species spanning 139 families (5). The family *Tarsonemidae* comprises around 530 species classified into 40 genera, many of which act as serious plant pests. Key species include *Polyphagotarsonemus latus* (chilli mite), *Phytonemus pallidus* (cyclamen mite), *Steneotarsonemus ananas* (pineapple mite), *S. bancrofti* (sugarcane mite) and *S. spinki* (rice mite), all of which have been linked to substantial agricultural damage (5). Among phytophagous mites, *Tetranychidae* is the most destructive

family, infesting ornamental plants, greenhouse crops and trees. Of the 1250 species identified, around 100 have been recognized as economically significant pests (10). Key species include *Tetranychus urticae, T. kanzawai, T. pacificus, Panonychus ulmi, P. citri, Oligonychus coffeae, O. punicae and Eutetranychus orientalis*. Within this group, *Panonychus citri, T. urticae and Panonychus ulmi* are particularly notorious for their devastating impact on global agriculture (10).

T. urticae is a generalist feeder, that infests approximately 1400 plant species from over 250 plant families including various horticultural and ornamental crops, particularly in greenhouse crops (9,10,11) such as tomatoes, hops, grapes, apples, strawberries, almonds, corn, cotton, ornamental crops and greenhouse vegetables (12-22). *T. urticae* has developed resistance to several acaricides, including abamectin (23), spiromesifen (24), chlorpyriphos (25), clofentezine, hexythiazox, etoxazole (26), cyenopyrafen, pyridaben (27), fenpyroximate (28) and spirodiclofen (29).

P. citri is an important cosmopolitan citrus pest (30, 31) which has been identified to be sustained in more than 111 different plant species, including broadleaf ornamentals, grapevine, almond, pear, palm and evergreen shrubs (17, 32, 33). The mite has developed resistance to pyridaben (34), spirodiclofen (35), fenpropathrin and abamectin (36). *P. ulmi* poses a threat to a wide range of crops and ornamental plants, including rose, elm, black locust, lilac, hawthorn, privet, chestnut, alder buckthorn as well as apple, plum, pear and cherry (37), has developed resistance to abamectin (38), spirodiclofen (39), chlorpyriphos, lambda-cyhalothrin (40), fenazaquin and tebufenpyrad (41).

Farmers predominantly rely on acaricides for mite management; however, their indiscriminate and prolonged use has led to the development of resistance in both open-field and polyhouse conditions. The first documented case of mite resistance to synthetic insecticides emerged in the 20th century when organophosphates failed to provide effective control on a global scale (42). Due to the widespread and intensive application of acaricides, several mite species have evolved resistance to a broad spectrum of chemical compounds worldwide (43, 44). Resistance mechanisms are often associated with the upregulation or structural modification of key detoxification enzymes, including glutathione-S-transferases (45), P450 monooxygenases (46) and esterases (47). These biochemical alterations enhance the sequestration or metabolism of acaricides, preventing them from reaching their intended target sites and thereby reducing their efficacy. *T. urticae*, *P. ulmi* and *P. citri* were selected as examples due to the high number of reported resistance cases across various acaricide classes. Their widespread occurrence, economic impact and ability to develop resistance rapidly make them key species for understanding acaricide resistance trends and management strategies.

The *T. urticae* genome, the first complete chelicerate genome, has expanded our understanding of arthropod genomics beyond Pancrustacea. The gene families responsible for detoxification, digestion and transport of xenobiotics are discovered in the complete genome of *T. urticae,* which is vital in resistance (48). However, resistance is complex, species-specific and not yet fully understood, particularly in non-model mites, making the effective management and overcoming of acaricide resistance an ongoing challenge. This review explores the present situation of acaricide resistance in key phytophagous mite species, mechanisms underlying the resistance (both physiological and biological) and management strategies aimed at mitigating acaricide resistance in phytophagous mites.

Acaricides used in phytophagous mites' management

Table 1 provides an overview of acaricides used for the management of mites, grouped based on their leading site of action and chemical (sub)-group, based on the IRAC Mode of Action classification (http://www.iraconline.org). (49), (IRAC, 2022)

Status of acaricide resistance problem in major phytophagous mites

Two-spotted spider mite - *Tetranychus urticae*

The arthropod pesticide resistance database has reported 558 resistant cases of *T. urticae* to 96 compounds globally, including some novel acaricide molecules like fenazaquin, propargite, spiromesifen, fenpropathrin, diafenthiuron and chlorfenapyr in around 263 sites (ARPD). Due to its diminutive size, rapid reproduction rate, short life cycle, high egg-laying capacity and unique reproductive strategy of arrhenotokous parthenogenesis, this species can easily develop resistance to acaricides (50). Some authors have extensively documented various molecular-level resistance mechanisms in *T. urticae* in their recent studies (51-54).

T. urticae populations in brinjal-growing areas of Punjab exhibited a decreasing trend in resistance to fenazaquin, with fold resistance values of 55.55, 30.00, 7.14 and 4.95 reported for the years 2010, 2011, 2013 and 2014, respectively (55). The field population of *T. urticae* displayed the highest resistance to fenazaquin, with a resistance ratio of 1320 in tomato and 1356 in field bean, while it exhibited the highest resistance ratio of 3822 to abamectin in greenhouse rose (56). Korean population of *T. urticae* showed a high degree of sensitivity to fenpyroximate (RR = 78), pyridaben (RR = 182) and dicofol (RR = 82) (57), while acaricides like propargite, fenpropathrin and azocyclotin showed the lowest resistant ratio in the range of 9-5 fold.

Fenazaquin demonstrated significant toxicity to *T. urticae*, with resistance ratios ranging from 2 to 8.62-fold based on a low baseline LC_{50} of 0.11 ppm, indicating emerging resistance. Resistance to fenpropathrin (1.86 to 37.28-fold, baseline LC_{50}: 0.12 ppm) and diafenthiuron (15.81 to 50.53-fold, baseline LC_{50}: 0.22 ppm) was observed at low to moderate and moderate to high levels, respectively. In contrast, high levels of resistance were recorded for propargite (45.16 to 65.10-fold, baseline LC_{50}: 0.91 ppm) and chlorfenapyr (54.67 to 100.14-fold, baseline LC_{50}: 0.15 ppm). Extremely high resistance was observed for buprofezin (377.97 to 514.44-fold, baseline LC_{50}: 5.17 ppm) and spiromesifen (193.04 to 452.61-fold, baseline LC_{50}: 2.00 ppm) (58). In some situations, *T. urticae* exhibited instability in resistance to spirodiclofen or spiromesifen (24, 59).

Citrus red mite - *Panonychus citri*

Resistance to acaricides in *P. citri* has been reported in various countries, including the United States, Japan, China, Taiwan, New Zealand and Turkey (60-62). The laboratory population of

Table 1. Acaricides are used to control phytophagous mites

Group	Sub-group	Active ingredient	Mode of action
1	1A - Carbamates	Formetanate, Methomyl, Aldicarb	Acetylcholinesterase inhibitor
	1B - Organophosphates	Chlorpyrifos, Diazinon, Dimethoate, Ethion, Malathion, Phosmet, Quinalphos	
3	3A - Pyrethroids and pyrethrins	Bifenthrin, Fenpropathrin, Cypermethrin, Lambda-cyhalothrin, Tau-fluvalinate, Dicofol	Sodium channel modulators
6	Avermectins, Milbemycins	Abamectin, Emamectin benzoate, Milbemectin	Chloride channel activator
10	10A - Clofentezine, Hexythiazox	Clofentezine, Hexythiazox, Etoxazole	Mite growth inhibitors
12	12A - Diafenthiuron, 12B - Organotin miticides 12C - Propargite 12D - Tetradifon	Diafenthiuron Fenbutatin oxide Propargite Tetradifon	Inhibitors of mitochondrial ATP synthase
13	Chlorfenapyr	Chlorfenapyr	Uncoupler of oxidative phosphorylation through the disruption of the proton gradient
15	Benzoylureas	Flufenoxuron	Chitin synthesis inhibitor
19	Amitraz	Amitraz	Octopamine receptor agonists
20	20B - Acequinocyl 20C - Fluacrypyrim	Acequinocyl Fluacrypyrim	Mitochondrial complex III electron transport inhibitors
21	21A - MET-I acaricides	Fenazaquin, Tebufenpyrad, Pyridaben, Fenpyroximate, Pyrimidifen	Mitochondrial complex I electron transport inhibitors
	21B - MET-II acaricides	Cyflumetofen, Cyenopyrafen, Pyflubumide	Mitochondrial complex II electron transport inhibitors
23	Tetronic and tetramic acid derivatives	Spirodiclofen, spiromesifen	Inhibitors of acetyl Coenzyme A carboxylase
Compounds of unknown	Dicofol	Dicofol	Uncertain mode of action
25	Mitochondrial Complex II Inhibitors	Bifenazate	Selective mitochondrial complex II electron transport inhibitor

P. citri in Japan displayed 2300-fold resistance to hexythiazox after treatment for three generations (63). Bifenazate and acequinocyl a frequently used acaricide in Japan, Spain and Belgium have encountered high resistance with the LC50 value of >10000 mg/L and 610 mg/L respectively in *P. citri* (64).

P. citri exhibited substantial resistance to spirotetramat, with resistance levels reaching 1668.4-fold in larvae and 135.6-fold in eggs, indicating a higher resistance potential compared to other acaricides such as hexythiazox and spirodiclofen (65), coinciding with the observations of (66), who documented an LC50 value of 418 mg L-1 for a Chinese population of *P. citri.* Further, under laboratory conditions, (67) reported that *P. citri* developed spirodiclofen resistance after 42 generations of selection, with an LC_{50} value of 422 mg/L, representing a 103-fold increase over the susceptible strain. Additionally, (65) observed that the Yakapinar population of *P. citri* exhibited an LC_{50} value of 2279.7 mg/L for pyridaben, indicating a 75.06-fold resistance ratio compared to the laboratory-susceptible population.

European red mite - *Panonychus ulmi*

Intense pressure from acaricide use on European red mite and other mite species has resulted in the development of resistance to various acaricides widely accessible in the market (39, 68, 69). *P. ulmi* resistance to parathion was first reported in 1951 by (70).

A laboratory-selected population of *P. ulmi* shows a significant resistance exceeding 2000-fold at the LC50 to clofentazine in apple orchards in Ontario (71). Resistance in *P. ulmi* to spirodiclofen was detected (39) subsequently, *P. ulmi* exhibited significantly increased resistance to spirodiclofen, with resistance ratios of 217 and 148 folds, respectively. A resistance ratio of 52.76-fold to pyridaben in *P. ulmi* was observed (72), with an LC_{50} value of 270.327 mg/L. While this indicates a substantial decline in susceptibility, the study did not assess whether this level of resistance would compromise field control efficacy relative to recommended application rates. Hexythiazox and clofentezine have been reported to exhibit cross-resistance in both *T. urticae* and *P. ulmi* (71, 73). Acaricide resistance cases reported in phytophagous mites are presented in Table 2 (74). A pie chart illustrating the distribution of acaricide resistance among major chemical groups was represented in Fig. 1.

Mechanisms of resistance: A mite can be able to withstand fatal concentrations of an acaricide through a variety of modifications. These are typically divided into two categories: mechanisms of decreased exposure or decreased responsiveness to the pesticides, depending on their biochemical and physiological properties (75,76). Various resistance mechanisms, such as behavioral adaptations, reduced penetration, sequestration and detoxification, collectively curtail the pesticide's efficacy by limiting its access to the target site. Conversely, alterations in the target site directly impede the pesticide's binding to its intended protein target (76-78). These mechanisms significantly influence shaping the dynamics and kinetics of pesticide toxicity that lead to the development of resistance (79, 80). Arthropod pests typically exhibit resistance to pesticides through four main broadly classified mechanisms *viz.,* behavioral resistance, metabolic resistance, penetration resistance and target site resistance.

Behavioral resistance: Behavioral resistance refers to modifications in arthropod behavior that reduce pesticide exposure and can be induced by prior encounters with pesticides (81, 82). This adaptive mechanism has been reported across multiple insecticide classes, including organochlorines, organophosphates, carbamates and pyrethroids. For instance, insects may cease feeding or relocate from treated areas upon exposure to certain insecticides (83). MET-I acaricides (fenpyroximate, pyridaben and fenazaquin) and mite growth

Table 2. Reports of acaricide resistance cases in phytophagous mites

Species	Taxonomy (Family)	Common name	No. of resistance cases	No. of active ingredients
Aculops lycopersici	Eriophyidae	Tomato rust mite	1	1
Aculops pelekaasi	Eriophyidae	Pink citrus rust mite	1	1
Aculus cornutus	Eriophyidae	Peach silver mite	3	3
Aculus fockeui	Eriophyidae	Plum rust mite	1	1
Aculus schlechtendali	Eriophyidae	Apple rust mite	1	1
Phyllocoptruta oleivora	Eriophyidae	Citrus rust mite	3	3
Brevipalpus chilensis	Tenupalpidae	False grape/red mite	3	3
Brevipalpus phoencis	Tenupalpidae	Passionvine mite	1	1
Panonychus ulmi	Tetranychidae	European red mite	203	48
Panonychus citri	Tetranychidae	Citrus red mite	106	29
Tetranychus atlanticus	Tetranychidae	Strawberry spider mite	7	5
Tetranychus cinnabarinus[a]	Tetranychidae	Carmine spider mite	37	19
Tetranychus kanzawai	Tetranychidae	Tea red spider mite	12	12
Acarus siro	Tetranychidae	Grain mite	5	3
Tetranychus urticae	Tetranychidae	Two-spotted spider mite	558	96
Rhizoglyphus echinopus	Tetranychidae	Bulb mite	6	5
Rhizoglyphus robini	Tetranychidae	Bulb mite	23	22
Tetranychus mcdanieli	Tetranychidae	Mcdaniel spider mite	19	13
Tetranychus turkestani	Tetranychidae	Strawberry spider mite	5	5
Tetranychus viennensis	Tetranychidae	Hawthorn spider mite	7	7

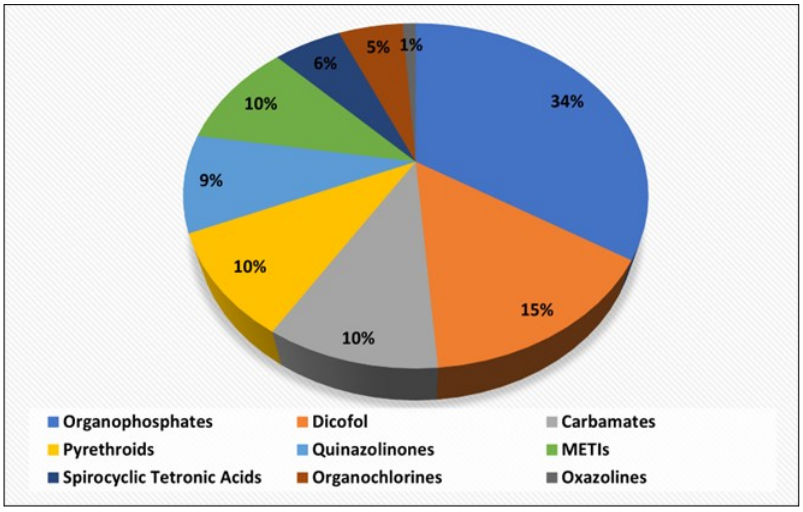

Fig. 1. Distribution of acaricide resistance development among major chemical groups in phytophagous mites.

inhibitors (etoxazole, hexythiazox and clofentezine) have been shown to induce dose-dependent irritant and repellent effects in *T. urticae*. Notably, MET-I–resistant *T. urticae* exhibit altered responses to these acaricides, influencing their behavior and oviposition preferences (80).

The behavioral responses of *T. urticae* and *P. ulmi* to various acaricides have been extensively investigated. The avoidance behavior in mites was first described (84), noting that populations previously exposed to acaricides exhibited increased movement away from treated surfaces. Exposure to organophosphates led to an immediate dispersal response in *T. urticae*, suggesting that sublethal exposure influences behavioral traits (85, 86). Certain acaricides induce irritability, leading mites to move away from residues (87), while altered oviposition behavior following exposure to pyrethroids also observed (88). The resistant *T. urticae* strains exhibit changes in movement patterns and feeding locations, reinforcing the role of behavioral changes in resistance development (89, 90).

Despite numerous reports of behavioral responses to insecticides and acaricides, there is ongoing debate regarding whether these responses constitute true behavioral resistance. Many studies infer resistance based on avoidance behaviors without clear evidence of heritable genetic changes (91). They argued that most cases labeled as behavioral resistance are instead aversion behaviors driven by repellency or learned avoidance, rather than genetic adaptation. This distinction is critical for accurately defining resistance mechanisms. The physiological and biochemical resistance mechanisms-such as cuticular thickening, target-site insensitivity and metabolic detoxification-have been well studied, behavioral resistance remains less understood (92). Although documented as early as the 1940s, research on this phenomenon has been limited due to the complexity of studying insect behavior and the absence of a clear definition.

Penetration resistance

Alterations in the target arthropod's physical characteristics, like decreased cuticular penetration or improved sequestration of pesticides, are examples of modifications (93). Changes in the cuticle associated with insecticide resistance primarily revolve around two key factors: the thickness and composition of the cuticle. While cuticular thickening has traditionally been linked with resistance, recent findings suggest a connection between cuticular composition and reduced penetration of xenobiotics (93, 94). The cuticle of organophosphate-resistant two-spotted spider mite (*Tetranychus telarius*) is thicker than that of the non-resistant strain, primarily due to increased thickness in the endocuticle component of the resistant strain (95). Similarly,the penetration rate as a key factor contributing to the resistance mechanism in citrus red mites (96).

Metabolic resistance

Elevated metabolic detoxification processes amplify the breakdown of a toxic substance, thereby diminishing the amount that reaches the intended target site. This enhancement may arise from an upsurge in the production or efficiency of various metabolic proteins operating across detoxification pathways. Metabolic detoxification encompasses Phase I, where enzymatic modification, like cytochrome P450s and carboxyl esterases, directly diminishes the toxicity of xenobiotics, Phase II, in which enzymes such as glutathione S-transferases (GST) increase the solubility of toxic xenobiotics and Phase III, where efflux pumps like ABC transporters expel toxic xenobiotics (97).

Metabolic resistance to insecticides primarily involves three main enzyme groups: glutathione S-transferases, esterases and mono-oxygenases. Esterases, a diverse group of enzymes, metabolize substrates containing ester linkages, whether they are of external or internal origin. Their association with insecticide resistance has been identified in more than fifty species of mites, ticks and insects (98). The predominant mechanism for up-regulation involves the amplification of esterase genes as outlined (99, 100). GST are proteins that link glutathione to various electrophiles, utilizing the cysteine sulfur atom. The polarity of glutathione plays a crucial role in traversing membranes, facilitating the elimination of conjugated toxins from cells and organisms through excretion (101, 102). Monooxygenases, alternatively known as mixed-function oxidases, P450s, or P450-monooxygenases, constitute a versatile enzyme group found in nearly all organisms. They are crucial in metabolizing both exogenous and endogenous chemicals (103).

Pesticide resistance has been associated with multiple resistance mechanisms, including elevated metabolic detoxification activity and increased expression of metabolic enzymes such as uridine diphosphate-glycosyl transferase (UGTs), glutathione S-transferase (GST), cytochrome P450-monooxygenase (P450), carboxylesterase (CarEs) and uridine diphosphate-monooxygenases (P450) (104-106). Although target-site mutations in resistance genes such as those affecting VGSC and GABA receptors play a critical role in resistance to specific acaricides like pyrethroids, metabolic resistance involving elevated activity of detoxification enzymes (e.g., cytochrome P450s, GSTs and carboxylesterases) is equally or more prominent in resistance to several other acaricides, such as abamectin, spirodiclofen and cyflumetofen in *T. urticae* (107).

Cytochrome P450 monooxygenase

Monooxygenases, also known to as cytochrome P450-dependent monooxygenases, play a vital role in regulating internal substances like steroids, hormones and fatty acids. This enzyme is essential for the breakdown and synthesis of external substances, including pesticides, plant toxins and drugs and is prevalent in aerobic organisms like insects, birds, plants, mammals and bacteria (108). The P450 gene superfamily comprises of seventy families comprising 127 subfamilies (109). Most of the functionally verified members of the CYPs are found in the CYP392 family, which belongs to the CYP3 clan. This group is notably enlarged in *T. urticae,* as highlighted (110, 111). Among these, CYP392A16 stands out as a significant enzyme, exhibiting high levels of expression in several strains resistant to multiple acaricides (112-114).

The overexpression of the CYP392A16 gene in a multi-resistant strain enables the metabolism of abamectin into a non-toxic form. CYP392A16's presence in a QTL region is linked to fenbutatin oxide resistance, but the exact metabolic process is unconfirmed. Additionally, RNA-sequence and Quantitative Trait Loci mapping studies associate CYP392A16 with pyflubumide resistance due to its proximity to a QTL and consistent five-fold higher expression observed in experimental evolution (105, 113, 114). An analysis combining genome-wide gene expression data indicated the significant involvement of two additional CYPs, CYP392A11 and CYP392A12, in a cyenopyrafen-resistant strain of *T. urticae*, given their elevated expression levels (115). RNA sequencing of abamectin-resistant populations revealed CYP392A11, CYP392A12, CYP389C10 and CYP392D8 as potential metabolic resistance-associated CYPs (116). Further, a genetic mapping for spirodiclofen resistance revealed a QTL containing a cluster of CYPs, specifically CYP392E4, CYP392E6, CYP392E7, CYP392E8, CYP392E9 and CYP392E10 (117). Additionally, CYP392E11, a member of the CYP392E family, was identified in fenbutatin oxide resistance population of *T. urticae* and it also provides resistance against pyflubumide/spirodiclofen (114).

Profiling the expression of fenazaquin-resistant *T. urticae* revealed nine distinct genes linked to mitochondrial electron transport inhibitors (METI) resistance, including CYP392A11, CYP392A12, CYP392A16, CYP392D2, CYP392D3, CYP392D6, CYP392D7, CYP392D8 and CYP392D10p (118). In *P. ulmi*, the PuP450-18 gene present within the CYP3 clan exhibits the most pronounced upregulation in expression when analyzing RNA-sequence data from populations susceptible and resistant to spirodiclofen, according to the previous findings (69). CYP4CF1 activated by pyridaben and CYP4CL2 (CYP4 clan) is activated by abamectin (119), azocyclotin and pyridaben in *P. citri*. Within the CYPs, each clan has the potential to confer resistance to distinct categories of acaricides.

Glutathione S-transferases

Glutathione S-transferases (GSTs) are a broadly distributed set of enzymes predominantly found in aerobic organisms. These enzymes metabolize insecticides primarily through conjugation with reduced glutathione, producing water-soluble metabolites that are easily excretable. Additionally, they help eliminate toxic oxygen-free radicals produced by pesticides (120). In insects, delta and epsilon class GSTs are commonly associated with resistance and surprisingly, mu class GSTs, once thought vertebrate-specific, are implicated in mite and tick resistance (121, 122).

An examination of gene expression throughout the entire genome revealed an association between TuGSTd05 and resistance (123). Multi-resistance phenotype of *T. urticae* is strongly connected to one mu class and two delta class GSTs (TuGSTm09, TuGSTd10, TuGSTd14). In a case of abamectin-resistant *P. citri* strain, PcGSTm5 (bearing the closest resemblance to TuGSTm02 in *T. urticae*) exhibited significant upregulation and showed inducibility upon exposure to abamectin (124). TuGSTm02 was also identified in *T. urticae*, where it plays a role in conferring resistance to cyflumetofen; this conjugation was validated (125) using high-performance liquid chromatography and mass spectrometry. TuGSTm04

has been proposed as a candidate gene potentially involved in cyflumetofen resistance based on gene expression and microRNA regulation studies, although the corresponding metabolite has not yet been identified (126).

Carboxyl or Cholinesterases

Carboxylesterases (CEs) are detoxification enzymes that contribute to pesticide resistance primarily by hydrolyzing ester bonds in insecticides and acaricides, thereby reducing their toxicity. In some cases, they may also act by sequestering xenobiotics. The role and efficiency of CEs in resistance mechanisms can vary depending on the mite species and the specific compound involved (68). Along with cytochrome P 450, CCEs play a crucial role in providing metabolic resistance to several key categories of acaricides (68). The carboxyl cholinesterase-mediated resistance for bifenthrin in *T. urticae* was first identified (104) and this was illustrated with evidence (127). Several carboxyl esterases, such as TuCCE27 in CarE6, TuCCE52 in CarE13, TuCCE57 in CarE15 and CCEincTu15 in CarE18 were upregulated in a laboratory-selected fenpropathrin-resistant *T. urticae* population, suggesting an association with resistance to pyrethroids (128).

Alternative splicing of CCE alleles, specifically TcCCE23 has been documented, with splice variants appearing to have varying impacts on the toxicity of fenpropathrin through dsRNA in *T. cinnabarinus* (129). Through RNAi, the overexpression of TCE2 (TuCCE55) and its role in resistance were reported in *T. urticae* populations against omethoate, abamectin and fenpropathrin (130, 131). Following this, a modified form of the CCE04 gene, designated as CCE04SR-VP, was detected with notable upregulation in two genetically distinct spirodiclofen-resistant populations of *T. urticae* (132) and multiple single nucleotide polymorphisms (SNPs) were also detected throughout the entire sequence, leading to various substitutions. The over-expression of diverse carboxylesterases (CCEs) has been linked to the resistance of *P. citri* populations to fenpropathrin and was substantiated (133) through toxicity assessments and reverse genetics studies. In the case of spirodiclofen-resistant *P. ulmi*, transcriptome analysis revealed the upregulation of CCEs, specifically PuCCE7 and PuCCE13, but their functional validation remains awaiting future studies (69).

UDP-glycosyl transferases

UDP-glycosyl transferase enzymes play a crucial role in facilitating the transfer of a glycosyl group to a nucleophilic molecule. Typically, this involves the attachment to the nucleophilic oxygen molecule of a hydroxyl group in various instances (134). This enzymatic process is fundamental for modifying molecules, adding glycosyl groups in a controlled and specific manner, contributing to a diverse range of biochemical reactions. Some UGT genes in *T. urticae* may have originated through lateral gene transfer from bacteria (135). However, this hypothesis does not apply to all UGTs and remains subject to further investigation. The over expression of UGT genes has been commonly observed in acaricide-resistant populations of *T. urticae* against abamectin, clofentezine, etoxazole, fenpyroximate, pyridaben, spirodiclofen and spirotetramat, cyenopyrafen and cyflumetofen, (136, 137). The UGT gene (UGT201D3) responsible for resistance through RNAi experiments and metabolic studies was identified (138). More recently, glycosylation activity of four UGTs-UGT10, UGT11, UGT28 and UGT29-towards abamectin and milbemectin has been explored, though direct biochemical evidence confirming their detoxification function is still emerging (137).

Target site resistance

Alterations in the target sites, which can impact the efficacy of applied acaricides, frequently arise due to point mutations within the genes responsible for the target proteins. These mutations bring about structural changes that impede or reduce the direct interaction between acaricides and their intended targets. These genetic modifications can influence the binding affinity and recognition between acaricides and the target proteins, leading to decreased effectiveness of acaricides (139, 140). Understanding these molecular changes is pivotal for developing strategies to manage acaricide resistance and improve agricultural pest control. The mutation arises from parallel evolution driven by intense acaricide selection forces and the functional limitations imposed by conserved target-site proteins (141).

Acetylcholinesterase

Acetylcholinesterase serves as a crucial enzyme involved in the breakdown of acetylcholine, which in turn inhibits synaptic transmission between nerve cells. In insects, excluding ticks and higher Diptera, most genomes typically contain a minimum of two genes resembling the *Anopheles gambiae* paralogous and orthologous genes, ace-1 and ace-2, which encode for AChE1 and AChE2 (142). The initial documentation of acaricide resistance associated with AChE in *T. urticae* traces back to Smissaert in 1964. Resistance mutations in AChE1 include G119S, A201S, G328A, F331W/C/Y (*Torpedo californica* numbering) and a novel A280T mutation found in organophosphate-resistant strains from Korea and Europe (53, 143). Combinations like F331W with G328A or G119S rarely occur and contribute to increased resistance in *T. urticae* (53, 110). The F331W mutation was observed in other mite species, highlighting the complexity of resistance mechanisms and the importance of understanding genetic mutations for effective pest management (69,144, 145).

Although early genomic analysis reported a single AChE gene in *T. urticae* (48), more recent studies suggest the presence of multiple acetylcholinesterase-like genes, potentially arising from gene duplication or functional divergence (52). The resistance-related point mutation was discovered in *T. urticae*'s AChE (146). AChE mutations in field populations of *T. urticae* in Europe, including A210S, G119S, F331W, G328A and T208A occurring at frequencies of 68, 41, 22, 21 and 18 %, respectively (107), while South Korean populations exhibited A391T, F331W and G228S AChE mutations with frequencies ranging from 3 to 76.6, 100 and 0 to 95.7 % (147). Site-directed mutagenesis in *T. urticae* showed E316, H369 and V105 active sites are important for AChE function (148).

Gene duplication serves as a compensatory mechanism to counteract the diminished catalytic activity caused by resistance mutations in AChE, as discussed (149). A nearly linear correlation was observed between the copy numbers of the AChE gene and the levels of resistance to monocrotophos across nine populations of *T. urticae* in South Korean apple

orchards (52). Beyond substitutions, copy number variation can contribute to increased expression, as evidenced by specific strains of *T. urticae* and *T. evansi* (52, 145), which exhibit the presence of multiple copies of AChE1. Notably, strains of *T. evansi* and *T. urticae* resistant to organophosphates (OPs) maintain both wild and mutant alleles of AChE, showcasing a dual allelic strategy to mitigate the fitness cost associated with the mutant allele. This dual allelic approach, as highlighted by (145) and (150), provides insights into the adaptive strategies employed by mite populations to cope with the challenges posed by organophosphate resistance.

Sodium channel modulator

The Voltage-Gated Sodium Channel (VGSC) is a well-characterized transmembrane protein that mediates the flow of sodium ions across cell membranes, thereby initiating and propagating action potentials. While its structure and function have been extensively studied in insects (139), direct molecular evidence in mites (Acari) remains relatively limited. However, VGSC mutations analogous to those found in insect pyrethroid resistance have been reported in some mite species, suggesting a potential, albeit not fully elucidated, role in acaricide resistance (68, 159). The VGSC serves a crucial role as a transmembrane protein, facilitating the movement of sodium ions across cell membranes and generating action potentials, as elucidated (139). This channel, which is the primary target of pyrethroids, exhibits numerous mutations across domains II, III and IV in various species of Acari. In *T. urticae*, resistance to pyrethroids is primarily associated with target-site mutations in the VGSC, such as L1024V and F1538I, which reduce insecticide sensitivity, alongside enhanced metabolic detoxification mediated by cytochrome P450 monooxygenases and carboxylesterases (52, 104, 151). Compared to insects, the sodium channel genes of mites and ticks show unique features upon sequencing, reflecting the greater diversity within Arachnida (152). In *T. urticae*, the presence of alternative splicing in the VGSC gene remains to be fully characterized and although mutually exclusive k/l exon usage has been documented in insects, direct evidence for a similar mechanism in mites is currently lacking (153).

Significant resistance-associated mutations identified in *T. urticae* include L1024V in domain II S6 (52) and F1538I in domain III S6 (167); however, these mutations alone may not fully account for high resistance levels, as combinatorial effects with other mutations and the genetic background also play crucial roles in determining resistance outcomes. As per previous results (152), the ATHRos-Bf strain in *T. urticae* mites exhibits two amino acid substitutions compared to susceptible mites: one is phenylalanine to isoleucine substitution in domain IIIS6 (F1538I in *Musca domestica*) and the other is an alanine to aspartic acid substitution present within the domain II or domain III intracellular interlinker (equivalent to A1215D) as per the report (154) F15381 was also present in *P. citri.* While the substitution A1215D is associated with F1538I, potentially enhancing resistance levels, populations carrying the A1215D mutation exhibit susceptibility to pyrethroids. This suggests that the A1215D mutation alone does not impart resistance, as highlighted (155). L1024V alone results in resistance to *P. ulmi* and *P. citri*. Both mutations, F1538I + A1215D and L1024V exhibited notably strong resistance to fluvalinate, bifenthrin and fenpropathrin (107).

In addition to F1538I, M918L and F1534S mutations was identified (156) in *T. urticae*, while reported variation at residue L925 in the VGSC was reported (157); however, the functional significance of L925 mutations, such as L925I, which is well known in insects like *Helicoverpa armigera*, remains to be clearly established in *T. urticae*. Copy number variation in the VGSC was found alongside the L1024V and F1538I substitutions in the *P. ulmi*-resistant population (158). However, the specific contribution of CNV to resistance and the original number of voltage-gated sodium channel copies remain puzzling. Acaricide resistance-conferring mutation on sodium channels in *T. urticae, P. ulmi and P. citri* are depicted in Fig. 2.

Glutamate and GABA-gated chloride channels

Glutamate- and GABA-gated chloride channels (GluCl) belong to the anion-permeable Cys-loop ligand-gated chloride channel family, also known as CysLGCCs (159). (159) reported that the *T. urticae* genome contains six GluCl genes (GluCl1–6). Target-site mutations, such as G326E in GluCl3 and G314D in GluCl1 and I321T have been identified for these GluCl genes (52, 137, 159).

Traditionally, cyclodienes were thought to impact the ion channel pore (TM2). However, recent studies have unveiled that the binding sites for newer acaricides are located in TM1 and TM3 of the rdl gene (160-162). Interestingly, despite mutations in TM2, which might not impact the efficacy of these novel acaricides (163, 164). This underscores the complexity of acaricide resistance mechanisms and highlights the importance of understanding the specific interactions between acaricides and their target sites. Furthermore, the link between abamectin resistance and metabolic resistance adds another layer of complexity to the resistance landscape (165, 166).

Abamectin resistance strain in *T. urticae* was initially explained by the identification of the G323D (also known as G314D) mutation in the conserved TM2 region of GluCl subunit 1 (GluCl1) in a Korean strain (167). Chinese populations exhibited G323D and G326E mutations, while European populations displayed I321T and L329T mutations in GluCl3, potentially leading to resistance against abamectin and milbemetcin. Abamectin resistance was linked to increased metabolic detoxification, characterized by the upregulation of cytochrome P450 and UDP-glycosyltransferase genes (137).

In another abamectin resistant strain identified through QTL mapping, V273I substitution in TM1 of GluCl3, was documented (168). Although the A301S mutation's functional influence on cyclodiene sensitivity has been verified through validation studies by (164, 169, 170) and its introduction *via* CRISPR/Cas9 into *P. xylostella* did not lead to increased resistance levels to dieldrin, fipronil or endosulfan (171). The cytochrome P450 gene (CYP392A16) and TuGSTd14 are linked to abamectin resistance, potentially serving as biomarkers (19, 172). Cross-resistance between milbemectin/abamectin has been observed in *T. urticae* populations in Brazil and Europe (137, 173).

Mitochondrial complexes

METI acaricides disrupt the mitochondrial electron transport chain by selectively inhibiting specific transmembrane complexes, most commonly complex I, though some

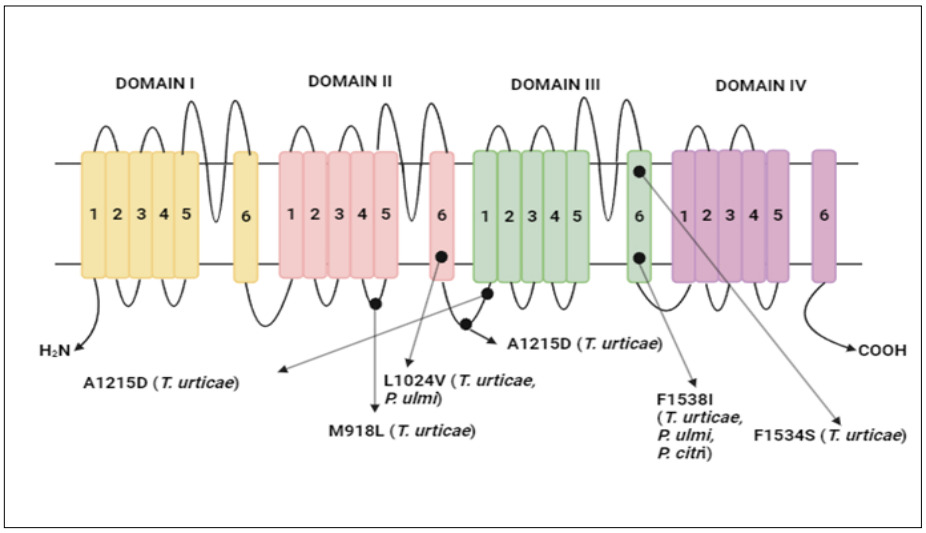

Fig. 2. Schematic depiction of the voltage-gated sodium channel, indicating genetic mutations linked to pyrethroid resistance in Acari. The illustration showcases the alterations at the target site caused by the mutation.

compounds may target complexes II or III, thereby impairing cellular respiration in mites. Acaricides like fenpyroximate, fenazaquin, pyridaben and tebufenpyrad target complex I and are categorized as METI site I inhibitors. Beta-keto nitriles (cyfumetofen, cyenopyrafen) and carboxanilides (pyfubumide) act on complex II, falling under METI site II. MET class III acaricides, including acequinocyl, bifenazate and fuacrypyrim, inhibit the cytochrome b Qo pocket within complex III. In contrast, diafenthiuron and propargite directly block ATP synthase. Mutational sites in mitochondrial complexes are depicted in Fig. 3.

The H110R mutation in the PSST subunit of METI-I complex I (according to *T. urticae* numbering) was discovered (174) in a strain of *T. urticae* resistant to tebufenpyrad, fenpyroximate and pyridaben. This mutation was also identified in fenpyroximate resistance strain of *P. ulmi* (175). Additionally, another mutation, A112V, was identified in a population displaying moderate resistance. In a population of *T. urticae* resistant to multiple agents, the potential mutation was identified (A55T substitution) associated with resistance (136). Nevertheless, this mutation is situated at a distance from the METI-I binding site and does not occur in a conserved region (176).

Inhibitors targeting succinate dehydrogenase in complex II, composed of subunits A, B, C and D (177), exhibit cross-resistance in *T. urticae* field populations to carboxanilides, cyfumetofen and cyenopyrafen acaricides (178). Preliminary genome-wide transcriptional expression studies associated CYP392A11 and CYP392A12 with cyenopyrafen and cyfumetofen resistance in *T. urticae* (115). Resistance mutations at specific positions (I260T/V and H258Y/L in sdhB and S56L in sdhC) were observed, with varying effects on cyflumetofen, pyflubumide and cyenopyrafen resistance (179-181). Notably, the H258Y mutation confers robust resistance to pyflubumide and cyenopyrafen but increases susceptibility to cyflumetofen (180).

Analyzing the sequences of bifenazate-resistant and bifenazate-susceptible strains of *T. urticae* revealed several mutations associated with resistance (G126S, I136T, S141F and P262T). These mutations were detected in the highly conserved cd 1 helix within cytochrome b (Complex III), as outlined (51). The G126S mutation is exclusive to *T. urticae* populations with substantial resistance (RR50 > 100) in South Korea, moderate resistance (10 < RR50 < 100) in the Pacific Northwest of the USA and populations in Finland, Japan and Cyprus (19, 107, 182). Moreover, G126S + S141F, P262T and

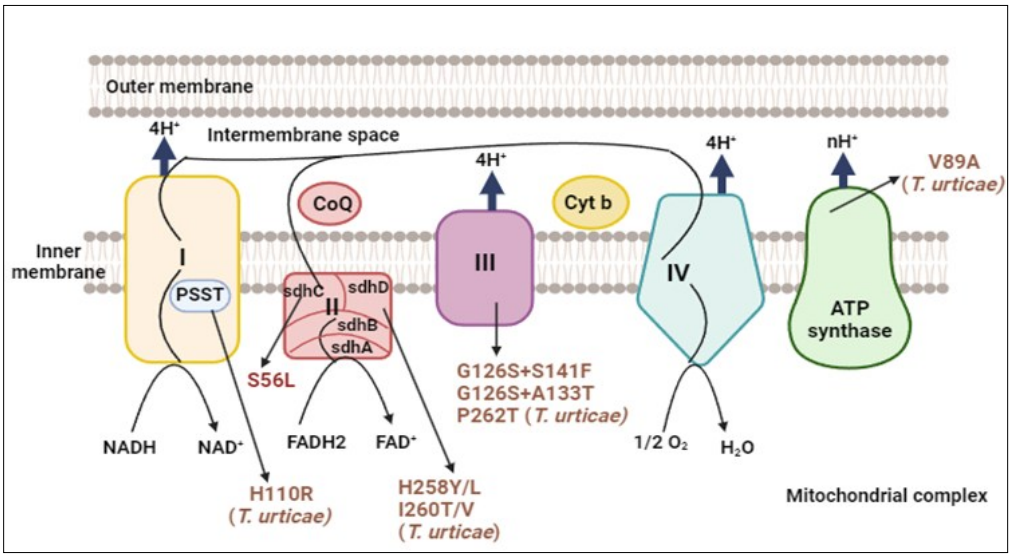

Fig. 3. Schematic illustration of mitochondrial electron transport chain with mutational sites which conferring resistance are depicted in red bold font.

G126S + A133T displayed elevated levels of cross-resistance to acequinocyl in *T. urticae* (64, 183). The G126S mutation in cytochrome b has been shown to confer high levels of resistance to bifenazate in *T. urticae*, even in the absence of other resistance mechanisms (179, 184). While additional metabolic factors, such as the activity of CYP392A11, may further enhance resistance or contribute to cross-resistance, G126S alone is often sufficient to cause substantial resistance.

Apart from the acaricides targeting mitochondrial electron transport, some acaricides like organotin compounds (cyhexatin and fenbutatin oxide) target the enzyme, ATP synthase responsible for ATP production. In these resistant strains, a non-synonymous single nucleotide polymorphism resulting in a V89A substitution was detected. The functional validation of this substitution was conducted through ATPase inhibition assays on isolated mitochondrial membrane preparations from both resistant and susceptible populations (114).

Octopamine receptor

Octopamine receptors, classified as G protein-coupled receptors (GPCRs), possess seven transmembrane domains, an extracellular N-terminus and an intracellular C-terminus, characteristic of GPCR structural features (185). They are grouped into α-adrenergic-like, β-adrenergic-like and octopamine/tyramine receptors (186). The octopamine receptor gene in *Rhipicephalus microplus*, explored to understand amitraz's target, revealed non-synonymous mutations (T8P and L22S) in highly resistant Brazilian and Mexican strains. Although lacking functional analysis, subsequent studies with mutation frequency calculations supported the connection between these mutations and amitraz resistance (187, 188). Additionally, non-synonymous mutations in the α-adrenergic octopamine receptor gene were associated with amitraz resistance (189). A relationship was observed between resistance ratio and base mutation in *P. citri* field populations employed in validating the T752C mutation (190). Further research is imperative to unveil the precise function of these mutations. In addition to SNPs, a duplication insertion has been identified in amitraz-resistant insects.

Acetyl CoA carboxylase (ACCase)

Acetyl CoA carboxylase, vital for eukaryotic lipid metabolism, features two primary catalytic sites: carboxyl transferase and biotin carboxylase (191). Spirodiclofen is designed to control economically significant phytophagous mite species such as *Brevipalpus*, *Aculus*, *Tetranychus* and *Panonychus*. Although *Phyllocoptruta oleivora* has sometimes been mentioned, it is important to note that spirodiclofen generally exhibits limited effectiveness against Eriophyid mites, including most *Phyllocoptruta* species (192). Spirodiclofen resistance in *T. urticae* is associated with an A1079T mutation in the ACCase gene, identified through genetic mapping (117). While a D384Y mutation in TuCPR and upregulated P450 genes like CYP392E4, CYP392E6, CYP392E7 and CYP392E8 have been observed, their specific role in keto-enol acaricide resistance remains unclear. The F1656L substitution found in resistant populations disappeared during selection with spiromesifen, suggesting a potential loss through genetic drift or selection-induced population bottleneck (181).

Chitin synthase

Chitin synthesis is essential for arthropod development, forming the exoskeleton and facilitating molting. Mite growth inhibitors (MGIs) such as etoxazole, hexythiazox and clofentezine disrupt this process, preventing mites from reaching maturity. In contrast to neurotoxic acaricides that target adult stages, MGIs act as insect growth regulators and are primarily effective as ovicides and/or larvicides. The molecular target of these compounds remained unclear until genetic mapping studies identified an I1017F substitution in the *chitin synthase 1* (CHS1) gene, which was strongly associated with resistance to MGIs (193, 194). This finding provided compelling evidence implicating CHS1 as a resistance-linked site for compounds like hexythiazox, etoxazole and clofentezine. However, functional and structural studies are still underway to confirm CHS1 as the definitive molecular target. A similar mutation, either alone or in combination with S1016L, was also identified in a *P. citri* population with high resistance to etoxazole (195). The I1017F mutation has been reported in *T. urticae* populations from several regions, including Australia, Belgium, Cyprus, Greece, Italy, Japan, Kenya, the Netherlands and Turkey and was detected in approximately 40 % of the *T. urticae* individuals surveyed in a recent study (107, 196).

Resistant management strategies in phytophagous mites

General resistance management strategies

Effective resistance management in arthropods relies on the judicious use of acaricides, incorporating strategies such as regular resistance monitoring, acaricide rotation and combination treatments. Monitoring resistance is a critical component in mitigating its development, as early detection allows for timely intervention through treatment adjustments or substitutions, thereby preserving susceptible populations (39, 197). Continuous and excessive application of acaricides significantly increases the likelihood of resistance emergence. For instance, amitraz resistance was notably prevalent in central Queensland, particularly when the frequency of applications exceeded five (198). Similarly, Rhipicephalus microplus exhibited a ninefold increase in resistance (RF = 9.2) after five generations of deltamethrin exposure, which escalated dramatically to an RF of 756 by the 11th generation (199).

Acaricide rotation, involving the systematic alternation of compounds with different modes of action, is a key strategy to delay resistance development. For example, alternating deltamethrin with coumaphos has been shown to slow down resistance evolution (199). However, some studies indicate that rotation alone may not provide long-term control (200). The effectiveness of this approach remains uncertain, as there is no definitive guideline on the optimal time intervals for switching between acaricides (201).

Another effective approach is the combination of acaricides with different mechanisms of action, which has been shown to delay resistance development (202). The combined use of carbamates, synthetic pyrethroids and chitin synthesis inhibitors has proven effective against various mite species (197, 203). However, for this strategy to be successful, the selected chemicals must not only be compatible but also exhibit similar persistence in the environment to ensure sustained efficacy against the target species. By integrating

these strategies: monitoring, rotation and combination treatments, resistance management programs can enhance the sustainability of acaricide use and reduce the risk of resistance development in arthropod populations.

Advanced Resistance Management Strategies

The continuous reliance on acaricides poses increasing risks to the environment, human health and livestock well-being, making it a growing public concern (204, 205). These challenges highlight the urgent need for safer, species-specific pest control strategies. Advanced molecular tools such as CRISPR/Cas9 and RNA interference (RNAi) are now being explored as potential solutions for managing acaricide resistance in a more targeted and sustainable manner.

CRISPR/Cas9 Technology

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9, a CRISPR-associated endonuclease, serve as a bacterial defense against viral infections. Cas9, guided by CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA), or synthetic single-guide RNA (sgRNA), induces precise double-stranded DNA breaks at the target site, which are subsequently repaired via Homology-Directed Repair (HDR) or Non-Homologous End Joining (NHEJ). However, off-target cleavage remains a known limitation, particularly in non-model organisms with less well-annotated genomes (206).

In pest control research, CRISPR/Cas9 has been used for genome editing in arthropods via microinjection into oocytes (174, 207, 208). While successful in *D. melanogaster*, this method proved lethal in Chelicerata embryos (209, 210). Alternative delivery methods, such as maternal germline injections, have shown promise in insects (211) and nematodes (57, 212). In *T. urticae*, a CRISPR/Cas9 mix was injected along with chloroquine, which was used to aid nucleic acid delivery; however, the resulting genome editing efficiency was low (<0.2 % modified offspring) (111). However, some researchers significantly improved knockout efficiency (>20 %) using SYNCAS, a formulation combining branched amphipathic peptide capsules with saponins (213). Additionally, Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) has emerged as a promising approach for delivering Cas9 into the ovaries of some microarthropods, though its applicability and efficiency in species like *T. urticae* remain under exploration (214). Harnessing CRISPR/Cas9 to disrupt resistance-related genes offers a promising strategy for acaricide resistance management in phytophagous mites.

RNA Interference (RNAi) Strategy

RNA interference (RNAi) is a natural gene-silencing mechanism that regulates gene expression through double-stranded RNA (dsRNA). In 1998, this breakthrough earned the Nobel Prize in 2006 and paved the way for RNAi-based pest management strategies. RNAi offers a potentially species-specific approach to suppress essential pest genes; however, off-target effects can still occur, particularly in organisms with gene redundancy or incomplete genome annotation. Additionally, RNA uptake and silencing efficiency can vary considerably among mite species, affecting the reliability of this strategy (215). In *T. urticae*, key RNAi-associated genes such as *Pasha, Ago, Drosha, RdRp, Loquacious, Dicer and Piwi/Aub/Ago3* have been identified (3, 48, 216-218). Unlike insects, *T. urticae* possesses five distinct *RdRp* genes instead of *SID-1*, with additional RNAi factors identified in other mites and ticks.

RNAi operates via post-transcriptional gene silencing mediated by small RNAs (18-30 nucleotides) that associate with Argonaute proteins (219). In animals, three major RNA classes, piRNAs, siRNAs and miRNAs, have been identified (220), with the siRNA pathway triggered both endogenously and exogenously (221). In arthropods, multiple Dicer enzymes enable separate pathways for siRNA and miRNA synthesis (222). The introduction of exogenous dsRNA induces rapid mRNA degradation, serving as an evolutionarily conserved defense against viruses and transposable elements (223, 224). RNAi has been experimentally applied to silence resistance-related genes in phytophagous mites, demonstrating potential as an eco-friendly strategy for acaricide resistance management; however, its practical application is limited by challenges such as inefficient dsRNA delivery, gut degradation and the absence of systemic RNAi components like SID-1 homologs (Table 3).

Conclusion

The examination of acaricide resistance in phytophagous mites has unveiled a complex interplay of genetic, molecular and physiological factors contributing to the evolution of resistance. The identification of key mutations, target site alterations and metabolic detoxification mechanisms has significantly enhanced our understanding of resistance mechanisms. According to the Arthropod Pesticide Resistance Database (APRD), organophosphates, METI, carbamates and pyrethroids has developed the highest number of resistance cases across various phytophagous mite species. Despite these insights, the persistent challenge of managing acaricide resistance requires a multifaceted approach. While RNAi holds promise as a gene-targeting tool, its field applicability in mites is currently limited; thus, emphasis should remain on integrated pest management, including sustainable practices, rotation of acaricides and the development of novel compounds targeting distinct vulnerabilities in mite populations. Future strategies should focus on integrated pest management, emphasizing sustainable practices and rotation of acaricides, while

Table 3. RNAi-mediated suppression of acaricide resistance genes in phytophagous mites

Species	Genes	Acaricides	Reference
T. urticae	NADPH- cytochrome P450 reductase	Bifenthrin, fenpyroximate and abamectin	97
	NADPH- cytochrome P450 reductase	Fenpropathrin	225
	Esterase gene	Fenpropathrin, cyflumetofen	132
T. cinnabarinus	3 aminobutyric acid chloride channel	Abamectin	226
	GABA transaminase	Abamectin	227
	Intradiol Ring-Cleavage Dioxygenase	Abamectin	228
P. ulmi	Glutathione-S-transferase mu-class 5	abamectin	229
	Carboxylesterases (CarEs-1, CarEs-7 and CarEs-9)	Fenpropathrin	133

recognizing that the development of novel compounds targeting specific vulnerabilities in mite populations is a long-term goal requiring extensive research, functional validation and overcoming significant regulatory and technical hurdles.

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Authors' contributions

SPS conceived and designed the initial draft and wrote the review. SE wrote and revised the manuscript. MM & RP revised the manuscript. HR contributed to visualization and PJSS, KKK & TSSP revised the manuscript. All authors reviewed the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest. The COI statement should only be indicated in the main manuscript file and not be uploaded as a separate file.

Ethical issues: None

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