ABSTRACT

DHAMMI, ANIRUDH. Effect of Imidacloprid on *Cotesia congregata,* an Endoparasitoid of *Manduca sexta,* and its Translocation from Host to Endoparasitoid. (Under the supervision of Dr. Clyde E. Sorenson.)

Field and lab studies were conducted to investigate the effect of imidacloprid on *Cotesia congregata* (Say) and its parasitism in tobacco hornworm, *Manduca sexta* (L.). Residue of imidacloprid and its metabolites were quantified in the hemolymph of *M. sexta* fed on imidacloprid treated diet and effect of the insecticide on parasitism by *Cotesia congregata* was also investigated. We also investigated the translocation of the insecticide from the host (*M. sexta*) to the endoparasitoid (*C. congregata*).

A two-year (2008 and 2009) field study undertaken to investigate the effect of foliar and systemic application of imidacloprid on parasitism indicates that at least foliar application (with the formulation Provado) has a negative impact on parasitism; percentage of parasitism was significantly lower in this treatment compared to the other treatments. In laboratory studies, direct contact with imidacloprid produced detrimental effects on *C. congregata* adults as did residue deposited on plant or food sources. However, rearing parasitized host larvae on diet with imidacloprid incorporated in it did not decrease *C. congregata* longevity. In a preliminary study of the negative effects of imidacloprid on parasitism of tobacco budworm, *Heliothis virescens (F.)* by its parasitoids (*Campoletis sonorensis* and *Cardiochiles nigriceps*), some suppression of parasitism by imidacloprid was observed.

We established that the QuantiPlate ELISA kit, which was originally developed for quantifying imidacloprid residues in water, can be used to measure residues in *M. sexta* and

C. congregata. There was no significant difference in residue in the hemolymph of *M. sexta* fed on imidacloprid treated plants at different rates and treatment timings in the field. However, non-parasitized *M. sexta* larvae do have significantly higher residues of imidacloprid as compared to parasitized larvae, suggesting an effect of imidacloprid on parasitism in *M. sexta*. We also established that imidacloprid and its metabolites do translocate from host *M. sexta* to *C. congregata*.

Effect of Imidacloprid on *Cotesia congregata*, an Endoparasitoid of *Manduca sexta*, and its Translocation from Host to Endoparasitoid

by
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BIOGRAPHY

Anirudh Dhammi was born to Ravinder and Sudarshan Dhammi on 25th November 1979 in Punjab, India. He was raised in the small town of Jagraon and enrolled at Punjab Agricultural University in 1998 for his Bachelor of Science. He graduated in 2002. He then completed a diploma in business from Crown Institute of Auckland, New Zealand in 2004, and then worked there for next two years. He was admitted as a graduate student at North Carolina State University under the supervision of Dr. Clyde Sorenson in 2007.

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INTRODUCTION

Manduca sexta (L.), commonly known as the tobacco hornworm, is one of the most destructive pests of tobacco. If left unchecked, *M. sexta* can destroy whole crops within weeks. Tobacco is the 7th largest cash crop in America and one of the most important crops in North Carolina and Kentucky, which together contribute about 2/3 of the total tobacco production the United States. This insect is potentially the most damaging pest of tobacco, particularly in southern states, where it can have 3 to 4 generations per growing season. *Manduca sexta* completes its life cycle in 30 - 50 days. Eggs hatch in 2 to 3 days and then proceed through 5 larval stages before pupation. It takes an average of about 22.8 days for pupation (Reinecke 1980). *M. sexta* is part of the hornworm complex along with *M. quinquemaculata* (Haworth) (the tomato hornworm). These are closely related species, but *M sexta* is the main focus for our study because it is easily available (it is reared at the NCSU insectary) and moreover is a model insect for many insect studies.

In commercial production systems, the tobacco hornworm is not very hard to control with insecticides; it is highly susceptible to acephate, spinosad (Tracer), methomyl (Lannate), *Bacillus thuringiensis* (Bt), emamectin benzoate (Denim), and flubendiamide (Belt) sprays (Burrack et. al. 2010). Control of insects with insecticides may be very effective, but it has become problematic because of the threat to the health of humans and wildlife (Carter 1966). In the last 40-50 years we have come to know about the negative effects of insecticides and insect resistance. DDT, one of the most potent insecticides, is now tolerated by numerous insects (Metcalf, 1989), and its effects on higher trophic levels has also been described (Kidd et. al. 2001). Insects have developed resistance not only against conventional insecticides like

organophosphates but also to the new generation insecticides like spinosad and *Bacillus thuringiensis* (Bt) toxin. Diamond Back Moth (DBM) has developed resistance against spinosad (Zeng-Mei et. al 2005) and Bt toxin in open fields (Heckel et. al 1999). Resistance in insects not only decreases crop production but also requires the discovery of new, alternative insecticides. Since 1980, 13% of US crops have been lost due to insect resistance as compared to 7% in 1940, even though we are using more insecticides (PBS, 2001). In order to counter this problem, Integrated Pest Management (IPM) has emerged as a powerful paradigm, in which multiple tactics (chemical, biological and cultural) are used in a compatible manner to keep pests below their economic injury level while providing protection for humans, animals, plants and the environment (Metcalf and Luckmann, 1994). Biological control and good agricultural practices with sensible use of insecticides can maintain insects at minimum levels. Biological control has great importance in controlling *M. sexta*. *M. sexta* has many natural enemies, including the predaceous *Polisties* spp. wasps. In addition, parasitoids, including *Trichogramma* spp., *Euplectrus platypenae* (Howard), and *Cotesia congregata* (Say) are important biological control agents (Gilmore 1938).

The adult *C. congregata* wasp oviposits in 2nd or 3rd stadia larvae of the host. It lays multiple eggs which hatch within 2 – 3 days of oviposition and undergo molting twice inside the host hemocoel. Emergence of 3rd instars takes place 12 – 17 days after oviposition. As soon as these larvae emerge from the host, they start spinning a cocoon, and the adult emergence takes place 3 – 8 days after cocoon formation (Fulton 1940).

Hymenopteran parasitoids coevolve with their host (Drezen et al. 2003). While over 500 insect species have documented resistance to pesticides (Swanson 2006), parasitoids still

remain effective. The symbiotic relationship between *C. congregata* and a bracovirus makes the wasp an even more effective parasitoid. CcBV (*C. congregata* Bracovirus) is injected at the same time as the wasp eggs in the host hemolymph. This virus suppresses the host immune system and promotes parasitoid development (Espagne et al. 2005).

C. congregata is an important insect not only for biological control but also as an important model for study. This host and parasitoid system has been the basis of many studies providing a better understanding of behavioral, physiological and biochemical aspects of host-parasitoid interactions. The caterpillar host exhibits a change in behavior after infection by parasites (Horton and Moore 1993). Feeding and locomotion of *M. sexta* decline 12 hrs before the emergence of *C. congregata* larvae, along with an increase in octopamine levels. Correlation between the change in host behavior and central nervous system octopamine contents infer the role of the octopaminergic system in depressing host feeding and locomotion (Adamo and Shoemaker 1998). It has been seen that high octopamine levels disrupt the functioning of the frontal ganglion, which decrease feeding by preventing swallowing (Miles and Booker, 1994, 2000). This biochemical interaction might lead to development of new ecologically acceptable insecticides. Moreover, the parasitoid *C. congregata* manipulates ecdysteroid levels of fifth instars of the host (Gelman et al., 1997).

Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine, is a member of the neonicotinoid insecticide class and highly effective against a variety of insects, including aphids, leafhoppers, planthoppers, thrips, and whiteflies (Elbert et al. 1991). It is also highly effective against insects such as *Amrasca devastans* (Dist.), *Bemisia tabaci* (Gennadius) (Razak el. al 2005) and *Schizaphis graminum* (Rondani) (Stone el. al

1999) which are resistant to conventional insecticides. Imidacloprid is xylem-mobile, which makes it especially useful for seed and soil treatments (Elbert et al. 1991). It acts on the nervous system of the insect and causes the blockage of postsynaptic acetylcholine receptors, leading to the death of the insect (Ware, 2000). Due to its acute toxicity for insects and low vertebrate toxicity, it has become one of the most widely used insecticides in the world (Matsuda et al 2001). By 2005 about 600,000 lbs of imidacloprid was used in the US. However, many studies have shown its negative effects on non-target insects and other arthropods. The predator *Hippodamia convergens* (Guérin-Méneville) (Coccinellidae) had significant mortality after exposure to imidacloprid residues (Mizell and Sconyers 1992). Similar effects were found in the predator *Orius tristicolor* (White) when confined with imidacloprid treated foliage (Sclar et al. 1998). Mortality of adult *Chrysoperla carnea* (Stephens) (Chrysopidae) was 83.3% in 24 h when exposed to imidacloprid in spray chamber experiments (Elzen et al., 1998). In addition to the effect of imidacloprid residue from foliar applications, systemic treatments of imidacloprid can also have significant effects on beneficial insects. *Coleomegilla maculata* (De Geer) (Coccinellidae) experience about 38% mortality just by feeding on flowers of plants treated with soil drenches of imidacloprid (Smith and Krischik 1999). Along with the direct contact mortality, sub-lethal effects of insecticides also need to be studied because these materials may reduce reproduction and reduced longevity, severely reducing the performance of biological control (Jacob et al., 1984; Roger et al., 1995).

Imidacloprid also has adverse effects on hymenopterans. Numerous studies have demonstrated its effects on *Apis mellifera* (Scutellata), the European honeybee. When

exposed to 24 parts per billion (ppb) of imidacloprid in artificial nectar, *A. mellifera* shows a 60% decrease in forging and decreased mobility in hives (Decourtye et al., 2004). In cucumber plants, 24 hrs of exposure of imidacloprid spray induced 30% mortality in *Bombus terrestris* (Linnaeus) (Incerti et al. 2003). A foliar application of imidacloprid decreases longevity by 25% and host finding by 77% in the wasp parasitoid *Microplitis croceipes* (Cresson) (Stapel et al. 2000). The adult mortality of *Diadegma insulare* (Cresson), a parasitoid of the diamond back moth, is also significantly higher after 24 hrs of imidacloprid treatment (Hill and Foster 2000)

Penetration of imidacloprid through insect cuticle is relatively low (Pfluger and Schmuck 1991), so in most cases it is considered to be safe for parasitoid larvae which reside inside their host. This might be true in case of foliar sprays, where beneficial insects are screened mainly by direct exposure to the insecticide, but indirect routes, like feeding on the contaminated prey, are often ignored (Granett and Weseloh, 1975; De Cock et al. 1996). Systemically applied imidacloprid moves through the soil and plant and then into insect hemolymph when they feed. Parasitoid larvae which feed on hemolymph of such insects could be affected by this insecticide. Hosts may survive or acquire resistance to an insecticide by sequestering or metabolizing it (Kadous 1983). In this case, the host may convert an insecticide to the metabolites which are toxic to internal parasitoids before it reaches the parasitoids, thus affecting the endoparasitoid (Croft 1990). When *M. sexta* fed on the leaves of tobacco systemically treated with imidacloprid, there was no observable increase in mortality (Bock 2010). If *M. sexta* sequesters or metabolizes imidacloprid, it is important to know if imidacloprid or its metabolites have any effect on the endoparasitoid *C.*

congregata; understanding the residues of imidacloprid and its metabolites in *M. sexta* is very critical in understanding any impacts on parasitism. Many studies have been done to look at the metabolism of imidacloprid in various biological systems. The quantification of imidacloprid and its metabolites is done mainly on environmental elements like soil and water (Baskaran 1997) where the concern is direct harm to non-target organisms.

Quantification of imidacloprid and its metabolites has been mostly done by HPLC-MS/MS, and, in some cases, with radioactive labeled compounds. HPLC is very sensitive and has been used most frequently. A HPLC protocol has been developed to quantify imidacloprid in potato and onion with a detection limit of 0.0075 and 0.0060 mg/kg respectively (Mandic et. al. 2004). A method to determine imidacloprid in tea was also developed with a sensitivity of 0.005 mg/kg (Sanyal et. al. 2006). Residue analysis of imidacloprid was conducted in tobacco with the limit of quantification of 0.04mg/kg. Imidacloprid was found to be in the range of 0.07mg/kg – 4.5mg/kg in baked tobacco using this method (Liu et. al. 2005).

Where many studies on the metabolism of imidacloprid have being conducted in plants, soil and water, few have been conducted in insects systems (Byrne et. al. 2003). Metabolites of imidacloprid were determined in *Musca domestica* (Linnaeus) through ^{14}C labeled imidacloprid (Nishiwaki et. al. 2004). HPLC – MS/MS was used to quantify imidacloprid and its metabolites in *A. mellifera* with the limit of quantification of 0.5 µg/kg (Suchail 2004). After 20 minutes of imidacloprid ingestion three residues (imidacloprid, 5-hydroxyimidacloprid and olefin) amounted to about 70% of the actual applied dose. Higher peak values of 5-hydroxyimidacloprid and/or olefin coincide with the mortality of *A.*

mellifera, which suggested that these metabolites were responsible for mortality instead of imidacloprid itself. Baked tobacco had an average of 1.47mg/kg of imidacloprid and its metabolites (Liu et. al. 2005), thus *M. sexta* feeding on treated tobacco might ingest enough imidacloprid to harm *C. congregata* larvae.

HPLC and radioactive labeling techniques are costly, time consuming and involve the use of toxic and radioactive materials. Recently, an enzyme linked immunosorbent assay (ELISA) has been used in quantifying pesticides in biological samples. ELISA was developed in 1971 by Engnall and Perlmann, even though full knowledge of this process was described by Wide and Porath in 1966. Since then, ELISA has been used in numerous clinical laboratories, in biomedical research and for quality control in many industries. ELISA has been used to quantify DDT and its metabolites in waste water **(**Valentini et.al. 2003), and chlorpyrifos (Cho et. al. 2002), isofenphos (Park et. al. 2002), and fenthion in fruit samples (Zhang et. al. 2008). An ELISA has also been developed for detection of imidacloprid in agricultural and environmental samples (Lee 2001), and is available in a commercial kit (ENVIROLOGIX, Portland, ME). This kit was developed to detect imidacloprid and its metabolites in water samples but has been successfully used in citrus (Castle et. al. 2005), grapes, and avocado (Byrne et. al. 2005).

We hypothesize that *M. sexta* fed on imidacloprid treated plants or artificial diet should have imidacloprid and/or its metabolites in their hemolymph. In normal circumstances when the host caterpillar is parasitized it releases an enzyme called FAD-glucose dehydrogenase (GLD). This enzyme surrounds and destroys the invader. CcBV in *C. congregata* renders these enzymes incapable of finding their target, and instead of surrounding the eggs they

aggregate to each other (Pruyne 1997). Thus, *C. congregata* eggs would be in direct contact with imidacloprid and its metabolites if present in the hemolymph of the host, and the insecticide could also have negative effects on the parasitoid larvae. If larval *C. congregata* come in contact with imidacloprid, it is also interesting to know if imidacloprid and its metabolites translocate from the host to the parasitoid.

Prior to our study, the effect of imidacloprid on *C. congregata* had not been studied. However, higher concentrations of nicotine in diet, having little or no effect on *M. sexta*, caused significant late-larval and pre-pupal mortality in *C. congregata* (Barbosa et al. 1991). Imidacloprid is structurally similar to nicotine, and we hypothesize that it might have a similar effect on *C. congregata*.

The focus of the research reported herein was to establish that the ELISA kit produced by ENVIROLOGIX was indeed applicable to insects and to determine if imidacloprid could be detected in the hemolymph of the host *M. sexta*. If residues of imidacloprid and its metabolites were present in host hemolymph, we then sought to measure any effects on parasitism by *C. congregata*. We investigated the translocation of imidacloprid and its metabolites from host (*M. sexta*) to endoparasitoids (*C. congregata*). We also investigated the effect of imidacloprid on parasitism in *M. sexta* under field conditions in both foliar and systemic applications and the parasitoid's adult mortality and longevity under laboratory conditions. The findings of our project not only help us to establish the effect of imidacloprid on one of the most effective parasitoids of *M. sexta,* but also provide a new insight into host - parasitoid relationships.

Our studies suggested that foliar application of imidacloprid has a negative effect on parasitism of *M. sexta*. Direct and residue contact of imidacloprid had detrimental effect on *C. congergata* adults, but longevity was not affected by exposure of *C. congregata* larvae to imidacloprid. Our investigation did not detect any difference in imidacloprid residue in *M. sexta* in different field treatments, but non-parasitized larvae did have higher residues of imidacloprid as compared to parasitized larvae. Translocation of imidacloprid and its metabolites from *M. sexta* to *C. congregata* was documented under laboratory conditions.

REFERENCES

Adamo, S. A . 1998. Feeding suppression in the tobacco hornworm, *M. sexta*: costs and benefits to the parasitic wasp *C. congregata Canadian Journal of Zoology.* 76: 1634–1640.

Adamo, S. A., and Shoemaker, K. L. 2000. Effects of parasitism on the octopamine content of the central nervous system of *Manduca sexta*: a possible mechanism underlying host behavioural change. *Canadian Journal of Zoology.* 78: 1580–1587.

Barbosa, P., Gross, P., and Kemper, J. 1991. Influence of plant allelochemicals on tobacco hornworm and its parasitoid, *Cotesia congregata. Ecology* 72: 1567–1575.

Baskaran, K., Kookana, R. S., and Naidu, R. J. 1997. Determination of the pesticide imidacloprid in water and soil using high-performance liquid chromatography. *Journal of Chromatography* 787: 271–275.

Baysoyu, D., Tiryaki, O., and Kilincer, N. 2005. Fate of ^{14}C-Aldicarb in Parasitized *Spodoptera littoralis* Boisd. Larvae. *Tarim Bilimleri Dergisi* 11: 316-320.

Bock, J. M. 2010. Insect Management in Burley Tobacco in Non-traditional Areas of North Carolina. MS thesis, North Carolina state University.

Burrack, H. J. 2010. "Tobacco Insect Management," *Flue-cured Tobacco Guide 2010.* 179–206 http://ipm.ncsu.edu/Production_Guides/Flue-Cured/flue_cured.pdf.

Castle, S. J., Bi, J. L., and Toscano, N. C. 2005. Application of competitive ELISA for the quantification of imidacloprid titres in xylem fluid extracted from grapevines. *Journal of Economic Entomology* 98: 182–187.

Byrne, J. F., Toscano, N. C., Urena, A. A., and Morse, J. G. 2005. Quantification of imidacloprid toxicity to avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), using a combined bioassay and ELISA approach *Pest Management Science* 61: 754–758.

Castle, S. J., Byrne, F. J., Bi, J. L., and Toscano, N. C. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Management Science* 61: 75–84.

Chareonviriyaphap, T., Prabaripai, A., and Bangs. M. J., 2004. Excito-repellency of deltamethrin on the malaria vectors, *Anopheles minimus, Anopheles dirus, Anopheles swadiwongporni,* and *Anopheles maculatus,* in Thailand. *Journal of American. Mosquito. Control Association.* 20: 45–54.

Cho, A. Y., Hye-Sung, L., Park, Y. P., Lee, T. Y., Hammock, B. D., Ahn, C. K., and Lee J. E. 2002. Development of an ELISA for the organophosphorus insecticide chlorpyrifos. *Bulletin of the Korean Chemical Society*.23:481-487.

Croft, B. A. 1990. Arthropod biological control agents and pesticides. Wiley and Sons: New York. 703 pp.

De Cock, A., De Clercq, P., Tirry, L., and Degheele, D. 1996. Toxicity of diafenthiuron and imidacloprid to the predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae). *Environmental. Entomology*. 25: 476-480.

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M. and Pham-Delègue, M. H. 2004. Effects of imidacloprid and deltamethrin on associative learning in honey bees under semi-field and laboratory conditions. *Ecotoxicology Environmental Safety* 57: 410–419.

Dhammi, A. 2010. Effect of Imidacloprid on *Cotesia congregata* an endoparasitoid of *Manduca sexta* and its translocation from host to endoparasitoid MS Thesis, North Carolina State University.

Drezen, J. M., Provost, B., Espagne, E., Cattolico, L., Dupuy, C., Poirie, M., Periquet, G., and Huguet, G., 2003. Polydnavirus genome: integrated *vs* free virus. *Journal of Insect Physiology* 49: 407–417.

Engvall, E., and Perlman, P. 1971 Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry*. 8: 871-878.

Eastham, H. M., Lind, R. J., Eastlake, J. L., Clarke, B. S., Towner, P., Reynolds, S. E., Wolstenholme, A. J. and Wonnacott, S. 1998. Characterization of a nicotinic acetylcholine receptor from the insect Manduca sexta. *European Journal of Neuroscience* 10: 879–889.

Elbert, A., Becker, B., Hartwig, J., and Erdelen, C. 1991. Imidacloprid-a new systemic insecticide. *Pflanzenschutz-Nachr. Bayer*.44: 113–136.

Elzen, G. W., Elzen, P. J., King, E. G. 1998. Laboratory toxicity of insecticide residues to *Orius insidiosus*, *Geocoris punctipes*, *Hippodamia convergens*, and *Chrysoperla carnea*. *Southwestern Entomology*. 23: 335–342.

Espagne, E., Dupuy, C., Huguet, E., Cattolico, L., Provost, B., Martins, N., Poirie, M., Periquet, G., and Drezen, J.M. 2004. Genome sequence of a polydnavirus: insights into symbiotic viral evolution**.** *Science* 306: 286-289.

Fulton, B. B. 1940. The hornworm parasite, *Apanteles congregatus* (Say) and the hyperparasite, *Hypopteromalus tabacum* (Fitch). *Annals of the Entomological Society of America* 33: 231-244.

Furusawa, T., Rakwal, R., Nam, H. W., Hirano, M., Shibato, J., Kim, Y.S., Ogawa, Y., Yoshida, Y., Kramer, J., Kouzuma,Y., Agrawal, G. K ., and Yonekura, M. 2008. Systematic investigation of the hemolymph proteome of *Manduca sexta* at the fifth instar larvae stage using one- and two-dimensional proteomics platforms. *Journal of Proteome Research.* 7: 938-959.

Garnett, J. and Weseloh, R. M.1975. Dimilin toxicity to the gypsy moth larval parasitoid, *Apanteles melanoscelus*. *Journal of Economic Entomology.* 68: 577–579.

Gelman, D. B., Reed, D. A., and Beckage, N. E. 1998. Manipulation of fifth-instar host (*Manduca sexta*) ecdysteroid levels by the parasitoid wasp *Cotesia congregata. Journal of Insect Physiology.* 44: 833-843.

Gilmore, J. 1938. Observations on the hornworms attacking tobacco in Tennessee and Kentucky. *Journal of Economic Entomology* 31: 706-712.

Hill, T. A., and Foster, R. E. 2000. Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Journal of Economic Entomology* 93: 763-768.

Heckel, G. D., Gahan, L. J., Liu, Y., Tabashnik, E. B. 1999. Genetic mapping of resistance to Bacillus thuringiensis toxins in diamondback moth using biphasic linkage analysis. Proceedings of the National Academy of Sciences USA. 96: 8373-837.

Horton, D. R., and Moore, J. 1993. Behavioral effects of Parasites and Pathogens in Insect Hosts. In: Parasites and Pathogens of Insects (Ed. by N.E. Beckage, S.N. Thompson, and B.A. Federici), pp. 107-124. San Diego: Academic Press.

Kadous, A. A., Ghiasuddin, S. M., Matsumura, F., Scott, S. G., and Tanaka, K. 1983. Difference in the picrotoxinin receptor between the cyclodiene resistant and susceptible strains of the German-cockroach. *Pest Biochemical Physiology* 19: 157–166.

Kidd, K. A.1998. Use of stable isotope ratios in freshwater and marine biomagnification studies. In *Environmental Toxicology: Current Developments*; Rose, J., Ed.; Gordon and Breach Science Publishers: Amsterdam, The Netherlands. Vol. 7.

Kumar, K., and Santharam, G. 1999. Laboratory evaluation of imidacloprid against*Trichogramma chilonis* Ishii and *Chrysoperla carnea* (Stephens), *Journal of Biological Control* 13: 18–73.

Lewis, G. A., and Madge, G. S. 1984. Esterase activity and associated insecticide resistance in the damson-hop aphid, *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) *Bulletin of Entomological Research.* 74: 227-238.

Li, Z. M., Wang, W. L., Wu ,H. M., Liu, S. S., Liu, Y. Q. and Tang, Z. H. 2006. Detection and analysis of spinosad residue in the body fluid of *Cotesia plutellae* larvae with HPLC, *Acta Entomologica Sin.* 49 : 137–141.

Lind, J. R., Martin, S. C., Stuart, E. R., and Fergus, G. P. 1998. [^{3}H]Imidacloprid Labels High- and Low-Affinity Nicotinic Acetylcholine Receptor-like Binding Sites in the Aphid *Myzus persicae*(Hemiptera: Aphididae). *Pesticide Biochemistry and Physiology.* 62: 13-14.

Liu, H., Song, J., Zhang, S., Qu, L., Zhao, Y., Wu, Y., and Liu, H. 2005. Analysis of residues of imidacloprid in tobacco by high-performance liquid chromatography with liquid–liquid partition cleanup *Pest Management Science.* 61: 511–514.

Mandic, A. I., Lazic, S. D., Ökrész, N., and Gaál, F. F. 2005. Determination of the Insecticide Imidacloprid in Potato (*Solanum tuberosum*L.) and Onion (*Allium cepa*)by High-Performance Liquid Chromatography with Diode-Array Detection. *Journal of Analytical Chemistry.* 60(12): 1134–1138.

Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., and Sattelle, D. B. 2001. Neonicotinoids insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences.* 22: 573–580.

Metcalf, R. L.1989. Insect Resistance to Insecticides, *Pesticide Science.* 26: 333-358

Metcalf, R. L., and Luckmann, W. H. 1994. Introduction to Insect Pest Management. John Wiley & Sons, NY.

Mizell, R. F., and Sconyers. M. C. 1992. Toxicity of imidacloprid to selected arthropod predators in the laboratory. *Florida. Entomologist* 75: 277–280.

Nishiwaki, H., Sato, K., Nakagawa, Y., Miyashita, M., and Miyagawa, H. 2004. Metabolism of imidacloprid in houseflies. *Journal of Pesticide Science*. 29: 110–116.

Park, H. J., Park, W. C., Jung, T. W., Ra, C. S., Lee, Y. T. 2002. Development of an Enzyme-Linked Immunosorbent Assay for the Organophosphrous Fungicide Tolclofos-methyl. *Bulletin of the Korean Chemical Society*, 24: 334-338.

Public Broadcasting Service 2001. Pesticide resistance. Retrieved on September 15, 2007.

Pfluger, W., and Schmuck, R., 1991. Ecotoxicological profile of imidacloprid.- *Pflanzenschutz-Nachrichten Bayer*, 44: 145-158.

Razaq, M., Suhail, A., Aslam, M., Arif, J., Saleem, A. M., and Khan, M. H. A. 2005. Evaluation of neonicotinoids and conventional insecticides against cotton jassid, *Amrasca devastans* (Dist.) and cotton whitefly, *Bemisia tabaci* (Genn.) on cotton. *Pakistan. Entomologist,* 27(1):75-78.

Reinecke**,** J. P., Buckner, J. S., Grugel, S. R. 1980**.** Life cycle of laboratory-reared tobacco hornworms, *Manduca sexta*, a study of development and behaviour, using time-lapse cinematography. *The Biological Bulletin*. 158:129–140.

Romero, A., Potter, M. F., Haynes, K. F. 2009. Behavioral Responses of the Bed Bug to Insecticide Residues. *Journal of Medical Entomology*. 46: 51-57.

Sclar, D. C., Gerace, D., and Cranshaw, W. S. 1998. Observations of population increases and injury by spider mites (Acari: Tetranychidae) on ornamental plants treated with imidacloprid. *Journal of Economic Entomology* 91:250–255.

Stone, S. B., Shufran, A. R., and Wilde, E. G. 1999. Imidacloprid Toxicity to Insecticide Resistance and Susceptible sSrains of the Greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) *Journal of the Kansas State of Entomological Society*, 72(2):248-250.

Smith, S. F. and Krischik, V.A. 1999.Effects of systemic imidacloprid on *Coleomegilla maculata*(Coleoptera: Coccinellidae). *Environmental Entomology* 28:1092–1100.

Swanson, T., 2006. Pest Resistance in Agriculture: An Economic Persp*ective The Economics of Managing Biotechnologies.* 22:51-69.

Sanyal, N., Hazra, D., Pal, R., Somchaudhury, A. K., and Chowdhury, A. 2006. Imidacloprid in processed tea and tea liquor. Journal of Zhejiang University-Science B, 7:619–622.

Suchail, S., Laurent, D., and Luc, P. B., 2004. Metabolism of imidacloprid in *Apis mellifera Pest Management Science*. 60:291–296.

Valentini, F., Compagnon, D., Giraudi, G., and Palleschi., G. 2003. Electrochemical ELISA for the screening of DDT related compounds: analysis in waste waters. *Analytica Chimica Acta,* 487(1):83-90.

Ware, G. 2000. *The pesticide book*, 5th ed. Thomson Publications, Fresno, CA.

Wide L, Porath J. 1966. Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. *Biochem Biophys Acta*. 30:257-260.

Zhang, Q., Sun, Q., Hu, B., Shen, Q., Yang, G., Liang, X., Sun, X., and Liu., F. 2008. Development of a sensitive ELISA for the analysis of the organophosphorous insecticide fenthion in fruit samples. *Food Chemistry* 106:1278-1284.

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Effect of Imidacloprid on *Cotesia congregata* and its Parasitism of *Manduca sexta*

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ABSTRACT

Field and laboratory studies were conducted to investigate the effect of imidacloprid on *Cotesia congregate* (Say) and its parasitism of tobacco hornworm, *Manduca sexta* (Linnaeus). Effects of foliar and systemic application of imidacloprid on parasitism by *C. congregata* were investigated in a study conducted in North Carolina through the summers of 2008 and 2009. These field studies indicate that foliar application of imidacloprid has a negative impact on parasitism in that the percentage of parasitized hornworm larvae was significantly less than in other treatments. We also observed negative effects of imidacloprid on parasitism of the tobacco budworm, *Heliothis virescens* (Fabricius) by its parasitoids (*Campoletis sonorensis* (Cameron) and *Cardiochiles nigriceps* (Viereck). In laboratory studies, direct contact with imidacloprid produced a detrimental effect on *C. congregata* adults. In addition, residues of imidacloprid also have a negative effect on *C. congregata* adults. Longevity of *C. congregata* doesn't decrease if parasitized host larvae were reared on diet with imidacloprid incorporated.

INTRODUCTION

The tobacco hornworm, *Manduca sexta (L.),* is one of the most important and potentially most destructive pests of tobacco (Furusawa et al. 2008). Although *M. sexta* is not hard to control by chemical means, ongoing concerns over the development of insecticide resistance suggest that reliance on insecticides is imprudent, and biological agents should play a prominent role in insect management (Crop profile for tobacco in West Virginia, 1999). Biological agents such as *Trichogramma* sp., *Euplectrus playthepenae* (Howard)., and *Cotesia congregata* can significantly reduce *M. sexta populations* (Gilmore 1938). Among these biological controls, *C. congregat*a, an endoparasitic wasp, is considered to be the most effective biological control agent against *M. sexta*. *Cotesia congregata* not only controls the host larvae by eventually killing it (thus decreasing reproduction for the next generation of host) but it also significantly decreases the feeding of the host, and therefore, economic damage; in some cases, host larvae cease to feed altogether (Adamo 1998).

While much research has been done on the effects of insecticides on non-target organisms, little has been done on the effects of insecticides on parasitoids. Imidacloprid is used extensively in tobacco due to its efficacy against pest arthropods including green peach aphids (*Myzus periscae* Sulzer); tobacco flea beetles (*Epitrix hirtipennis* Melsheimer) and thrips, (*Frankliniella* sp.), and due to its low toxicity to humans (Matsuda et al 2001). In North Carolina 80% of flue-cure tobacco was treated with imidacloprid in 2008 (Burrack unpublished data). As a neonicotinoid, imidacloprid acts on the actylcholine receptor and paralyzes the insect (Ware 2000).

Imidacloprid is effective against pests but can also be quite detrimental to beneficial insects. Severe mortality has been seen in *Hippodamia convergens* Guérin-Méneville (Coccinellidae) after exposure to imidacloprid residue (Mizell and Sconyers 1992). Similar effects have been found in *Orius tristicolor* (White) and *Chrysoperla carnea* Stephens (Chrysopidae) (Sclar et al. 1998 and Elzen et al., 1998). These insects are predators and act as natural enemies to many pests, and negative effects of imidacloprid on these insects can disturb crop ecology. Imidacloprid has also been documented as detrimental to some hymenopterans, including *Apis mellifera* (Scutellata)*;* a 60% decrease in forging and mobility in hives have been detected when bees are exposed to 24 ppb of imidacloprid in artificial nectar (Decourtye et al., 2004). In addition, foliar application of imidacloprid has induced 30% mortality in *Bombus terrestris* (Linnaeus) after 24 hrs of exposure (Incerti et al. 2003). Imidacloprid also reduced longevity by 25% in *Microplitis croceipes* Cresson (Stapel et al. 2000).

Imidacloprid is applied to tobacco plants via foliar or systemic application, and it has shown little or no toxicity to *M. sexta* (Lind et al. 1998, Bock 2010). We hypothesized that *M. sexta* is exposed to this insecticide via ingestion of treated foliage from foliar application or systemic applications targeting other insects. Since *M. sexta* ingest treated foliage, they could contain residues of imidacloprid or its metabolites in the hemolymph. If this is the case, imidacloprid and its metabolites might come in direct contact with developing *C. congregata* larvae inside the host and thus have negative effects on *C. congregata* larvae. We also hypothesized that imidacloprid directly applied to adult *C. congregata*, or contact with residues, would have detrimental effect on the parasitoid; moreover, *C. congregata* longevity

would decrease if larvae are exposed to imidacloprid or its metabolites. The objectives of the studies reported herein were to look at the effects of imidacloprid on parasitism in *M. sexta* by *C. congregata* under field conditions with both foliar and systemic applications, and examine the effects of imidacloprid on adult *C. congregata* mortality and longevity. We conducted a two-year field study to examine the effects of different treatments of imidacloprid on parasitism of *M. sexta* by *C. congregata*. The mortality of adult *C. congregata* in direct or residue contact with imidacloprid by foliar application was assessed under laboratory conditions. The wasp's longevity when exposed to imidacloprid in the hemolymph of the host was also investigated in the lab.

MATERIALS AND METHODS

Laboratory study: The effect of imidacloprid on adult *C. congregata* was studied under laboratory conditions, and *C. congregata* were reared to ensure a stable supply of insects. The *C. congregata* colony was maintained in the laboratory at 25^{o}C and a photoperiod of 16:8, light:dark. Relative humidity of 35 – 45 % was maintained in the colony. A diet of agar and honey in distilled water was provided by braided roll submerged in the reservoir flask. "BugDorm" cages, 29.8 cm X 29.8 cm (Bioquip, Rancho Dominguez, CA) were used to rear adult wasps. Female *C. congregata* are ready to lay eggs two days after emergence. Second instar *M. sexta* were put on tobacco leaves and exposed to adult *C. congregata* females in the cage for parasitization. After parasitization, *M.sexta* larvae were reared on artificial diet (Yamamoto 1969) at 25^{o}C and a photoperiod of 16:8, light:dark until parasitoid emergence and cocoon formation.

Effect of Direct Contact of Imidacloprid on adult *C. congregata* mortality

To evaluate the effect of direct contact with imidacloprid on adult wasp mortality, thirty newly emerged *C. congregata* were placed in each of two cages. Water and honey diet were also provided in each cage. Imidacloprid formulated as Provado® (Bayer CropScience Research Triangle Park, NC) was used to make a preparation equivalent to 3.75ml/L as recommended for treatment in the field. This solution was sprayed on the adults in a petridish in one cage; the other adults were sprayed with water alone. The spray was done with a T- Jet D233 nozzle dispensing an average of 1.6 ml of solution per cage, which translates to about 0.11 ml of insecticide solution per petridish containing adults. Mortality was checked every hour. Three replicates were conducted.

Effect of imidacloprid residue on *C. congregata* adult mortality

In order to investigate the effect of imidacloprid residues from foliar application on adult *C. congregata,* tobacco leaves and agar-based honey diet were sprayed with the Provado® solution (3.75ml/L) and then allowed to air dry. With a T- Jet D233 nozzle an average of about 0.36 ml of insecticide solution was applied to diet and 1.45 ml of insecticide solution was applied to the leaf and the spray allowed to dry. In this experiment, adult *C. congregata* were exposed to imidacloprid by contact and feeding. Thirty newly emerged adult wasps were placed in a cage along with the treated diet and leaf. A second set of thirty adults were placed in another cage with agar based honey diet and tobacco sprayed with water alone and allowed to dry. Adult mortality was assessed every 6 hours until all adults were dead in the treated cage. Three replicates were conducted.

Effect of imidacloprid exposure to *C. congregata* larvae on *C. congregata* adult mortality

The effect of parasitoid larval exposure to imidacloprid on adult longevity was assessed by rearing *M. Sexta* on insecticide treated diet. *M. sexta* diet (Yamamoto 1969) was prepared with imidacloprid incorporated at a concentration of 4.5mg/kg. This was the maximum concentration of imidacloprid found in oven-dried flue cured tobacco leaves which were collected from the different locations by Liu et. al. in 2005. Residue in this study might have come from environmental soil and water or from direct application of imidacloprid. *M. sexta* larvae were exposed to *C. congregata* wasps, parasitized by them, and then reared on the treated diet. Another set of *M. sexta* were similarly parasitized and fed on non-treated diet. Sixty newly emerged parasitoids adults from both these treatments were placed in separate cages and mortality was assessed every 24 hrs up to 9 days. Behavior of *C. congregata* was not investigated.

Field Trials: In order to assess the effect of imidacloprid on parasitism of *M. sexta* by *C. congregata* under field conditions, trials were conducted in 2008 and 2009 at the Central Crops Research Station in Clayton, North Carolina. In 2008, a randomized complete-block experiment with 4 treatments (two systemic imidacloprid treatments, one foliar imidacloprid treatment and an untreated control) and 4 blocks, for a total of 16, 4- row plots (22 plants/row), was established. Greenhouse grown flue cured tobacco (*Nicotiana tabacum* L, var. NC 71) plants were transplanted on 22 Apr. Systemic treatments of imidacloprid formulated as Admire Pro were applied on the same day; insecticide was delivered to the crown of the transplant in 118 ml of water as a soil drench. Admire Pro was applied at a rate

of 0.192 LB A/A (the low systemic rate which simulates an application of 0.8fl oz/1000 plants with the population of 6700 plants per acre) and 0.384 LB A/A (a high systemic rate which is double of lower rate) to appropriate plots. Treatments were applied to the middle two rows of the plot with side rows acting as buffers.

Manduca sexta numbers were very low in this test until late August; in order to enhance the probability of obtaining useable data, we ratooned a portion of the tobacco in each plot on 24 Jul. We cut the stalk of the tobacco plant, leaving about 6" of tobacco stem above ground level, and then removed all but one of the axilar buds released by the topping approximately 14 days later. Since plants were cut we re-treated this ratoon crop with a simulated transplant treatment on 25 July. We re-treated the ratooned crop because we assumed that the titer of insecticide in the remaining plant would have declined between the initial treatment and the cutbacks. Residues of imidacloprid and its metabolites were not assessed in plants because we didn't have the resources available to validate the ELISA both for plant and insect samples, and therefore concentrated on insect samples. The foliar treatment was applied to appropriate plots at the rate of 0.05 LB ai/A on 15 Jun in the original crop, and on 25 Jul in the ratoon crop. A single T- Jet D233 nozzle was used to deliver the equivalent of 22.5gal/acre spray solution. The natural *M. sexta* population in the field was supplemented on 2 Sept. by releasing two second instar larvae (NCSU Insectary, Raleigh, NC.) on each plant in the middle two row of each plot. Larvae older than the second instar were collected on 9 Sept from the middle two rows of plots, counted, and reared in the lab, to observe endoparasitism.

In 2009 a similar study was conducted, again at the Central Crops Research Station. Plants were transplanted on 27 May. Admire Pro at rate of 0.192 LB ai/A and 0.384 LB ai/A was applied on 4 June and an application of Provado® was conducted on 20 June. Several hundred larvae and pupae were uniformly released in the field as availability permitted (about an average of 200 larvae/release date), due to very low natural tobacco hornworm population levels through the middle of the summer. We started putting out *M. sexta* on 7 July and we distributed larvae (depending on the availability) 2- 3 times every week up to first week of August. Systemic treatments (Admire Pro 4.6 SC) were re-applied after establishment of a ratoon crop on 5 August. Admire Pro was applied at rates of 0.192 LB ai/A and 0.384 LB ai/A to appropriate plots. Foliar applications (Provado® 1.6 F) were made on 26 August with the same spray equipment and set-up as indicated above. The total number of 4^{th} and 5^{th} stadia larvae and the number parasitized were counted in middle two rows of each plot on 3 September, 10 September, 17 September and 24 September; no parasitism was observed in the field prior to late August.

Manduca sexta were not found in the early season of 2009, so we also conducted a preliminary study to investigate the effect of imidacloprid on parasitism of tobacco budworm, *Heliothis virescens* by two its parasitoids *Campoletis sonorensis* and *Cardiochiles nigriceps*. Plants in plots received one neonate/plant on 27 June. Third stadium larvae were collected after 2 weeks, and these larvae were reared on artificial diet and the percentage of parasitism to both species of parasitoids calculated.

Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC). Data was subjected to ANOVA using Proc GLM except field trials conducted in year 2009 where Proc Mixed was used.

RESULTS

We found that imidacloprid has detrimental effects on adult *C. congregata*. All adults were dead within 15 minutes after being sprayed with imidacloprid, while only 10% of adults in the control were dead after 24 hrs (Table 1); this represents a significant difference between treatments ($df = 1, 152:, F = 1848.79$ $p < 0.0001$, $\alpha = 0.05$). When adult *C. congregata* were exposed to imidacloprid treated leaves and diet, all the adults were dead within 24 hrs, while only 15% of adults died after 24 hrs in the untreated control (Table 1). Mean time of survival for treated *C.* wasps was 20.14 hrs, significantly less than that of the control ($df = 1,144: F = 427.92: p < 0.0001$, $\alpha = 0.05$).

Longevity of *C. congregata* adults was not affected by the type of diet on which parasitized caterpillars were fed. Mean longevity of adult *C. congregata* was 4.60 days when parasitized caterpillars were fed on artificial diet without incorporated imidacloprid. In parasitized host larvae fed on imidacloprid diet, the longevity of adult *C. congregata* was 4.55 days, which was not significantly different ($df = 1, 158, F = 0.03$ $p = 0.8705$, $\alpha = 0.05$) from the control treatment (Figure 1).

The studies at Central Crops Research Station in 2008 and 2009 indicated that imidacloprid in a foliar application as Provado® has negative effects on parasitism in the

field. In 2008, the percentage of parasitism in Provado® plots was significantly less (Figure 2) than other treatments (df = 3, 12,: F = 4.61:p = 0.0322 and $\alpha = 0.05$). However, the systemic applications did not appear to reduce parasitism compared to the untreated control (Figure 2). The average percentages of parasitism in the lower and higher rates of systemic imidacloprid (Admire Pro) are 43.11% and 41.11%, respectively. In the summer of 2009, we assessed parasitism in the field, rather than rearing field-collected larvae in the lab, and this procedure produced similar results. We observed 44.47% parasitism in Provado® treated plots across sampling dates, which was significantly less than the other treatments (df =3, 44 F = 5.05 and p= 0.0043 and $\alpha = 0.05$).; these other treatments, including the control (60.80%), Admire Pro (Low) (54.58%) and Admire Pro (High) (54.94%) did not differ in percentage of parasitism (Figure 3). Weekly evaluations of effects were informative. In the first week, Provado® treated plots had only 30.97% parasitism (Table 2), less than other three treatments (significantly different at $\alpha = 0.10$). In the second (significantly different at $\alpha = 0.10$) and third weeks, parasitism increased in Provado® plots to 39.92% (Table 2) and 45.48 % (Table 2), respectively. By the fourth week, parasitism in the Provado® plots reached 61.53% and was not significantly different from the other treatments (Table 2).

In our preliminary study of the effects of imidacloprid on budworm parasitism we observed that imidacloprid applied in either fashion (Admire Pro or Provado®) reduced parasitism compared to untreated plots. Percentage of parasitism in control plots was 75.88%, while in the Provado® , Admire Pro (Low), and Admire Pro (High) plots it only reached 56.70%, 54.53% and 58.48% respectively (Figure 4).

DISCUSSION

Our laboratory studies documented contact and residual toxicity of imidacloprid to adult *C. Congregata* wasps. Translating this to the field studies, it is likely that adult *C. congregata* may come in contact with imidacloprid directly during application of the foliar insecticide or be exposed to its residues after application. Subsequent population reduction of adult wasps could be the reason behind the decrease in percentage of parasitism in Provado® treated plots. Additionally, many insects are repelled or irritated by insecticides and therefore avoid prolonged exposure (Romero et. al. 2009; Chareonviriyaphap et. al. 2004); these behavioral responses can lead to decreased exposure to lethal doses of insecticide, but could also substantially alter the wasps' ability to effectively seek and sting hosts. Adult wasp exposure to imidacloprid in the systemically treated plots was probably much lower since the chemical is internal to the plant, and therefore repellency or irritancy may not be much of a factor in these treatments. We have observed *M. sexta* movement between plots (Dhammi, 2010), and thus an individual insect may feed on plants treated with different rates of imidacloprid or on the untreated border plants (2 rows of plants in between treated plots), potentially affecting our ability to discern differences between treatments.

We re-treated our plots after cut-back, where we assumed that titer in the remaining plant would have declined. It is likely that titer in re-treated plants would be higher than that of initially treated plants. Ratooned plants are older and have a well-established root system, which might lead to higher intake of imidacloprid as compared to younger transplants. On the other hand, translocation of imidacloprid depends on transpiration and the rate of

transpiration in the plant; since ratooned plants had been cut, their transpiration rate in the plant would be low compared to intact plants of the same age, suggesting that uptake of imidacloprid in ratooned plants would not be high even though they have larger root systems.

The two years of field studies suggest that foliar imidacloprid had a significant effect on parasitism of *M. sexta* by *C. congregata*, while systemic application (Admire Pro) didn't appear to reduce parasitism by this species. One explanation is that the residues of imidacloprid are diluted by plant growth and metabolic decay between application and the time hornworms attack the plant some weeks later. Another possible reason for these observations could be that *C. congregata* larvae are exposed to different metabolites in systemic and foliar applications of imidacloprid. Some of the imidacloprid metabolites found in plants and mammals are imidacloprid, olefin, 5-hydroxy- imidacloprid, guanidine, 4,5-dihydroxy- imidacloprid, 6-chloronicotinic acid and urea (Suchail 2004). It should also be considered that when *C. congregata* develops in a larva (*M. sexta*) which feeds on a plant treated systemically with imidacloprid, two biological systems are involved in the metabolism of imidacloprid. In plants imidacloprid is metabolized by three pathways which includes hydroxylation, reduction or loss of nitro groups and bridge cleavage of C-N bonds (Kagabu 1997). This leads to the formation of 5-hydroxy- imidacloprid, 4,5-dihydroxy-imidacloprid, olefin, urea imidacloprid, Guanidine and 6-Chloronicotinic acid. Caterpillars feeding on foliage from systemic applications may potentially metabolize the insecticide and metabolites further before endoparasitic larvae are exposed to them. On the other hand, in the case of foliar application, imidacloprid may potentially be metabolized only in the *M. sexta*

before *C. congregata* larvae are exposed to it. In these two different cases *C. congregata* may be exposed to different metabolites; it is possible that in systemically treated plots *C. congregata* may be exposed to less toxic metabolites as compared to Provado® plots.

Percent parasitism in the foliar treated plots steadily increased over the four weeks until it was similar to the rate observed in the control, suggesting that residues potentially affecting parasitoid larvae were eventually decaying to a non-toxic level. In Provado® treated plots in the first week, we observed 30.97% parasitism and 39.92 in the second week; by the fourth week we saw 61.53% of parasitism. This suggests a decline in Provado® effect in the field. Weekly variations could be because of increase in wasp activity (since parasitism was observed to increase in the control and systemically treated plots as well), but the diminishing effect of Provado® cannot be discounted.

Our preliminary study shows that both foliar and systemic treatment of imidacloprid seems to decrease parasitism in budworms. Imidacloprid treated plots had higher numbers of budworms in a concurrent study of insect pest management in burley tobacco at Cunningham Research Station in Kinston, North Carolina (Bock 2010); this may have been in part due to possible negative effects of imidacloprid on parasitism. More research needs to be done to fully understand the effect of imidacloprid on parasitism in tobacco budworms.

We also observed that parasitized hornworm larvae reared on imidacloprid treated artificial diet produced fewer cocoons as compared to those reared on untreated diet. This observation requires further investigation. There is also a need to determine if imidacloprid

has any effect on the forging capacity, fertility and host-finding capability of adult *C. congregata*. Additional research on these questions will provide a more sound understanding of the relationships between insecticides and biological control agents.

CONCLUSION

Our results strongly suggest that foliar applications of imidacloprid significantly reduce endoparasitism of *M. sexta* by *C. congregata*. This is due not just to direct deposits on wasps during application but may also be due to contact with residues of imidacloprid by adults and effects on larvae in caterpillar. Even though systemic treatments do not produce negative effects on parasitism, further research is needed, particularly into the sub lethal effects of imidacloprid residues. This research documents the impacts of an insecticide targeting one suite of pests on parasitoids important in suppressing non-target pests. These results suggest that systemic application methods be used for neonicotinoid insecticides in tobacco whenever possible, rather than foliar applications, to protect *C. congregata* suppression of the hornworm complex.

REFERENCES

Adamo, S. A. 1998. Feeding suppression in the tobacco hornworm, *M. sexta*: costs and benefits to the parasitic wasp *Cotesia congregata Canadian Journal of. Zoology*. 76(9): 1634–1640.

Furusawa, T., Rakwal, R., Nam, H. W., Hirano, M., Shibato, J., Kim, Y.S., Ogawa, Y., Yoshida, Y., Kramer, J., Kouzuma,Y., Agrawal, G. K ., and Yonekura, M. 2008. Systematic investigation of the hemolymph proteome of *Manduca sexta* at the fifth instar larvae stage using one- and two-dimensional proteomics platforms. *Journal of Proteome Research.* 7: 938-959.

Chareonviriyaphap, T., Prabaripai, A., and Bangs, J. M. 2004. Excito-repellency of deltamethrin on the malaria vectors, *Anopheles minimus, Anopheles dirus, Anopheles swadiwongporni*, and *Anopheles maculatus*, in Thailand. *Journal of the American Mosquito Control Association.* 20: 45–54.

Dhammi, A. 2010. Effect of Imidacloprid on *Cotesia congregata* an endoparasitoid of *Manduca sexta* and its translocation from host to endoparasitoid, MS Thesis, North Carolina State University.

Lind, J. R., Martin, S. C., Stuart E. R., and Fergus, G. P. 1998. [^{3}H]Imidacloprid Labels High- and Low-Affinity Nicotinic Acetylcholine Receptor-like Binding Sites in the Aphid *Myzus persicae* (Hemiptera: Aphididae*). Pesticide Biochemistry and Physiology 62:1 3-14.*

Liu, H., Song, J., Zhang, S., Qu, L., Zhao, Y., Wu, Y., and Liu, H. 2005. Analysis of residues of imidacloprid in tobacco by high-performance liquid chromatography with liquid–liquid partition cleanup *Pest Management Science.* 61: 511–514.

Bock, J. M. 2010. Insect Management in Burley Tobacco in Non-traditional Areas of North Carolina. MS thesis, North Carolina state University.

Gilmore, J. 1938. Observations on the hornworms attacking tobacco in Tennessee and Kentucky. *Journal of Economic Entomology* 31: 706-712.

Kagabu, S. 1997. Chloronicotinyl Insecticides – Discovery, Application and Future Perspective.

Reviews in Toxicology 1: 75-129.

Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., and Sattelle, D. B. 2001. Neonicotinoids insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci* 22: 573–580.

Romero, A., Potter, M. F., Haynes, K. F. 2009. Behavioral Responses of the Bed Bug to Insecticide Residues. *Journal of Medical Entomology*. 46(1):51-57.

Yamamoto, R. T. 1969. Mass rearing of the tobacco homworm.2. Larval rearing and pupation. *Journal of. Economic. Entomology*. 62:1427-1431.

Ware, G. 2000. *The pesticide book*, 5th ed. Thomson Publications, Fresno, CA.

Table 1. Effect of foliar treatment and residue of imidacloprid on longevity of *C. congregata* adults

Experiment	Average longevity of *C. congregata* adult exposed to imidacloprid	Average longevity of *C. congregata* adult in control condition
Foliar Treatment	<15 min.	>24 hrs
Residue Treatment	20.14 hrs Standard Error = 1.04	101.56 hrs Standard Error= 3.76

Foliar treatment df = 1, 152:, F = 1848.79 $p < 0.0001$ and for residue treatment df = 1,144: F = 427.92: $p < 0.0001$where $\alpha = 0.05$.

Table 2. Percentage of endoparasitism in *M. sexta* by *C. congregata* in different weeks of sampling in different treatments of imidacloprid.

Imidacloprid treatments	Percentage of parasitism in different weeks			
	Week I	Week II	Week III	Week IV
Control	52.86 a	58.67 a	59.19 a	72.48 a
Admire (Low)	49.05 a	57.42 a	53.89 a	57.95 a
Admire (High)	44.92 a	53.91 a	55.13 a	65.80 a
Provado	30.97 a*	39.92 a*	45.48 a	61.53 a
F- value and p-value *df* (3, 11) at $\alpha = 0.05$	3.02, 0.0754	2.80, 0.0896	0.52, 0.6780	1.34, 0.3110

* Provado treatment is significantly different at $\alpha = 0.10$.

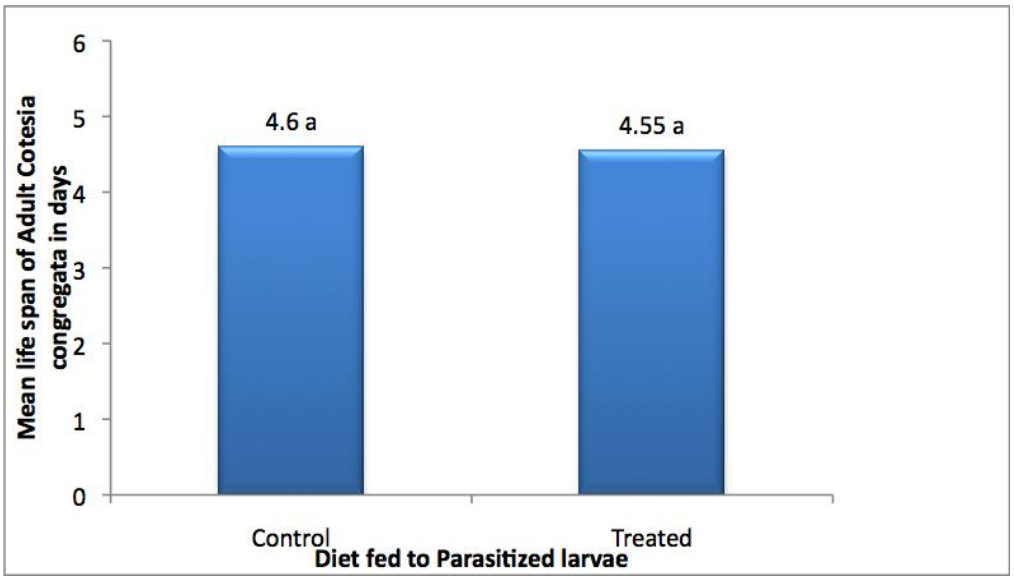

Figure 1: Mean life span of Adult *Cotesia congregata* in relation to parasitized *Manduca sexta* larvae feeding on treated or control diet (df = 1, 158, F = 0.03 p = 0.8705 and $\alpha = 0.05$).

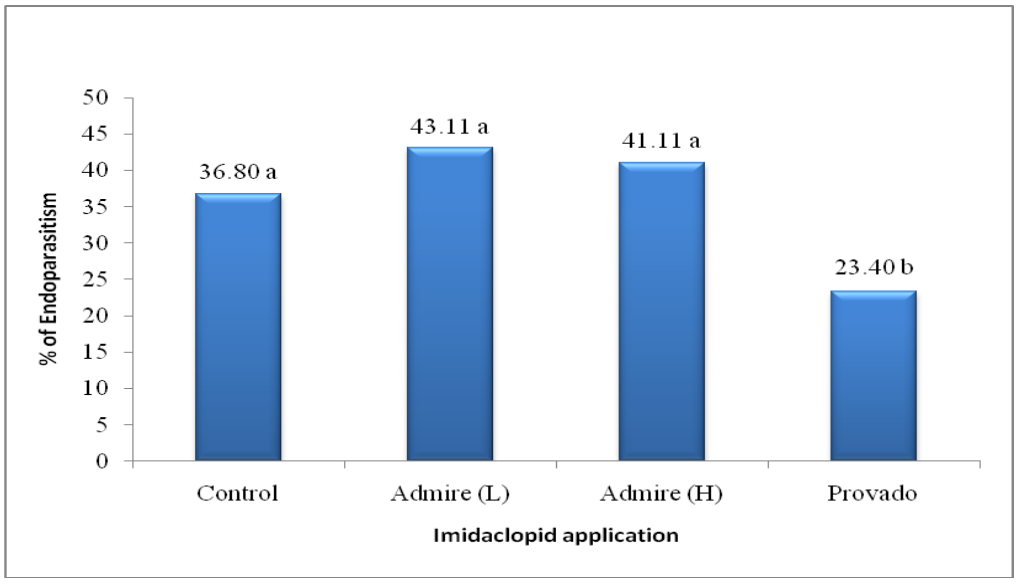

Figure 2: Percentage of endoparasitism of *Cotesia congregata* in relation to different applications of imidacloprid in 2008 (df = 3, 12,: F = 4.61:p = 0.0322and $\alpha = 0.05$).

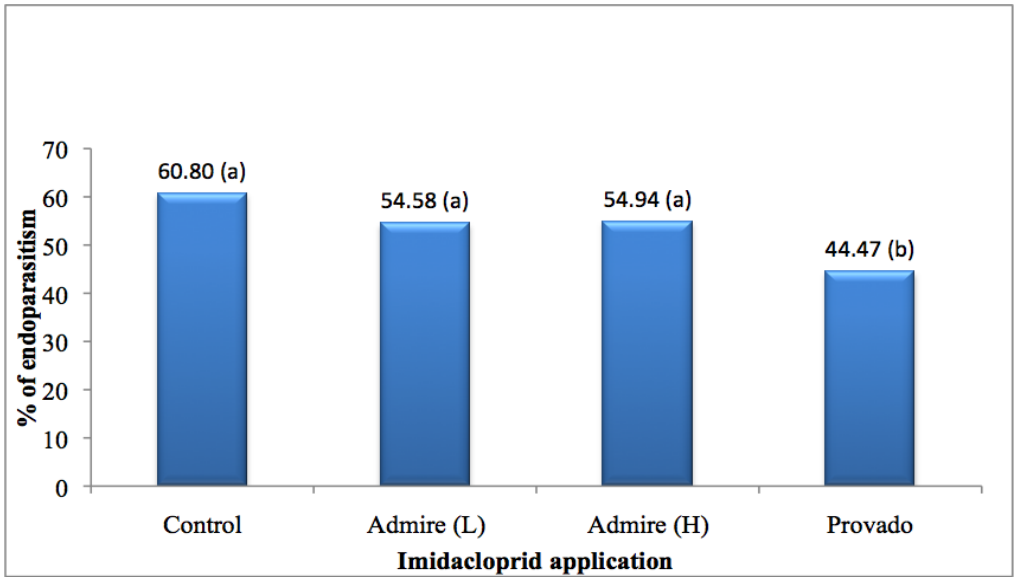

Figure 3: Percentage of endoparasitism of *Cotesia congregata* in relation to different application of Imidacloprid in 2009 where Admire (L) = Admire Pro Low concentration ; Admire (H) = Admire Pro High concentration (df =3, 44 F = 5.05 and p= 0.0043and α = 0.05).

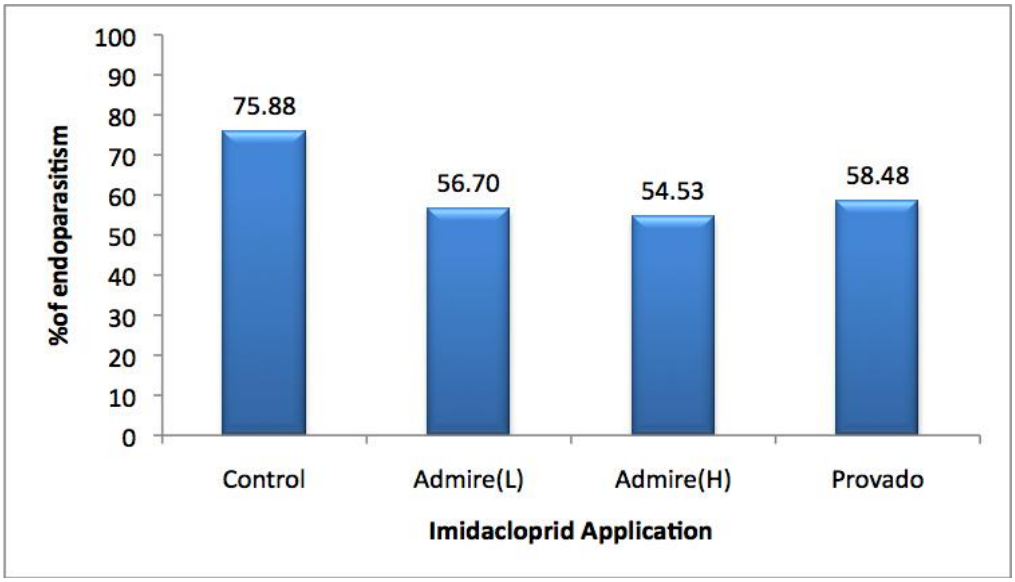

Figure 4: The percentage of endoparasitism in Tobacco budworm in relation to different applications of imidacloprid in 2009.

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Investigation of Imidacloprid and its Metabolites in *Manduca sexta* and its Translocation from *M. sexta* to the Endoparasitoid *Cotesia congregata*

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ABSTRACT

A study to quantify residues of imidacloprid and its metabolites in *Manduca sexta* (Linnaeus) when fed on imidacloprid-treated diet (artificial or tobacco), and the insecticide's effects on the endoparasitoid, *Cotesia congregata* (say) was undertaken. We also investigated translocation of imidacloprid and its metabolites from the host to parasitoid larvae. We established that the QuantiPlate ELISA kit (ENVIROLOGIX,Portland, Me), which was originally developed for quantifying imidacloprid in water, can be used to measure residues in *M. sexta* and *C. congregata.* No significant difference in residue was seen in the hemolymph of *M. sexta* which fed on imidacloprid-treated plants at different rates and treatment timings in the field. However, non-parasitized *M. sexta* larvae did have significantly higher residues of imidacloprid as compared to parasitized larvae, suggesting an effect of imidacloprid on parasitism of *M. sexta.* We also demonstrated that imidacloprid and its metabolites translocate from host *M. sexta* to *C. congregata.*

INTRODUCTION

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine) is one of the most widely used insecticides in the world, mainly due to its high toxicity towards insects and low toxicity against vertebrates (Matsuda et al 2001). Moreover, it is very effective against insects which have shown resistance against other conventional insecticides (Lewis and Madge 1984). It is highly effective against aphids, leafhoppers, planthoppers, thrips and whiteflies (Elbert et al. 1991).

Imidacloprid has excellent xylem-mobility, which makes it useful for seed and soil treatment (Elbert et al. 1991). Because of this, it is useful applied both as a foliar and soil-applied systemic; it is used both ways in tobacco, although the systemic use pattern is prevalent. Tobacco hornworm, *Manduca sexta*, is one of the most destructive pests of tobacco but is unaffected by imidacloprid (Lind et al. 1998, Bock 2010). Penetration of imidacloprid through insect cuticle is relatively low (Pfluger and schmuck 1991), so it is thought that in most cases endoparasitoid larvae are relatively safe inside the host. However, one of the most effective endoparasitoids of *M. sexta*, *Cotesia congregata*, could be exposed to imidacloprid when its host feeds on a foliarly or systemically treated plant. The effect of imidacloprid on *C. congregata* can only be effectively investigated if a technique for residue analysis of the insecticide in host larvae can be established.

Imidacloprid and its metabolites have been analyzed and quantified in many biological systems. HPLC protocols have been developed to quantify imidacloprid in potato

and onion (Mandic et. al. 2004), tea (Sanyal et. al. 2006) and also in cured tobacco (Liu et. al. 2005). However, not much has been done in insects. Imidacloprid and its metabolites have been quantified in *Apis mellifera* Scutellata (Suchail 2004). The HPLC MS/MS technique used in these studies is expensive and time consuming. On the other hand, the Enzyme linked ImmunoSorbent Assay (ELISA) is much less expensive and faster, and it recently has been used to quantify pesticides in many biological systems. ELISA has been developed to quantify DDT in waste water **(**Valentini et.al. 2003), and chlorpyrifos (Cho et. al. 2002), isofenphos (Park et. al. 2002), and fenthion for fruit samples (Zhang et. al. 2008). An ELISA to measure imidacloprid in water samples has also been developed by ENVIROLOGIX, Portland, Me; this has been successfully used to quantify imidacloprid and its metabolites in citrus (Castle et. al. 2005), grapevines (Byrne et. al. 2005) and avocado (Byrne et. al 2005), but, up to now, it has not been used in insects.

The translocation of pesticides from hosts to endoparasitoids is a very under-studied area of science. A residue analysis of spinosad in the body of *Cotesia plutella* (Kurdjumov), an endoparasitoid of *Plutella xylostella* (Linnaeus) by HPLC (Li et. al. 2006) has been developed, and ^{14}C-aldicarb translocation has been used to identify movement of that insecticide from *Spodoptera littoralis* (Boisduval) to its parasitoid *Chelonus oculator* (Panzer) (Baysoyu et. al. 2005).

We hypothesized that *M. sexta* fed on imidacloprid treated diet (tobacco and artificial diet) would have imidacloprid and its residue in their hemolymph, and, further, that developing *C. congregata* larvae would be exposed to it. Thus imidacloprid residues in *M.*

sexta could have negative effects on parasitism by *C. congregata*. We also hypothesized that if imidacloprid is present in the hemolymph of the host it could be translocated to endoparasitoid larvae.

The purpose of this study was to establish if an ELISA kit could be used to quantify imidacloprid and its metabolites in insect hemolymph. Studies were also conducted to quantify the residue of imidacloprid in *M. sexta* from the field and the relationship, if any, between detected titers and observed parasitism. Finally, we used this ELISA kit to establish presence of imidacloprid in *C. congregata* larvae and thus infer translocation of imidacloprid from the host to the parasitoid.

While we did not document significant differences in residues of imidacloprid in the hemolymph of *M. sexta* from different treatments in the field, we did find that non-parasitized larvae have significantly higher residues of imidacloprid as compared to parasitized larvae. We also established that imidacloprid and its metabolites can be translocated from the host to the parasitoid.

MATERIALS AND METHODS

In order to conduct residue analyses of imidacloprid and its metabolites we used a competitive ELISA technique, where residues in unknown samples compete with horseradish peroxide-labeled insecticide for a limited number of antibody binding sites. This conjugation is quantified colorimetrically and is inversely proportional to the residue present in the sample. This ELISA kit is commercially available from ENVIROLOGIX, Portland, Me Inc

(500 Riverside Industrial Parkway, Portland,ME 04 103, USA) as the QuantiPlate kit for imidacloprid. This kit has a detection range of 0.2 (parts per billion) ppb – 6 ppb.

The QuantiPlate kit was developed to measure imidacloprid and its metabolites in water, so our first experiments were done to establish that this kit was also capable of detecting residue in *M. sexta* hemolymph and in *C. congregata* supernatant. The kit is provided with three standards: 0.2 ppb, 1ppb and 6 ppb. In order to see if imidacloprid residues could be detected in *M. sexta* hemolymph , 10, 20, 30, 40 and 50 µl of *M.sexta* (from the NCSU insectary) hemolymph was centrifuged at 10,000g for 1 min and the supernatant was collected. These were added to separate 100 µl aliquots of the 1ppb standard and then the ELISA was run (as per kit manual catalog number Ep 006). Negative control and standards (100 µl) were loaded along with prepared samples (110, 120, 130, 140 and 150 µl) in respective wells. The 1ppb standard was also run alongside the different concentrations of hemolymph. Each concentration was replicated three times. Then imidacloprid-enzyme conjugate (100 µl) was added to each well and incubated for 1 hour at room temperature on an orbital shaker. After incubation, contents were shaken out, wells were flooded with distilled water, and flushed again. This step was repeated four times. Then substrate (100 µl) was added and incubated for 30 minutes at room temperature on the orbital shaker. After incubation stop solution was added to each well and absorbance was read within 30 minutes at λ 450 nm. Similar experiments were done with *C. congregata* larvae, but here instead of hemolymph, whole larvae were homogenized. As soon as third instar *C. congregata* larvae emerged from their host caterpillars, they were collected and stored at -80^{o}C. These larvae

were washed with distilled water (rinsed three times) and dried with clean sterile Kim Wipe® lab tissues. One hundred *C. congregata* larvae were then homogenized (all from a single host), the homogenate mix was centrifuged, and 30 and 40 µl of the resulting supernatant was then added to 100 µl of the 1ppb standard imidacloprid solution. The ELISA was run under the conditions described above for *M. sexta* samples. In both these experiments, no deviation from the 1ppb concentration in the prepared samples would demonstrate the ability of this ELISA kit to accurately identify the concentrations of residues in insect products. This assay also provided valuable information about the concentrations of samples we should use to avoid a matrix effect. This is a competitive ELISA, so the absorbance is inversely proportional to the concentration of imidacloprid. Certain concentrations of hemolymph can give lower absorbance and thus falsely indicate higher imidacloprid concentration, known as the matrix effect. In all the experiments a positive control (hemolymph from larvae which had no imidacloprid in their diet) was also run.

Residue analyses for imidacloprid and its metabolites from field-collected larvae were also conducted. For this, three parasitized and three non-parasitized larvae were collected from the 16 plots used in a parallel field study (Dhammi 2010). Insects were collected from plots treated systemically with a lower rate of Admire Pro (0.192 LB A/A), a higher rate of Admire Pro (0.384 LB A/A), foliarly with Provado® (0.05 LB A/A), and the untreated (control). These larvae were taken to the lab and hemolymph was collected and stored at -80^{o}C for further use. Collected hemolymph was centrifuged and 25 µl of supernatant was

diluted to 100 µl with distilled water; the ELISA was then run on these prepared samples in order to quantify imidacloprid and its metabolites.

In order to investigate the translocation of imidacloprid from the host caterpillar to the parastioid, *C. congregata* were collected in the field and then reared in the lab at 25^{o}C, photoperiod of 16:8 and relative humidity of 35 – 45 %. Wasps were reared in 11-3/4" X 11-3/4" BugDorm cages (Bioquip) and fed on agar and honey based diet. Distilled water was provided by braided roll submerged in the flask. First and second instar *M. sexta* larvae provided by the NCSU insectary were placed in the cage on a tobacco leaf with two-day old female wasps. *M. sexta* larvae were thus parasitized and the parasitized larvae were then reared in the lab on artificial diet having imidacloprid incorporated in it at a concentration of 4.5 mg/kg of dry diet weight; another cohort was reared on normal hornworm diet as the negative control. *M. sexta* larvae was reared at 25^{o}C and a photoperiod of 16:8. As soon as 3rd instar *C. congregata* larvae emerged from the host, they were collected and stored at -80^{o}C. One hundred *C. congregata* larvae were homogenized and centrifuged, then 25 µl of supernatant was diluted to 100 µl with distilled water. Similar sample preparation was done with *C. congregata* from parasitized *M. sexta* reared on normal diet. Samples from the hemolymph of respective hosts were prepared and ELISA was run along with other samples. In this experiment eight hosts and their parasitoids were used. Two replications of a positive control were also run along with other samples.

Data was analyzed with SAS version 9.1 (SAS Institute, Cary, NC). Data was subjected to ANOVA using Proc GLM except field samples for year 2009 where Proc Mixed was used.

RESULTS AND DISCUSSION

Our experiments indicate that the QuantiPlate ELISA kit, which was originally developed for quantifying imidacloprid and its metabolites in water samples, can also be used to detect such residues in samples generated from insects. We also established that the concentration of hemolymph in a sample does have a significant effect on the assay ($df = 7$, 10:, $F = 164.71$: $p < 0.0001$ and $\alpha = 0.05$); pair-wise analysis demonstrated that when hemolymph was diluted three times, i.e. 50µl was added to 100µl of the 1ppb imidacloprid standard, a matrix effect occurred that exaggerated the concentration of imidacloprid and metabolites present in the sample. At all other, more dilute concentrations no significant differences from the control stock solution were obtained (Figure 1). These results strongly suggest that hemolymph should be diluted at least four to one for accurate assays with *M. sexta* samples. Based on these *M. sexta* results, we assessed only two dilutions for *C. congregata;* (30 µl of homogenate added to 100 µl of 1ppb of imidacloprid and 40 µl of homogenate added to 100 µl of 1ppb of imidacloprid) both concentrations were not significantly different than the 1 ppb imidacloprid control, suggesting that these concentrations represent an acceptable range of dilution for samples generated from this species ($df = 4, 4$, $F= 0.61$ and $p= 0.6788$ and $\alpha = 0.05$) (Figure 2).

Our experiments also documented that imidacloprid is indeed present in the hemolypmph of *M.sexta* exposed to dietary imidacloprid, with no imidacloprid found in negative controls. When *M. sexta* is reared on artificial diet incorporating imidacloprid at a concentration of 4.5 mg/kg, an average of 26 ppb of imidacloprid was found in the insect's hemolymph. On the other hand, when caterpillars were reared on plants treated with imidacloprid (Admire Pro @ 0.384 LB A/A) we observed an average of 16.1 ppb of imidacloprid in insect hemolymph. No imidacloprid was detected in a negative control, where larvae were reared on tobacco leaves not treated with imidacloprid, suggesting that nicotine present in larval hemolymph doesn't produce any cross-reactivity in the assay. Concentrations of imidacloprid in the range we observed from hornworms collected from field grown, treated tobacco have been documented to have negative effects on beneficial arthropods in other studies; for instance, 10 ppb of imidacloprid in sugar water shorten longevity of green lace wing by 60% (Kumar and Santharam, 1999), and 24 ppb imidacloprid incorporated in nectar decreases foraging and mobility of *Apis mellifera* (Decourtye et al., 2004). It is therefore conceivable that imidacloprid in these concentrations in *M.sexta* larvae could have deleterious effects on endoparasitoids such as *C. congregata* inhabiting them. Similar speculations were made by Barbosa et. al. (1991) about nicotine.

Residue analysis of imidacloprid and its metabolites in field collected *M. sexta* larvae collected from plots treated with different rates of imidacloprid produced no significant differences between insecticide treatments ($df = 1, 24.5$; $F = 1.12$ $p= 0.3597$ and $\alpha = 0.05$). Larvae from untreated control plots had the lowest amounts of imidacloprid with an average

of 0.87 ppb (Figure 3). Larvae from Provado® treated plot had the highest imidacloprid residues in their hemolymph with an average of 2.53 ppb; however, this was not significantly different from the other treatments.

This lack of difference among treatments could be due to at least two factors. First, we collected these larvae very late in the growing season. Study conducted by Byrne et. al. in 2010 suggested that up to 4 weeks after application of imidacloprid, the concentration of imidacloprid remained stable but then starts to decline. In Poinsettia after four weeks imidacloprid was not detectable by ELISA (Byrne et. al. 2010). It is thus likely that imidacloprid residues in the soil and plants had degraded substantially by this time. In the previous experiment establishing the presence of imidacloprid in the hemolymph of *M. sexta* feeding on the higher rate of Admire Pro in the field, we observed about 16.4 ppb of imidacloprid; larvae used in this study were collected earlier in the season when residues in the plants were likely higher. It should be noted here that we did not see any parasitism in the field at that point (whereas parasitism was observed in other parts of the field containing the trial). Secondly, parasitized larvae have decreased feeding rates, and this might reduce intake of imidacloprid. It should also be noted that we saw 0.87 ppb of imidacloprid in larvae from untreated control plots where theoretically there should not be any imidacloprid. This may indicate possible movement of *M. sexta* larvae between plots; it is quite possible that some of the larvae we found in control plots had already fed on treated plants in other plots. This kind of movement could also obscure differences in residues of imidacloprid between different insecticide treatments.

Residues of imidacloprid and its metabolites in non-parasitized *M. sexta* larvae were significantly higher than those in larvae parasitized by *C. congregata*. We observed only about 0.5 ppb of imidacloprid in parasitized larvae while we saw 3.15 ppb of imidacloprid ($df= 1, 24.5$; $F = 13.27$; $p = 0.0013$ and $\alpha = 0.05$) (Figure = 4) in non-parasitized caterpillars. This suggests that these higher residues of imidacloprid may have significant, negative effects on parasitism. The lower limit of this ELISA kit is 0.2ppb; any residue lower than this would be identified as no residue. At very low residue levels, HPLC MS/MS is probably a preferable analytic technique; however, the ELISA technique appears to be useful for biologically significant residue levels. We observed one outlier in this study where one sample from parasitized larvae produced a reading of 22.09 ppb, which is substantially higher than other samples from parasitized larvae. This outlier may have been the result of human error on the part of the first author; it is also possible that the larvae represented by this sample moved from an adjacent field study.

We identified the translocation of imidacloprid from host *M. sexta* larvae to *C. congregata* larvae in our laboratory- reared parasitized caterpillars. Parasitized caterpillars had an average of about 2.48 ppb of imidacloprid residue in their hemolymph, while endoparasitoid supernatant contained an average of 1.41 ppb of imidacloprid. This suggests that some of the imidacloprid is transferred from *M.sexta* to *C. congregata*. Imidacloprid can have detrimental effect on endoparasitoids, but the concentration of imidacloprid seems low as compared to the positive control (non-parasitized *M.sexta* fed on imidacloprid diet), where we had 26.46 ppb of imidacloprid. This could be explained by the fact that parasitized

caterpillars in later stadia dramatically decrease feeding; this could lead to decreased intake of imidacloprid through diet. It may be desirable, in future investigations of the translocation of imidacloprid from host to parasitoids, to concentrate efforts on the earlier life stages of *M.sexta,* when larvae still eat significant amounts of foliage. This will necessitate working with smaller volumes of hemolymph from *M.sexta* and smaller *C. congregata* larvae; these smaller sample volumes may not be amenable to assay through ELISA, and HPLC MS/MS could again be a better analytical option for such investigations.

Our studies document that the QuantiPlate ELISA kit can be used to quantify imidacloprid in *M.sexta* and *C. congregata*. Hemolymph of *M.sexta* should be diluted more than three times in order to avoid a matrix effect in the hemolymph. No statistical differences in the concentration of imidacloprid and its metabolites were seen in the field among different treatments, but a significant difference in residues was detected in parasitized versus non-parasitized *M. sexta* larvae in the field. Non-parasitized larvae have significantly higher residues which suggest imidacloprid can reduce parasitism in *M.sexta*. We also observed translocation of imidacloprid from host to parasitoid. The results of these studies should help refine the use of this important insecticide in tobacco.

REFERENCES

Barbosa P., Gross P., Kemper J. 1991 Influence of plant allelochemicals on tobacco hornworm and its parasitoid, *Cotesia congregata. Ecology* 72:1567 – 1575.

Baysoyu, D.,Tiryaki, O.,and Kilincer, N. 2005. Fate of ^{14}C-Aldicarb in Parasitized *Spodoptera littoralis* Boisd. Larvae. *Tarim Bilimleri Dergisi* 11(3): 316-320.

Bock, J. M. 2010. Insect Management in Burley Tobacco in Non-traditional Areas of North Carolina. MS thesis, North Carolina state University.

Byrne , F. J., Castle, S. J., Bi, J. L., and Toscano, N. C. 2005. Application of competitive ELISA for the quantification of imidacloprid titres in xylem fluid extracted from grapevines. *Journal of Economic Entomology* 98:182–187.

Byrne, F. J, Toscano, N. C., Urena, A. A., and Morse, J. G. 2005. Quantification of imidacloprid toxicity to avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), using a combined bioassay and ELISA approach *Pest Management Science* 61:754–758.

Byrne, J. F., Oetting, D. R., Bethke, A. J., Green, C., and Chamberli, J. 2010. Understanding the dynamics of neonicotinoid activity in the management of *Bemisia tabaci* whiteflies on poinsettias crop protection. 29(3):260-266.

Castle, S. J., Byrne, F. J., Bi, J. L., and Toscano, N. C. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Management Science* 61:75–84.

Cho, A. Y., Hye-Sung, L., Park, Y. P., Lee, T. Y., Hammock, B. D., Ahn, C. K., and Lee ,J. E. 2002. Development of an ELISA for the organophosphorus insecticide chlorpyrifos. *Bulletin of the Korean Chemical Society*. 23:481-487

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M., and Pham-Delègue, M. H. 2004. Effects of imidacloprid and deltamethrin on associative learning in honey bees under semi-field and laboratory conditions,.*Ecotoxicology and Environmental Safety* 57: 410–419

Dhammi, A. 2010. Effect of Imidacloprid on *Cotesia congregata* an endoparasitoid of *Manduca sexta* and its translocation from host to endoparasitoid, MS Thesis, North Carolina State University.

Elbert, A., Becker, B., Hartwig, J., and Erdelen, C 1991. Imidacloprid-a new systemic insecticide. Pflanzenschutz-Nachr. Bayer 44: 113–136.

Kumar, K., and Santharam, G, 1999. Laboratory evaluation of imidacloprid against*Trichogramma chilonis* Ishii and *Chrysoperla carnea* (Stephens), *Journal of Biological Control* 13: 18–73.

Lewis, G. A., and Madge, G. S 1984. Esterase activity and associated insecticide resistance in the damson-hop aphid, *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) *Bulletin of Entomological Research* ,74: 227-238.

Li, Z. M., Wang, W. L., Wu, H. M., Liu, S. S., Liu, Y. Q. and Tang Z.H 2006, Detection and analysis of spinosad residue in the body fluid of *Cotesia plutellae* larvae with HPLC, *Acta Entomologica Sinica* 137–141.

Lind, J. R., Martin, S. C., Stuar,t E. R., and Fergus, G. P. 1998. [^{3}H]Imidacloprid Labels High- and Low-Affinity Nicotinic Acetylcholine Receptor-like Binding Sites in the Aphid *Myzus persicae* (Hemiptera: Aphididae). *Pesticide Biochemistry and Physiology* 62:1 3-14.

Liu, H., Song, J., Zhang, S., Qu, L., Zhao, Y., Wu, Y., and Liu, H. 2005. Analysis of residues of imidacloprid in tobacco by high-performance liquid chromatography with liquid–liquid partition cleanup *Pest Management Science.* 61: 511–514.

Mandic, A.I., S. D. Lazic, S.D., Ökrész, N., and Gaál, F.F. 2005. Determination of the Insecticide Imidacloprid in Potato (*Solanum tuberosum*L.) and Onion (*Allium cepa*)by High-Performance Liquid Chromatography with Diode-Array Detection. *Journal of Analytical Chemistry,*. 60(12): 1134–1138.

Matsuda, K., S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso, and D. B. Sattelle. 2001. Neonicotinoids insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci* 22:573–580.

Park, H. J.; *Park*, W. C.; Jung, T. W.; Ra, C. S.; Lee, Y. T. 2002. Development of an Enzyme-Linked Immunosorbent Assay for the Organophosphrous Fungicide Tolclofos-methyl. *Bulletin of the Korean Chemical Society,* 24:334-338.

Pfluger, W., and Schmuck, R., 1991. Ecotoxicological profile of imidacloprid.- *Pflanzenschutz-Nachrichten Bayer*, 44: 145-158.

Valentini, F., Compagnon, D.,. Giraudi, G., and Palleschi., G. 2003. Electrochemical ELISA for the screening of DDT related compounds: analysis in waste waters. *Analytica Chimica Acta*, 487(1): 83-90.

Sanyal, N., Hazra, D., Pal, R., Somchaudhury, A. K., & Chowdhury, A. 2006. Imidacloprid in processed tea and tea liquor. *Journal of Zhejiang University-Science B*, 7, 619–622.

Suchail, S., Laurent, D., and Luc, P. B., 2004. Metabolism of imidacloprid in *Apis mellifera Pest Management Science*,. 60(3): 291 – 296.

Zhang, Q., Sun, Q., Hu, B., Shen, Q., Yang, G., Liang, X., Sun, X., and Liu., F., 2008 Development of a sensitive ELISA for the analysis of the organophosphorous insecticide fenthion in fruit samples. *Food Chemistry* 106(3): 1278-1284.

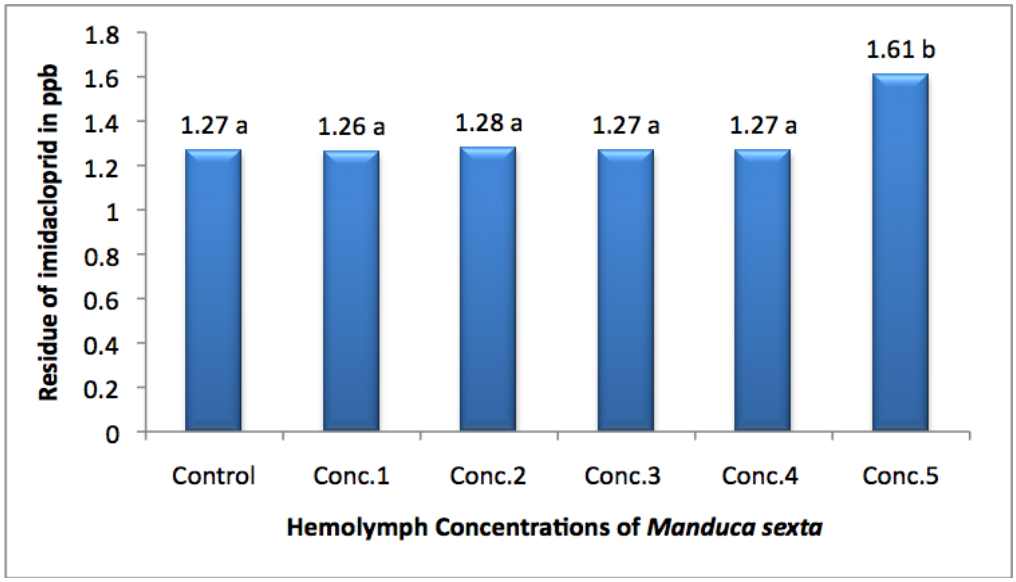

Figure 1. Concentration of imidacloprid and its metabolites in *M. sexta* hemolymph at different concentrations (diluted with 1ppb of imidacloprid) where Conc.1= 10µl of hemolymph + 100 µl of 1ppb of Imidacloprid; Conc.2 = 20µl of hemolymph + 100 µl of 1ppb of Imidacloprid; Conc.3 = 30µl of hemolymph + 100 µl of 1ppb of Imidacloprid; Conc.4= 40µl of hemolymph + 100 µl of 1ppb of Imidacloprid and Conc.5= 10µl of hemolymph + 100 µl of 1ppb of Imidacloprid. Control= 1ppb of imidacloprid (df = 5, 12:, F =229.13: $p < 0.0001$ and $\alpha = 0.05$).

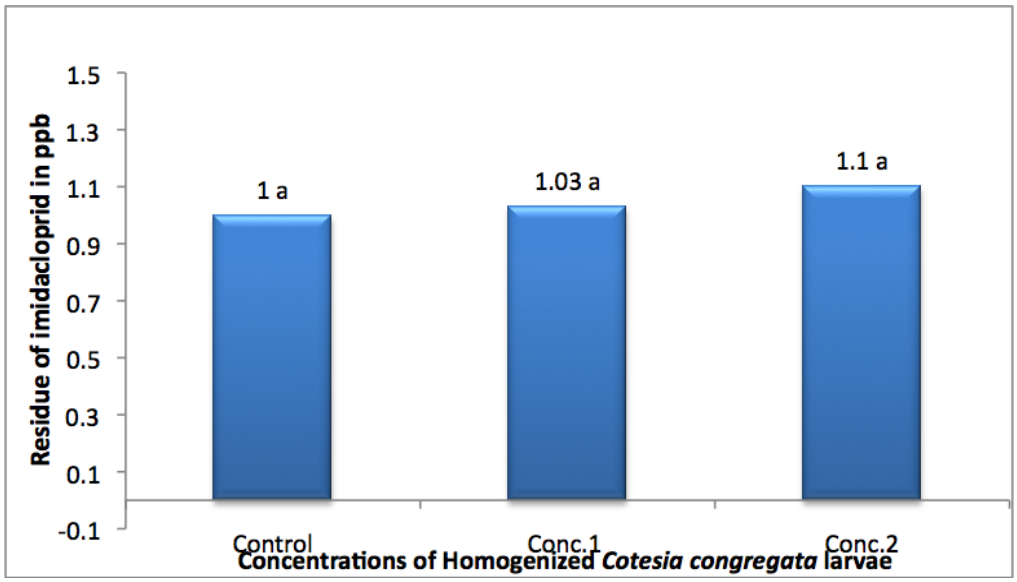

Figure 2. Concentration of imidacloprid and its metabolites in C. congregata homogenate concentration diluted with 1ppb of imidacloprid where Conc.1= 30µl of homogenate + 100 µl of 1ppb of Imidacloprid and Conc.2 = 40µl of hemolymph + 100 µl of 1ppb of Imidacloprid; Control = 1ppb of Imidacloprid. ($df = 2, 6$; $F= 0.61$ and $p= 0.5878$ and $\alpha = 0.05$).

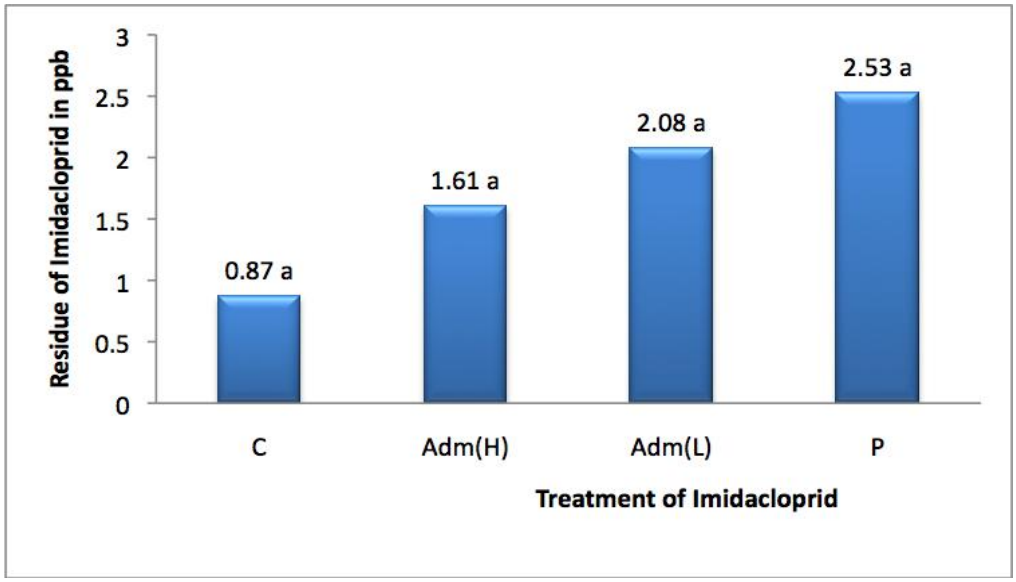

Figure 3. Imidacloprid Residue present in hemolymph of larvae collected from field for different applications of Imidacloprid where C = Control; Adm(H) = Admire Pro high concentration; Adm(L)= Admire Pro low concentration and P = Provado (df = 1, 24.5 ; F = 1.12p= 0.3597 and $\alpha = 0.05$).

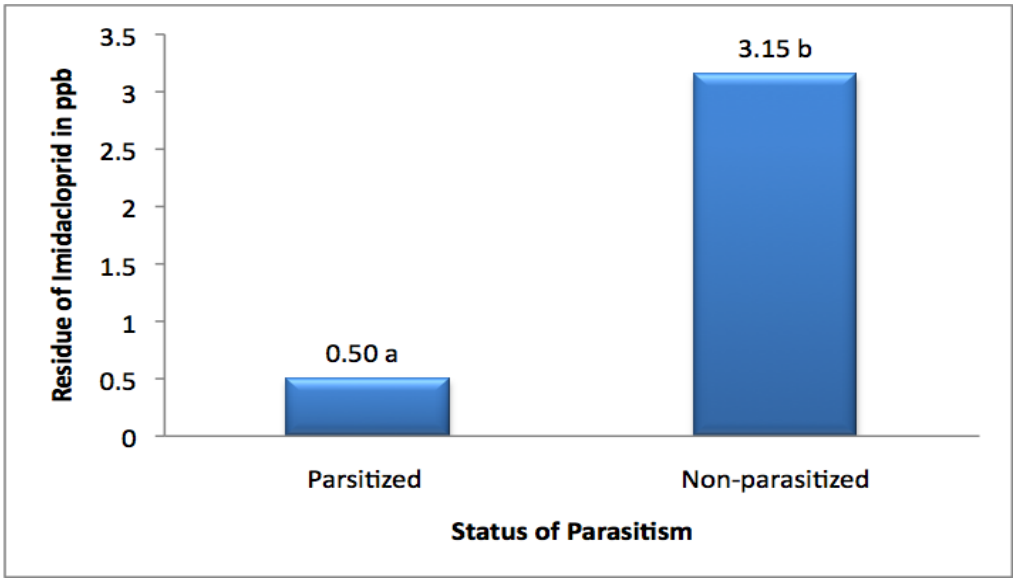

Figure 4. Presence of Imidacloprid residue in relation to parasitized and non-parasitized larvae. (df= 1, 24.5; F = 13.27; p = 0.0013 and $\alpha = 0.05$)