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Spirotetramat — An Alternative for the Control of Parasitic Sucking Insects and its Fate in the Environment

Norma-Julieta Salazar-López, María-Lourdes Aldana-Madrid, María-Isabel Silveira-Gramont and José-Luis Aguiar

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Abstract

Spirotetramat is an insecticide derived from tetramic acid, a systemic material, for the control of sucking insects in their juvenile, immature stages, including aphids, scale insects, and whitefly. It produces growth inhibition of younger insects, reduces the ability of insects to reproduce, resulting in mortality. It acts to inhibit the biosynthesis of lipids and represents a new alternative for the control of problematic insects such as *Planococcus ficus* and *Aphis gossypii*. After a foliar application of spirotetramat, it enters the plant and transforms to its metabolite enol, along with the metabolite ketohydroxy, which are the two main products of degradation.

Studies on the 90% degradation (DT_{90}) in the soil under field conditions demonstrates the velocity of dissipation of spirotetramat and its main metabolites, BYI08330-enol and BYI08330-ketohydroxi, was from 1.1 to 3.5 days and from 16.7 to 77.8 days, respectively. Given these results, ground water contamination by spirotetramat is not very probable, and there is no evidence of accumulation in the soil or in the air. Spirotetramat has been used by itself for the control of aphids in grapevine, and combined with imidacloprid in walnut; a reduction in the control efficiency of spirotetramat alone, possibly due to a change in the aphid population genetic makeup of the population, which resulted in a higher tolerance to the control dose was observed. However, when combined, it was possible to achieve up to 90% control 5 days after application. For this reason, it is important to establish a permanent sampling program for insects, and to apply insecticides only when the insects reach the action threshold; to prevent resistance building up, it is recommended to use materials with different modes of action, insecticide rotation, or alternative compounds.

Keywords: Spirotetramat, systemic, sucking pests, tetramic acid, degradation

1. Introduction

On a worldwide level, farmers' crops are being attacked by a wide variety of insect pests, these results in increased costs of production and can even result in the total loss of the crop. For this reason, there is a constant search for compounds or formulations for the control of new pests or for those that have developed resistance. The pesticides that are effective and are also environmentally friendly are highly valued; these versatile products are able to be part of the best agricultural practices and biological control leading to an integrated pest management program without the need for compounds that also harm non-target organisms. Based on the need to offer new and better products, in 2008 Bayer synthetized from tetramic acid a compound that had already demonstrated insecticidal properties, a new compound called spirotetramat [1].

Spirotetramat acts as an inhibitor of the biosynthesis of lipids and represents a new alternative for the control of problematic insects, such as apple wholly whitefly and whitefly biotype Q, which cause severe damage in agricultural crops and have developed resistance to the commonly used pesticides used for their control [1, 2]. For these reasons, this research was initiated into a review of the origin and chemical properties of spirotetramat, looking into possible uses, its fate in the environment (soil, air, and water), its metabolism in plants, and the possibility of developing resistance.

2. The origin of spirotetramat

Evidence exists indicating that compounds derived from the structural unit of tetramic acid have biological activities across a wide spectrum; antibiotic tirandamicin A and the phytotoxin of tenuazonic acid are examples of this type of compounds found in nature [3]. Furthermore, there are synthetic compounds that are utilized as herbicides and insecticides as in the case of spirotetramat (commercial name Movento®) developed by Bayer CropScience as an insecticide [3, 4]. The discovery of this compound came about through research into improving myticide and herbicide activity by Bayer with compound derived from tetramic acid. As a first step in this research, compounds were synthesized with the Bucherer-Bergs reaction of tetramic acid (compound 1a in Figure 1] and its acetyl derivative (compound 1b in Figure 1), splitting the 1-amino-4-methyl-carboxylic acid methyl ester cyclohexane. According to the research, they observed a significant improvement in herbicidal activity in comparison with analogous compounds where spirocyclic were not substituted. In another research, t they also reported that the compound 1b demonstrated excellent myticide activity and was highly effective against the aphid *Myzus persicae* [5].

Previous evaluations in the investigation led to derivatives of spirocyclic tetramic acid alkoxy-substituted. In this case, the mechanism is the synthesis of Strecker and the splitting of the 4-metoxy-1-amino-ciclohexane-carbonitrile (compound 2 in Figure 2) and they were able to obtain and isolate isomer mixes; the least present isomer had good control of *Myzus persicae*, which at that time was close to the efficacy of the best aphicide, imidacloprid. However, the
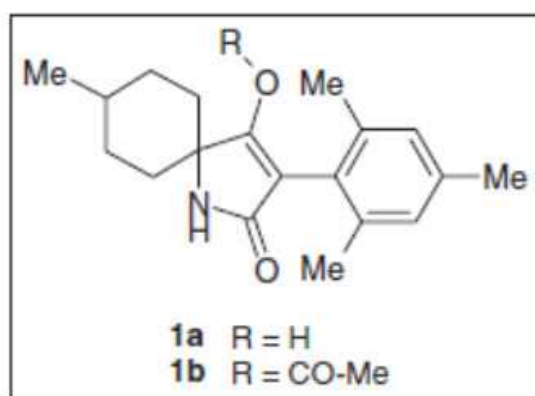
Figure 1. Compounds derived from tetramic acid with improved herbicidal activity.

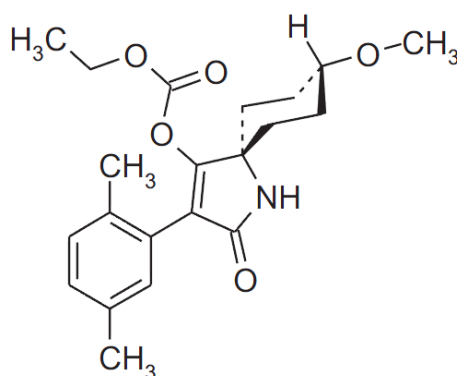results demonstrated a disadvantage in that they saw an increase in the herbicidal activity of the sample [5].

Figure 2. Molecular structure of spirotetramat.

3. Pest control

Spirotetramat is an insecticide that targets sucking insects in their juvenile stage such as aphids (*Aphis spp.*, *Myzus spp.*, *Dysaphis* spp., *Toxoptera spp.*, *Phorodon humuli*), rice aphids (*Phylloxera spp.*), psyllids (*Psylla spp.*, *Paratrioza cockerelli*), mealybugs (*Pseudococcus spp.*, *Planococcus spp.*), and whiteflies (*Bemisia spp.*, *Trialeurodes vaporariorum*). Table 1 lists the studies into the relationship of the effectiveness of spirotetramat in different insects [1, 6].

As mentioned earlier, the aphicide activity of spirotetramat is effective in the immature stages where incomplete ecdysis can be observed, the insect cannot completely shed its exoskeleton, thus impeding its growth; however, what has been observed in nymphs is that they appear to be immobile and they dry up quickly. The efficacy of spirotetramat on adult insects is reduced due to their mobility; they tend to produce nymphs that die within 24 hours or the nymphs

are non-fertile, thus reducing the procreation and fertility of the future generations [7]. Spirotetramat has demonstrated excellent efficacy on peach, cotton, and plum aphids that are 3-4 days old. It has also been observed that on female adult whiteflies (*B tabacco*) treated with spirotetramat (40 and 200 ppm), the number of eggs produced is a function of the applied doses (major reduction 90% and 60%), including a concentration of 8 ppm, 80% of the eggs do not hatch. It was also observed that the way of contact of the insecticide influences its effectiveness on the control of the insects; it has a major effect if spirotetramat is ingested orally than if it is by direct contact with the insect [1, 8].

Another laboratory study suggested that spirotetramat can be utilized in a safe integrated pest management program for the control of the cabbage aphid, as there is less mortality in comparison of other insecticides of the marmalade hoverfly *Episyrphus balteatus*, which is a natural aphid predator; furthermore, the fertility of the treated adult syrphids is not affected [9]. In another study to determine the collateral damage of spirotetramat on the wasp *Anagyrus*, a grapevine mealybug parasite, it was found that there was no detectable mortality on the parasite after 24 hours of application; there were no adverse effects on the development of the parasite in the pupa stage inside the mummified mealybug, nor were there any effects on the emergence of the new *Anagyrus* [10]. It must be pointed out that in an integrated pest management program where the arthropod *Galendromus occidentalis* is used for biological control, the use of spirotetramat is not recommended given that at concentrations of 0.228 g a.i.L^{-1}, there was a mortality rate of 90% for the eggs, and 100% for the larvae [11]. This was also similar for the toxicity results for *Tamarixia radiata*, a parasitoid of citrus Asian psyllid (*Diaphorina citri Kuwayama*), that with an application dose of 0.8 mL L^{-1} with water did not present favorable conditions for its development and it was highly toxic [12].

Reference	Dose applied	Organism controlled	Pest location
Moens et al., 2011 [9]	75 g a.i.ha^{-1}	*Brevicoryne brassicae L.*	Cabbage
Jamieson et al., 2010 [13]	3.36 g a.i.100 L^{-1}	*Orchamoplatus citri).*	L. C.
Mansour et al., 2011 [10]	120 mL h L^{-1}	*Planococcus ficus*	L. C.
Page-Weir et al., 2011 [14]	40 mL a.i.100 L^{-1}	*Bactericera cockerelli*	Tomato & potato
Smiley et al., 2011 [15]	88-110 g a.i.ha^{-1}	*Heterodera avenae*	Wheat roots
Duvaresch et al., 2008 [16]	120 g a.i.ha^{-1}	*Aphis gossypii*	Cotton
Kay & Herron, 2010 [17]	144 g a.i.ha^{-1}	*Frankliniella occidentalis*	Peppers
Fu & Del Real, 2009 [18]	60-120 g a.i.ha^{-1}	*Planococcus ficus*	Vine
Marcic et al., 2012 [19]	200, 60, 18 mg a.i.L^{-1}	*Tetranychus urticae*	L. C.
Frank & Lebude, 2011 [20]	1.7 oz 100 gal^{-1}	*Adelges tsugae*	Fir
Elizondo & Murguido, 2010 [21]	0.5 and 0.6 L ha^{-1}	*Myzus persicae Sulzer, Bemisia tabaci Gennadius,* *Thrips palmi Karny*	Potato (*Solanum tuberosum L*)

*a.i. Active ingredient

*L. C. Laboratory conditions

Table 1. Organisms controlled with spirotetramat under different conditions.

4. Fate of spirotetramat in the plant and environment

It is important to note that after applying insecticides on crops or on the soil, it is possible that the active ingredient is not absorbed permanently by the soil or that it can mobilize to bodies of water. There exist a lot of physical and chemical and microbiological factors that can determine the fate of the products used in plant protection, some are: hydrolytic degradation and photochemical, biological transformation and mineralization, absorption and movement of the active ingredient, as well as the degraded products in the soil. It is important to note that the above mentioned processes depend on the chemical structure and the physical properties of the compound used, as well as the soil, the vegetation, and the climatic conditions [22].

4.1. Factors affecting the fate of spirotetramat

Considering that spirotetramat has no acidic properties or alkaline in aqueous solutions, it also stands out that soil pH and that of the aqueous systems have no influence on the physico-chemical properties of the spirotetramat. The solubility and lipophilicity of water are important because they provide us with information on the mobility and solubility of spirotetramat in water; if they are low (0.0299 g L^{-1}), this indicates good soil absorption, resulting to very low risk of infiltrating into aquifers. With a base vapor pressure of 5.6×10^{-9} Pa and Henry's constant 6.99×10^{-8} Pa, it can be concluded that there is no possibility of spirotetramat volatizing in any significant form [22, 23]. It is important to consider the properties of the metabolite BYI08330-enol (referred as "enol" from now on); the enol form possesses properties slightly acidic (pK_a=5.2). Furthermore, due to its high solubility in water (2.7 g L^{-1} at pH 7), it presents a risk of possible leaching into subterranean waters; same as with spirotetramat, the volatility of the enol form possesses no significant role [22].

4.2. Plant metabolism of spirotetramat

Before the creation of spirotetramat, there were only systemic insecticides that were only capable of moving in one way, those that enter the plant then move to different locations within the plant; however, this travel was only one way going up the xylem. The advantage of spirotetramat is that once it penetrates under the leaf of a plant, it is transformed by a hydrolytic split to spirotetramat-enol that due to its physiochemical properties is capable of moving up and down through the phloem, which allows it to reach and access pests that are difficult to reach, such as the grape mealy bug (*Planococcus ficus*) [18]. In contrast with systemic insecticides that travel only one way, such as the case with imidacoprid, spirotetramat-enol being a systemic metabolite with double lanes can protect new leaves generated after the application and it can even protects the roots [1, 18].

Reference [24] determined the metabolism of spirotetramat in apple, cotton, lettuce, and potato (Table 2). In the cultivars analyzed, the main residues found were the father (BYI08330) and three dominant metabolites, BYI08330 enol, BYI08330 enol-glucoside, and BYI08330 cetohydroxy, which is in accordance with those reported by [25] (Figure 3). However, in apples, they detected a fourth metabolite, BYI08330 monohydroxy, with a considerable percentage (around

15.6%); meanwhile in the potato tuber, the main metabolite was BYI08330-enol, along with the absence of the father compound. To conduct those studies, a foliar application of spirotetramat OD-100 was applied, where the dose administered was 167 g a.i.ha^{-1} for lettuce, which was equivalent to the maximum recommended by the manufacturer; the same was administered for apple, potato, and cotton, with rates of 576, 308, and 264 g a.i.ha^{-1}, respectively, which were equivalent to 2.5, 1.1 at 1.8 and 0.85 times the dose recommended per each season.

It is important to consider that even using higher doses than the recommended, none of the residual concentrations found will surpass the maximum residual limit (MRL) established by the Environmental Protection Agency (EPA) and the Codex Alimentarius of the FAO/OMS [25]. It was observed that the residual concentration of insecticide on apple leaves, potato leaves, and lettuce was superior to the apple fruit, potato (tuber), and the cotton seed. In the three leaves analyzed (lettuce, apple, and potato leaves) the father compound was found to be above 49% of the total residues, which may indicate that the major part of the compound recovered as residue remains in the leaves without being metabolized.

| Compound (mg kg^{-1})	Apple	Apple (leaves)	Lettuce	Cotton (seed)	Potato (tuber)	Potato (leaves)
Spirotetramat (BYI08330)	0.32	26.37	1.75	<0.001	-	5.455
BYI08330-enol	0.01	4.26	0.56	0.047	0.168	0.870
BYI08330-enol glc	0.03	-	0.36	0.004	0.006	0.395
BYI08330-cetohydroxy	0.05	1.09	0.20	0.011	0.018	2.745
BYI08330-monohydroxy	0.10	-	-	-	-	-
Total residues	0.61	36.63	3.13	0.119	0.225	11.057
MRL[a]	0.7	-	8	0.3	1.6	-
MRL[b]	0.7	-	7	-	0.8	-

[a]MRL, [26]

[b]MRL, [25]

Source: [24], modified

Table 2. Proportions and principal metabolites of spirotetramat in apple, cotton, lettuce, and potato.

4.3. Fate of spirotetramat in the soil

It is necessary to investigate the degradation of the active compounds in the soil since it is possible that part of the insecticide will reach the soil directly or indirectly after being applied to a crop. The most important process to consider in the soil is the degradation by microorganisms under aerobic conditions. However, there are other factors that could contribute, such as the abiotic chemical degradation expressed as photolysis on the soil surface and also hydrolysis; other physical processes involved such as leaching, a translocation that can make it more profound in the soil; volatility; and the evaporation from the plant or from the soil
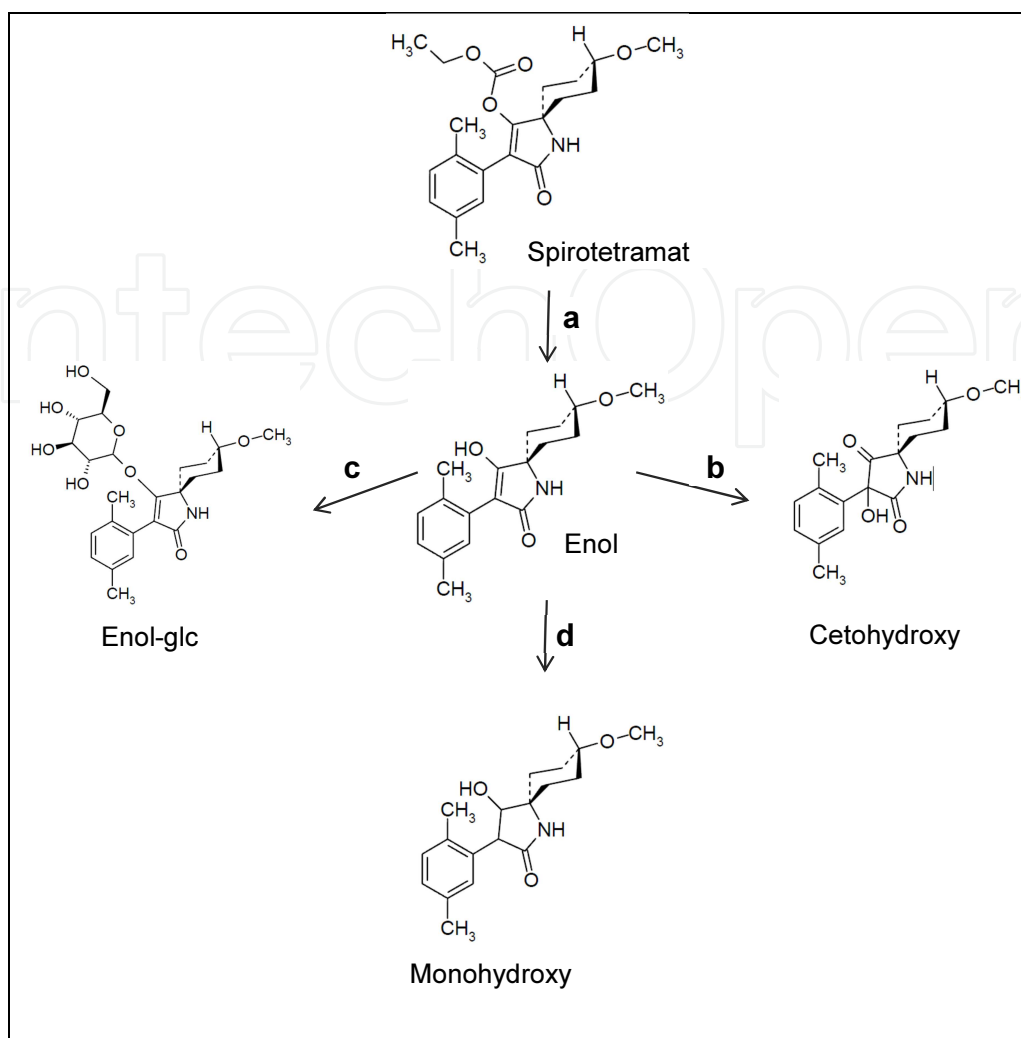
Figure 3. Principal reactions and metabolite of spirotetramat in plants: a) Hydrolytic split, b) Oxidation of the Pyrrole group, c) Conjugation of the hydroxyl group BYI08330-enol with glucose, and d) Reduction.

surface [22]. However, these same researchers [22] observed that spirotetramat under aerobic soil conditions will degrade rapidly after 1-2 days, dissipating more than 90%. At the same time, during the testing period the two major metabolites generated were BYI08330-enol (maximum 24.3%) and BYI08330-cetohydroxy (maximum 16.3%), two of the dimers enol BYI08330-MA-amida (maximum 6.4%), and lastly two minor metabolites, BYI08330-desmethyl-enol (maximum 3.7%) and BYI08330-oxo-enol (maximum 1.2%) [23, 27].

In the study designed for 127 days, under aerobic conditions, spirotetramat was degraded rapidly; a day after the application, only 53.6% and 72.2% of the substance was detected. There were two principal metabolites identified, BYI08330-cetohydroxy (maximum 25.3%) and BYI08330-enol (maximum 7.8%); there were also three minor metabolites detected, these were confirmed using the previous method established in laboratory studies. It was also observed that for the aerobic soil metabolism, under acidic extraction, the metabolite BYI08330-enol was partially unstable, and that like spirotetramat it dissipated using a two-phase kinetic [22].

As was mentioned earlier, the velocity of degradation of spirotetramat in the soil under aerobic conditions was very rapid. Under laboratory conditions, the degradation time (DT_{50}), was from 0.14 days (geometric average); for the majority of the scenarios it was 0.21 days. In situations with trails under outside climatic conditions, spirotetramat also degraded rapidly, with a DT_{50} average of approximately 2 days. The velocity of degradation for BYI08330-enol in the soil under aerobic conditions was 0.08 days (DT_{50}); this information allows us to conclude that this metabolite is the one that will degrade rapidly [22].

The soil degradation studies under field conditions with spirotetramat demonstrate that the dissipation velocity DT_{50} was between 0.3 and 1.0 days; the dissipation of 90% (DT_{90}) was between 1.1 and 3.5 days. In the case of the combined residues of spirotetramat (BYI08330-enol, BYI08330-cetohydroxy), the DT_{50} was between 5.0 and 23.4 days, the DT_{90} had a range of 16.7 to 77.8 days. The residues of spirotetramat were not found to be below the shallow layer (0-15 cm), due to the possibility of the presence of leaching in subterranean waters was not probable. Considering that within 14 days after the application of spirotetramat it degraded to concentrations below 0.5 µg kg^{-1}, the possibility of the accumulation of residues in the soil one year later after the first application is low [22, 27].

The photo-transformation of spirotetramat on the soil surface does not represent a process of degradation relevant to conditions of solar radiation. The trials undertaken to evaluate photo-transformation on the soil surface reveal that there are no different products derived from this effect after the application of spirotetramat [22, 23].

On the other hand, the anaerobic degradation in the soil follow almost the same route as under aerobic conditions, that is to say that no different metabolites are formed than those observed under aerobic conditions and it is concluded that it degrades rapidly [22, 23, 27].

Based on the literature discussed previously, the main route of spirotetramat dissipation in soil is the degradation to enol-BYI08330 and BYI08330-cetohydroxy; these followed by a degradation to non-extractable residues and mineralization to CO_2. Concerning the mobility of the spirotetramat, the results showed that this pesticide can be classified as low mobility in soil. In the case of the BYI08330-enol, the strongly retained portion is considered stationary, while the weak form, as well as the BYI08330-cetohydroxy bound fraction possesses an intermediate leaching potential through the soil [22, 27].

4.4. Fate of spirotetramat in the aquatic environment

The research trials conducted demonstrate that spirotetramat is susceptible to degradation under biotic and abiotic processes in darkness as well as solar light. With reference to the abiotic degradation, the hydrolytic degradation becomes a relevant mechanism for the degradation of spirotetramat in the environment, especially under neutral and alkaline conditions. The half-life under hydrolytic conditions (20°C) at pH 7 is from 13 days, and at pH 9 it is less than half a day. On the other hand, hydrolysis does not represent a relevant degradation mechanism with regards to BYI08330-enol in the environment, the half-life at the pH range of 4 to 9, at 25°C is expected to be about a year [22].

The results of the photo-transformation in water demonstrate that this mechanism contributes in a significant way to the elimination of spirotetramat in natural water. In systems with water/ sediment, spirotetramat is degraded rapidly through the metabolites BYI08330-enol and BYI08330-cetohydroxy. In the same system under anaerobic conditions, spirotetramat de‐grades rapidly, mainly into the metabolite BYI08330-enol. From the previous information and the evaluation of drinking water exposure, the use of spirotetramat does not represent a risk to human health [22, 23].

According to the results of toxicological studies isolated in *Ceriodaphnia dubia*, it was observed that mixing spirotetramat with an agricultural adjuvant (Destiny) caused more damage together than each one separately; this does not indicate synergy, but that each compound causes a certain level of mortality, and together the effect of the mixture is additive. This suggests that no further study is needed to determine which mixes of insecticides and adjuvants are causing damage to aquatic organisms [28].

4.5. Interaction of spirotetramat with the air

With a base vapor pressure of 5.6×10^{-9} Pa for spirotetramat and 1.2×10^{-10} Pa for l BYI08330-enol, it is expected that none of the two compounds will volatize when applied to the leaves or to the soil surface. Furthermore, considering the estimated life of these compounds in the air (maximum 3 hours); they are not expected to be able to travel in a gaseous state over large distances and as a result they cannot accumulate in the air [22, 27].

5. Field studies of spirotetramat in grapevine

It is essential to understand the course of action and toxicity of pesticides through the devel‐opment of methods and procedures of bioassays because some chemicals (such as organo‐phosphates, carbamates, and pyrethroids) can express their toxicity in a maximum time frame of 48 hours; other reduced risk pesticides such as spirotetramat, spinetoram, novaluron, chlorantraniliprole, and flubendiamide express their toxic effects several days after treatment [11]. For the majority of systemic insecticides, the primary route of entry is through the xylem, normally through the roots after directed applications. A foliar treatment of spirotetramat can be translocated acropetally and basipetally; it can also be ambimobil (movement across the xylem and phloem) and by this manner it can supply systemically to the top and bottom of the plant with a great potential for control of grape *phylloxera* [29].

The profile of the insecticide residuals in grape is influenced not only by its penetration and properties of translocation, but also by the active growth pattern of the vine that will produce an effect of dilution of the residues. If the compound is applied on the mature leaves through a foliar application, the drop in the residues will depend on the environmental degradation of the compound, given that the life size is constant during this stage [30]. Spirotetramat is an insecticide compatible with an integrated pest management approach; it has provided a new mode of action against sucking insects such as whiteflies, psyllids, and aphids [22]; it is effective

in reducing the stages of *A aurantii* (citrus pest) and allows for the survival of the primary parasite *A melinus* [31].

In reference [29], they concluded that the goal of obtaining laboratory data would be useful for the implementation of a strategy to implement when using pesticides; it is essential to understand the attributes of the pesticide in question, the target organism (pest or beneficial), and the ecosystem that these organisms are present in.

6. The possible generation of resistance to spirotetramat

Resistance to insecticides and myticides is one of the serious obstacles in the effective management of pests, and is a clear example of evolution and natural selection. True resistance is produced when there is a structural genetic change that could be hereditary. In contrast, tolerance is the natural ability of a population of arthropods to tolerate the toxic effects of a specific insecticide. This can occur through a physiological adaption in just one generation but by the same toxin, it can lose the effect if the insects are not exposed again to this toxin. Actually, the insecticide and myticide resistance in grape in North America is not a problem due to the existence of management programs [29].

Two types of resistance are recognized—behavioral and physiological. Behavioral resistance is defined as the capacity of the arthropod to avoid toxic doses that ordinarily would be lethal. On the other hand, physiological resistance is a question of hypersensitivity of the arthropod exposed to the compound, which depends on three factors: reduction in the penetration of the toxin, a better way to detoxify, and desensitizing the target destination [29].

The cases of documented insecticide resistance in aphids within the group of ketoenols is for spirodiclofen, where there have been strains observed in the laboratory and field populations of mites *Tetranychus urticae* [32-34], *Panonychus citri* [35], and *Panonychus ulmi* [36]. This information indicates a possible risk of resistance in aphids to spirotetramat. Recent studies by Pan and collaborators [37] report a strain of cotton aphid that develops spirotetramat resistance of 11.97 times by adults and 441.26 times by adult nymphs, in comparison with the susceptible strain. However, these lack the cross resistance to existing insecticides and for this reason it is considered a new tool in the management of insecticidal resistance for cotton aphid.

A proteomics study based on identification and analysis of proteins associated with the mechanism for tolerance to spirotetramat in *Aphis gossypii Glover* detected approximately 493 associated protein points that possibly may confer resistance to spirotetramat for the cotton aphid [38]. Knowledge generation involving proteomic resources are expected to contribute to a better understanding of the development of resistance to spirotetramat.

7. Conclusion

Spirotetramat acts as a biosynthesis inhibitor of lipids and presents good activity against the most important aphids including whitefly. The activity of spirotetramat predominates in the

immature stages of development in aphids, causing on occasion an incomplete ecdysis and causing a reduction in productiveness and fertility. The results suggest that spirotetramat can be used in a secure program of integrated pest management involving biological control, however, in this respect there is contradictory information; each case should be evaluated independently depending on the organism used for biological control.

Once spirotetramat is found inside the plant, it is transformed to its enol form and to the metabolite cetohydroxy, both are two main degradation compounds. The possibility of leaching of spirotetramat into subterranean waters is low according to the results; the probability of accumulating in the soil is also very low. On the other hand, based on the results in aquatic environment, under certain conditions there can exists the possibility of accumulation in this system; for this reason adequate care should be taken in applications near aquatic systems. Furthermore, no reports were located that indicated the possibility of spirotetramat accumulation in the air. Lastly, up to this moment there have been proteomic studies suggesting certain forms of resistance to spirotetramat in cotton aphids.

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