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FLONICAMID - A NOVEL MODE OF ACTION FOR PIERCING SUCKING INSECTS

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Introduction

Flonicamid is a novel pyridine-carboxamide insecticide (N-cyanomethyl-4-trifluoromethyl nicotinamide, CAS 158062-67-0) that is particularly effective against aphids, *Lygus* spp. and other piercing/sucking insects. It has been approved for use on a variety of crops at rates varying from 0.044 to 0.089 lb/acre, varying with the insect pest and crop. Flonicamid has plant systemic activity). Aphids and plant bugs respond quickly upon exposure to flonicamid with feeding ceasing within 15 – 30 minutes and death following some time later depending on environmental conditions. In addition, flonicamid's selectivity for Homoptera and Hemiptera confers an unusually safe profile against non-target beneficial insects as well as for other environmental concerns.

Flonicamid possesses a novel mode of action that is distinct from the neonicotinoids (Hancock et al. 2003, Black et al. 2006, and Hayashi et al. 2006. .Ishihara Sangyo Kaisha (ISK) discovered it and initial development was conducted by ISK and ISK Biosciences. Flonicamid is now being co-developed by ISK and FMC Corporation. Flonicamid will be marketed in the Americas by FMC and will be sold in cotton as Carbine™ 50 WG.

A number of studies have been conducted to determine the mechanism of action of flonicamid. The results of this research clearly show that flonicamid blocks the A-Type potassium channel. This paper is a brief summary of this research.

Methods

Exclusion, binding and electrophysiology studies

A series of assays were conducted to determine if the mode(s) of action of flonicamid differs from that of the most common commercial insecticides used in US crop protection today. These included: exclusion studies to determine if flonicamid's target site was the same as known target sites, nicotinic receptor site studies that involved binding studies, nicotinic cross-resistance work and symptomology studies. In addition, electrophysiology studies utilizing whole-cell patch-clamp recording from living insect neurons, were conducted to determine if flonicamid has any effect on the nicotinic acetylcholine channel. The specific details regarding the methods used in this research are reported by Black et. al 2006 and Hayashi et al. 2006.

Cross-resistance studies

The cross-resistance studies were conducted using a foliar dip bioassay. In these tests the foliage was dipped into aqueous solutions of the test materials, the foliage was allowed to dry and then the foliage was infested with aphids. The tests were assessed for mortality 72h after the aphids were exposed to the treated foliage.

Electronic feeding monitor assays

In addition, electronic feeding monitor assays (Figure 1) were conducted on *Lygus hesperus* to determine the effects of flonicamid on *Lygus* feeding and behavior. Briefly, these studies measure changes in electrical resistance in the circuit that is completed when the insect makes contact with the plant with its mouthparts. The electrical resistance waveform patterns produced are correlated with actual feeding events.

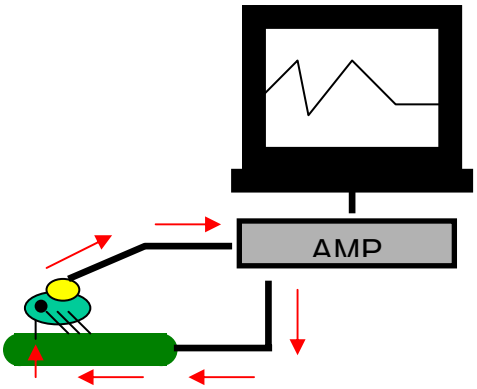

Figure 1. Diagram of setup of electronic feeding assay.

Symptomology studies

The effects of flonicamid on *Aphis gossypii* and *Lygus spp*. were determined by exposing the insects to a dry film of flonicamid on glass or foliage; removing the insects; placing them on untreated plant foliage and then observing .the insects for behavioral effects. These effects included walking, antennal and body movements and feeding.

Results

Exclusion studies

A number of studies were conduct to exclude known target sites. The data from these tests demonstrated how flonicamid does not work. The target sites examined included the following known target sites: acetylcholinesterase, nicotinic acetylcholine receptor, octopamine receptor, nitric oxide synthase, nitrous oxide receptor, Complexes I-V and Uncouplers, GABA-receptor, Na+ channel and insect growth regulators.

The commercial reference standards performed as anticipated while flonicamid did NOT inhibit/bind to the targets in these assays

Cross-resistance studies

A number of evaluations of flonicamid on strains of *Aphis gossypii* that were known to be resistant to carbamates, organophosphates and/or pyrethroids were conducted by ISK. High levels (>100 fold) of resistance were present in these strains. However, flonicamid was virtually unaffected by the resistance present in these strains (Figure 2) [Need to fix Figure info].

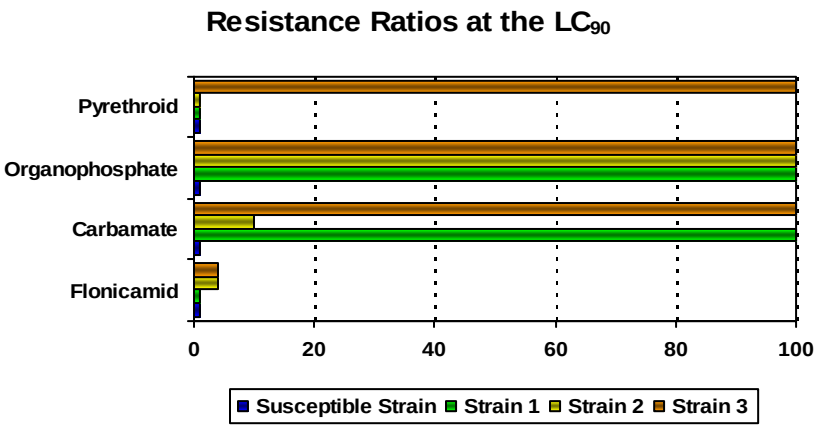

Figure 2. Resistance levels for chemical classes shown in Aphis gossypii determined in foliar bioassays (ISK data).

In another cross-resistance study, flonicamid was evaluated against susceptible and imidacloprid resistant *Bemesia tabaci* collected in Spain. Flonicamid is not highly effective on this species of whitefly but it has sufficient activity to conduct cross-resistance bioassays. Only a small difference in activity was observed between the susceptible and resistant strains for flonicamid but a rather large difference was noted for imidacloprid (Figure 3).

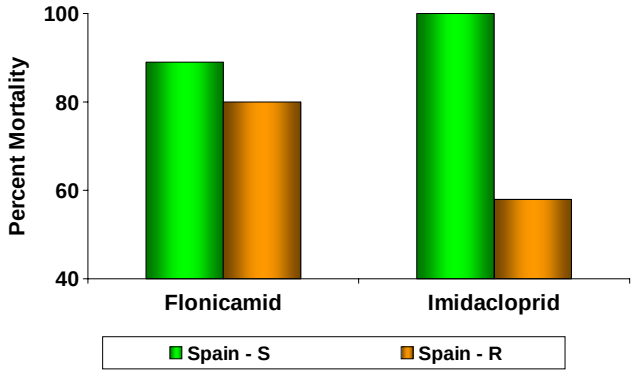

Figure 3. Percent Mortality (96h) of susceptible and neonicotinoid resistant *Bemesia* strains from Spain (U. Almeria) after exposure to 100 ppm flonicamid and imidacloprid

Neonicotinoid Binding Studies

Imidacloprid and acetamiprid displaced bound imidacloprid with nanomolar potency. Additionally, thiamethoxam and clothianidin also displaced bound imidacloprid. In contrast, even at concentrations as high as 100 µM, flonicamid failed to compete with imidacloprid or acetamiprid for nACh receptor binding sites in aphid receptor membranes.

In addition, it was found that flonicamid did not compete with methyllycaconitine, the most potent small molecule nACh receptor antagonist.

The results of this work are shown in Table 1.

Table 1. Results of competition studies with bound imidacloprid.

	Concentration (nM) required to compete with the compounds shown at nACh Receptor binding sites		
Compound	Imidacloprid	Acetamiprid	Methyllycaconitine
Imidacloprid	2.5	2.5	2.5
Acetamiprid	3.98	3.1	3.1
Clothianidin	7.94	5.01	5.01
Thiamethoxam	316	160	160
Methyllycaconitine	0.79	0.79	0.79
Flonicamid	Not Active (>100,000)	Not Active (>100,000)	Not Active (>100,000)

Electrophysiology Studies

This research utilized whole-cell patch-clamp recording from living insect neurons to assess flonicamid mechanism-of-action. Direct recordings of ionic current flowing through nicotinic acetylcholine channels (nACh) demonstrated that flonicamid failed to alter flow of current through this channel in three species: *Heliothis virescens*, *Peripalaneta americana* and *Drosophila melanogaster*, i.e., the high dose of flonicamid had no effect (Figure 4).

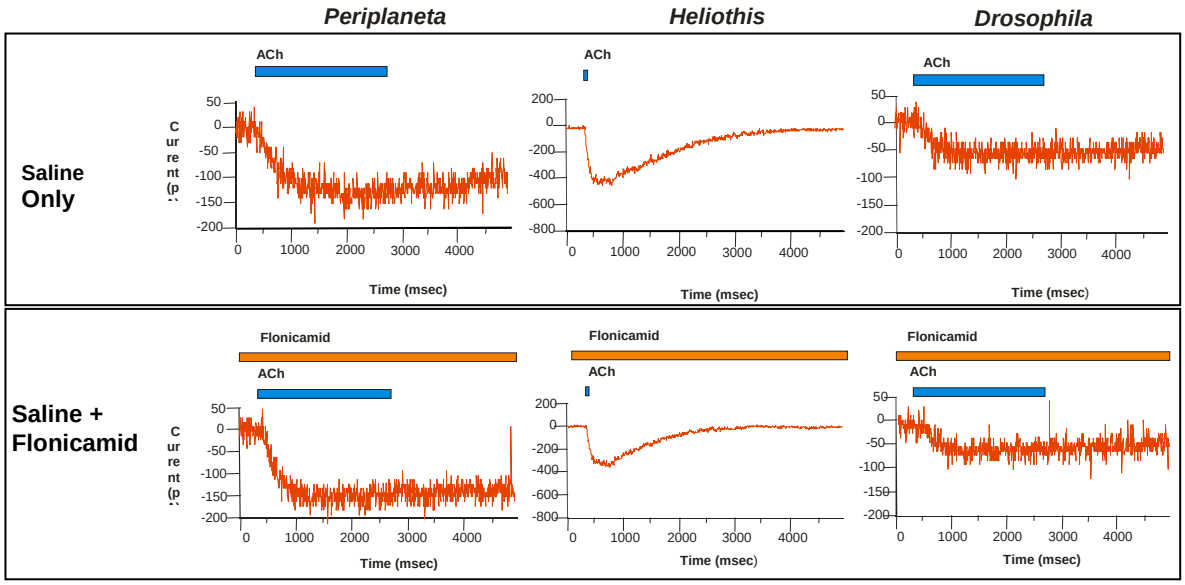

Figure 4. The response of the nicotinic ACh channel to a high dose (10 uM) of flonicamid.

A similar test was conducted with imidacloprid. In the presence of imidacloprid the cell no longer responded to a pulse of acetylcholine Figure 5.

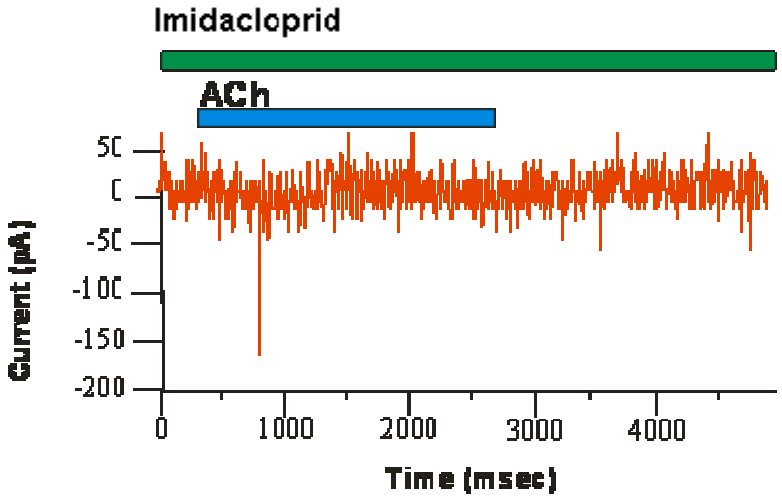

Figure 5. Lack of response of the cell to a pulse of acetylcholine in the presence of imidacloprid, the cell can no longer respond to the pulse of ACh (blue bar).

Electrical feeding studies

The electrical feeding studies clearly showed that flonicamid shut down feeding within about 30 minutes or less (Figure 6).

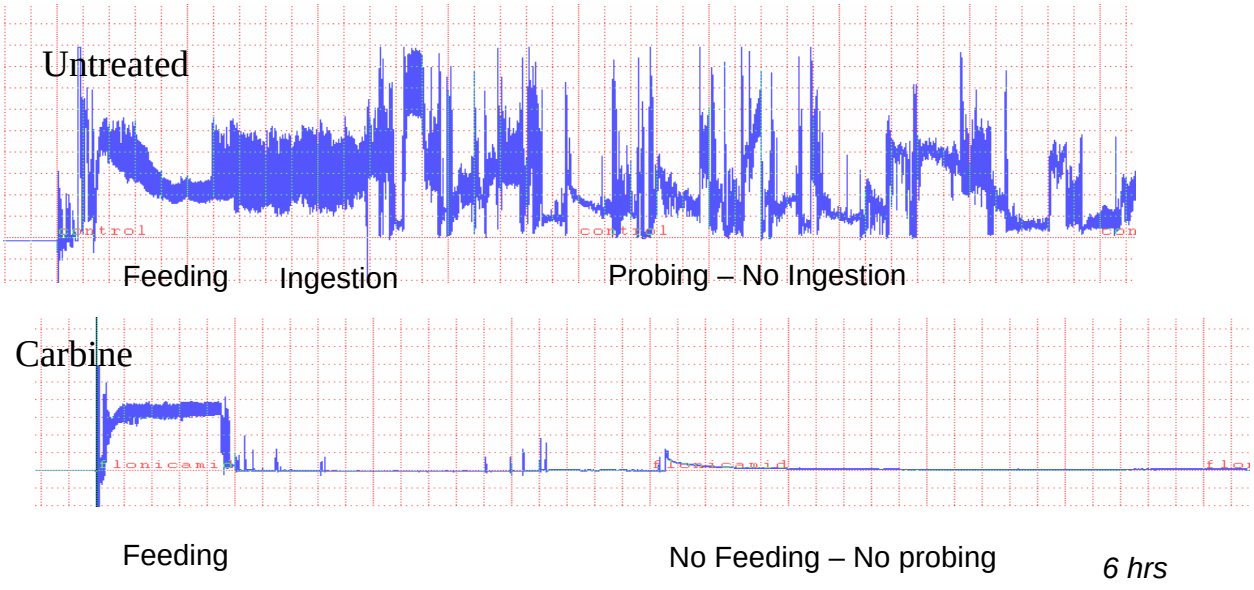

Figure 6. The results of electrical feeding studies showing that flonicamid stops feeding within about 30 minutes or less.

Stopping feeding reduces ingestion by plant bugs and also significantly reduces the number of feeding scars caused by the feeding (Figures 7 and 8).

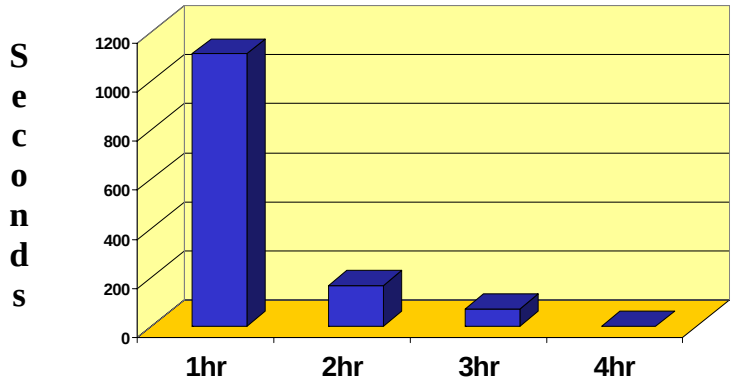

Figure 7. Reduction in the time of *L. hesperus* feeding after treatment with flonicamid..

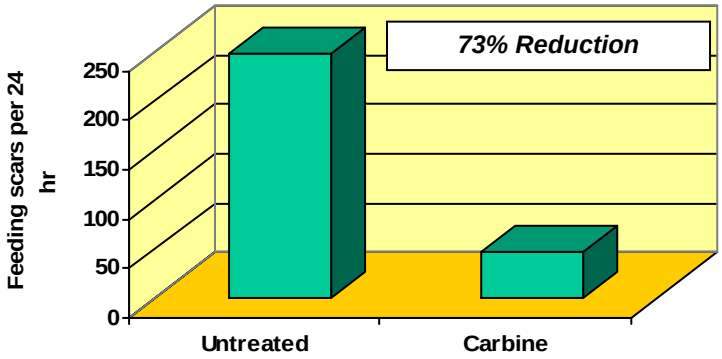

Figure 8. Comparison of feeding scars between treated and untreated plant foliage.

Symptomology

Each chemical class produces unique symptoms of intoxication in insects that are affected by the chemistry. Consequently, observation of the symptoms can be used to determine if two compounds belong to the same chemical class. The symptoms produced by flonicamid are quite different from those evoked by the neonicotinoids.

After 15 – 30 minutes of exposure to flonicamid plant bugs and aphids results in a halting of feeding, characteristic leg flicks and swivels, increased antennal movement, difficulty in righting, staggered walk, quivering and enhanced light sensitivity. Conversely, imidacloprid produces fast, violent twitching, falling over, uncoordinated movements and the legs are bent over the thorax.

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The effect of flonicamid on A-type potassium currents:

Hayashi et al (2006) conducted A-type potassium current experiments using *H. virescens* neurons because these currents are better characterized than those of *Periplaneta* or *Drosophila*. By recording from cultured *Heliothis* neurons after 1 to 2 days in culture, large outward, potassium currents in normal AIS saline were observed (Figure 9) . When the current family included an inactivating A-type outward current, the addition of 10 uM flonicamid caused the current to be abolished. After switching back to normal AIS saline, the A-type current was recovered . Flonicamid antagonizes an A-type potassium current in cultured Heliothis neurons.

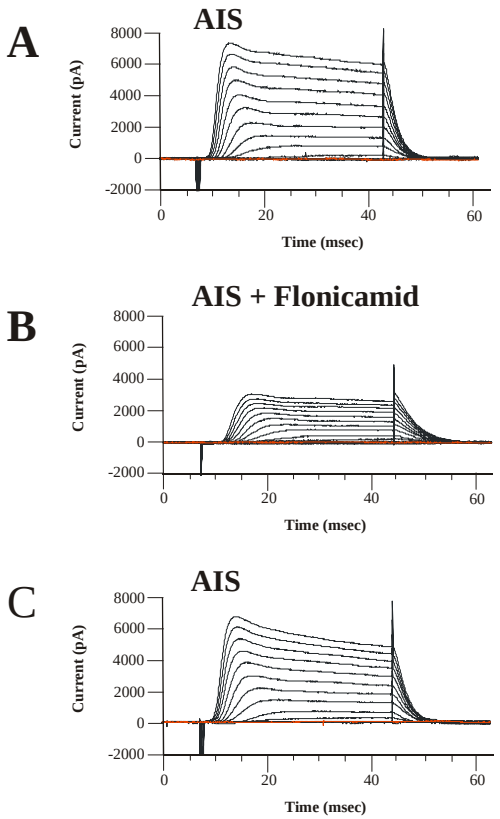

Figure 9: Voltage gated outward currents were exhibited by H. virescens neurons in whole-cell patch-clamp. The outward current, identified by kinetics, include aninactivating A-type potassium current and a non-inactivating delayed rectifier type current. A) Outward currents observed in normal AIS saline. B) The addition of 10 uM flonicamid to the superfusing saline caused loss of the A-type potassium current. C) When normal AIS superfusing saline was resumed, the A-type potassium current returned in 15 minutes.

The putative mechanism of flonicamid's lethal effect on insects is the blockade of the A-Type potassium channel at the presynaptic terminal. A blockade of this channel would disrupt normal synaptic transmission and lead to a loss of nervous system control. This is supported by the observation that aphids, when treated with flonicamid, exhibit a loss of directed movement characterized by a cessation of feeding and a continuous non-directed behavior

Conclusions

- At least one of the mechanisms of flonicamid's lethal effect on insects is the blockage of the A-Type potassium channel.
- In aphids and plant bugs, treated with flonicamid, this is exhibited by a loss of directed movement characterized by a continuous non-directed behavior and a cessation of feeding.
- Cessation of feeding prevents plant damage.
- Flonicamid's lethal effect on insects does not resemble that of imidacloprid
- Does nNot act on nicotinic receptor binding site.
- Flonicamid is inactive on the nACh receptor.
- Studies confirmed that flonicamid does not compete with the neonicotinoid receptor binding sites or with any site within the nACh Receptor complex.
- Novel target site - no cross-resistance issues.
- Flonicamid is NOT a neonicotinoid.

References

Black, Bruce, Joe Argentine, Rathnam Chaguturu, Ratna Dargar, Leo Dungan, Russ Eldridge, Jeff Herron, Lyle Kinne, Russ Peters, Amy Wrzesinski, Debbie Yuhas. 2006. Flonicamid Mode of Action Studies. In Proc. Beltwide Cotton Prod. Conf., National Cotton Council, Memphis, TN.

Hancock, Gary H.. 2003. M. Fustaino, M. Morita. Flonicamid (F1765, IKI220): Novel Insecticide Chemistry for Cotton and Other Crops. 2003 Beltwide Cotton Conference, Nashville, TN. pp 83-88.

Hayashi, Jon H., Geralyn Kelly and Lyle P. Kinne. 2006 Flonicamid mechanism-of-action studies using while-cell patch-clamping of cultured insect neurons. In Proc. Beltwide Cotton Prod. Conf., National Cotton Council, Memphis, TN.

Joost, Houston. 2006. Precise Feeding Cessation Characterization of *Lygus hesperus* exposed to Carbine. In Proc. Beltwide Cotton Prod. Conf., National Cotton Council, Memphis, TN.

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