RESPONSE OF THE WHITEFLY *Bemisia tabaci* B BIOTYPE TO REPEATED EXPOSURES OF IMIDACLOPRID

By

Mamy L Rakotondravelo

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ABSTRACT

RESPONSE OF THE WHITEFLY *Bemisia tabaci* B Biotype TO REPEATED EXPOSURES OF IMIDACLOPRID

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The whitefly, *Bemisia tabaci* (Gennadius), is among the most damaging of all pests in cropping ecosystems. Among the different biotypes found in this complex species, B biotype is one of the most widespread in agricultural systems. Within a few years of its initial introduction imidacloprid became the standard product for control of whitefly. This study evaluates the effect of frequent applications of imidacloprid to a population of whitefly. B biotype whitefly collected from a commercial greenhouse in Michigan, was cultured and put under imidacloprid selection to determine the rate of development of resistance to this insecticide. Before imidacloprid treatments started this population already had a reduced susceptibility to imidacloprid with a resistance ratio RR of 11-fold compared with that of a reference strain that we obtained from University of California, Riverside. When adults of this resistant population were exposed to an increasing concentration of imidacloprid, the resistance level increased to 218-fold in the F11 generation, indicating a rapid development of resistance after repeated exposure to high doses of imidacloprid. The realized heritability h^2 of the resistance was also determined. The overall mean estimate of realized heritability for imidacloprid resistance in this B biotype population was 0.10, suggesting that phenotypic variation in imidacloprid resistance in this population was accounted for by an additive genetic variation. The response of the resistant population to treatment with other neonicotinoids was then assessed. The imidacloprid resistant population

exhibited a low level of cross resistance to thiamethoxam (RR=9fold) and to acetamiprid (7-fold). To have a complete picture of our assessment, the effect of exposure of whitefly to a very low concentration of this insecticide was also determined. Three different populations were used:a reference strain, a resistant and a rebuilt reference strain. The exposure of immatures of the resistant strain to a low priming concentration of imidacloprid (0.0012 mg a.i. per pot), followed by a subsequent exposure to imidacloprid as adults elicited an increase in fecundity when compared with non-primed females (77.0 eggs/female compared with a 66.4 eggs/female). Resistant females pre-exposed to low priming concentration (0.0012 mg a.i per pot) were also found to exhibit greater egg production than resistant females primed with a high concentration of imidacloprid (12 mg a.i. per pot) (54.4 and 46.0 eggs per female). F1 female progeny of the reference strain from parents primed with imidacloprid at 0.0012 mg a.i. per pot produced more eggs than F1 female progeny from non-primed parents (19.6 and 13.6 eggs/female). The test was repeated on the rebuilt reference strain using two priming concentrations (0.0012 and 0.00012 mg a.i. per pot). The test on the parental generation of this rebuilt reference strain showed that females primed with the two low concentrations of imidacloprid 0.0012 and 0.00012 mg a.i. per pot have greater egg production than the non-primed females for the rebuilt reference strain (191.8, 186.7 and 169.3 eggs per female, respectively). However, no difference was found in the fecundity of the F1 progeny of females for this rebuilt reference strain. All the results suggest that treatment with a low amount of imidacloprid applied during an early immature stage of development has an effect on adult whitefly fecundity. The result of this study has provided more insight into the strategy for whitefly management and into the effects of residual amount of insecticide in the organism.

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CHAPTER I. Introduction.

I Whitefly *Bemisia tabaci* B biotype.

Bemisia tabaci (Gennadius) (Insecta: Hemiptera: Homoptera: Aleyrodidae) is a polyphagous insect found on over 500 plants species in every continent except Antartica (Oliveira *et al.,* 2001). Whitefly *Bemisia tabaci* is a complex species that comprises up to 20 biotypes (Perring, 2001). Among the different biotypes, Q biotype and B biotype are the most studied. Recently, it was reported that *Bemisia tabaci* is a cryptic species complex consisting of at least 24 species which cannot be distinguished morphologically (DeBarro *et al*., 2011). Based on the new classification, the B biotype whitefly is identified as Middle East- Asia Minor whereas the Q biotype as Mediterranean (De barro *et al.*, 2011). However, the term biotype is still frequently used in published studies when addressing these whiteflies (Kontsedalov *et al.,* 2012). These two whitefly biotypes vary in fecundity, host range, and efficiency of virus transmission (DeBarro *et al.,* 2006; Gottlieb *et al.,* 2010). The Q and B biotype are reproductively incompatible, probably due to a prezygotic barrier (Sun *et al.,* 2011). Both biotypes may spread rapidly through the sale of ornamental plants (Debarro *et al.,* 2011). B biotype is the most widespread in agricultural ecosystems (Byrne *et al.,* 2010). It is also known as *Bemisia argentifolii* and may be referred to as silverleaf or sweetpotato whitefly (Debarro and Driver, 2007). The B biotype whitefly may displace indigenous biotypes due to its high fecundity, its mating behavior, and its higher fitness on several taxa of host plants (De Barro, 2011). However, the Q biotype whitefly tends to develop higher levels of resistance to insecticides than the B biotype whitefly, so that Q biotype may be more widespread in production systems where insecticides are used intensively (Horowitz *et al*., 2005; Kontsedalov *et al.,* 2012). For example, a monitoring study in Israel demonstrated that Q biotype was the predominant biotype in greenhouses where chemical use was more frequent whereas B biotype

whitefly constitutes the majority of the whitefly found in open fields due to their higher fitness under field conditions (Kontsedalov *et al.*, 2012). This trend was also noticed with neonicotinoids. For example, B biotype was the predominant biotype in China in 2006 (Chu *et al.*, 2007), and the subsequent monitoring study reported that Q biotype displaced B biotype due to the increasing use of neonicotinoids (Chu *et al.*, 2010).

Bemisia tabaci is among the most damaging of all pests in cropping ecosystems. It feeds on plant phloem, excretes honeydew upon which sooty mold grows, and also transmits viruses (Oliveria *et al.*, 2001). The first reported damage caused by B biotype whitefly in the USA was on poinsettia, *Euphorbia pulcherrima* in Florida in 1986 (Price and Schuster, 1991). It soon spread to other southern states including California, Texas and Arizona (McAuslane, 2010)**.** If left uncontrolled, whitefly infestations can induce significant crop loss. The agricultural loss associated with whitefly in Arizona, Florida, Texas and California was estimated at $500 million in 1991 alone (Perring *et al.*, 1993).

II Whitefly pest management.

Different strategies have been adopted to control whitefly. Among them is the use of natural enemies, including fungi such as *Ascherosnia, Beauveria,* and *Metarzhium,* and predators like *Amblyseius swirskii, Macrophlophus calliginosus,* or the parasitoids in the genera *Encarsia* or *Eretmocerus* (Horowitz *et al.*, 2011). However, despite many attempts to protect, augment or release many different natural enemies, the use of chemicals is still the main strategy adopted for whitefly control (Palumbo *et al*., 2001). Conventional insecticides such as carbamates, organophosphates and pyrethroids were among the chemical classes most heavily used for whitefly control before 2000 (Debarro, 2011). However, numerous studies reported the

development of resistance to these insecticides (Denholm *et al*., 1998). The use of mixtures of pyrethroids with other classes of insecticides such as organophospahtes or carbamates was also commonly adopted due to their efficacy for whitefly control especially in the southwestern United States (Palumbo *et al.*, 2001; Horowitz *et al.*, 2011). Similar to the other conventional pesticides, resistance to these pyrethroid mixtures was also reported after a few years of their use (Dennehy and Wiliams, 1997). The diversity of chemicals for the whitefly control was increased with the introduction of insect growth regulators IGR. Pyriproxyfen and buprofezin are among the most heavily used IGR's for whitefly control (Toscano *et al*., 2001). Buprofezin interferes with chitin formation and affects the development of immature insects (Ishaaya *et al*., 1988), whereas pyriproxyfen is a juvenile hormone analog that affects embryogenesis and metamorphosis (Ishaaya and Horowitz, 1992).

In the early 1990s, imidacloprid, belonging to a different class of insecticide called the neonicotinoids, was introduced into the market (Nauen and Denholm, 2005). This compound is very effective in controlling sucking insects such as planthoppers, aphids and whiteflies due to its systemic properties (Tomizawa and Casida, 2003). After imidacloprid, several additional neonicotinoid products also became available to growers, including thiomethoxam, acetamiprid, and dinotefuran. In 2006, neonicotinoids made up 17% of the global insecticide market with an annual sale of 1.5 billion US dollars (Jeschke and Nauen, 2008). Imidacloprid is one of the most successful neonicotinoid insecticides; it has been registered for use on 140 crops in more than 120 countries, and it was predicted to continue to be one of the leading insecticides in a few years to come (Elbert *et al.*, 2008). Imidacloprid acts on the central nervous system as an agonist of the nicotinic acetylcholine receptors (nAChR) in insects (Liu and Casida, 1993). Since imidacloprid had a new mode of at the time of the introduction, it was quickly adopted as the

best insecticide to use on resistant whiteflies (Rauch and Nauen, 2003). However, due to its overuse high levels of resistance were reported in B biotype whitefly in various countries. For example, a high resistance factor to imidacloprid (RR = 230 fold) was documented in B biotype whitefly collected from the field in Mexico and in Guatemala in 2005 (Gorman *et al.*, 2010). Vassiliou *et al.* (2011) also found a wide range of imidacloprid resistance levels (RF= 67 to 392-fold) in B biotype whitefly collected from different regions in Cyprus. Rauch and Nauen, (2003) documented a high resistance level (874-fold) in a B biotype whitefly collected from roses in greenhouses in Israel. In contrast to these results, no report of whitefly control failure was documented for outdoor crops in Arizona and Florida despite the reliance on imidacloprid for whitefly control in these regions in 1990s (Palumbo *et al.,* 2001). Castle *et al.* (2010) suggested that biological, ecological and operational factors could explain this modulation of the development of insecticide resistance in whiteflies. For example, the authors reported that the presence of untreated crops of alfalfa in Imperial Valley, California, which comprises 70% of the area, could serve as refugia for susceptible whitefly and slowed down the development of resistance to imidacloprid in this region despite its heavy use. This differential level of imidacloprid resistance in whitefly has also been observed in different regions across the world with different cropping systems, and the resistance level was found to be not necessarily associated with the amount of use of the pesticides. For example, Vasillou *et al.* (2011) reported resistance levels varying from moderate to high in B biotype whitefly populations collected from different regions in Cyprus, and the resistance level was not correlated with the history of neonicotinoid application. In addition, different biotypes in the same regions in China also showed a different levels of resistance to imidacloprid, with B biotype whitefly being susceptible to this compound whereas Q biotype whitefly being resistant (Luo *et al.*, 2010). All

these observations seems to hint that in addition to environmental factors present in the field, other factors such as biological, physiological or genetic factors may also contribute to the development of different resistance levels in the field populations of whitefly.

III Realized heritability h^2

As a matter of fact, Tabashnik (1992) argued that the rate of development of the resistance to insecticides could be predicted through the analysis of the heritability of resistance to the insecticide in the species of interest. The phenotypic variation (VP) of a threshold trait such as insecticide resistance is a combination of additive genetic variation (VA) and environmental variation (VE) (Falconer, 1989, Firko and Hayes, 1990). Heritability in a narrow sense is defined as the proportion of the total phenotypic variation attributable to the additive genetic variation (Falconer, 1989; Tabashnik, 1992). Thus, heritability enables an estimation of the relative contribution of the gene and the environment to the variation of the trait of interest (Visscher *et al*., 2008). Heritability depends on different factors such as the allele frequency of the resistant level but also to the environmental conditions under which the population is studied (Falconer, 1989, Tabashnik, 1992). Indeed, laboratory populations which are likely to have less environmental variation have a much higher heritability than field populations (Bull *et al.*, 1982). Thus, the heritability of laboratory populations may not necessarily reflect the situation in the field (Falconer, 1989, Tabashnik and McGaughey 1994). However, several studies also suggested that heritability in the laboratory could still predict the time of development of resistance in field. For example, Li *et al.* (2012), starting with a field-collected population of *Musca domestica* in China, selected for imidacloprid resistance for 21 generations, and estimated a relatively low heritability ($h^2 = 0.10$) in the houseflies. The authors noted that no reports of

resistance in housefly were yet documented in China. Wang *et al.* (2008) also estimated a very low h^2(0.08) in the planthopper, *Nilaparvata lugens*, collected in China and selected for imidacloprid resistance for 27 generations. The authors also noted that the field population of planthopper remained susceptible to imidacloprid from 1996 to 2003. Resistance was only detected in 2005, some 13 years after the first use of imidacloprid in China. These studies are in contrast to the results found by Sivasupramanian and Watson (2000) who estimated a high heritability of resistance to the mixture of acephate and fenpropathrin on B biotype whitefly in Arizona, and this high heritability estimate was consistent with the rapid observation of control failure of field populations, which occurred within two years (Dennehy and Wiliams, 1997). A relatively high heritability (h^2 = 0.40) was also estimated in the greenhouse whiteflies, *Trialeurodes vaporarium*, selected for dichlorovos resistance in the laboratory. This value was also found to be in agreement with a rapid development of resistance in field (Omer *et al.*, 1993). However, it is also noteworthy that some other reported heritability values for insecticides in other pests were found to be not consistent with the rate of resistance development in field populations. For example, Firko and Hayes (1991) reported a very high heritability of 0.85 for cypermethrin in the tobacco budworm, however no field control failure was documented until 10 years after the introduction of this insecticide. In brief, heritability could be one factor that may partially explain the wide variation in how rapidly resistance develops among different populations of one species or biotype.

IV Resistance and cross resistance to neonicotinoids in whitefly.

In addition to a pronounced difference in levels of resistance to imidacloprid among different populations of B biotype whitefly, a wide variation in the observed levels of cross

resistance to other neonicotinoid has also been observed. For example, the level of cross-resistance to neonicotinoids in populations of B biotype whitefly was found to vary from very low (RRs of 5 and 2 for acetamiprid and thiamethoxam, respectively) to a high (RRs of 78 and more than 900) (Prabhaker et al., 2005; Rauch and Nauen, 2003). Resistance to imidacloprid was attributed to the P450 detoxification system in whiteflies (Rauch and Nauen, 2003). An overexpression of one of the P450 genes, CYP6M1PvQ, was found in imidacloprid-resistant populations of both B and Q biotypes (Karunker *et al.,* 2008). In a subsequent study, Roditakis *et al*. (2011) demonstrated that indeed CYP6M1PvQ has a potential to metabolize imidacloprid in whiteflies. However, the same study showed that thiamethoxam was not metabolized by the protein of this P450 gene. In fact, Nauen *et al.* (2003) showed that thiamethoxam was converted to clothianidin in cotton plants and in the insects ingesting it, and now clothianidin is known to be a byproduct of thiamethoxam. Interestingly, Roditakis et *al.* (2011) found that as opposed to thiamethoxam but similar to imidacloprid, indeed clothianidin was metabolized by the gene responsible of imidacloprid resistance in whiteflies, CYP6M1PvQ. In addition, Roditakis *et al*. (2011) demonstrated that similar to thiamethoxam, the protein of the P450 gene CYP6M1PvQ also did not metabolize acetamiprid in whiteflies. In agreement with the metabolism study, most of the reports on cross resistance levels in B biotype whitefly resistant to imidacloprid showed a high level of resistance to thiamethoxam but a moderate level of resistance to acetamiprid. For example, Gorman *et al.,* (2010) showed that population of B biotype whitefly from Guatemala having a resistance ratio (RR) of 230 to imidacloprid exhibited a resistance ratio of 150-fold to thiamethoxam whereas it was only 15-fold for acetamiprid. The same study also reported that the B whitefly population collected from Mexico having a resistance ratio of 230-fold to imidacloprid exhibited 73-fold resistance to thiamethoxam but only 15-fold resistance to

acetamiprid. Similarly, different B biotype whitefly populations collected from different regions in Cyprus, and having a moderate to high resistance level to imidacloprid (RR of 77 to 392), also showed a moderate level of resistance to thiamethoxam (RR = 50 to 164) and a low level of resistance to acetamiprid (RR = 7 to 12) (Vasillou *et al.*, 2011). The same observation of higher resistance to thiamethoxam than to acetamiprid was even noted in several imidacloprid resistant populations of Q biotype whitefly. For example, Rao *et al.*, (2012) reported that a population of Q biotype collected from China which has a similar high resistance level for thiamethoxam and imidacloprid (244 and 273-fold respectively) showed only 68-fold of resistance to acetamiprid. The selection history was already shown to influence the resistance level to other neonicotinoids in B biotype whitefly. Horowitz *et al.* (2004) found that whitefly population selected for a thiamethoxam resistance showed a very low resistance to acetamiprid whereas an acetamiprid-selected population was highly resistant to thiamethoxam. In contrast, Feng *et al*. (2009) selected B biotype whitefly for thiamethoxam resistance (RR: 25.6) found a similar level of resistance to acetamiprid (RR 35-fold) and imidacloprid (47.3-fold). Interestingly, Karunker *et al.*, (2008) found the CYP6CM1 as the P450 gene that was over-expressed in imidacloprid-resistant whiteflies Q and B biotype. However, Xie *et al.* (2012) observed an overexpression of three other P450 genes, CYP6a8, CYP4v2, and CYP6v5, in thiamethoxam-resistant whitefly. These results seem to suggest that a different history of selection could also select a different gene, causing a different level of resistance between neonicotinoids in whiteflies.

Dinotefuran is also another neonictoinoid used for growers for whitefly control. However, this insecticide has a different structure as it does not have the pyridine or thiazole ring similar to the previously introduced neonicotinoids such as imidacloprid, acetamiprid and thiamethoxam (Wakita *et al.*, 2005). Prabhaker *et al*. (1997) demonstrated that the population of

B biotype whitefly selected for imidacloprid resistance in laboratory did not exhibit an appreciable cross resistance to this compound. Similarly, this non-significant cross resistance to dinotefuran was also observed in some Q biotype whitefly. Three populations of Q biotype collected from three different regions in China with a high resistance level to imidacloprid (201, 244, 219-fold) showed a resistance level of 2, 4 and 6- fold to dinotefuran, respectively (Rao *et al.,* 2012).

Besides the different metabolism of neonicotinoids, an increasing number of studies also demonstrated that the bacterial symbionts hosted by both B and Q biotype *Bemisia tabaci* also could influence the susceptibility of these whiteflies to neonicotinoids. Ghanim and Kontsedalov (2009) showed that Q biotype was double-infected by the bacterial symbiont *Ricketssia* and *Arsenophonus* on one hand, or *Wolbachia* and *Arsenophonus* on other hand, were more susceptible to imidacloprid and thiamethoxam than the Q biotype whitefly infected by a single symbiotic bacterium, *Arsenophonus*. Kontsedalov *et al.* (2008) showed that B biotype whitefly harboring the symbiotic microorganism *Rickesttsia* was more susceptible to thiamethoxam and acetamipird than individuals not infected by these microorganism, however the presence of these *Rickettsia* did not affect susceptibility to imidacloprid. The compositions of these facultative symbionts can vary between regions or biotypes (Xie *et al.,* 2012). All of these studies suggest that differential susceptibility to insecticides between strains or biotypes could also be partially explained by the presence of different symbiotic microorganism in these whiteflies.

V Hormesis.

Bonmatin *et al.* (2003) reported that imidacloprid can still be found 12 months or longer after its application in the field. The same authors detected the amount of imidacloprid to be as

low as 0.5-20 µg/kg in soils, plants, and pollens. Exposure of pests to a low amount of imidacloprid is then expected for the entire crop production period. Some toxicants are known to induce a stimulatory response at low doses, and this phenomenon is known as hormesis in the toxicological literature (Calabrese and Blain, 2005). Hormesis is an adaptive response which is characterized by biphasic dose response, with a stimulatory effect at the low doses of the toxicant and an inhibitory effect at the high doses (Calabrese and Baldwin, 2002). The pioneer studies on this biphasic dose response were conducted in the 1880s by a German pharmacologist, Hugo Schluz, who observed a stimulation of yeast metabolism at low dose of various chemical agents whereas the chemicals have inhibitory effects at high doses (Calabrese, 2002). As Schulz and his colleague psychiatrist Rudolph Arndt developed the concept of this dose response, this phenomenon was previously known as the Arndt-Schluz- law after the publication of Schluz' work in 1912 (Calabrese, 2002). Several investigations on this phenomenon were then conducted by different researchers in the 1920's and 1930's (Calabrese, 2002). After the 1930's the concept drew less attention from scientists due to some limitations related to the concepitself and due to the fact some investigators associated the phenomenon with the unpopular homeopathy concept (Calabrese, 2002, Calabrese, 2005, Calabrese *et al*., 2010, Rozman and Dull, 2003). The term hormesis (from Greek word which means to excite) was used for the first time by Chester Southam and John Ehlrich in 1943 (Calabrese, 2009). A resurgence of interest in hormesis was noted again in late 1970s due to the needs in risk assessment and the availability of improved methodology for detecting low doses of various chemicals (Calabrese *et al.*, 2010). Over the last two decades, Edward Calabrese, a toxicologist in Massachusetts University, has been one of the leading researchers in the study of hormesis and has published several documents on this concept. Through an exhaustive analysis of a huge volume of literature,

Calabrese and his colleagues set some criteria, principles and properties that characterize hormesis in his several documents (Calabrese *et al.*, 1999, Calabrese and Blain, 2005). For example, hormesis was found to be induced by a wide variety of chemical and physical agents in different organisms from bacteria to mammals (Calabrese and Blain, 2005). Calabrese and Baldwin (2003) reported that in most cases hormetic stimulation has an amplitude of 130 to 160% of the untreated controls. The hormetic dose that induces stimulation above the control is known as the hormetic zone, and is usually located just below the No Observed Adverse Effect Level (NOAEL). The width of the zone is usually in the 10 to 100-fold dosage range , but 70% of the studies are about in 20-fold (Calabrese and Baldwin, 2003). Calabrese and Baldwin (2002) also defined two types of hormesis: first, the overcompensation stimulation hormesis (OCSH) which is a result of an overcompensatory response following a disruption of homeostasis, and second, the direct stimulation hormesis (DSH). In contrast to OCSH, the direct hormesis is an adaptive response that is associated with the regular function of metabolism, thus with no disruption of homeostasis to be repaired (Calabrese and Baldwin, 2002). The hormetic effect may be detected with a single treatment, or sometimes it can only be determined after challenging the cell or organism with a subsequent treatment. When a subsequent treatment is required the effect is known as 'conditioning hormesis' (Calabrese *et al.*, 2007). In conditioning hormesis, the first treatment can protect the organism against the toxic effects of a subsequent more severe treatment (Calabrese *et al.*, 2007; Calabrese and Mattson, 2011). In fact, Stebbing, (2002, 2009) viewed hormesis as an acquired tolerance. If the subsequent treatment does not occur, the overcorrection response would be seen as stimulation above the control (Stebbing, 2009).

Hormesis has some characteristic properties. One of them is a lack of response of the

organism depending on the similarity or difference between the stress agent used in pre-exposure and the subsequent exposures. For example, one inducing agent used for the pre-exposure could protect the organism against the damaging effect caused by a subsequent higher dose of the same stress agent only (Crysper and Johnson, 2002). However in some cases, one stress agent can elicit an adaptive response to cope with the detrimental effects of the subsequent exposure to more other stress agents (Chujo *et al.,* 2012), a phenomenon that was also known as cross-adaptation or cross-tolerance (Zhao and Wang, 2012; Chujo *et al.,* 2012).

The dose used for the pre-exposure can also influence hormesis. For example, Chujo et *al.,* (2012) found that in *C. elegans* only the low but not the high dose of the stress agent such as heat stress, osmotic stress and oxidative stress can elicit an adaptive response that protects the worm against the damaging effect of the subsequent higher dose of these stress agents. However, Wang and Xing (2010) demonstrated that pre-treatment of *C. elegans* with UV irradiation at 15, 20 and 30 J/m^2/min elicited an adaptive response, thus it protected the worm against the reproductive toxicity of a subsequent exposure to cadmium. However the pre-exposure to a lower dose of this UV irradiation (5 J/m^2/min) did not induce such a response. These results suggest that the pre-exposure is only effective within a certain range of dose. Stebbing (2009) already proposed in his model the absence of response of the organism if the dose of the inducing factor is too low to trigger the phenomenon, but at too high of a dose the damaging effect would prevail.

Another important characteristic of hormesis is that this phenomenon can be observed when the inducing factors are applied at specific life stages. For example, Lebourg (2005) demonstrated adaptive hormesis in *Drosophila melanogaster* by pre-exposing young males to hypergravity, which induced a longer lifespan than the non-pre- exposed individuals when they

were later exposed to heat shock. Nascarella *et al.* (2003) also reported that the larva of the blow fly, *Phormia regina,* fed a diet with a low dose of cadmium exhibited a high pupation rate. However the treatment adversely affected the adult emergence of this species, indicating an occurrence of hormesis at the larval stage only. But in some cases, the hormetic effect can occur in adulthood when the mild stress was applied at an early stage of development of the individuals (Costantini *et al*., 2010).

As far as insects are concerned, one remarkable study of stimulation caused by low doses of insecticides was conducted by Luckey in 1968. Luckey (1968) demonstrated a stimulation of growth of the house cricket *Acheta domesticus* to sublethal concentrations of 14 insecticides. This stimulation was suggested to be associated with hormoligosis, a term used first time by Luckey in 1955 to describe the biphasic dose response that the author found, thus a stimulatory effect at low doses of the toxicants and an inhibitory effect at higher doses (Luckey, 2008). Hormoligosis and hormesis indicate a stimulation induced by low doses of different stressors, but until now there has not been any evidence that the two concepts describe the same phenomenon, thus induced by the same mechanism, or that they are different from each other. Different authors already attempted to differentiate the two effects. For example, Cutler (2012) and Cohen (2006) pointed out the suboptimal conditions to be present for hormoligosis to occur to separate the two concepts. In a different approach, Rozman and Doull (2003) proposed that hormesis refers to a homeostatic overcompensation response triggered by an initial inhibition whereas hormoligosis is associated with the overcompensation response induced by an initial stimulation.

Because hormesis induces a modest response (30-60% higher than the control) its biological significance is sometimes overlooked (Calabrese, 2000). In agricultural ecosystems, some investigators support the idea that hormesis may contribute to a pest resurgence or

secondary outbreak following an insecticide application (Guedes *et al*., 2010; Morse, 1998, Cohen, 2006). Recently an increasing number of studies have investigated potential stimulatory effects of sublethal doses of pesticides on populations of insects. For example, Guedes *et al*. (2010) demonstrated that low doses of deltamethrin induced the maize weevil, *Sitophilus zeamais*, to increase the intrinsic rate of population growth. A list of studies reporting an occurrence of hormesis on different endpoints in arthropods was documented by Cohen (2006). Recently, Cutler (2012) also published a more specific review on this concept by giving the main focus of hormesis induced by insecticides in insects. Longevity and fertility were the biological endpoints often reported in these studies (Cohen, 2006, Cutler, 2012). In some cases, the cause of the stimulation was not well determined whether it was through a direct effect of the toxicants or through an indirect effect such as through a change in physiology of the treated host plants or other factors (Cohen, 2006, Cutler, 2012). For example, Van der Laan, (1961) reported an increase in populations of whitefly after the application of DDT, and the author suggested that this might have been related to a physiological change in the cotton host plants after the DDT application, favoring the rate of viability of eggs. The authors excluded the reduction of natural enemy as the main cause of the increase because others chemicals including endrin and toxaphan which are as toxic to the natural enemy as DDT were studied simultaneously, however no increase of whiteflies were observed in the plots treated with these chemicals.

The mechanism of hormesis is not yet elucidated, but Calabrese and Baldwin (2002) argued that the occurrence of hormesis on different biological measures across different taxa suggest that the induction of hormesis could not be explained by a single mechanism. These authors argued that despite the unlikely occurrence of a single mechanism, it seems that the hormesis results from a common strategy which consists of allocation of resources with the main

objective to conserve homeostasis in the organism (Calabrese and Baldwin, 2002). Several studies also suggested that hormesis is a phenomenon that is related to an occurrence of stress (Stebbing, 2009; Chujo et *al.,* 2012). Calabrese *et al.,* (2010) also reported several stress responsive genes that were involved in the hormesis phenomenon in mammals. Zhao and Wang (2012) reported that hormesis in the nematode *Caenorhabditis elegans* was also often associated with a regulation of various metabolic and signaling pathways in addition to the involvement of the antioxidant system triggered by various stress agents. An increasing number of authors working on mammals suggested that hormesis might also be associated with an epigenetic mechanism (Scott *et al.,* 2009; Vaiserman, 2010, 2011).

An epigenetic phenomenon is suggested by the observation of hormesis on adult life when treatment was applied at an early stage of development (Costantini *et al.,* 2010). In fact, an increasing body of evidence shows that an exposure of mammals to various chemical factors during early stages of development can influence the phenotype of individuals when they become adults, and this phenomenon is believed to be associated with an epigenetic mechanism (He *et al.,* 2006; Zawia and Basha, 2011). Epigenetics are the processes causing a heritable change of gene expression without an alteration in the DNA sequence. Instead, the heritable changes are caused by changes in DNA methylation, histone modification, and remodeling of chromatin which consequently causes activation or repression of the genes (Jaenisch and Bird, 2003; Decluve *et al.,* 2009). In fact, the epigenetic status of a cell or an organism is vulnerable to modification triggered by environmental factors during critical periods of development (Janish and Bird, 2003, Reik et *al.,* 1993). Once established, the epigenetic modification can be inherited by the daughter cells after cell division (Ng and Gurdon, 2008), and the associated phenotype can therefore be observed during the adult life of the individuals. In mammals, for example, this sensitivity to modification may occur during embryo development or during the post natal period (Decluve *et*

al., 2009). The epigenetic modification induced by environmental factors can affect the somatic cells or the germ cells as well. In mammals, one of the periods of sensitivity of the germ cell is during the period of gonadal sex determination of the embryo when there is an epigenetic reprogramming event (demethylation and then remethylation of the DNA) in the germ cells (Skinner, 2008). It was noticed that the timing of the epigenetic reprogramming differs between sexes and species. For example, remethylation of germ cells occurs so late in female mice that it doesn't happen until after birth (Reik *et al.,* 2001). In rats, the demethylation of primordial germ cells (PCG) occurs when they migrate to the precursor embryonic gonads, and the remethyaltion of the germ cells starts in the gonads (Anway *et al.,* 2005). Any treatment after this short period of time did not have any effect on the germ cell (Anway *et al.,* 2005). An epigenetic modification that affects the germ cell can be inherited by the subsequent generation (Jabalonka and Raz, 2009). Skinner (2008) proposed two definitions to categorize inheritance of the traits by the subsequent generation: multigenerational and transgenerational effects. In the multigenerational effect, the F1 offspring are exposed directly to the modifying agent when they are inside the treated pregnant mother. In the transgenerational effect, the effect was inherited by individuals of the subsequent generation that were not directly exposed to the modifying agent, like the F3 generation in some studies with mammals. Pesticides are among the stress agents that can induce transgenerational effects in various organisms. An increasing number of studies have shown a transgenerational inheritance of the effect of pesticides on the reproductive system of mammals. For example, the pyrethroid insecticide permethrin and the insect repellent N,N-Diethylmeta-toluamide (DEET) were shown to induce a decrease of the primordial follicle pool size in the ovary of the F3 generation in rats (Manikkam *et al.,* 2012). Anway *et al*. (2005) also showed that the fungicide vinclozolin and the pesticide methoxychlor reduced the number and motility of

sperm in rats and the effects persist until the F4 generation of the males.

Little is known about the sensitivity to the environmental factors or epigenetic reprogramming and its timing in insects. However some studies on honeybees help elucidate this phenomenon in insects. Changing the DNA methylation of honeybees during larval stage was found to elicit a phenotypic plasticity in the adults; silencing of methyltransferases dnmt3 (enzyme responsible for the de novo DNA methylation) by treatment with small interfering RNA of new hatched larva generated a high proportion of queens which have a well-developed ovaries whereas the control individuals gave workers with rudimentary ovaries (Kukcharski *et al.,* 2008). Similarly, feeding honeybee larva with a royalactin, a protein of the royal jelly, during larval stage can also induce a queen phenotype in honeybee and housefly adults, thus exhibiting an increased fecundity and longevity (Kamakura, 2001). All of these studies seem to suggest that the larval stage in insects might be vulnerable to change triggered by different environmental factors. In addition, the effect of treatment applied during the larval stage of insects can even persist in a few subsequent generations. For example, a change of the length of the antennae from a short phenotype to a long phenotype in the Mediterranean flour moth *Ephestia kuehniella* was observed when the fifth larval instar or pupae of this species was treated with a temperature at 25 C or lithium, and the change was inherited by the offspring and persist until the F4 generation (Pavelka and Koudelova, 2001). Similarly, treatment of the third instar larva of moth *Spodoptera exigua* with fenitrothion also caused a malformation of eggs in this species, and the effect was observed in three unexposed generations (Adamski *et al.,* 2005).

Reproduction is a key component of fitness of an organism, thus a study of effects of insecticides on reproduction is important. It was already known that treatment of some insect and mite species with insecticides could enhance their fecundity. For example, imidacloprid was found to enhance the fecundity of the spider mites and this effect was suggested to be mediated by

some change in the treated plants (Szczepaniec *et al.*, 2011). However, an elevated juvenile hormone (JH) was also reported in species of insect exhibiting an increased fecundity after treatment with imidacloprid during immature stage. For example, Yu *et al.* (2007) showed that *Chilo suppresalis* treated with imidacloprid since neonate exhibited an elevated JH and an increased fecundity of this species. Fecundity and JH titer of *Tryporyza incertulas* were also reported to increase when this pest was treated with imidacloprid since egg stage (Wang *et al.*, 2005). These results seem to suggest that the effect of imidacloprid might be mediated by a physiological change in the insect rather than in the treated host plants. The effect of low dose of imidacloprid on fecundity in insects seems to support this hypothesis. Indeed, several studies indicated that a low dose of imidacloprid applied during adult stage elicited an inhibitory effect on the reproduction in various insect species. For example, He *et al.*, (2012) found that low doses of imidacloprid reduced the fertility of the *B. tabaci* whitefly treated with this insecticide as adults. Boina *et al*. (2011) also reported a decrease in fecundity of the citrus psyllid, *Diaphorina citrius* treated with imidacloprid as adults. However, in studies on some other insects, a variability in results was noticed in the same species treated with the same insecticide depending on the stage treated. For example, Bao *et al.*, (2009) found a decrease in fecundity of brown planthopper, *Nilaparvata lugens* exposed to sublethal dose of imidacloprid applied as topical treatment during the adult stage. In contrast, Yin *et al.*, (2008) reported an increase in fecundity of females of the same species that grew on plant rice treated with imidacloprid since third-instar. This discrepancy in result was also observed with aphids *Myzus persicae.* For example, Devine *et al.*, (1996) showed that the number of nymphs produced by *M. persicae* treated with a low dose of imidacloprid as adults declined when comparing with these of the untreated aphids. In contrast, Ayyanhath *et al.* (2013) found an increase in fecundity of the same species of aphids

when the immature stages was treated. All these results across different species of insects seem to suggest that the increase of fecundity induced by low amount of imidacloprid happens only under specific conditions, which is when the treatment is applied during immature stage of the insects, and the inhibitory effect was observed when the treatment is carried out on the adult stage. However, Yu *et al.*, (2010) also demonstrated an increase in reproduction of *M. persicae* when the newly emerged adults of this species were treated with imidacloprid, thus even without treating the immature stage. Therefore, it might be that the effect of imidacloprid can also be inherited by the following generation; so that an increase in fecundity might be observed even the individual offspring was not exposed during larval stage as long as the parents was already exposed during their larval stage. A study on hormesis in fecundity, thus the study of effect of low dose, will be then an adequate approach to help elucidate this phenomenon.

VI Objectives

In order to develop a more complete picture of the effect of imidacloprid on the population of silver leaf whitefly repeatedly exposed to this compound, we initiated experiments to meet the following objectives:

1. Determine the rate of development and heritability of imidacloprid resistance in whiteflies heavily exposed to high doses of this compound.

2. Screen the response of other pesticides that are registered for control of whiteflies in commercial greenhouses to determine levels of cross resistance.

3. Determine whether or not a continuous exposure to a low amount of imidacloprid can induce hormesis in whitefly that might contribute to maintaining and promoting whitefly population.

CHAPTER II. Selection and heritability of imidacloprid resistance in *Bemisia tabaci* B biotype.

I Introduction.

The whitefly *Bemisia tabaci* (Gennadius) (Insecta: Hemiptera: Homoptera: Aleyrodidae) is an important pest of greenhouse plants, vegetables, legumes, grains and cotton, and may cause significant crop losses (Oliveria *et al*., 2001, De Barro, *et al.*, 2011). Chemical sprays are the primary method used for its control in various cropping system (Horowitz *et al*., 2011). After a series of control failures on many crops in the 1980's when organochlorines, pyrethroids, and organophosphate insecticides were heavily used, imidacloprid became the most widely used insecticide for whitefly control **(**Palumbo *et al.*, 2001). Due to extensive use of this insecticide, high levels of resistance to this insecticide were documented in many different countries, including Guatemala, Mexico, and Israel (Raunch and Nauen, 2003; Gorman *et al.*, 2010). In contrast to these results, and despite the reliance on imidacloprid for whitefly control on cotton in Arizona in the 1990s, no report of whitefly control failure was documented there or in Florida (Palumbo *et al*., 2001). However, selection of imidacloprid resistance in a laboratory in California suggested a potential development of resistance in the B biotype whitefly **(**Prabhaker *et al*., 1997). Moreover, in previous research where the level of imidacloprid resistance was monitored in field populations, susceptibility varied considerably over time. Schuster *et al*. (2010) reported reduced susceptibility to imidacloprid in some field populations collected in Florida in 2001, 2002 and 2003, followed by increased susceptibility in the populations of the same regions in 2004 and 2005. Dennehy *et al.* (2004) also found a high susceptibility of whitefly to imidacloprid on cotton in Arizona in 2000 and 2003 after reduced susceptibility was reported in previous years. This variation suggested an occurrence of reduction of susceptibility

to the imidacloprid in B biotype whitefly followed by a reversion of the insecticide resistance.

These observations from field suggest that several factors could influence the development and loss of imidacloprid resistance in field populations. Biological, ecological and operational factors in field production were suggested to modulate or accelerate insecticide resistance in whitefly and in arthropods in general (Castle *et al.*, 2010; Rosenheim and Tabashnik, 1992). However, determining the most important factors that can cause change in the susceptibility of natural populations of whitefly to insecticides is not easy due to the complexity of the environmental factors involved. Alternatively, selection for resistance in a laboratory is an effective method to study insecticide resistance to provide information such as the potential rate for the development of the resistance and stability of the resistance (Brown and Payne, 1988). Another advantage of selection experiments in the laboratory is that they enable researchers to estimate the heritability *(h^2)* of the resistance trait, thus the proportion of total phenotypic resistance variation that is associated with the additive genetic variation (Falconer, 1989), and to evaluate the risk of resistance to a pesticide (Tabashnik, 1992). Because populations of B biotype whitefly are known to vary considerably among different geographic regions and cropping systems (Tabashnik, 1992; De Barrow *et al*., 2011), it is also important for the greenhouse floriculture industry to determine the heritability of imidacloprid resistance from B biotype whitefly collected from a commercial greenhouse in the United States.

The initial objective of this study was to determine the rate of development of imidacloprid resistance in a population of B biotype whitefly collected from a commercial greenhouse in Michigan, by putting the population under imidacloprid selection pressure. Initially adults were used in the selection regime. However, cultures in large cages failed to thrive due to infestations of thrips and mites. This selection process was then replaced by a more controlled selection

approach using adults and immatures. The study enabled us to determine the heritability of resistance as a tool for understanding the risk of development of imidacloprid resistance in *B. tabaci* B biotype.

II Materials and methods.

II-1 Plants.

Cotton was used as a host plant for the whitefly culture and the experiments in this study. The variety ST5599B was used initially; and a second variety, FM958LL, was used after February 2009. The cotton seeds were provided by Cotton Incorporated, Cary, NC. Seeds were planted first in a 48-cell tray with standard potting medium soil in the laboratory or in the greenhouse with a 16:8 h (light/dark) photoperiod and grown until they reached the two true-leaf stage. After, the cotton seedlings were planted in a 6' pot with sterilized potting soil in the greenhouse where they were grown. The young cotton plants at the three to four true-leaf stage were introduced into the caged culture to maintain the whitefly population. Cotton seedlings at the two-true leaf stage were also used for the bioassays.

II-2 Insecticide.

The formulated product Merit 75 WP was used as the source of imidacloprid for our tests, the insecticide used was less than two years old. Tap water was used to dilute the formulation to the concentrations needed in this study no physicochemical of the water was used.

II-3 Insects.

II-3-1 Resistant strain.

We obtained a few poinsettia plants infested with silverleaf whitefly from a commercial greenhouse in Michigan in September 2006. The poinsettia plants were put in a cage at the MSU greenhouse. The whiteflies were allowed to grow on these plants. Cotton plants were put in the culture cage for the emerging adults to colonize. Colonies were then maintained on cotton plants from January 2007 to April 2007. Cotton plants were systemically treated with a soil drench of imidacloprid at the rate of 6 mg a.i. per 6' pot before placing them into the culture cage (Table 2.1). The resistant strain collected from poinsettia was reared and selected for resistance with increasing concentrations of imidacloprid from May 2007 until April 2008. This resistant strain is referred to as MI-R1. Details of the selection procedure are described below in Table 2.1 and Figure 2.1. Between April 2008 and March 2009 the culture became infested with mites and thrips and was nearly lost. In April 2009 the resistant culture was split into two resistant cultures. In one culture resistance was selected for from April 2009 to December 2009 with increasing concentrations of imidacloprid. This resistant strain is referred to as MI-R2. Details of the selection procedure are in Table 2.1 and Figure 2.2. A second resistant strain, referred to as MI-r2a, was maintained on cotton plants treated with a constant concentration of imidacloprid (12 mg a.i. per pot). This resistant strain MI- r2a has been maintained from May 2009 until the present time.

II-3-2 Reference strain.

We also maintained a population provided to us by Dr. Nilima Prabhaker, at the University of California Riverside, CA. The population was started from a few cotton leaves infested with whitefly pupae. The infested leaves were placed in a cage containing young cotton

plants, so that emerging adults would oviposit on them. This culture was maintained as a reference strain from April 2007 to January 2009. This reference strain is referred to as CA-s1. The reference population also became heavily infested with thrips and mites between September and December 2008, and failed to thrive. The culture was replaced in February 2009 by a second set of susceptible whitefly provided to us by Dr. Nilima Prabhaker and reared on a new variety of cotton plants (FM958LL). This second reference strain, referred to as CA-s2, was also started from a few cotton leaves infested with whitefly pupae. This reference strain was used from February 2009 until present time. All whiteflies were reared in double-walled walk-in screen-cage with double-doors in a section of the Plant Sciences greenhouses at Michigan State University. High intensity lamps are positioned above the cages to provide adequate lighting for the plants.

II-4 Selection.

II-4-1 History of selection and the resistant strain.

The resistant strain and reference strain of whitefly were identified as B biotype by Dr. Frank Byrne at the University of California, Riverside, CA. The timetable of the selection is summarized in Table 2.1. Two sequences of selection were made to build-up resistance levels. The first set of selections was made by exposing the resistant whitefly adults to imidacloprid using systemic uptake bioassay method, and moving survivors to new non-treated plants. The second selection procedure was conducted by exposing both adults and immatures to imidacloprid. Immatures were exposed by rearing resistant whitefly on plants systemically treated with imidacloprid. The emerged adults were collected and treated with successively higher rates of imidacloprid using the systemic uptake bioassay, and moving survivors to new

plants that were systemically treated with imidacloprid.

II-4-1-a) Exposure of adult whitefly.

The first set of selections was made by exposing whitefly adults (MI-R1) from May 2007 to April 2008 to increasing concentrations of imidacloprid (Figure 2.1, Table 2.1). Whitefly adults were selected for imidacloprid resistance by using a systemic uptake bioassay as described by Cahill *et al.* (1996) and Li *et al.* (2000) with a slight modification. Cotton seedlings at the two true-leaf stage were cut at the main stem, and the stems immersed in a 20ml-glass vial containing imidacloprid (Merit 75 WP) at different selecting concentrations (from 30 to 500 mg/L) (Table 2. 1) for 24 h. After 24 h of imidacloprid uptake by the cotton seedlings, leaf discs were cut and put in scintillation vials containing 2 ml of agar (1.3%) in the bottom of the vial. The leaf was put upside down on the agar in the bottom of the vial so that the underside of the leaf faces up. Fifteen to thirty six seedlings were used for each selection. Whitefly adults from the resistant strain MI-R1 were collected into vials containing imidacloprid-treated leaf discs; the collection was conducted during the adult emergence peak. For the collection, the mouth of the vial was put onto the cotton leaf in a way that surrounds the adults to be collected, and then the cotton leaf was shaken so that the whiteflies flew into the vial. Then the vial was capped with parafilm which was punctured several times with an insect pin to allow some aeration. Fifty to 126 vials were used for each selection and each vial contained 30 to 224 whitefly adults, the total of number of adults collected and then treated was shown in Table 2.2 for each selection. The adults were then placed on treated leaf discs for 48h at room temperature, after which the surviving adults were collected and released on 18-30 new non-treated cotton plants in a clean cage. Eggs produced by these females were allowed to grow until adulthood, and a new selection was made

Table 2.1. History of selections for the resistant whitefly culture.

	Date	Generation	Selecting concentration for adult (mg/L)	Soil drench concentration (mg a.i./6"pot)
	From September 2006 to April 2007	Population build up	-	6 (one treatment per month)
MI-R1	8 May 2007	Parental P	30	-
	4 June 2007	F1	30	-
	1 July 2007	F2	100	-
	2 August 2007	F3	200	-
	26 August 2007	F4	200	-
	27 September 2007	F5	350	-
	30 October 2007	F6	500	-
	17 December 2007	F7	500	-
	17 January 2008	F8	500	-
	18 February 2008	F9	500	-
	19 March 2008	F10	500	-
	23 April 2008	F11	500	-
	From May 2008 to August 2008			6 (one treatment per month)
	From September 2008 to March 2009	Population near extinction		
MI-R2	April 2009	Parental P'	100	6
	May 2009	F'1	no adult selection	12
	June 2009	F'2	no adult selection	12
	July 2009	F'3	no adult selection	12
	August 2009	F'4	100	-
	September 2009	F'5	150	12
	October 2009	F'6	250	12
	November 2009	F'7	400	12
	December 2009	F'8	500	12

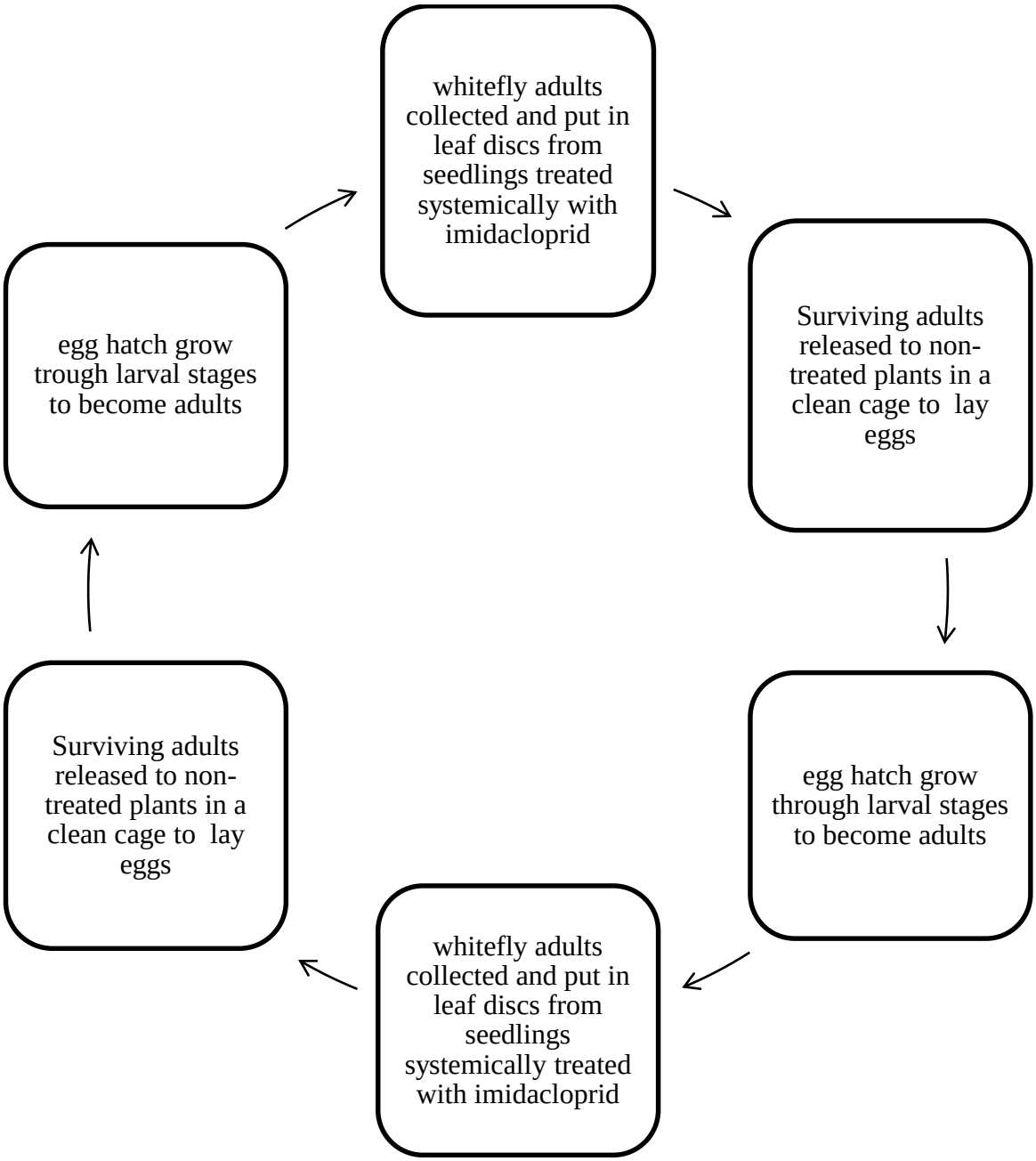

Figure 2.1. Selection of adults of the resistant strain MI-R1 from May 2007 until April 2008 using a systemic uptake bioassay

(Figure 2.1). The selection procedure was repeated for 11 generations of the cultured whitefly from May 2007 to April 2008.

The selection process was interrupted from May 2008 to March 2009 as the population was heavily infested by mites and thrips, and the resistant culture was nearly lost. From May to August 2008 the culture was maintained on cotton plants treated with imidacloprid at half of the label rate (6 mg a.i. per pot), and from September 2008 to March 2009 the culture was maintained on non-treated plants to allow the population to recover. Because there was an interruption of selection during this period, the reversion of resistance was documented during that time period.

II-4-1-b) Exposure of both adults and immature whiteflies.

A new variety of cotton (FM958LL) was used after the first set of selections. This second selection procedure was conducted on the resistant strain MI-R2 from April 2009 to December 2009. Both adults and immatures of MI-R2 were exposed to imidacloprid (Figure. 2.2, Table 2.1). Whitefly adults were selected by using the same systemic uptake bioassays as described above. Adults of the resistant strain MI-R2 were collected into 20 ml- scintillation vials and exposed for 48 h to the leaf discs systemically treated with imidacloprid at different selecting concentrations (100, 100, 150, 250, 400, 500 mg/ L) for 24 h (Table 2.1, 2.3). The collection was performed when adult emergence reached a generation peak. Thirty two to 66 vials were used for the selection and each vial contained 36 to 127 adults. The total numbers of collected adults were shown in Table 2.3. Meanwhile, 12-18 new young cotton plants were treated with the selecting concentration of imidacloprid (6 and 12 mg a.i per pot). Imidacloprid was applied as a soil drench by pouring the solution of imidacloprid into the soil around the base of the plants. After the collection of the emerged adults, the cotton plants were removed from the cage and the new

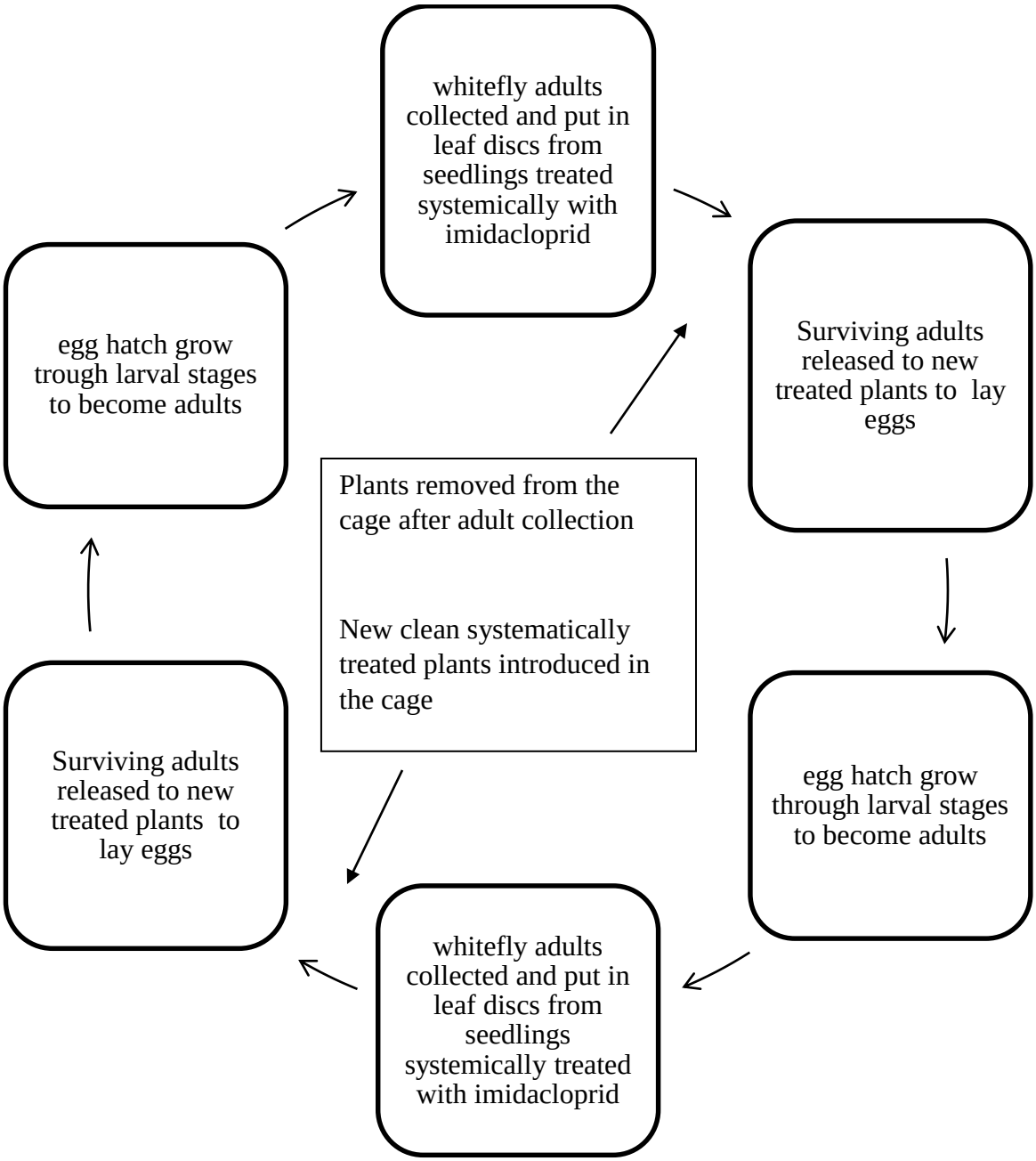

Figure 2.2. Selection of the resistant strain MI-R2 from April 2009 to December 2009 using systemic uptake bioassay and soil drench treatment

treated young cotton plants were introduced in the same cage. The surviving adults after the 48 h exposure to imidacloprid were released on these new treated plants to lay eggs and this new generation was allowed to become adults for a new selection. This selection procedure was repeated for 8 generations of this resistant strain from April 2009 until December 2009, the population was lost afterwards . Also, the number of surviving adults of F'4 after the adult selection was very low; therefore the host cotton plants were not treated in order to obtain more emerged individuals in the next generation.

II-5 Exposure of the resistant strain MI-r2a to soil-treated plants.

A second resistant strain, referred to as MI-r2a, has been reared from May 2009 until the present time and studied at the same time with the selected resistant strain MI-R2. However as opposed to the first resistant strain (MI-R2), this population (MI-r2a) was not selected for resistance with increasing concentrations of imidacloprid but was maintained on cotton plants treated with imidacloprid at 12 mg a.i. per pot. This second resistant strain MI-r2a was initiated from eggs deposited on cotton plants by the new parental generation of the first resistant strain MI-R2 recovered in April 2009. These eggs and few remaining resistant adults were allowed to grow on these plants. Clean cotton plants treated with imidacloprid at 12 mg a.i per pot were introduced monthly in the same cage to maintain the population. The imidacloprid was applied as soil drench by pouring the solution of imidacloprid at the base of the plants. This rate was adopted with this product in this study as it is the label rate recommended for whitefly control with the imidacloprid Marathon 60WP. Cotton plants were only removed from the cage when they are died (Figure 2.3). For this resistant strain M-r2a, generations of whitefly were not separated and individuals of all ages could be found at any time, thus no exact number of

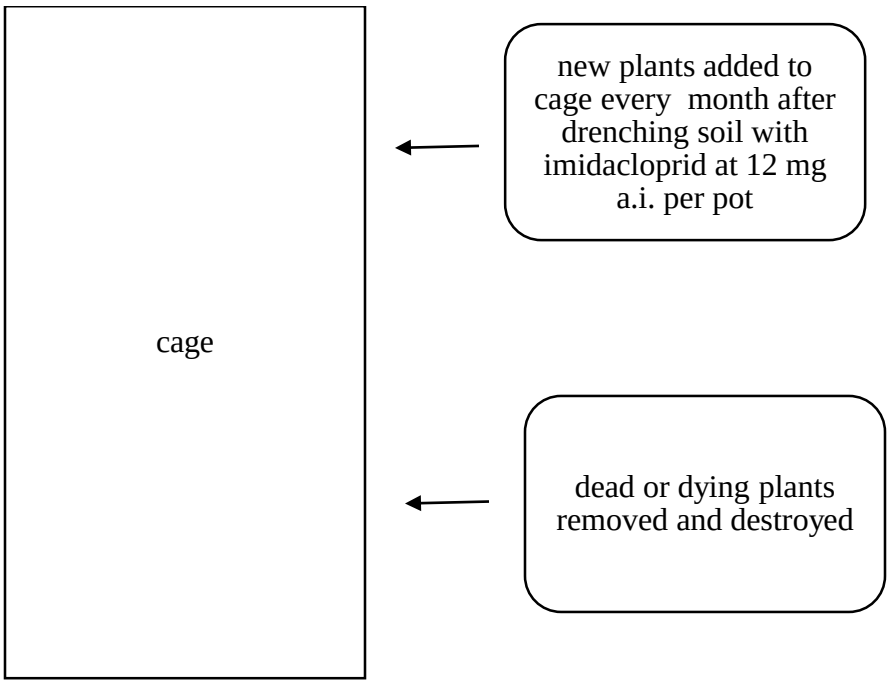

Figure 2.3. Maintenance of the resistant whitefly MI-r2a on plants treated with imidacloprid applied as soil drench from May 2009 until present time. This resistant strain MI-r2a was used for the insecticide efficacy test and the study on sublethal effects of imidacloprid on whitefly.

generations could be determined in such a continuous culture.

II-6 Bioassay for determining LC_{50}.

The susceptibility of the whitefly (resistant strain and reference strain) to imidacloprid was evaluated by using the systemic uptake bioassay method as described above. Cotton seedlings at the two true-leaf stage were cut at the main stem, and the stems were immersed in 20 ml of imidacloprid (Merit 75 WP) at different concentrations for 24 h. Six to ten different concentrations of imidacloprid giving more than 0% and less than 100% mortality were used for each test, and three to four cotton seedlings per concentration were used. Whitefly adults from the resistant strains were collected into the vials as described above. Three to four replicates were used for each concentration and each replicate contained 30-80 individuals. Mortality was checked after the 48h- exposure. The same systemic uptake bioassay was also conducted on the reference strain.

II-7 Data analysis.

II-7-1 Estimation of resistance ratio RR.

Data from the resistance bioassay were used to determine the slope and LC_{50} (SAS version 9.1 software) for the reference strain and the resistant strain. The resistance ratio was determined as the LC_{50} of the resistant strain divided by the LC_{50} of the reference strain. The LC_{50} for the reference strain for the first regime of selection was the average of the two LC_{50} determined during this selection procedure. The LC_{50} for the reference strain for the second regime of selection was also the average of the two new LC_{50} determined during this selection

procedure.

II-7-2 Estimation of realized heritability (h^2).

The realized heritability (h^2) for this resistant strain of *Bemisia tabaci* B biotype was estimated based on the method used by Tabashnik (1992) according to the following formula:

$$h^2 = R/S$$

where R is the response to selection (Falconer, 1989) which is estimated as :

$$R = [\log (\text{final } LC_{50}) - \log (\text{initial } LC_{50})] \times 1/n$$

In this formula final LC_{50} is the LC_{50} of offspring after n generations of selection, and initial LC_{50} is the LC_{50} of the parental generation before n generations of selection. Returning to the original formula, S is the selection differential (Falconer, 1989) which is estimated as:

$$S = i\sigma_p$$

where *i* is the intensity of selection estimated from p, the percentage of population with values above the selection threshold (percentage surviving selection) using Appendix A of Falconer (1989). The phenotypic standard deviation (σ_p) is estimated as:

$$\sigma_p = [1/2(\text{initial slope} + \text{final slope})]^{-1}$$

To determine a change of the rate of resistance development during the course of selection, the heritability of first half (F0 to F5) and second half (F5 to F11) of the selection regime by treating adults of the resistant strain MI-R1 were determined (Tabashnik, 1992). The heritability in the

second set of selection by treating adults and immatures of the resistant strain MI-R2 was not estimated as no adult selection (thus no mortality data) could be carried out in a few generations (F'1, F'2, and F'3) during the selection due to the low number of emerged adults.

The number of generations G required for a 10-fold increase in LC_{50} is also estimated. For that, the response to selection R was estimated with the mean slope obtained from F0 to F11 by using the following formula:

$$R = h^2 S$$

Then, the prediction of number of generation required for an increase of LC_{50} was estimated according to the Tabashnik' s formula (1992) :

$$G = R^{-1} = (h^2 S)^{-1}$$

III Results.

The population of silverleaf whitefly (B biotype) MI-R1 collected from a commercial greenhouse in Michigan showed a reduced susceptibility to imidacloprid (LC_{50} = 23.42 compared to a LC_{50} of 1.97 for our reference strain CA-s1 (Table 2.2), indicating a development of resistance to imidacloprid under greenhouse conditions. When adults of this population were exposed to an increasing concentration of imidacloprid, the LC_{50} level of resistance increased from 23.42 mg /L in the parental generation to 284.47 mg /L in the F5 generation, and to 431.08 mg /L in the F11 generation (Table 2.2). However, the LC_{50} of the untreated reference strain

Table 2.2. Summary of the selection for imidacloprid resistance in the resistant strain MI-R1 by exposing the whitefly adults to imidacloprid.

Strain	Generation	Selecting dose (ppm)	No. adults treated	% Mortality	Slope ± SE	LC50 (ppm)	95% FL[a]	RR[b]
CA-s1	RF[c]	-	-	-	1.03 ± 0.16	1.77	0.80-3.11	-
MI-R1	Parental	30	7471	63	1.15 ± 0.10	23.42	16.68-33.51	11
MI-R1	F1	30	7981	50	nd[d]	nd	nd	nd
MI-R1	F2	100	11244	57	0.80 ± 0.06	57.87	38.20-85.22	29
MI-R1	F3	200	21756	87	nd	nd	nd	nd
MI-R1	F4	200	5820	62	nd	nd	nd	nd
MI-R1	F5	350	7179	57	0.71 ± 0.07	284.87	197.62- 418.50	144
MI-R1	F6	500	5288	45	nd	nd	nd	nd
CA-s1	RF	-	-	-	0.60 ± 0.08	2.18	1.16-3.87	-
MI-R1	F7	500	4885	47	nd	nd	nd	nd
MI-R1	F8	500	3975	46	1.22 ± 0.20	411.07	240.97-786.11	208
MI-R1	F9	500	5966	46	nd	nd	nd	nd
MI-R1	F10	500	8343	47	nd	nd	nd	nd
MI-R1	F11	500	10328	51	0.62 ± 0.04	431.08	274.26-692.53	218

a) FL: fiducial limits

b) RR: resistance ratio =LC_{50} of resistant strain / LC_{50} of reference strain (where LC_{50} of reference strain is the average of the two LC_{50} values of RF (1.97)).

c) RF: reference strain CA-s1

d) nd: not conducted

CA-s1 remained similar to the parental population when checked after six months (LC_{50} from 1.77 to 2.18 mg /L) (Table 2.2, Figure A1). When compared with the average LC_{50} (1.97 mg /L) of our reference strain CA-s1, the level of resistance increased from 11-fold in the parental generation to 29-fold in the F3 generation, 144-fold in the F5 generation, and to 218-fold in the F11 generation (Table 2.2).After the F11 generation, the selection was interrupted for one year due to a heavy infestation of the culture by thrips and mites. The culture was not exposed to imidacloprid for 7 months to allow the population to recover. After this 7 month period, the average LC_{50} of the new reference strain C-s2 was 4.16 mg/L whereas the LC_{50} of the resistant strain MI-R2 was 48.48 mg/L (Table 2.3). This indicated a resistance ratio (LC_{50} resistant/LC_{50} reference strain) of 11- fold. This suggests that resistance can be significantly reduced (from 218–fold to 11-fold) in a short period of time (7 months) when selection pressure is not applied, but not lost. Apparently imidacloprid resistance is not very stable in *B. tabaci* B biotype.

After this time, when selection was initiated again by exposing both immatures and adults of MI-R2, the LC_{50} increased slightly from 48.48mg /L to 58.1 mg/L in F'3 generation, then to 193.75 mg/L in the F7 generation (Table 2.3, Figure A2). During the same period of time the LC_{50} reference strain CA-s2 remained stable (4.12 and mg/L and 4.21 mg/L) (Table 2.3).

Comparing the LC_{50} of the resistant strain MI-R2 to the reference strain CA-s2, the resistance ratio of the resistant strain MI-R2 increased from 11-fold in the first generation after the 7-month hiatus in selection to 14-fold in the F3 generation, and to 46-fold in the F7 generation.

The response to imidacloprid exposure of resistant strain MI-r2a that has been reared continuously on plants treated with imidacloprid at 12 mg a.i. per pot was shown in Table 2.4.

Table 2.3. Summary of the selection for imidacloprid resistance in the resistance strain MI-R2 by exposing whitefly immatures and adults to imidacloprid.

Strain	Generation	Soil - applied dose a.i.(mg)	Adult-selecting dose (ppm)	No. adults treated	% Mortality	Slope ± SE	LC_{50} (ppm)	95% FL[a]	RR[b]
CA-s2	RF[c]	-	-	-	-	1.15 ± 0.16	4.12	1.58-11.87	-
MI-R2	P’	6	100	5187	63	0.94 ±0.20	48.48	8.90-386.88	11
MI-R2	F’1	12	nd[d]	nd	nd	nd	nd	nd	nd
MI-R2	F’2	12	nd	nd	nd	nd	nd	nd	nd
MI-R2	F’3	12	nd	nd	nd	1.01 ± 0.13	58.1	28.55-111.87	14
CA-s2	RF	-	-	-	-	0.96 ± 0.11	4.21	2.13 - 7.92	
MI-R2	F’4	-	100	2851	74	nd	nd	nd	nd
MI-R2	F’5	12	150	2027	42	nd	nd	nd	nd
MI-R2	F’6	12	250	2552	53	nd	nd	nd	nd
MI-R2	F’7	12	400	3113	62	0.82 ± 0.10	193.75	112.39- 87.25	46
MI-R2	F’8	12	500	4421	62	nd	nd	nd	nd

a) FL: fiducial limits

b) RR: resistance ratio =LC_{50} of resistant strain / LC_{50} of reference strain (where LC_{50} of reference strain is the average of the two LC_{50} values of RF (4.16).

c) RF: reference strain CA-s2

d) nd: not conducted

Table 2.4. Resistance level of the resistant strain MI-r2a reared continuously on plants treated with imidacloprid at a recommended dose (12 mg a.i. per pot) for whitefly control.

Date	Strain	Number of adults	Slope ± SE	LC_{50} (ppm)	95% FL[a]	RR[b]
July 2009	Reference CA-s2	632	0.96 ± 0.11	4.21	2.13 - 7.92	
	Resistant MI-r2a	563	0.95 ± 0.07	30.54	22.62 - 40.39	7
June 2010	Reference CA-s2	834	1.09 ± 0.06	4.08	3.31 - 5.02	
	Resistant MI-r2a	783	1.03 ± 0.01	37.47	23.62 - 56.12	9
April 2011	Reference CA-s2	841	1.28 ± 0.15	6.17	3.90 - 10.63	
	Resistant MI-r2a	944	1.18 ± 0.07	59.41	49.84 - 70.74	9

a) FL: fiducial limits

b) RR resistance ratio (LC_{50} of resistant strain / LC_{50} of reference strain)

The resistance level in this resistant strain MI-r2a was relatively stable over two years (Table 2.4). The resistance ratio RR was slightly increased from 7-fold on July 2009 to 9-fold two years later, indicating that the level of resistance increase much slower with this type of imidacloprid pressure.

The overall mean estimate of realized heritability for imidacloprid resistant in this B biotype whitefly population was 0.10, with a mean response of 0.10 and a mean selection differential (S) of 0.99 (Table 2.5). This suggests that phenotypic variation for imidacloprid resistance in this population of B biotype whitefly was accounted for by an additive genetic variation. The realized heritability in the first half of the selection was higher (0.16) comparing with the second half of the selection (0.02) (Table 2.5). This suggests that the additive genetic variance declines over the course of selection. The projected rate of resistance evolution is proportional to the value of heritability h^2 and the selection intensity. For example, a 10-fold increase of LC_{50} would be obtained in 3 generations with an intensity of selection causing 90 % mortality and h^2 of 0.16 whereas the same rate of increase would require 10 generations with an intensity of selection causing 50% mortality and with a h^2 of 0.10 (Figure 2.4).

IV Discussion.

The population of whitefly (*B. tabaci* B biotype) collected from a commercial greenhouse in Michigan showed a reduced susceptibility to imidacloprid compared to our reference strain, indicating that resistance to imidacloprid developed under commercial greenhouse conditions. By putting the population under selection pressure, the resistance level increase considerably. Prabakher *et al.* (1997) also demonstrated development of imidacloprid resistance in a B biotype

whitefly population initially collected from the field in California and put under selection pressure in a laboratory. Similarly, Wang *et al.* (2009) reported a high level of resistance in a B biotype whitefly collected from a greenhouse in China after selection for imidacloprid resistance for 30 generations in the laboratory. All of these results suggest that *B. tabaci* B biotype have a potential to develop imidacloprid resistance when exposed to selection pressure.

After a 7-month hiatus from imidacloprid treatments, the resistance ratio RR of our imidacloprid-resistant strain decreased 95%. This drop could be explained by the loss of resistant alleles and an increase in susceptible alleles during this time. The rapid decline of resistance levels suggests that imidacloprid resistance is not stable in this strain B biotype whitefly. Other authors also reported the instability of imidacloprid resistance in *B. tabaci* B biotype. For example, Schuster *et al*. (2010) reported a reduction by 80% of imidacloprid resistance in a lab population of B biotype whitefly that was not exposed to the insecticide for four to six generations. Gutierrez-Olivares *et al*. (2007) also reported a significant decrease of resistance level in the B biotype whitefly without exposure to imidacloprid. Raunch and Nauen (2003) reported a significant drop of the resistance level of the population of B biotype whitefly from 870-fold to 361-fold within six months. These relatively fast declines in resistance in unexposed B biotype whitefly is in contrast to what has been observed for the Q biotype whitefly where an elevated level of resistance is maintained even without exposure to imidacloprid for 2 years (Nauen *et al.*, 2002). Theoretical models indicate that the stability of insecticide resistance depends on different factors including the level of the allele frequency in the population, the size of untreated refuges and the fitness cost associated with the resistance (Carriere and Tabashnik, 2001). For example, Wilson *et al.* (2007) demonstrated that a reduction of pyriproxyfen resistance in Q biotype whitefly was caused by the fitness cost associated with the resistance.

Whatever is the cause of decline, this instability of the imidacloprid resistance associated with this temporal refuge when there was no exposure might partly contribute along with other factors to the considerable variation of susceptibility of field populations of B biotype whitefly to imidacloprid.

When imidacloprid treatments were initiated again, and a new regime of selection was performed, the resistance ratio (RR) of our resistant strain MI-R2 increased again from 11-fold in the parental generation P' to 46-fold in the F'7 generation. This suggests that resistant alleles were still present in the population despite the temporal refuge without exposure, and the population could still respond to the new selection. Wilson *et al.* (2007) demonstrated that the pyriproxyfen resistance in Q biotype whitefly was also found to be unstable but was not lost completely even without the insecticide exposure, and the simulation study projected that the pyriproxyfen resistant allele would remain in the unexposed Q biotype population for 55 generations, thus for 5 years (Crowder *et al*., 2005).

The size of the resistant population used in the first set of selections of MI-R1 (May 2007 to April 2008) was much larger than the population used in the second set of selections of MI-R2 (April 2009 to December 2009, Tables 2.2, 2.3). The low number of emerged adults prevented us from performing a selection in the first three generations, F'1, F'2 and F'3 (April 2009 to June 2009), of the resistant strain MI-R2 (Table 2.3). The small size of the population may be due to prolonged exposure of sessile immatures, which are also more susceptible to imidacloprid than the adults. Indeed, the resistance in the current study is likely associated with the P450 detoxification system (Chapter 3 Table 3.1). Nauen *et al.* (2008) demonstrated that the expression of the P450 gene depends on the age in whitefly B and Q biotypes, and these

authors stated that the level of expression in the immature stage was so low that whitefly was vulnerable at this early stage. Thus, high mortality in the immature was not surprising.

The second resistant strain, MI-r2a, has been maintained on plants treated with imidacloprid at 12 mg a.i. per pot, a label rate for whitefly control when using imidacloprid Marathon 60WP. Generations overlapped in this culture as plants were only removed when they died. Resistant levels remained relatively stable for two years (Table 2.4). This suggests that the regular use of imidacloprid as a soil drench will maintain resistance in the population but that the level of resistance increases slowly when compared with the resistant strain MI-R2 selected with an increasing concentration of imidacloprid (Table 2.3, 2.4).

The study of realized heritability is commonly used to summarize the results of selection for insecticide resistance (Tabashnik, 1992). Rosenheim (1991) did a thorough analysis of different factors that were not controlled in the current study and that may cause an underestimation or overestimation of the realized heritability value. He reported that the h^2 estimate was underestimated if there is a differential uptake of the toxicants between all individuals in the population studied, especially between the more tolerant and less tolerant individuals. A difference of tolerance to the toxicant between male and female could also induce an underestimation of the realized heritably, especially if the selection is carried out in adults that have been already mated (Rosenheim, 1991; Bloch and Wool, 1994). No investigation of the effect of imidacloprid on male and females were performed in this study. Conversely, an overestimation of realized heritability can occur if the sublethal effect of the toxicant induces difference in the reproduction of the surviving individuals with less fecundity in the less tolerant individuals. Thus, the interpretation of the realized heritability h^2 estimate needs caution due to

these biases and limits that could be associated with the different factors mentioned above (Bloch and Wool, 1994).

The overall realized heritability h^2 estimate in this study was 0.10. Heritability of a population can change over time, and the heritability value during the time when the most significant change in resistance occurs was suggested to represent better the heritability of the population (Huang *et al*., 1999). Thus by using the heritability in the first half of the selection, the heritability estimate in this population was 0.16 under the conditions used in this study, this estimate was similar to that found by Sethi *et al.* (2008) with a different population in an independent study (h^2 = 0.16). The phenotypic variation (VP) of a threshold trait such as insecticide resistance is a combination of the additive genetic variation (VA) and environmental variation (VE) (Falconer1989, Firko and Hayes, 1990). And heritability (in a narrow sense) is defined as the proportion of the total phenotypic variation attributable to the additive genetic variation (Falconer, 1989; Tabashnik, 1992). Thus, the heritability analysis in this study indicated that 10% of the total imidacloprid tolerance variation in this population of B biotype whitefly is accounted for by an additive genetic variation.

An analysis of h^2over a course of selection was also performed. Additive genetic variation, thus the heritability, has a maximal value when the allele of the trait of interest is at intermediate frequency and is zero when the alleles of interest is absent or fixed in the population (Falconer, 1989). Similar to the result found by Sethi *et al.* (2008) who also selected for imidacloprid resistance in B biotype whitefly, the h^2 estimates in the current study declined from the first half to the second half of the first set of selections, suggesting a decrease in additive genetic variation, thus an increase of the resistant alleles as the selection proceeds.

Table 2.5. Estimation of realized heritability of resistance to imidacloprid in *Bemisia tabaci* B biotype collected from a commercial greenhouse in Michigan.

Generation	Estimation of response to selection			Estimation of selection differential				h^{2e}
	Initial LC_{50} (Log)	Final LC_{50} (Log)	R[a]	i[b]	Mean Slope	σp[c]	S[d]	
F0-F5	1.369	2.454	0.180	1.020	0.930	1.075	1.096	0.164
F5-F11	2.454	2.634	0.025	0.766	0.665	1.503	1.152	0.022
F0-F11	1.369	2.634	0.105	0.880	0.885	1.129	0.994	0.106

a) R: Response to selection

b) i: intensity of selection

c) σp: phenotypic standard deviation

d) S: selection differential

e) h^2: realized heritability estimate

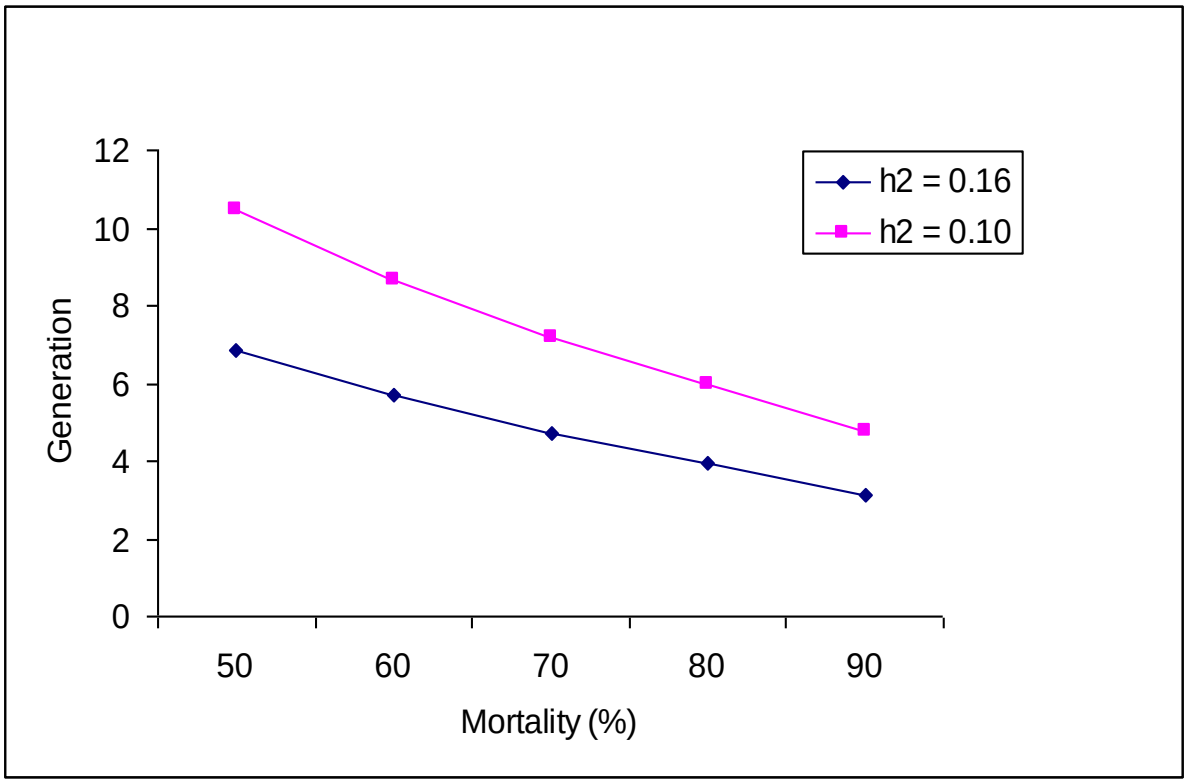

Figure 2.4. Effect of heritability (h^2) on number of generations of *Bemisia tabaci* B biotype required for a 10-fold increase in LC_{50} of imidacloprid at different selection intensities.

**** For interpretation of references to color in this and other figures, the reader is referred to electronic version of this dissertation.*

The main purpose of heritability study is to be able to forecast the rate of resistance development, thus to assess the risk of resistance to an insecticide (Tabashnik, 1992). The projected rate of the resistance development in this study predicted that a 10-fold increase of LC_{50} would be reached in 7 generations with a heritability h^2 of 0.16 and a mortality of 50% per generation (Figure 2.4). Interestingly, the result of selection in laboratory carried out by Prabhaker *et al.* (1997) in California falls within this range. These authors selected for imidacloprid resistance in B biotype whitefly by using 40 to 50% mortality per generation and an increase of 10-fold of LC_{50} was observed between 5 and 7 generations of selection. Our results also predicted that as few as 3 generations may be needed to develop a 10-fold increase of LC_{50} under higher selection intensity (90% mortality per generation) and a heritability h^2 of 0.16 (Figure 2.4). This high mortality of 90% per generation was used by Wang *et al.* (2009) for imidacloprid resistance selection in B biotype whitefly collected from cotton greenhouse in China. Wang *et al.* (2009) did not show all of their results; however they reported a 254-fold increase in LC_{50} after fifteen generations of selection. Thus, the results in this study seem to be in agreement with those found from other independent laboratory experiments for imidacloprid selection on B biotype whitefly. The laboratory results suggest that B biotype whitefly has a potential to develop imidacloprid resistance and the resistance could be detected after a few generations depending of the intensity of selection.

Despite this consistency of results among laboratory experiments, it may not necessarily reflect the situation in field as the value of heritability h^2 is specific to one particular population under the specific environmental conditions to which they are subjected (Falconer, 1989;

Tabashnik and McGaughey, 1994). Moreover, heritability in laboratory is likely to have less environmental variation than in the field (Bull *et al.*, 1982). In addition, the h^2 estimate may have limitations and biases as explained previously; therefore caution should be used when using it as a reference for field populations.

Consistent with many other reports studying quantitative genetic of insecticide resistance in different pests (Lai and Su, 2011; Tabashnik, 1992), the current study found that the response to selection, thus the rate of development of resistance in this whitefly population, depends on the heritability of resistance and the intensity of selection (Figure 2.4). From this viewpoint, assuming field population having even lower heritability found in the present study, the whitefly population showing high level of resistance in field might have been experienced a very high intensity of selection for several reasons such as the cropping system and pest management practice. That would be probably the case for the whitefly population collected from the greenhouse in Israel reported by Nauen *et al.* (2008).

The low realized heritability (0.10) observed in this study also suggests an importance of the environmental variation in the imidacloprid tolerance phenotype variation in this population. It was emphasized that the heritability parameter does not provide information on the exact magnitude of the contribution of the environmental components and the genetic components in the trait of interest; however environment can have some influence on the expression of the phenotype (Visscher *et al*., 2008). Consistent with the low heritability value in the current study, several studies have demonstrated previously that different environmental factors could influence the susceptibility of whitefly to imidacloprid. For example, Castle *et al.* (2010) reported that the quality of the host plant could influence the expression of imidacloprid resistance in B biotype whitefly. These authors showed that the population of *B. tabaci* collected from cotton plants in

Arizona at the end of the season, thus when the host plant cottons were already in a low quality, was found to be susceptible to imidacloprid; however a resistance to this insecticide was revealed when the same population was reared on untreated cotton plants with a higher quality in the laboratory. Huang *et al.* (2011) also reported that the susceptibility of B biotype whitefly to imidacloprid increased with the temperature. Several studies also demonstrated that the susceptibility of B biotype whitefly to several insecticides varies depending on the host plants on which they were reared (Castle *et al.*, 2009, Liang *et al.*, 2006; Xie *et al*., 2010). A wide variability of the level of imidacloprid resistance depending on the location where the whitefly was collected was already reported previously. For example, Vasillou *et al.* (2011) reported some resistance level varying from moderate to high in B biotype whitefly populations collected from different regions in Cyprus, however the authors did not found a significant correlation between the history of neonicotinoid application and the resistance level. This is in contrast to the results found on Q biotype whitefly as demonstrated by Roditakis *et al.* (2008) where the variability of the level of resistance in Q biotype populations collected from different locations in Crete was well correlated with the number of imidacloprid applications. Besides the identified environmental factors, some source of variations that have effect on the phenotypic variation are unobserved. Recently, increasing number of studies working on heritability of quantitative traits suggested that epigenetics might be the mechanism behind the observed or non-observed environmental variation and genetic variation, thus on the heritability of the trait (Feninberg and Irizarry, 2010, Petronis *et al.*, 2010; Wong *et al*., 2005). All of these studies suggested that many other factors might influence the evolution of resistance to insecticides in field population. Therefore, in concurrence with several previous reports, this study suggest to take into account

the environmental situation in field, along with the results generated from laboratory, when assessing and managing the risk of pesticide resistance in B biotype whitefly .

V Conclusion.

The study of the realized heritability suggests that the imidacloprid resistance was genetically based and can be inherited by the upcoming generations. The B biotype whitefly can develop a high level of resistance to imidacloprid with an intensive use of this compound. This could explain the observation of high resistance levels in some field populations. Similar to the results found of a previous study, the realized heritability was found to be relatively low suggesting that a small proportion of imidacloprid tolerance variation only was attributable to the genetic variation in this population under the conditions used in the current study. This might also explain partially the wide variability of the susceptibility of resistance in different fields. However, due to different limitations and biases that can be associated with the realized heritability estimate; this explanation remains only suggestive but not conclusive. Another important characteristic of resistance is its stability after an arrest of the insecticide exposure. Consistent with other previous findings, this study showed that the imidacloprid resistance was not stable in B biotype whitefly as it was significantly reduced within a few months without imidacloprid exposure. This instability of the resistance might also play an important role to moderate the development of resistance. That could be another additional cause of the observed variability of imidacloprid susceptibility in field population over time. However, despite the increase in susceptibility after a temporal refuge, the resistance was not completely lost and the population was able to respond with a new set of selections after seven months. Therefore, it would be advisable to give enough time for the population to regain susceptibility before using

imidacloprid again. In brief, this experiment in laboratory suggests that in addition to the ecological, operational factors which are already known to influence the evolution of resistance in field, some other factor such as heritability, instability of resistance can influence evolution of resistance. Thus, a combination of results from both the laboratory and field assessment would be the better strategy to manage the imidacloprid resistance in field population.

CHAPTER III. Response of imidacloprid-resistant whitefly B biotype to pesticides used for whitefly control.

I Introduction.

Bemisia tabaci is a complex species of whitefly that comprises up to 20 biotypes (Perring *et al*., 2001). Among them, biotype B and biotype Q are the most invasive and most damaging. These two biotypes vary in their biology such as fecundity, host range, efficiency to virus transmission (DeBarro *et al.*, 2006, Gottlieb *et al.*, 2010) and also insecticide resistance where Q biotype is prone to develop higher resistance levels than B biotype (Horowitz *et al.*, 2005, Kontsedalov *et al*., 2012). *Bemisia tabaci* B biotype was first found to cause economic damage on poinsettia in Florida in 1986, followed by reports from Arizona, California and other states (McAuslane, 2010). The impact of whitefly feeding, virus transmission and sooty mold on cultivated plants has caused enormous annual crop losses worldwide (Oliveria *et al*., 2001). As a result of widespread use of chemicals for its control, whitefly has become resistant to many conventional insecticides including many organophosphates and pyrethroids (Cahill *et al.*, 1995; Dennehy and William, 1997). In the early 1990s, imidacloprid, belonging to a different class of insecticide called the neonicotinoids, was introduced to growers as one option of chemicals to control whitefly (Nauen and Denholm, 2005; Horowitz *et al.*, 2011). Imidacloprid acts on the central nerve system as an agonist of the nicotinic acetylcholine receptors (nAChR) in insects (Liu and Casida, 1993). Because imidacloprid had a mode of action that was different from insecticides being used at the time, it gave excellent control of resistant populations of whitefly (Rauch and Nauen, 2002). Imidacloprid is very effective in controlling sucking insects due to its systemic properties (Tomizawa and Casida, 2003). Within a few years imidacloprid became the standard product for control of whitefly in North American greenhouses, and by 2004 resistance

to imidacloprid had been well documented in the Q biotype of *Bemisia tabaci*, which originated from overseas. Between 1995 and 2011 several additional neonicotinoid products also became available to greenhouse growers, including thiamethoxam, acetamiprid, and dinotefuran. Since these insecticides are in the same chemical class and share a common target sites as imidacloprid (Honda *et al.*, 2006), entomologists working with the greenhouse industry were wary of cross-resistance problems. Most of the research on whitefly resistance to imidacloprid was done with the *Bemisia tabaci* Q biotype (Nauen and Denholm, 2005*),* and recommendations for growers to use insecticides in other chemical classes became widespread, despite the fact that most growers had problems with the B biotype, not the Q biotype (Byrne *et al.*, 2010).

Research projects focusing on whitefly resistance to imidacloprid demonstrated that populations of both Q and B biotype could develop resistance to imidacloprid. However, a wide variation in the response of imidacloprid resistant whitefly to other neonicotinoid insecticides was noticed. For example, different populations of B biotype were found to vary from having a resistance ratio (RR) of 2 and 5 in the most susceptible populations to a ratio of 78 and > 900 in the most resistant populations, for acetamiprid and thiamethoxam, respectively (Prabhaker *et al*., 2005; Rauch and Nauen, 2003). Elucidating the amount of cross resistance in an imidacloprid-resistant population of B biotype whitefly in the Midwestern USA would be very helpful to greenhouse growers considering the use of neonicotinoid insecticides to control whitefly.

Because the level of cross-resistance in imidacloprid-resistant populations of B biotype is uncertain, we conducted our research with a B biotype culture that was started from infested plants in a commercial greenhouse in Michigan. The susceptibility of this population to other insecticides of different classes registered for whitefly control was also compared with a reference strain.

II Material and Methods.

II-1 Plants.

Cotton plants were used in the study (FM958LL). Cotton seeds were planted in a 48-cell tray with potting soil and the trays held in a laboratory or in the greenhouse until plants reached the two true-leaf stage. Seedlings in the two true-leaf stage were used in a systemic uptake bioassay or they were planted in 6" pots with potting soil in the greenhouse as described previously. Cotton plants in the four to six true-leaf stage were used to test the susceptibility of the whitefly to different insecticides. Seedlings were treated with Pylon (chlorfenapyr, 0.98 ml/L) when they were in the cotyledon stage to prevent infestations of thrips and mites.

II-2 Insecticides.

Formulated products registered for control of whitefly on greenhouse or other crops were used in the present study. Ten insecticides belonging to six different chemical classes and one microbial insecticide were tested. The insecticides tested, their product name and the chemical class and group according to Insecticide Resistance Action Committee (IRAC) are: imidacloprid (Merit 75WP, neonicotinoid 4A); acetamiprid (Tristar 30SG, neonicotinoid, 4A); dinotefuran (Safari 20G, neonicotinoid 4A); thiamethoxam (Meridian 25WG, neonicotinoid, 4A); lambda-cyhalothrin (Scimitar GC, pyrethroid, 3A); fenpropathrin (Tame 2.4 EC, pyrethroid, 3A); flonicamid (Aria 50WG, novel chemistry, 9C); pyriproxyfen (Distance, insect growth regulator [IGR], 7C); buprofezin (Talus 40SC, buprofezin inhibitors of chitin biothesythisis, 17); pymetrozine (Endeavor 50WG, pymetrozine, 9B); and *Beauveria bassiana* (Botanigard 22WP, live fungal spores). All insecticides used were less than two years old. All formulated products were diluted with tap water to the needed dose, no physicochemical

properties of the water were tested.

II-3 Whitefly.

II-3-1 Reference strain.

We maintained a reference strain, CA-s1, of the B biotype whitefly in the greenhouse (with an LC_{50} of 1.97 mg /L for imidacloprid) from April 2007 to January 2009 (see Chapter I). This reference strain was used for testing for cross-resistance among neonicotinoid insecticides using systemic uptake bioassay. The CA-s1 population was replaced in February 2009 by a new reference strain, CA-s2, (with an LC_{50} of 4.26 mg /L for imidacloprid) from the same source as the first one (from Dr. Prabhaker, University of California Riverside, CA.) due to mites and thrips infestation (see chapter I). This second reference strain (CA-s2) was used in the insecticide efficacy testing under greenhouse conditions from June 2009 until October 2009.

II-3-2 Resistant strain.

The resistant strain, MI-R1, was initially obtained from a commercial greenhouse in Michigan. This population was subjected to selection for imidacloprid resistance from May 2007 to April 2008 (Table 2.2 of chapter I). It was maintained and split into two cultures in April 2009, as described in Chapter I. The two resistant strains MI-R1 and MI-r2a were used in this study. Tests on cross resistance to neonicotinoid insecticides using a systemic uptake bioassay method in the laboratory were conducted on generation F5 of the selected resistant strain MI-R1. In September 2007, this population was 144-fold more resistant compared with the reference strain CA-s1, with an LC_{50} for imidacloprid being 284.87 mg/l (Table 2.2 of chapter I). The test of the synergistic effect of pyperonyl butoxide (PBO) which is an inhibitor of

P450 detoxification enzyme was also performed on the F8 generation (in January 2008) and on F11 generation (in April 2008) of this selected resistant strain MI-R1.

Tests on insecticide efficacy and cross resistance under greenhouse conditions were made with the resistant strain, MI-r2a, between June 2009 and October 2009. This population was 7-fold more resistant to imidacloprid when compared with the reference strain CA-s2. The LC_{50} value for imidacloprid of this resistant strain MI-r2a was 30.54 mg/L (Table 1.4 of chapter I).

II-4 Systemic uptake bioassay for determining LC_{50}.

A systemic uptake bioassay method as described in the previous section (II-6 of Chapter I) was used for the study of cross-resistance of our imidacloprid-resistant whitefly colony to thiamethoxam, acetamiprid and dinotefuran. Briefly, cotton seedlings at the two true-leaf stage were systemically treated with different concentrations of imidacloprid for 24h. Six to nine concentrations giving more than 0% and less than 100% mortality were used. Whitefly adults from the generation F5 of the resistant strain MI-R1 were collected as described previously into the scintillation vials. Four replicates were used for each concentration and each replicate had thirty to fifty adults. Mortality was checked after 48h. The same experiment was conducted with the reference strain.

II-5 Efficacy of insecticides to the resistant strain MI-r2a.

II-5-1 Test on adults.

The experiment was conducted in the greenhouse. Cotton plants in the four to six true-leaf stage were planted in 6’ pots in the greenhouse for this test. Cotton plants were treated with

insecticides at the highest labeled rate. If products are labeled for use as a soil drench, they were applied that way. However, if they are only labeled as a foliar spray, they were sprayed. Four neonicotinoid insecticides were tested: imidacloprid (Merit 75WP) applied as a soil drench at a rate of 12 mg a.i./pot; acetamiprid (Tristar 30SG) applied as a foliar spray at 5.3 oz /100 gal; dinotefuran (Safari 20SG) applied as a soil drench at a rate of 24 oz /100 gal; and thiamethoxam (Meridian 25WG) applied as foliar spray at 8.5 oz/100 gal, buprofezin (Talus 40SC) applied as a foliar spray at a rate of 9 fl. oz /100gal, pyriproxyfen (Distance IGR) applied as a foliar spray at 8 fl. oz. /100 gal; *Beauveria bassiana* (Botanigard 22WP) applied as a foliar application at 1 lb /100 gal; and lambda-cyhalothrin (Scimitar GC) applied as a foliar spray at 148 ml /100 gal. For soil drench treatments, insecticide was applied by pouring 100 ml of solution around the base of each plant. The treatment was performed 24 h before the beginning of the test to allow cotton plants time to uptake the insecticide. For the foliar treatment, one liter of solution of insecticide was prepared and the insecticide was applied with a garden plant sprayer. The upper side and lower side of the leaf were sprayed until first runoff to ensure good coverage. Plants were sprayed three hours before caging whiteflies on the plants to allow time for the spray to dry on the leaves. Water was sprayed for the control treatment.

Unsexed whitefly adults from the resistant strain MI-r2a and reference strain CA-s2 were collected in 20 ml scintillation vials by filling them with CO_2, and keeping them capped with a thumb until the vial's mouth was placed over whiteflies on a leaf. The leaf was shaken to encourage the whiteflies to move into the vial. Anesthetized whitefly adults were counted and transferred into a clip-cage by pouring them from the vial into the clip-cage.

The clip cage was attached to a wooden stake as a support and was placed on the underside of well-developed leaves of treated plants. Each plant supported two clip-cages, one

on each of its two opposite leaves. On each plant one of the clip cages contained reference adults and the other one resistant adults. Each treatment was replicated four times and each replicate contained between thirty and ninety adults. Adult mortality was checked after 3 days.

II-5-2 Test on immature.

Adults from the resistant strain MI-r2a and the reference strain CA-s2 were collected in vials as previously described. Thirty unsexed adults were transferred with a thin brush into one clip-cage for oviposition. Clip-cages containing the whiteflies were then put on plants so that each plant supported one cage for susceptible adults and one cage for resistant adults. Adults were released after two days, and the clip-cages re-established to cover the eggs which had been deposited. The number of larvae emerging from the deposited eggs was counted under a dissecting microscope after eight days. Clip-cages were re-established after counting and treatment. Cotton plants with four to six true-leaves, grown in 6' pots in the greenhouse were used. Plants were treated with insecticide at the highest labeled rate. The same insecticide treatments used in the adult assay were used again for this test on immatures. Three additional insecticides were also tested: flonicamid (Aria 50WG) applied as a soil drench at a rate of 120 g/100 gal, fenpropathrin (Tame 2.4 EC) applied as a foliar spray at 16 fl. oz./gal, and pymetrozine (Endeavor 50WG) applied as a foliar spray at 20 g/100 L. The clip cage was removed for the foliar application and then re-established after the treatment. Treatments were made on 20 and 21 June 2009 for the four neonicotinoids (imidacloprid, dinotefuran, thiamethoxam and acetamiprid); on 30 June 2009 for buprofezin and pyriproxyfen; on 24 July 2009 for lambda-cyhalothrin and *Beauveria bassiana* and on 20 September 2009 for flonicamid, fenpropathrin and pymetrozine. Water was used for control.

Mortality was checked under the microscope one week after treatment. Immature mortality at

the end of the test was also determined by subtracting the number of empty pupal cases left by emerged adults from the number of treated first instars; this count was performed from 17 to 20 days after the treatment. Cotton plants used in the test for cross resistance to flonicamid, fenpropathrin and pymetrozine became so heavily infested with mites and thrips that the test was ended one week after treatment. Each treatment was replicated four times; each replicate contained 92 to 418 larvae.

II-6 PBO synergism test.

A piperonyl butoxide (PB0) synergism test was also conducted for determining the possible involvement of P450 monooxygenases in the expression of resistance. Cotton seedlings at the two true-leaf stage were collected from the laboratory. Discs from the cotton leaves were cut and immersed for 10 s in 10 ml of 500 ppm PBO solution containing 10 µl of Triton X-100. Then, the leaf discs were air-dried at room temperature. After drying, leaf discs were transferred into scintillation vials containing 2 ml of agar (1.3%) in the bottom of the vial. The leaf disc was put upside down so that the lower side of the leaf faces up. Whiteflies from the generation F11 of the reference strain MI-R1 were collected into the vial as described previously (II 4-1a of chapter I). Then the adults were exposed to these PBO-treated leaf discs for 7 hours for F11. After, the PBO pre-exposed adults were transferred to new vials with imidacloprid-treated leaf discs for the systemic uptake bioassay described previously. For the transfer, the mouth of two vials was connected to each other so that the vial with whitefly adults was placed below the other vial. After shaking the vials gently the whitefly adults moved upward into the vial above. Six concentrations giving more than 0% and less than 100% mortality were used for the assay. Four replicates were used for each concentration and each replicate contained thirty to fifty adults.

The mortality was checked after 48h. The same procedure was performed with adults pre-exposed for 7 h to leaf disc immersed for 10 s in 10 ml of water containing with 10 µl of X-triton. Then, the same systemic uptake bioassay for the exposure of the whiteflies to imidacloprid for 48h was performed. The same test was also conducted on generation F8 of this resistant strain MI-R1 but with a shorter exposure time of 2 hours to a higher concentration of the PBO solution (1000 ppm). This test was conducted during the trial for determination of the right dose to be used for PBO test.

II-7 Data analysis.

SAS (version 9.1) software was used to determine the LC_{50} value of the neonicotinoids. The ratio of LC_{50} of the resistant strain and the reference strain was calculated to determine the cross resistance level. Two LC_{50} are considered significantly different when their 95 % fiducial values do not overlap.

The number of individuals tested were different between treatments, thus the efficacy of insecticides on immatures and adults was determined by using Henderson -Tilton formula (1955):

$$Efficacy\ (\%) = [1 - (T_a \times C_b / T_b \times C_a)] \times 100$$

in which T_a = live individuals in the treatment after treatment

T_b = live individuals in the treatment before treatment

C_a = live individuals in the control after treatment

C_b = live individuals in the control before treatment

In order to determine whether imidacloprid resistant whiteflies exhibit resistance to other insecticides registered for whitefly control, the efficacy of the resistant strain MI-r2a and reference strain CA-s2 was compared with *t*-student test by using SPSS 17.5.

III Results.

In fall of 2007 the LC_{50} of imidacloprid for the resistant strain CA-s1 was found to be 1.97 mg/l when building a high level of resistance in the imidacloprid-resistant strain MI-R1. Two years later in April 2009 after the population decline due to mite and thrips infestations, new cotton variety and new reference strain CA-s2 were used in the study. The LC_{50} value of imidacloprid for the reference strain CA-s2 was 4.16 mg/L when we tested for insecticide efficacy under greenhouses conditions.

When the generation F8 of the resistant strain MI-R1 were pre-exposed to PBO, the LC_{50} value decreased from 411.07 mg/L (no PBO exposure) to 81.59 mg/L (pre-exposed to PBO at 1000 ppm) (Table 3.1). Similarly, the LC_{50} of imidacloprid to generation F11 of this resistant strain dropped from 431.08 mg/L to 32.47 mg/l when they were pre-exposed to PBO at 500 ppm for 7 hours (Table 3.1). These results suggest a synergistic effect of PBO and suggest that cytochrome P450 monooxygenases could be involved in the imidacloprid resistance mechanism for this population.

Cross-resistance of whitefly in the imidacloprid-resistant strain MI-R1 to other neonicotinoids was also studied. When the resistant strain MI-R1 and reference strain CA-s1 were treated with thiamethoxam, the LC_{50} for resistant strain was 29.20 mg/l, compared with

Table 3.1. Synergistic effect of PBO on the toxicity of imidacloprid to F8 and F11 generation of the resistant strain MI-R1 of *Bemisia tabaci* B biotype.

Generation	Treatment	Number of adults	Slope ± SE	LC_{50} (ppm)	95% FL[a]	RR[b]
F8	Imidacloprid without PBO	959	1.22 ± 0.20	411.07	240.87-86.11	208
F8 [c]	Imidacloprid with PBO	773	1.02 ± 0.11	81.59	54.17-124.84	45
F11	Imidacloprid without PBO	1602	0.62 ± 0.04	431.08	274.26-92.53	218
F11[d]	Imidacloprid with PBO	1204	0.79 ± 0.14	32.47	4.17-142.57	16

a) FL fiducial limits

b) RR resistance ratio (LC_{50} of resistant strain / LC_{50} of reference strain (1.97)). Resistance strain MI-R1 and reference strain CA-s1 were used in the test

c) whiteflies were pre-exposed to PBO at 1000 ppm for 2 hours

d) whiteflies were pre-exposed to PBO at 500 ppm for 7 hour

Table 3.2. Response of the reference strain (CA-s1) and the generation F5 of the imidacloprid-resistant strain (MI-R1) of *Bemisia tabaci* B biotype to three neonicotinoid insecticides in a systemic uptake bioassay.

Insecticide /Strain	Number of adults	Slope ± SE	LC_{50} (ppm)	95% FL[b]	RR[c]
Acetamiprid					
Reference	1252	0.81 ± 0.11	1.21	(0.79 – 1.78)	
Im-Resistant[a]	674	1.06 ± 0.09	8.65	(5.60 – 12.68)	7
Thiamethoxam					
Reference	960	0.93 ± 0.15	3.18	(2.19 – 4.61)	
Im-Resistant	561	1.51 ± 0.42	29.20	(6.10 – 60.36)	9
Dinotefuran					
Reference	816	0.72 ± 0.06	0.16	(0.09 - 0.26)	
Im-Resistant	739	0.74 ± 0.10	0.61	(0.20 – 1.35)	4

a) Im-Resistant: F5 generation of the imidacloprid resistant strain MI-R1

b) FL: fiducial limits

c) RR: resistance ratio (LC_{50} of imidacloprid resistant strain / LC_{50} of reference strain).

3.18 mg/L for the reference strain, indicating a low level of cross-resistance (9-fold, Table 3.2).A similar level of resistance (7-fold) was also observed with acetamiprid as indicated by the difference in LC_{50} of the resistant strain MI-R1 and reference strain CA-s1 (8.65 and 1.21 mg/l, respectively) (Table 3.2). No significant difference in the LC_{50} values was found among the resistant MI-R1 and reference strain CA-s1 for dinotefuran as their 95% fiducial limits overlapped (Table 3.2).

Results of a standard efficacy test on the whitefly populations for imidacloprid at the labeled rate indicate that mortality of whitefly from the resistant strain MI-r2a was significantly lower than that of the reference strain CA-s2 at one week (79.1% and 21.9% mortality, respectively, $p < 0.0001$) and 18 days (80.1% and 27.2% mortality, $p = 0.002$) after the treatment (Table 3.3). A second efficacy test gave similar results for adults (83.4% and 3.7% mortality, respectively, $p < 0.0001$) (Table 3.7). For thiamethoxam, no difference in mortality was observed for the immature stages one week and two weeks after treatment (Table 3.3). However, a significant difference in mortality was observed with the adults between the two strains (92.8 and 62.3%, $p = 0.004$), indicating a reduced susceptibility of the resistant whitefly adults MI-r2a to thiamethoxam (Table 3.7). No difference of efficacy of the two other neonicotinoids (acetamiprid and dinotefuran) was observed for both immature and adult whiteflies of MI-r2a and CA-s2 (Table 3.3, 3.7). Results of efficacy tests of the non-neonicotinoid insecticides buprofezin, pyriproxyfen and *Beauveria bassiana* indicate no significant differences in efficacy for immatures and adults between the resistant strain MI-r2a and reference strain CA-s2 (Table 3.4, 3.5, 3.8). The only observed difference in efficacy for any non-neonicotinoid insecticide was for lambda-cyhalothrin, where a slight but non-significant difference in mortality of immatures and adults was observed (Table 3.8, 3.5). No differences in

Table 3.3. Efficacy ± SE of neonicotinoids against immatures of the resistant strain (MI-r2a) and the reference strain (CA-s2) of *Bemisia tabaci* B biotype whitefly on cotton plants. *: Mean number of immatures from four replicates.

Insecticide /strain	Immature alive *	Efficacy (%)	Immature alive*	Efficacy (%)	Immature alive*
	Before treatment	7 days after treatment (6/28/09)		18 days after treatment (7/9/09)	
Imidacloprid[d]					
Reference	312.2	79.1 ± 5.7a[c]	35.7	80.1 ± 5.6a	31.7
Resistant	204.2	21.9 ± 5.2b	75.5	27.2 ± 8.6b	71.2
Acetamiprid[e]					
Reference	212.5	98.8 ± 0.8a	1.2	99.3 ± 0.6a	0.5
Resistant	227.5	97.8 ± 0.8a	2.1	97.9 ± 0.8a	1.7
Thiamethoxam[f]					
Reference	249.5	98.8 ± 0.5a	1.5	99.2 ± 0.4a	1.0
Resistant	187.0	94.5 ± 2.6a	4.5	94.5 ± 2.3a	4.5
Dinotefuran[g]					
Reference	233.7	99.8 ± 0.2a	0.3	99.7 ± 0.2a	0.2
Resistant	235.2	97.7 ± 1.2a	1.7	97.9 ± 0.8a	0.5
Untreated control					
Reference	255.2	-	157	-	146.7
Resistant	233.7	-	126	-	119.2

c) Same letter within the same insecticide in the same column indicates lack of statistical difference in insecticide efficacy between the resistant strain and the reference strain according to Student t test at 0.05 level

d) Imidacloprid (Merit 75WP) applied as a drench treatment at 12 mg a.i. per pot

e) Acetamiprid (Tristar 30SG) applied as a foliar spray at 5.3 oz/100gal

f) Thiamethoxam (Meridian 25WG) applied as foliar spray at 8.5 oz/100 gal

g) Dinotefuran (Safari 20G) applied as a soil drench at a rate of 24 oz/100gal

All treatments were conducted on June 21, 2009, thus eight days after eggs deposition when all eggs hatched.

Table 3.4. Efficacy of buprofezin and pyriproxyfen against clip-caged immatures of the resistant strain (MI-r2a) and the reference strain (CA-s2) of whitefly *Bemisia tabaci* B biotype on cotton plants. *: mean number of immature whitefly from four replicates.

Insecticide /strain	Immature alive *	Efficacy (%)	Immature alive*	Efficacy (%)	Immature alive*
	Before treatment	7 days after treatment (7/7/09)		20 days after treatment (7/20/09)	
Buprofezin[d]					
Reference	237.5	97.9 ± 0.9a[c]	3.5	100 ± 0.0a	0
Resistant	294.7	98. 2 ± 0.4a	4.0	100 ± 0.0a	0
Pyriproxyfen[e]					
Reference	186.2	75.4 ± 3.5a	33.0	99.8 ± 0.2a	0.2
Resistant	162.0	72.6 ± 2.9a	33.7	99.7 ± 0.2a	0.2
Untreated control					
Reference	168.0	-	121.2	-	115.2
Resistant	172.2	-	130.0	-	125.7

c) Same letter within the same column indicates statistically different between the insecticide efficacy against the resistant strain and the reference strain within the same insecticide according to Student t test at 0.05 level

d) buprofezin (Talus 40SC) applied as a foliar spray at a rate of 9 fl. oz/100gal

e) pyriproxyfen (Distance IGR) applied as a foliar spray at 8 fl. oz./100gal

All treatments were conducted on June 30, 2009, thus eight days after eggs deposition when all eggs hatched.

Table 3.5. Efficacy ± SE of lambda- cyhalothrin and Beauveria bassiana against clip-caged immatures of the resistant strain and the reference strain (CA-s2) of whitefly *Bemisia tabaci* B biotype on cotton plants. *: Mean number of immatures from four replicates.

Insecticide /strain	Immature alive *	Efficacy (%)	Immature alive*	Efficacy (%)	Immature alive*
	Before treatment	7 days after treatment (7/31/09)		17 days after treatment (8/10/09)	
Beauveria bassiana[c]					
Reference	174.0	73.2 ± 3.7a[b]	23.7	90.8 ± 0.5a	8.0
Resistant	193.2	82.0 ± 4.0a	19.7	90.9 ± 0.8a	9.0
Lambda-cyhalothrin[d]					
Reference	184.5	88.6 ± 3.9a	10.7	90.1 ± 4.2a	8.7
Resistant	204.5	82.2 ± 2.1a	19.7	82.8 ± 2.2a	17.5
Untreated control					
Reference	176.0	-	89.5	-	84
Resistant	149.7	-	82.0	-	76

b) Same letter within the same column indicates statistically different between the insecticide efficacy against the resistant strain and the reference strain within the same insecticide according to Student t test at 0.05 level

c) *Beauveria bassiana* (Botanigard 22 WP) applied as a foliar spray at 1 pound/100gal

d) lambda-cyhalothrin (Scimitar GC) applied as a foliar spray at 148ml /100 gal

All treatments were conducted on July 7, 2009, thus eight days after eggs deposition when all eggs hatched.

mortality of immatures were also observed between the two strains MI-r2a and CA-s2 with the other insecticides tested (flonicamid, fenpropathrin and pymetrozine), suggesting an absence of resistance to these insecticides in this resistant strain MI-r2a (Table 3.6).

IV Discussion

Resistance to insecticides can be caused by different mechanism in insects. Several published studies reported that cytochrome P450 enzymes were involved in imidacloprid resistance in Q and B biotype of *Bemisia tabaci* (Rauch and Nauen 2003, Karunker *et al*., 2008). The results of this study also suggest that cytochrome P450 monooxygenases are an important part of the resistance mechanism of whitefly in our resistant strain because pre-treatment with the P450 inhibitor, PBO, reduced the level of imidacloprid resistance from an RR of 218 to 16. However, it is also noteworthy that resistance was not completely eliminated by the PBO pre-treatment, suggesting an additional mechanism is involved (Table 3.1). In a previous study a mutation of the target site nAChR and an overexpression of P450 gene have been shown to cause imidacloprid resistance in a population of aphids, *Myzus persicae,* from southern France (Bass *et al.*, 2011). A mutation on the target site nAChR was also observed in imidacloprid-resistant brown planthoppers *Nilaparvata lugens* (Liu *et al.*, 2005).

When imidacloprid was applied to potted plants in an efficacy test designed to determine what level of control would be obtained by greenhouse growers using products at a rate of 12 mg a.i. per pot, which is the label rate recommended for the imidacloprid product Marathon 60WP, it was effective (83.4 % mortality) on whitefly from the reference strain CA-s2 but had little effect on immature or adult whiteflies (27.2 or 3.7% mortality) from the resistant strain (MI-r2a) with a level of resistance RR of 7-fold (LC_{50} = 30.54 mg/L) (Table 3.3, 3.7). These results are

Table 3.6. Efficacy ± SE of flonicamid, fenpropathrin, and pymetrozine against clip-caged immatures of the resistant strain (MI-r2a) and the reference strain (CA-s2) of whitefly *Bemisia tabaci* B biotype on cotton plants. *: Mean number of imatures from four replicates.

Insecticide/ Strain	Immatures* alive	Efficacy ± SE (%)	Immatures* alive
	Before treatment	7 days after treatment (9/27/09)	
Flonicamid[c]			
Reference	240.2	42.6 ± 5.7a[b]	94.2
Resistant	240.5	41.9 ± 6.0a	96.5
Fenpropathrin[d]			
Reference	252.7	71.4 ± 6.8a	48.7
Resistant	214.5	66.6 ± 7.3a	47.2
Pymetrozine[e]			
Reference	184.5	48.6 ± 7.0a	10.7
Resistant	204.5	42.6 ± 12.8a	19.7
Untreated control			
Reference	195.0	-	66.7
Resistant	139.2	-	51.5

a) Same letter within the same insecticide in the same column indicates lack of statistical difference in insecticide efficacy between the resistant strain and the reference strain according to Student t test at 0.05 level

b) flonicamid (Aria 50WG) applied as a soil drench at a rate of 120g/100gal

c) fenpropathrin (Tame 2.4 EC) applied as a foliar spray at 16 fl. oz./gal

d) pymetrozine (Endeavor 50WG) applied as a foliar spray at 20g/100l

All treatments were conducted on September 20, 2009, thus eight days after eggs deposition when all eggs hatched.

Table 3.7. Efficacy ± SE of neonicotinoids (imidacloprid, acetamiprid, thiamethoxam, dinotefuran) against clip-caged resistant adults (MI-r2a) and reference adults (CA-s2) of whitefly *Bemisia tabaci* B biotype exposed for 3 days to the treated cotton plants. *: Mean number of adults from four replicates.

Insecticide/ Strain	Adult* alive	Efficacy ± SE (%)	Adult* alive
	Before exposure	3 days after insecticide exposure (6/11/09)	
Imidacloprid[d]			
Reference	54.2	83.4 ± 3.5a[c]	8.7
Resistant	48.0	3.7 ± 0.9b	45.5
Acetamiprid[e]			
Reference	63.0	100 ± 0.0a	0.0
Resistant	53.5	100 ± 0.0a	0.0
Thiamethoxam[f]			
Reference	41.7	92.8 ± 3.4 a	3.2
Resistant	49.0	62.3 ± 5.9 b	18.0
Dinotefuran[g]			
Reference	50.2	100.0 ±0.0 a	0.0
Resistant	50.0	97.8 ±1.2a	1.0
Untreated control			
Reference	77.7	-	76.5
Resistant	66.7	-	67.5

c) Different letter within the same insecticide in the same column indicates statistical difference in insecticide efficacy between the resistant strain and the reference strain according to Student t test at 0.05 level

d) Imidacloprid (Merit 75WP) applied as a soil drench at a rate of 12 mg a.i. per pot

e) Acetamiprid (Tristar 30SG) applied as a foliar spray at 5.3oz/100gal

f) Thiamethoxam (Meridian 25WG) applied as foliar spray at 8.5 oz/100 gal

g) Dinotefuran (Safari 20G) applied as a soil drench at a rate of 24oz/100gal

All treatments for the drench application (imidacloprid and dinotefuran) were conducted on June7, 2009 and on 8 June, 2009 for foliar application (thiamethoxam and acetamiprid).

Table 3.8. Efficacy ± SE of pyriproxyfen, *Beauveria bassiana*, buprofezin and lambda-cyhalothrin against clip-caged resistant adults (MI-r2a) and reference adults (CA-s2) of whitefly *Bemisia tabaci* B biotype exposed for 3 days to the treated cotton plants. *: Mean mean number of adults from four replicates.

Insecticide/ Strain	Adult* alive	Efficacy ± SE (%)	Adult* alive
	Before exposure (7/6/09 and 7/7/10)	3 days after insecticide exposure (7/9/09 and 7/10/09)	
Pyriproxyfen[c]			
Reference	48.5	29.2 ± 9.1a[b]	32.2
Resistant	50.2	15.1 ± 4.0a	40.5
Beauveria bassiana[d]			
Reference	44.7	19.1 ± 5.2a	34.0
Resistant	47.7	20.0 ± 7.7a	36.2
Buprofezin[e]			
Reference	47.5	10.7 ± 3.4a	39.7
Resistant	44.0	11.9 ± 4.8a	36.5
lambda-cyhalothrin[f]			
Reference	45.7	63.5 ± 11.6a	15.7
Resistant	42.0	55.4 ± 8.8a	17.5
Untreated control			
Reference	37.5	-	35.2
Resistant	42.7	-	40.5

b) Different letter within the same insecticide in the same column indicates statistical difference in insecticide efficacy between the resistant strain and the reference strain according to Student t test at 0.05 level

c) pyriproxyfen (Distance IGR) applied as a foliar spray at 8 fl.oz./100gal

d) *Beauveria bassiana* (Botanigard 22 WP) applied as a foliar spray at 1 pound/100gal

e) buprofezin (Talus 40SC) applied as a foliar spray at a rate of 9 fl.oz/100gal

f) lambda-cyhalothrin (Scimitar GC) applied as a foliar spray at 148 ml/100 gal

All treatments were conducted on July, 6 2009 except for lambda- cyhalothrin applied on July 7, 2009.

consistent with many reports by greenhouse growers over the last 15 years, that imidacloprid failed to provide adequate control of whitefly. Our colony of resistant B-biotype was started from whitefly collected from a production greenhouse in southeast Michigan. The initial test of the whitefly population before selecting for higher levels of imidacloprid resistance indicated an LC_{50} of 23.42 mg/L (RR=11). Although this is a low level of imidacloprid resistance, our standard efficacy tests with products at labeled rates indicate that at a similar resistance level (LC_{50} = 30.54 mg/L, RR = 7) efficacy is already affected. Control in production greenhouses may be even more affected because growers are expecting at least 6 weeks of protection after a single soil drench of imidacloprid or other neonicotinoid insecticides.

We observed high mortality of immature whitefly from the resistant strain MI-r2a (27% at 18 days after imidacloprid treatment) (Table 3.3), indicating that this population is not homogenously resistant and that susceptible alleles still exist at a certain frequency in the population, which is consistent with its low level of resistance (RR = 7). In addition, whitefly immatures were suggested to be more susceptible to imidacloprid than whitefly adults (Jones *et al.*, 2011; Nauen *et al*., 2008). All individuals in the reference strain CA-s2 was also not susceptible to imidacloprid at the time of the test. Instead of seeing 90-100% mortality after treatment with imidacloprid, only 80% of immature whiteflies died after treatment, suggesting that some individuals from the resistant strain may have found their way into the reference strain.

The level of cross-resistance of imidacloprid resistant populations to other neonicotinoids has been a subject of controversy due to variation in the results obtained from several studies. In the current study, the resistant strain MI-R1 with a relatively high level of resistance (LC_{50} = 284.47 mg/L, RR = 144) was used to study the cross resistance to these

neonicotinoids using a systemic bioassay. In addition, the response of the resistant strain MI-r2a with a lower resistance level (LC_{50} = 30.54 mg/L, RR =7) to three neonicotinoids was also used and compared to the response of the reference strain CA-s2 under the greenhouse conditions. The results of cross resistance in these studies are consistent with those of Prabhaker *et al*. (2005) who found a low level or no cross resistance to neonicotinoids with a B biotype whitefly population. In our tests, three neonicotinoids (acetamiprid, thiamethoxam and dinotefuran) caused as much mortality of immatures of the resistant whitefly MI-r2 a as they did for immatures of the reference strain CA-s2 (Table 3.3). This is not surprising given the fact that P450 monooxygenases might play an important role in the resistance in this population as described above, and previous study demonstrated an age specific expression of the P450 gene responsible of imidacloprid resistance in whitefly, with a low level of expression in immatures, leaving this stage more vulnerable to the neonicotinoids than the whitefly adults (Jones *et al.*, 2011; Nauen *et al*., 2008).

When the adult whiteflies were tested for insecticide efficacy, the resistant whitefly showed some cross-resistance to one of three neonicotinoids tested. Resistant adults of MI-r2a with a low level of resistance (RR =7) were less susceptible to thiamethoxam than reference adults CA-s2 (Table 3.7). This is consistent with results obtained with the resistant strain MI-R1 with a high resistance level (RR =144) by using the systemic bioassay method, where a low level of cross resistance (9-fold) was found (Table 3.2). Recently, an overexpression of one P450 gene, CYP6M1PvQ, was observed in an imidacloprid-resistant population of both B and Q biotypes (Karunker *et al*., 2008). In a subsequent study, Roditakis *et al*. (2011) demonstrated that indeed CYP6M1PvQ has a potential to metabolize imidacloprid in whitefly. However, the same study showed that thiamethoxam was not metabolized by the protein of this P450 gene. In fact,

Nauen *et al*. (2003) showed that thiamethoxam was converted to clothianidin in cotton plants and in the insects after its application, clothianidin is a byproduct of thiamethoxam. Interestingly, Roditakis *et al.* (2010) found that as opposed to thiamethoxam but similar to imidacloprid, clothianidin was metabolized by the protein of the P450 gene, CYP6M1PvQ, responsible for imidacloprid resistance in whitefly. All these studies seem to support the findings in this study showing a resistance to thiamethoxam; thus the thiamethoxam could be first converted to clothianidin in the plants and whitefly, and then the protein of the P450 gene of the whitefly could degrade the metabolite clothianidin that was generated, which consequently makes the mother compound thiamethoxam less toxic to the whitefly. A low level of resistance (7-fold) was also observed with acetamiprid using the systemic bioassay with the resistant strain MI-R1 having a high level of resistance to imidacloprid (144-fold) (Table 3.2). However, the result of the efficacy test shows no difference in response of the adults of the resistance strain MI-r2a with low RR= 7 and the reference strain C-S2 to this compound (Table 3.7). This discrepancy could be explained by the fact that acetamiprid has a high level of insecticidal activity when applied as foliar treatment (Horowitz *et al*., 1988). In addition, Roditakis *et al*. (2010) demonstrated that similar to thiamethoxam, the protein of the P450 gene CYP6M1PvQ also did not metabolize acetamiprid in whitefly. These authors also suggested that this could be another explanation of the observation of low level of cross-resistance to neonicotinoids in some whitefly populations.

Apparently in agreement with the results on the metabolism of these two compounds (acetamiprid and thiamethoxam) by the protein of P450 gene, several published studies on cross resistance to neonicotinoids using a leaf dip assay found that the level of resistance was much higher for thiamethoxam than for acetamiprid in imidacloprid-resistant whiteflies. For example, the B biotype whitefly having a resistance ratio (RR) of 230 to imidacloprid exhibited a

resistance ratio of 73-fold to thiamethoxam whereas it was only 15-fold for acetamiprid (Gorman *et al*., 2010). Different B biotype whitefly populations collected from different regions in Cyprus having a moderate to high resistance level to imidacloprid (RR of 77 to 392) also showed a moderate level of resistance to thiamethoxam (RR = 50 to 164) but a low level of resistance to acetamiprid (RR = 7 to 12) (Vasillou *et al*., 2012). The same observation of higher resistance to thiamethoxam than to acetamiprid was also noticed in several imidacloprid resistant populations of Q biotype whitefly (Rao *et al*., 2012). The LC_{50} for thiamethoxam in the resistant strain MI-R1 (LC_{50} =29.20 mg/L) was higher than that for acetamiprid (LC_{50} = 8.65 mg/L) in the current study, however, these values are not significantly different based on their 95 % fiducial limit and the resistance ratio was similar for both compounds (9-fold and 7-fold for thiamethoxam and acetamiprid respectively). Prabhaker *et al*. (2005) also observed this. It is noteworthy that systemic bioassays were used in these two studies (this study and Prabhaker *et al.*, 2005) and that might partially explain the difference in the results. It is also likely that difference in level of resistance could be caused by the predominant gene or mechanism that was selected in different populations. For example, Horowitz *et al*. (2004) found that a whitefly population selected with thiamethoxam showed a very low resistance to acetamiprid whereas acetamiprid-selected population was highly resistant to thiamethoxam. In contrast, Feng *et al.* (2009) selected B biotype whitefly for thiamethoxam resistance (RR: 25.6) found a similar level of resistance to acetamiprid (RR 35-fold) and imidacloprid (47.3-fold). Interestingly, Xi *et al*. (2012) observed an overexpression of three other P450 genes (CYP6a8, CYP4v2, and CYP6v5) in thiamethoxam-resistant whitefly *B. tabaci* but not the P450 gene CYP6CM1 that was found previously in imidacloprid-resistant whiteflies Q and B biotype (Karunker *et al*., 2008). Thus, a different history of insecticide treatments can generate a difference in the predominant genes or

mechanism responsible for resistance in different whitefly populations. All these studies suggest that variation in cross resistance level between the three neonicotinoids (imidacloprid, thiamethoxam and acetamiprid) is not unexpected. In addition, the bacterial symbionts hosted by both B and Q biotype *B. tabaci* were recently found to influence the susceptibility of these whiteflies to acetamiprid, thiamethoxam and imidacloprid and other insecticides as well (Ghanim and Kontsedalov, 2009; Kontsedalov *et al*., 2008). The compositions of these facultative symbionts can vary between regions or biotypes (Xie *et al*., 2012). That could be one more possible cause that might partially explain the variation in level of resistance to these compounds in different populations.

Results from a systemic bioassay test and from a standard greenhouse efficacy test indicate that imidacloprid-resistant whitefly from our cultures did not exhibit any cross-resistance to dinotefuran. Prabhaker *et al.* (2005) also detected no cross resistance to dinotefuran in B-biotype whitefly selected for imidacloprid resistance. Similarly, different Q biotype whitefly populations collected from fields in China having high resistance level to imidacloprid also showed very low or no resistance to dinotefuran (Rao *et al*., 2012). Dinotefuran has a different structure compared with the other neonicotinoids. Thus, it would not be unlikely that the same protein of the P450 gene responsible for imidacloprid resistance in whitefly may metabolize dinotefuran in a different way than for other neonicotinoids. Studies of binding target sites demonstrate that even dinotefuran shares the same target site with other neonicotinoids such as imidacloprid, thiamethoxam and acetamiprid in several different insects including whitefly, dinotefuran may have a different interaction with the target site as shown in the assay with a *Homalodisca* leafhopper (Honda *et al*., 2006). Liu *et al*. (2006) studied effects of the mutation Y151S on the nicotinoic acetylcholine receptor, which causes imidacloprid

resistance in brown planthoppers, on the agonist potency of a few neonicotinoid compounds. These authors found that the impact of this mutation Y151S on the agonist potency of dinotefuran was the smallest among all the neonicotinoids in the study. Considering the results of all these studies, a low or non-observation of cross resistance to dinotefuran is not unexpected.

The standard efficacy test also did not show a difference between the resistant strain MI-r2a and reference strain CA-s2 for the other non-neonicotinoid insecticides tested. It is noteworthy that the efficacy testing was conducted with a population having a low resistance level (7-fold). It is very likely that a low or a moderate resistance to other insecticides might not be detected with this level of resistance in the population used for the test. Thus, the results observed with these insecticides are not conclusive. Indeed, no difference was observed between resistant MI-r2a and reference strain CA-s2 with pymetrozine in the current study (Table 3.6). However, a good correlation of level of resistance between pymetrozine and the neonicotinoids imidacloprid and thiamethoxam was already observed in whitefly having a resistance ratio of 10,000 fold (Gorman *et al.*, 2010). A slight difference in efficacy between the resistant strain MI-r2 and the reference strain CA-s2 (82% and 90% respectively) was observed with lambda-cyhalothrin (Table 3.5), however, this small difference is not significantly different. The two insect growth regulator compounds, pyriproxyfen and buprofezin caused the lowest efficacy in adults among the insecticides tested in the present study (Table 3.8). In fact, these compounds which act mainly on the immature stages were known to have less activity to the adults (Palumbo *et al.*, 2001). Buprofezin interferes with chitin formation in the development of immature insects (Ishaaya et *al*., 1988), whereas pyriproxyfen which is a juvenile hormone analog affects embryogenesis, metamorphosis and formation of adults in insects (Ishaaya and Horowitz, 1992). Consistent with their mode action, these two IGR compounds caused a high level of mortality to

the immature stages of both strains (Table 3.4). Elbert and Nauen (2000) even suggested the two IGR compounds as alternative insecticides in a pesticide rotation with neonicotinoids. Indeed, pyriproxyfen was found to cause 100% mortality of eggs of Q biotype whitefly population which has highly resistance to imidacloprid, whereas buprofezin caused an appreciable mortality rate (68%) to the immature of the same population (Elbert and Nauen, 2000).

Similar to the result found by Oetting (2007) conducted on B biotype whitefly, *Beauveria bassiana* was found to cause high mortality (90%) to immatures two weeks after treatment in the current study (Table 3.5). No difference in efficacy for immatures or adults was observed among the two strains MI-r2a and CA-s2. Efficacy of flonicamid against immatures was similar to that observed by Parella (2006), with a moderate effect of this insecticide on whitefly immatures one week after treatment, and no appreciable differences among the reference strain and CA-s2 and resistant strain MI-r2a was observed in the current study (Table 3.6).

V Conclusion.

The current study found that resistance in this B biotype of *Bemisia tabaci* population was mainly mediated by P450 monooxygenases as suggested by the significant synergistic effect of imidacloprid in the resistant whitefly pre-treated with the P450 inhibitor, PBO. The population showed a low level of cross resistance to thiamethoxam. This is consistent with the recent study that found protein of the P450 gene responsible for imidacloprid resistance in whitefly also metabolizes clothianidin, a direct metabolite of thiamethoxam (Roditakis *et al*., 2010, Nauen *et al*., 2003).

In contrast no appreciable cross resistance was observed with dinotefuran in this

population. A low level of cross-resistance to acetamiprid was also observed (Table 7). However the product of the same P450 gene did not metabolize this compound in a recent study Roditakis *et al.* (2010). This suggests that a different P450 gene or even an additional mechanism might have been involved in the imidacloprid resistance in this population. In fact, as in previous studies, the imidacloprid resistance was not completely removed by PBO in the current study, a result that supports the occurrence of other mechanisms of resistance. Thus, the wide variation in the level of resistance to imidacloprid observed in different populations of whitefly might be attributed to differences in the mechanisms of resistance that were selected for, and that might be related to the history of insecticide treatments. Regardless of the mechanism of resistance, the results of this study suggest that greenhouse growers should avoid the use of thiamethoxam and acetamiprid in their rotation of insecticides for whitefly control because of the history of widespread use of imidacloprid over the past 15 years, and because of the documented cross resistance to acetamiprid, and especially thiamethoxam. The response of the resistant whitefly to insecticides with different modes of action was also studied and no appreciable resistance to these insecticides was observed. However, the results were not conclusive as the level of resistance of the imidacloprid-resistant population used in the study was too low to detect a low level of cross resistance to these compounds. Still, the result is informative because the level of resistance (7-fold) was similar to an imidacloprid resistant population of B biotype found in a commercial greenhouse in southeast Michigan (LC_{50} = 30.54 mg/L), and therefore may be representative of resistance levels that greenhouse flower growers are likely to have.

CHAPTER IV. Induction of hormesis in whitefly *Bemisia tabaci* B biotype by low priming doses of imidacloprid.

I Introduction.

Numerous studies have assessed the effects of sublethal doses of insecticides on insects. Most of the investigations were made with adult insects and most of the results demonstrate an inhibitory effect (Desneux et al. 2007, Tan et *al.*, 2012; Boina *et al*., 2009). However, toxicants may also cause different responses at very low doses that do not cause an observable adverse effect, and this phenomenon is known as hormesis in toxicology (Calabrese and Blain, 2005). In hormesis, the low dose of the stressors including insecticides may elicit a stimulatory effect whereas higher doses of the same stressors are toxic or inhibitory (Calabrese and Baldwin, 2003a; Calabrese and Blain, 2005). There are two types of hormesis: first, an overcompensation stimulation hormesis (OCSH) which is a result of an overcorrection response following a disruption of homeostasis, and second, a direct stimulation hormesis (DSH) (Calabrese and Baldwin, 2002). The stimulation can be observed at a low dose where the stressor does not elicit any observable toxicity (Calabrese and Blain (2003a), and it may also be observed over a wide range of doses, thus even at the dose where the stressors elicit adverse effects (Stebbing, 2009; Chujo *et al.* 2012). The hormetic effect may be detected with a single treatment, but sometimes it can only be determined after a pre-exposure or priming of the cell or organism. When a pre-exposure is needed the effect is known as 'conditioning hormesis' (Calabrese *et al.*, 2007). In conditioning hormesis, the first treatment can protect the organism against the toxic effects of a subsequent and more severe treatment (Calabrese *et al.*, 2007, Calabrese and Mattson, 2011). Therefore, hormesis can be viewed as an acquired tolerance (Stebbing, 2002, 2009). The underlying mechanism of hormesis is not fully understood. However, several investigators

reported that different biological and physiological processes including stress response systems, signaling pathways and metabolic mechanisms could be involved in hormesis (Zhao and Wang, 2012; Calabrese *et al.*, 2010). Other authors suggest that hormesis might be associated with epigenetic modification in the organism (Vaiserman, 2011; Costantini *et al.*, 2011). Indeed, increasing number of studies have shown that epigenetics are involved in hormesis and the phenomenon is mediated by oxidative stress (Schroeder *et al*., 2013).

Hormesis can be induced by different toxicants across a wide range of taxa on several endpoints. Recently, hormesis has gained interest among pest management researchers. Some investigators support the idea that hormesis may contribute to a pest resurgence or secondary outbreak following an insecticide application (Guedes *et al*., 2010; Morse, 1998). In insects, the hormetic effect can happen at different stages of development including early adulthood (Kristensen *et al*., 2003; Lebourg, 2005) or during the larval stage (Nascarella *et al.*, 2003). When conditioning hormesis is applied to management of insect pests of cultivated crops some important questions arise: if insects in immature stages are exposed to a trace amount of insecticide, will this stimulate the fecundity of the same individuals when they encounter the same insecticide during their adult stage, and will it protect the individuals from a subsequent exposure to higher doses of the same insecticide?. In addition, previous studies demonstrated that side effects of a single exposure to pesticides applied at an early stage of the development can persist in a few subsequent generations of mammals and insects (Manikkam *et al*., 2012, Adamski, *et al*., 2005, Anway *et al.*, 2005). The question could be then asked whether a stimulation induced by a single exposure of insects to pesticides during immature stage can reappear in their offspring.

Whitefly is a major pest in both greenhouses and agricultural fields. The life cycle of a

whitefly starts with the egg which has a pedicel used to obtain water from the host leaf to which it is attached (Byrne *et al.,* 1990). After egg hatch, whitefly immatures go through four instars. The last stage of the fourth instar is a pharate adult which is often mistakenly called a pupa (Gelman *et al.,* 2007). Then as adults, whitefly can reproduce parthenogenetically. Being arrhenotokous, the virgin females can lay eggs, which become male if unfertilized or become female if fertilized (McAuslane, 2007).

The neonicotinoid insecticide imidacloprid has been commonly used for control of sucking insects such as whiteflies, aphids, and planthoppers since its introduction to the market in the early 1990s (Elbert *et al.,* 2008). Imidacloprid may persist up to 12 months or longer after its application to soil (Bonmatin *et al.,* 2003). Therefore, pests on plants in fields where a soil application of imidacloprid is made can be exposed to low doses of imidacloprid for the entire crop production period. Because of this long exposure period it is important to know if trace amounts of imidacloprid in plants have any effect on the fitness of the pest population. In previous research with the green peach aphid some studies showed that a low dose of imidacloprid can inhibit fecundity (Devine *et al.,* 1996), while in other studies fecundity was increased (Yu *et al.,* 2010; Cutler *et al.,* 2009). Other authors reported no effect of a low dose of imidacloprid on the fertility of green peach aphid (Janmaat *et al.,* 2011). This variability of results was also observed with other pest species such as brown planthopper, *Nialaparvata lugens*, treated with imidacloprid (Bao *et al.,* 2009; Yin *et al.,* 2008). This discrepancy in results suggests that imidacloprid can induce a stimulation or hormesis in the fecundity of insects under specific circumstances.

This study investigates the potential of low dosages of imidacloprid to induce an increase in whitefly fecundity, and the heritability of the hormetic effect. Because the long-term persistence of resistant populations of whitefly is of critical importance to flower production, vegetable production and cotton production, the current study was conducted on *B. tabaci*

biotype, the most widespread resistant-biotype on these crops. This study focuses mainly on an imidacloprid-induced increases in fecundity, because fecundity is a key component of fitness.

II Materials and methods

The study on hormesis was conducted at two stages: the first stage was conducted between 2009 and 2012 with two cultures: a resistant whitefly strain and a reference strain. The second stage was conducted in 2013 with a rebuilt population from the original reference strain.

II-1 Plants

II-1-1 Plants used between 2009 and 2012

Cotton seeds were planted in 48-cell trays filled with a soil-less medium (Baccto, Michigan peat company, Texas), and grown in the greenhouse until they reached the two true-leaf stage. Cotton seedlings were then planted in 6" pots filled with the same growing medium. Seedlings were treated with Pylon (chlorfenapyr, 0.98 ml/L) when they were in the cotyledon stage to prevent thrips and mites infestation. Cotton plants at the four to six true-leaf stage were used for the experiment.

II-1-2 Plants used in 2013

The same cotton seeds used in previous study (2009-2012) were used. However, cotton plants were grown with a different type of soil medium, Supermix perlite, (Michigan grower products, Inc, Michigan) provided by the greenhouses at Michigan State University. In addition, in previous experiments no fertilizer was used for growing cotton plants, but because unfertilized cotton plants grow very slowly, in 2013 soluble fertilizer (Everris, NA Inc., Ohio) was applied so that more plants could be grown for the final set of experiments. One hundred ml of a fertilizer

solution at 500 ppm N was applied twice per week to each plant.

II-2 Insecticides.

The formulated product Merit 75 WP (imidacloprid) was used in this study. Tap water was used to dilute the imidacloprid into the needed concentrations. The physicochemical properties of the water were not tested. Solutions were prepared immediately before each experiment.

II-3 Whitefly.

II -3-1 Whitefly used between 2009 and 2012.

II -3-1 a) Reference strain.

We have maintained a reference strain of the B biotype in the greenhouse (Chapter II section II-3). The second susceptible culture, initiated on February 2009, was used as a reference population for this study. Its LC_{50} value for imidacloprid determined between April 2009 and April 2011 ranged from 4.08 to 6.17 mg/L (Chapter II Table 2.2, 2.4).

II -3-1 b) Resistant strain.

The resistant strain was initially obtained from a commercial greenhouse as described in an earlier section (Chapter II section II-3). The recovered resistant population has been continuously reared on plants treated with imidacloprid at 12 mg a.i. per pot since May 2009. This population was 9-fold more resistant to imidacloprid when compared with the reference strain in June 2010 (LC_{50} was 37.47 mg/L) and April 2011 (LC_{50} was 54.19 mg/L, (Chapter II Table 2.4).

II-3-2 Whitefly used in 2013.

After July of 2012, when the initial research project was coming to an end the two whitefly cultures used for this research were reduced to the whiteflies on few plants in each cage. As the study was re-initiated in 2013, the resistant culture and susceptible culture used in previous years were re-built from January to March of 2013 for the next round of experiments. It is noteworthy that some mixing of these two populations occurred before and during the period of rebuilding because of holes in the screen cages. The population used for experiments in 2013 is from the original reference culture, after some immigration from the resistant culture between September 2012 and March of 2013. The LC_{50} to imidacloprid was not determined with a full range of test doses, but results from the first preliminary experiments in 2013, described below, indicate that this population now appears to have a low level of resistance to imidacloprid. It is also noteworthy that a different type of soil medium provided by the greenhouses in Michigan State University was used in the study. In addition, in previous experiments no fertilizer was used for growing cotton plants, but because unfertilized cotton plants grew very slowly, in 2013 soluble fertilizer was applied at the standard rate as a drench so that more plants could be grown for the experiments. Thus, the experiments in 2013 were conducted under conditions different from that of the previous years, and this required some preliminary experiments to determine the appropriate treatment concentrations.

II-4 Hormesis on the resistant and reference strains between 2009 and 2012.

Priming of an organism with a low dose of a toxicant can induce a stimulation of various processes (Stebbing, 2002, Chujo et *al*., 2012). In addition, a mild stress applied at early stage of an organism can have effect on the adult stage (Costantini *et al*., 2010). Therefore, in the current

study whitefly immatures were primed with imidacloprid, followed by a subsequent exposure of the same individuals to imidacloprid after they became adults.

II-4-1 Assay for the collection and priming of immature.

II-4-1-a) Assay with priming of immature.

Four to six new cotton plants in the four to five true-leaf stage were placed in the reference culture or the resistant culture to allow adults to lay eggs on them. Plants were removed after three days when they had enough eggs and were transferred into two separate cages. These plants were then treated with imidacloprid (Merit 75WP) applied as a soil drench by pouring 100 ml of the imidacloprid solution at the needed priming concentration (see each experiment below) around the base of the plants. The eggs were allowed to grow on the treated plants until the end of fourth stage, which occurs about 20 days after treatment, then pupae were collected from mature leaves. When collecting, the largest pupae of any group were selected as they usually turned out to be females. Pupae were collected by cutting a small piece (35-50 mm^2) of the cotton leaf under each pupa. Most of the pupae were collected individually on separate leaf discs, but when they were very close together, 2 or 3 pupae were collected on the same leaf disc. The small leaf disc with the pupa was then transferred into a 1.7 ml microcentrifuge tube and held at room temperature until adult emergence (one to two days).

II-4-1-b) Assay without priming of immature.

The procedure for the experiment without priming of immature was the same as the one with priming of immature as described above. The only difference is that the cotton plants containing eggs laid by the adult females were not treated with imidacloprid in this assay so that the whitefly nymphs grew and fed on untreated plants from egg hatch to end of the fourth instar. Then females were exposed to imidacloprid after pupation and emergence as described on the

assay for the adult exposure section below (II-4-2).

II-4-2 Assay for the collection and exposure of adults.

Upon emergence, the sex of the adults from the collected pupae was determined by checking the tip of their abdomen under a microscope to ensure they were females. The few males that were collected by mistake were discarded. Emerging females were transferred to treated leaf discs cut from cotton plants in the four to five true-leaf stage after they were treated systemically with imidacloprid (Merit 75WP) for three days at different concentrations (see each experiment) as a soil drench. Three plants were used for each treatment so that leaf discs used for each treatment could be cut from the same leaf of the same plant to minimize the effect of variation in the amount of imidacloprid in different plants. Each of the three plants provided replicate leaf discs for all treatments. Young upper leaves in the same stage of development were always used because the imidacloprid concentration in leaves can vary depending on their age and location on a plant (Olson *et al,* 2004). The leaf discs were placed in a 1.7 ml microcentrifuge tube containing a 1.25 ml layer of agar (1.3%) on the bottom. The microcentrifuge tubes were punctured several times with an insect pin for aeration before the experiment. Females were transferred as previously described (Chapter II) from the tube where they emerged to the new tube containing the treated leaf disc. The number of eggs laid by these females was monitored daily until the female's death. The longevity of the female was also recorded. Initially, each treatment consisted of fifteen female adults. Females which died due to the transfer, fungi or because of a decayed leaf disc were eliminated from the test, thus each treatment consisted of 8 to 15 replicates with each adult female on a separate leaf disc in a microcentrifuge tube being a replicate. The death of females was considered to be due to the imidacloprid when the females were seen to exhibit symptoms of toxicity including paralysis or

long lasting inertia before death, or if the death occurred with no injury in the microcentrifuge tube with a very clean leaf disc (no fungi, decay or excessive moisture that traps the female).

II-4-3 Test for hormesis in the parental generation.

II-4-3-a) Response of females of the reference and resistant colonies to imidacloprid.

For this experiment, pupae were either collected from the reference culture where immatures were not exposed to imidacloprid or from the resistant culture, where immatures grew on plants that were treated with imidacloprid (Merit 75 WP) at 12 mg a.i. per pot. The assay for the collection of pupae for adult emergence described above was followed (see II- 4-1-b). Then, the emerged adults were collected and exposed to leaf discs taken from plants treated with imidacloprid at different concentrations.

In hormesis, stimulation can be seen as a stimulation above the level of the control at doses where the agent does not elicit an observable adverse effect (Calabrese and Baldwin, 2003b) but the stimulation can also be seen over a wide range of dosage even at high dose that causes an adverse effects (Wang and Xing 2010, Crysper and Johnson, 2002, Stebbing, 2002). Thus in the current study, a wide range of concentrations of imidacloprid were used, from a very low dosage that would not be expected to show adverse effects in whitefly to relatively high doses.

The lowest concentrations used were estimated based on the published studies. Indeed, a decrease in fecundity induced by imidacloprid was often associated with reduction of excretion of honeydew in insects, thus through the feeding behavior (Devine *et al.*, 1996, Boina *et al*., 2009). The EC_{50} for the suppression of honeydew is the concentration that causes a 50% reduction of honeydew excretion in the insects. The EC_{50} for the suppression of honeydew

excretion by whitefly adults in a systemic assay was estimated at 0.037 mg/L with a 95% fidicual limit (FL) of 0.0059-0.11 (Nauen *et al.,* 1998). The concentration of imidacloprid that did not elicit any observable depression of honeydew excretion in another sucking insect, the aphid species, using a systemic assay was shown to be at 0.0001 mg/L (Nauen, 1995). Based on these published results, the lowest concentration of imidacloprid chosen was 0.00012 mg a.i per pot in the current study. The concentration of 6 mg a.i per pot was selected as the highest concentration, which is half of the rate of 12 mg a.i. per pot, a rate recommended for whitefly control in greenhouses. Four more concentrations of imidacloprid between these two concentrations were first used including 0.012, 0.012, 0.12, 1.2 mg a.i. per pot. These six concentrations were then used to study the response in fecundity of whitefly adults to imidacloprid exposures. The imidacloprid was applied according to the aforementioned method (see II- 4- 2). Water was used on control plants.

II-4-3-b) Response of resistant and reference females with different priming concentrations.

As no stimulation in fecundity was observed in the test described above, the effect of priming was tested by applying imidacloprid at an early stage of development of the whitefly. The test was then conducted on whiteflies from the reference strain and resistant strain that were exposed to imidacloprid during their immature stage and adult stage.

The dosage for the priming exposure is a factor that can affect the occurrence of hormesis (Stebbing, 2002). Perhaps because the mechanism causing hormesis is not well understood, little information is available on how to select a priming rate. The study of Stebbing *et al.* (2002) showed that doses below the threshold for an observable adverse effect could be used for priming exposure to induce stimulation. Similarly, the result of the study of Davies *et al.* (1995)

on yeast suggested that the low concentration of a stressor that induces a stimulation above the untreated control level could be used as a priming dose to study a conditioning hormesis. In the current study, whitefly individuals were first exposed to a single dosage through their whole life. For that, immatures were primed with the wide range of concentrations of imidacloprid used in the previous experiment (0.00012, 0.0012, 0.012, 0.12, 1.2, 6 mg a.i. per pot) by using the method described above (II-4-1-a). Then, the emerged adults were subsequently exposed to the same concentration used during their immature stage (0.00012, 0.0012, 0.012, 0.12, 1.2, 6 mg a.i. per pot) following the method described above (II-4-2) and their fecundity was assessed. Water was used for the control (0 mg a.i. per pot).

II-4-3-c) Response of resistant and reference females primed with 0.0012 mg a.i. per pot.

The concentration of 0.0012 mg a.i. per pot was selected as priming concentration based on the results of the previous experiment. The effect of priming of whitefly immatures with this concentration of 0.0012 mg a.i. per pot was further investigated by expanding the range of concentrations of the subsequent exposure by adding one lower concentration of imidacloprid and one higher concentration. The experiment followed the assay described above (II-4-1-a and 4-2). Therefore, emerged adults were subsequently exposed to eight imidacloprid concentrations (0.000012, 0.00012, 0.0012, 0.012, 0.12, 1.2, 6, and 12 mg a.i. per pot) as adults. Water was used for the control (0 mg a.i. per pot).

II-4-3-d) Response of resistant females with and without priming.

To determine if priming is needed for resistant whitefly to exhibit hormesis on fecundity, a comparison was made between the responses of resistant adults that were pre-exposed to the priming concentration of imidacloprid at 0.0012 mg a.i. per pot and those that were not primed

with imidacloprid (no priming: 0 mg a.i per pot) during their immature stage. The experiment was conducted as described above (II -4-1 and 2). Immatures of the resistant strain were either reared on plants treated with imidacloprid at 0.0012 m a.i. g per pot from eggs to pupae or they were reared on plants that were not treated with imidacloprid. Emerged adults from the collected pupae were then subsequently exposed to different concentrations of imidacloprid. Three additional concentrations were added to determine if resistant females exhibit a biphasic dose response in fecundity: two concentrations 0.0009 and 0.003 a.i per pot, thus near the concentration 0.0012 a.i per pot where stimulation was found, and one very high concentration 48 mg a.i per pot. Thus, the concentrations used were: 0.00012, 0.0009, 0.0012, 0.003, 0.012, 6, 12 and 48 mg a.i per pot. Water was used for the control (0 mg a.i. per pot).

II-4-3-e) Response of resistant females with high and low priming concentrations.

This test aimed to determine the effect of priming concentration of imidacloprid on hormesis. Immature resistant whiteflies were primed with a concentration of 0.0012 mg a.i per pot or 12 mg a.i per pot (a 1,000-fold greater concentration). The experiment was conducted by using the assay on the immature and adult exposure as described above (II -4 -1-a and 4-2). More concentrations for the subsequent exposure were added in this test to determine if resistant females exhibit a biphasic dose response in fecundity: 0.00012, 0.0003, 0.0009, 0.0012, 0.003, 0.006, 0.009, 0.012, 12 and 48 mg a.i per pot. Water was used for the control (0 mg a.i. per pot).

II-4-4 Test for hormesis in the F1 generation of resistant and reference strains.

A test on the F1 generation was conducted to determine whether hormesis can occur in the first generation progeny of the primed immatures. A new parental cohort was used in this study. Ten plants in the four to six true-leaf stage were placed in the reference culture and five

cotton plants were placed in the resistant culture for adults to lay eggs on. Plants were removed from the cultures after three days and transferred in two different cages. These plants with whitefly eggs were then treated with the priming concentration of imidacloprid (0.0012 mg a.i. mg per pot), so that the immatures of this parental generation grew and fed on treated plants. The other five plants introduced in the reference culture were not treated with imidacloprid (no priming) and were used as negative controls. Eggs were allowed to grow to pupae on all the treatments. When most of the eggs reached the pupal stage, at twenty days after eggs were deposited, five new untreated plants were introduced in each three cages (for primed and non - primed reference strain, and primed resistant strain). The newly emerged adults (parental generation) were allowed to fly to the untreated plants where they could lay eggs (F1 generation). The F1 immatures grew on untreated plants (no priming). Then, the old plants on which the immatures of parental generation grew were removed from the cage after one week, after almost all the adults had emerged. At this point the experiment followed the procedure described in section II 4-2. Pupae (F1) were collected for adult emergence, and upon emergence, the female adults (F1) were subsequently exposed to imidacloprid at different concentrations (0, 0.0012, 0.12, 0.12, 12, 24, 48 mg a.i mg per pot). Fecundity of females from three treatments groups was determined: F1 females of the resistant strain generated by primed parents (denoted as $F1_{RP)}$, F1 females of the reference strain generated by primed parents (denoted as $F1_{SP),}$ and F1 females of the reference strain generated from parents without priming exposure (denoted as $F1_{SN}$).

II-5 Hormesis in the rebuilt reference strain in 2013.

Results from experiments conducted between 2009 and 2012 suggest that the fecundity of female whiteflies can be stimulated by a very low priming dose given to the immature stage. The experiments gave some information about the range of priming doses of imidacloprid and the range of doses for subsequent exposures that result in the increased fecundity of primed individuals. The priming effect was observed consistently throughout experiments conducted from 2009 to 2012, but the experiments were not repeated to determine the reproducibility of the results. Therefore, a set of experiments was conducted in 2013 where a comparison could be made of the fecundity of primed and non-primed females. Instead of testing a wide range of subsequent exposure concentrations, more replications were added to a few selected subsequent exposure concentrations. Hormesis experiments from 2009 to 2012 also gave some interesting results about the F1 offspring of primed whiteflies. In the experiment the F1 offspring of adults that were primed as larvae also showed an increase in fecundity when they were given an additional exposure as the F1 adults. These F1 experiments also need to be repeated with more replicates per treatment. The goal for 2013 was to repeat the original priming experiment and the F1 experiment, each three times, for a total of six new experiments, after appropriate treatment doses were determined for the re-built culture.

Experiments in 2013 were conducted on the rebuilt population from the reference strain to determine whether priming of whitefly females with a low concentration of imidacloprid could protect them against the adverse effect of a subsequent and more severe imidacloprid exposure. Because the culture of whitefly used in 2013 is a re-constituted reference culture, I first tested subsequent exposure doses at concentrations expected to be just below levels that

cause mortality. However, because a new growing medium was used in 2013, and a fertilizer was used for the first time (no fertilizer was used in to grow plants between 2009 and 2012), the appropriate range of concentrations for the subsequent exposure still needed to be determined. Similarly, the effect of the priming concentration used in the previous years was assessed again under these new conditions.

II-5-1 Response of the rebuilt population from the reference strain without priming.

The experiments in 2013 were conducted under conditions different from that of the previous years, and this required some preliminary experiments to determine the relevant subsequent concentrations, thus first determining the concentrations that elicit reductions in fecundity due to a mild effect of imidacloprid but not a toxic effect causing mortality. Therefore, the response in fecundity of the rebuilt population of whitefly adults to a wide range of imidacloprid concentration was assessed first. The assay described in section II -4-1b and II-4-2 was followed. Initially seven concentrations (0, 0.00012, 0.0012, 3, 6, 9, 12, 15 mg a.i. per pot) were used based on results from experiments conducted in 2012. Because no significant decrease in fecundity was observed, another experiment was conducted with higher concentrations (12, 24, 48, and 60 mg a.i. per pot). Water was used as control.

Initially, each treatment consisted of twenty female adults with each adult female on a separate leaf disc in a microcentrifuge tube being a replicate. Females which died due to the transfer, fungi or decay of the leaf disc were eliminated from the test.

II-5-2 Response of the rebuilt population from the reference strain with priming.

Priming concentrations used in the 2013 experiments were selected from the priming concentrations that gave the greatest and most consistent hormesis effect when individuals were given a subsequent exposure as adults: 0.0012 imidacloprid per pot. However due to the new

conditions in 2013, the assessment of the same priming concentration was conducted. Another priming concentration close to 0.0012 mg a.i. per pot was added (0.00012 mg a.i. per pot) because this priming concentration was also shown to elicit a non-significant increase in fecundity in the 2009-2012 study.

A test on the fecundity of the rebuilt population was then conducted. Immature whiteflies were pre-exposed to the priming concentration 0.00012 and 0.0012 mg a.i per pot. Then, the emerged adults were collected and subsequently exposed to leaf discs treated with imidacloprid at subsequent concentrations as following the method described above (see II- 4-2). Subsequent exposure concentrations of imidacloprid of 36 and 72 mg a.i. per pot were used due to the results of the experiment above. To make sure that the imidacloprid drench did not leak through the pots the solution was applied uniformly over the surface of the soil.

The two concentrations were very toxic. However, the experiment was still carried out to provide information on the effect of a very severe subsequent exposure. Since the experiment was primarily intended to assess the effect of priming on the sublethal effect of imidacloprid on fecundity, another experiment with less toxic effect was also conducted. Therefore, as soon as it was observed that the two subsequent concentrations were highly toxic to the collected female adults, the extra primed and non-primed female pupae (primed as larvae, but not used in the experiment) were used for another experiment. Females were emerging rapidly from pupae, so drenches (12 mg a.i. per pot) made as a second exposure had to be limited to a 36 hour period before leaf discs were cut for the experiment instead of the standard 72 hour drench and uptake period. A lower subsequent exposure concentration (12 mg a.i. per pot) was used to avoid female mortality. Initially, each treatment consisted of twenty five female adults with each adult female on a separate leaf disc in a microcentrifuge tube being a replicate. Females which died due to the

transfer, fungi or a decayed leaf disc were eliminated from the test.

II-5-3 Test for hormesis on the parental generation of the rebuilt reference strain.

After the aforementioned experiments on the response of fecundity under the new cotton growing conditions, a test on hormesis was carried out. The effect of the priming exposure during the immature stage on the fecundity of the same individuals subsequently exposed as adults to the imidacloprid was then tested. Immatures were exposed to the two priming concentrations of imidacloprid (0.00012 and 0.0012 mg a.i per pot) and water was used for the non- primed females. The assay for the collection of pupae for adult emergence described above was followed (see II- 4-2) with a slight modification. Twelve plants in the four to six true-leaf stage were placed in the rebuilt reference culture for adults to lay eggs on. Plants were removed from the cultures after three to five days and transferred into three different rearing cages (24 X 24 X 72") made of polyester/nylon netting, mesh size 48 x 48. These plants with parental eggs were then treated with the priming concentration of imidacloprid (0.0012, 0.00012 or 0 mg a.i. mg per pot). Emerging adults were exposed to the subsequent exposure concentrations. Two subsequent exposure concentrations (6 and 12 mg a.i. per pot) were used due to the results of the previous experiment and the same volume of water was used in the drench for the control. Fifteen plants were used for each treatment. The imidacloprid solution was applied by pouring imidacloprid uniformly over the surface of the soil for both the priming exposure and the subsequent exposure. The experiment was placed in growth chamber set at 22 °C. The eggs were counted every other day except for the two first experiments during which eggs were counted daily. Eggs were removed from each tube every week to facilitate the counting. For that, the females were transferred into an empty microcentrifuge, the eggs on the leaf discs were removed with a white tissue and forceps, and then the female was transferred back to the tube

after the cleaning. Initially, each treatment consisted of thirty female adults with each adult female on a separate leaf disc in a microcentrifuge tube being a replicate. Females which died due to the transfer, fungi or decayed leaf disc were eliminated from the test. This experiment was repeated three times with three different cohorts. The first parental generation is referred to as $P1_1$. The second parental generation is referred to as $P1_2$. A third a parental generation is referred to as $P1_3$.

II-5-4 Test for hormesis on the first generation of the rebuilt reference strain.

A test on generation F1 was conducted to determine whether the stimulatory effect can be transferred to the first generation of offspring of the primed immatures. The assays followed the procedure described in section II-4-4 with a slight modification. When eggs of the primed (0.00012, 0.0012 a.i. per pot) or non- primed parental generation reached the pupal stage, four new untreated plants in the four to six true-leaf stage were introduced in each of the three rearing cages. The old plants on which the immatures of parental generation grew were removed from the cage after one week. The experiment followed the procedure described in section II 4-2. Female adults of the first generation were exposed to two subsequent exposure concentrations (6 and 12 mg a.i mg per pot) or a water drench.

This experiment on the F1 generation was repeated three times. The first generation progeny of the parental generation $P1_1$ is referred to as $F1_1$. Likewise, progeny of $P1_2$ is referred to as $F1_2$, and progeny of $P1_3$ as $F1_3$. Initially, each treatment consisted of thirty female adults with each female being placed on a separate leaf disc in a microcentrifuge tube.

II-6 Data analysis.

The analysis of effect of exposure to imidacloprid on fecundity and longevity of females was determined by using a generalized linear mixed model, PROC GLIMMIX with a log link function (SAS 9.3). As treatment with imidacloprid can affect the longevity of females, longevity was not included in the model for the analysis of the fecundity and the fecundity was expressed as number of total eggs laid by females. In these experiments the effect of exposure (priming exposure or subsequent exposure) was considered as a fixed effect, with the other exposure and the female replicates being considered as random effects.

For experiments with the rebuilt reference strain that were repeated three times, each experiment was treated as an experimental block. The degrees of freedom in the analysis were adjusted by using Kenward- Roger method. The priming exposure and subsequent exposure are fixed effects, and the experimental run and whitefly replicate are random effects. The interaction between the effects of priming and subsequent exposures was determined as well. The post-hoc Tukey-Kramer was used following detection of a significant difference among treatments.

III- Results.

III-1 Hormesis with the resistant strain.

III-1-1 Study of the effect of priming exposure on the resistant adults.

The response in fecundity of the resistant strain to an exposure of imidacloprid was assessed first. Since the resistant colony was continuously maintained on cotton plants treated with imidacloprid at 12 mg a.i. per pot, the immature stages of the tested females had been exposed to imidacloprid. The mean fecundity and longevity of these females are shown in the Table 4.1a. No significant difference was observed among the total egg production of females

Table 4.1a. Mean ± SE total egg production and longevity of females of *Bemisia tabaci* B biotype collected from the resistant colony and exposed to different concentrations of imidacloprid as adults.

Imidacloprid treatment (mg a.i. per pot)		Resistant strain		
Priming exposure (Single drench , continuous leaf exposure)	Subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
12	0	13	113.0 ± 5.2	22.0 ± 1.3
12	0.00012	12	101.9 ± 9.9	21.2 ±1 .3
12	0.0012	13	104.0 ± 7.6	20.4 ± 1.2
12	0.012	15	109.5 ± 8.6	21.9 ± 1.2
12	0.12	13	114.3 ± 6.9	22.0 ± 1.3
12	1.2	14	113.7 ± 8.3	21.6 ± 1.2
12	6	12	102.0 ± 10.5	21.5 ± 1.3

Table 4.1b. Summary of the generalized linear mixed model for the data in Table 4.1a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	6	85	0.52	0.79
Longevity					
	Imidacloprid exposure	6	85	0.20	0.97

Differences of means

Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	111.7a	9.8	0	22.0a	1.3
0.00012	96.5a	8.9	0.00012	21.2a	1.3
0.0012	100.5a	8.9	0.0012	20.4a	1.2
0.012	104.8a	8.6	0.012	21.9a	1.2
0.12	112.0a	9.8	0.12	22.0a	1.3
1.2	109.4a	9.3	1.2	21.6a	1.2
6	96.9a	8.9	6	21.4a	1.3

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer .

Table 4.2a. Mean ± SE total egg production and longevity of females of the resistant strain *Bemisia tabaci* B biotype primed with seven different concentrations of imidacloprid as immatures, then exposed to the same concentrations of imidacloprid as adults.

Imidacloprid treatment (mg a.i. per pot)		Resistant strain		
Priming exposure (single drench, continuous leaf exposure)	Subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0	0	15	70.6 ± 4.3	18.6 ± 1.3
0.00012	0.00012	14	78.0 ± 4.9	19.2 ±1.3
0.0012	0.0012	14	92.7 ± 5.7	20.9 ± 1.4
0.012	0.012	14	81.0 ± 5.0	20.5 ± 1.4
0.12	0.12	11	71.0 ± 5.1	19.6 ± 1.5
1.2	1.2	13	66.9 ± 4.4	19.9 ± 1.4
6	6	15	82.1 ± 4.9	18.8 ± 1.3

Table 4.2b. Summary of the generalized linear mixed model for the data in Table 4.2a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	6	89	2.81	0.01
Longevity					
	Imidacloprid exposure	6	89	0.54	0.77

Differences of means

Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	68.7b	4.5	0	18.6a	1.1
0.00012	76.4ab	5.1	0.00012	19.2a	1.1
0.0012	91.7a	6.0	0.0012	20.9a	1.2
0.012	79.4ab	5.3	0.012	20.5a	1.2
0.12	70.4ab	5.4	0.12	19.6a	1.3
1.2	65.6b	4.6	1.2	19.9a	1.2
6	80.8a	5.2	6	18.8a	1.1

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.3a. Mean ± SE total egg production and longevity of females of the resistant strain *Bemisia tabaci* B biotype primed with imidacloprid at 0.0012 mg a.i. per pot as immatures, and then exposed to different concentrations of imidacloprid as adults.

Imidacloprid treatment (mg a.i. per pot)		Resistant strain		
Priming exposure (single drench, continuous leaf exposure)	Subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0.0012	0	10	47.1 ± 4.6	14.9 ± 1.2
0.0012	0.000012	15	58.6 ± 4.6	16.8 ± 1.0
0.0012	0.00012	15	54.9 ± 4.4	16.6 ±1.0
0.0012	0.0012	15	64.9 ± 5.1	16.8 ± 1.0
0.0012	0.012	14	57.9 ± 4.7	16.2 ± 1.0
0.0012	0.12	8	51.2 ± 5.5	17.5 ± 1.4
0.0012	1.2	15	40.0 ± 3.3	15.8 ± 1.0
0.0012	6	15	53.6 ± 4.3	15.9 ± 1.0
0.0012	12	14	48.8 ± 4.1	16.8 ± 1.1

Table 4.3b. Summary of the generalized linear mixed model for the data in Table 4.3a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	8	112	3.26	0.002
Longevity					
	Imidacloprid exposure	8	112	0.92	0.92

Differences of means

Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean Longevity	SE
0	47.0ab	4.4	0	14.9a	1.2
0.00012	58.5a	4.3	0.00012	16.8a	1.0
0.00012	54.93ab	4.1	0.00012	16.6a	1.0
0.0012	64.9a	4.8	0.0012	16.8a	1.0
0.012	57.2a	4.4	0.012	16.2a	1.0
0.12	51.2ab	5.3	0.12	17.5a	1.4
1.2	39.9b	3.1	1.2	15.8a	1.0
6	53.5ab	4.0	6	15.9a	1.0
12	48.8ab	3.8	12	16.8a	1.0

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

treated with a wide range of concentration of imidacloprid from 0.00012 to 6 mg a.i. per pot ($p = 0.79$) (Table 4.1b). Similarly, imidacloprid treatments had no effect on the longevity of these resistant females ($p = 0.97$) (Table 4.1b).

After that test, the resistant females were treated with various concentrations of imidacloprid as immatures and then subsequently exposed to the same concentration as adults. The mean fecundity and longevity of these females are shown in the Table 4.2a. The analysis indicates a significant difference in the egg production of these resistant females ($p = 0.01$) (Table 4. 2b). A follow-up Tukey-Kramer test indicates that females primed with the imidacloprid concentration of 0.0012 mg a.i. per pot as immatures and then subsequently exposed to the same concentration as adults produced more eggs than the control females (91.7 eggs/female and 68.7 eggs/female respectively, $p = 0.04$) and that of the treatment at 1.2 mg a.i. per pot ($p = 0.01$) (Table 4.2b). No significant difference was observed in the longevity of these resistant females ($p = 0.77$) (Table 4. 2b).

Stimulation of egg production above the control level was found following a priming concentration of 0.0012 mg a.i. per pot. This concentration of imidacloprid 0.0012 mg a.i. per pot) was selected and studied further in the next round of experiments. Females primed with 0.0012 mg a.i. per pot were subsequently exposed to a wide range of imidacloprid concentrations. The mean egg production and longevity of these females are shown in Table 4. 3a. The test on the fecundity indicates a significant difference ($p = 0.002$) among treatments (Table 4.3b). The mean total number of eggs laid by females that were treated as adults at 1.2 mg a.i was lower than that of females treated with low concentrations from 0.00012 to 0.012 mg a.i per pot (Table 4.3b). The fecundity was found to peak at the subsequent exposure concentration of 0.0012 mg a.i. per pot (64.93 eggs/female) (Table 4.3a, b). The longevity of adult females

treated with all subsequent exposure concentrations of imidacloprid was slightly higher than the untreated adults , however, the analysis indicates no significant difference among treatments ($p = 0.92$) (Table 4.3b).

III-1-2 Comparison of fecundity and longevity of primed and non-primed resistant females.

The egg production and longevity of resistant females primed with imidacloprid at 0.0012 and 0 mg a.i per pot and then subsequently exposed to different concentrations of imidacloprid are shown in the Table 4.4a. The test on the effect of priming exposure on fecundity of these whitefly females indicates a significant difference ($p < 0.001$) (Table 4.4b). The mean number of eggs laid by females primed with imidacloprid at 0.0012 a.i. mg per pot was higher than that of the non-primed females (77.0 and 66.4 eggs/female, $p < 0.001$) (Table 4.4b). Thus, the result indicates an overall significant increase of 15% in egg production of the primed females. The results also indicate a significant difference in fecundity among the subsequent concentrations ($p < 0.001$) (Table 4. 4b). The follow-up Tukey Kramer test indicates that the number of eggs laid by females subsequently exposed to imidacloprid at 48 mg a.i per pot was lower than that of all other treatments (Table 4.4b). The test also indicates that the effect of priming on longevity of these female adults is not significant ($p = 0.65$) (Table 4.4 c). Subsequent exposure of females to imidacloprid also did not affect the longevity of females ($p = 0.20$) (Table 4.4c). This result also suggests that a low uptake of imidacloprid might happen with the highest treatment at 48 mg a.i per pot so that no decrease in longevity for the two groups of females (primed and non-primed) was seen at this high concentration 48 mg a.i per pot.

Table 4.4a. Mean ± SE fecundity and longevity of the resistant strain primed with imidacloprid at 0 and 0.0012 mg a.i. per pot as immatures, then exposed as adults to 9 concentrations of imidacloprid centered about 0.0012 mg ai per pot

Subsequent exposure concentration (single drench, leaf disc collected 72 h after application (mg a.i. per pot)	Resistant strain with no priming (0 mg a.i. per pot)			Resistant strain primed with 0.0012 mg a.i. per pot (single drench, continuous leaf exposure)		
	Adult female count	Eggs per female	Longevity (days until mortality)	Adult female count	Eggs per female	Longevity (days until mortality)
0	14	72.3. ± 3.9	18.5 ± 4.9	15	74.8 ± 4.4	18.4 ± 0.8
0.00012	12	68.7 ± 3.8	18.8 ± 5.4	15	78.4 ± 5.4	17.4 ±0.8
0.0009	13	82.9 ± 6.1	20.6 ± 5.7	14	92.6 ± 5.0	20.5 ± 0.7
0.0012	14	84.4 ± 5.3	20.2 ± 5.4	12	100.0 ± 3.0	20.9 ± 0.7
0.003	12	69.0 ± 5.1	19.6 ± 5.6	13	80.23 ± 6.0	19.3 ± 0.7
0.012	13	68.8 ± 3.6	19.3 ± 5.3	14	76.21 ± 3.0	19.0 ± 1.0
6	14	63.9 ± 5.3	17.4 ± 4.6	12	79.4 ± 5.4	18.8 ± 1.0
12	13	69.0 ± 4.8	19.5 ± 5.4	15	81.6 ± 3.9	21.0 ± 0.9
48	15	41.7 ± 3.9	18.5 ± 4.9	15	52.5 ± 5.0	20.2 ± 1.5

Table 4.4b. Summary of the generalized linear mixed model for the data on fecundity in Table 4. 4a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	244	19.68	<0.0001
Subsequent exposure	8	125	11.98	< 0.0001

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE
0	66.4a	4.5	0	73.1ab	6.3
0.0012	77.0b	5.2	0.00012	72.3ab	6.3
			0.0009	84.3ab	7.3
			0.0012	90.2a	7.9
			0.003	72.1ab	6.3
			0.012	71.4ab	6.2
			6	70.3b	6.1
			12	74.7ab	6.5
			48	45.2c	4.0

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.4c. Summary of the generalized linear mixed model for the data on longevity in Table 4. 4a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	244	0.25	0.61
Subsequent exposure	8	112	1.39	0.20

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	18.4a	0.7	0	18.4a	0.7
0.0012	18.0a	0.8	0.00012	18.0a	0.8
			0.0009	20.5a	0.8
			0.0012	20.5a	0.8
			0.003	19.5a	0.8
			0.012	19.2a	0.8
			6	18.1a	0.8
			12	20.3a	0.8
			48	19.6a	0.8

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer .

III-1-3 Comparison of the fecundity and longevity between resistant females primed with imidacloprid at 0.0012 and 12 mg a.i. per pot.

It was found in the previous experiment that a priming of the resistant females with 0.0012 mg a.i. imidacloprid per pot could stimulate their fecundity when they become adults. An experiment was designed to determine if a high priming concentration can also induce a stimulation of fecundity of the female whitefly. In this regard, a comparison between the effect of priming concentrations of imidacloprid at 0.0012 and 12 mg a.i. per pot on fecundity was performed. The mean fecundity and longevity of these females are shown in the Table 4.5a. The analysis indicates a significant effect of the priming concentration on the fecundity of the resistant females ($p = 0.01$) (Table 4.5b). The overall egg production by females primed with imidacloprid at 0.0012 mg.a.i per pot was significantly higher than that produced by females primed with imidacloprid at 12 mg a.i per pot (54.49 and 46.09 eggs/female, $p = 0.001$) (Table 4.5 b). The priming exposure of females with a low level of imidacloprid (0.0012 mg a.i. per pot) elicits an 18 % increase in egg production when compared to the females primed with a high concentration of imidacloprid (12 mg a.i. per pot). The effect of subsequent exposure also shows a significant difference between treatments ($p < 0.0001$) (Table 4.5 b). The fecundity of females exposed as adults to imidacloprid at 48 mg (32.37 eggs/female) was lower than that of the other treatments (Table 4. 5b). The fecundity of females treated with 12 mg a.i per pot was also lower than these of the females treated with low level of concentrations from 0.0003 to 0.009 mg a.i per pot (Table 4. 5b).

The priming exposure did not have a significant effect on the longevity of female adults ($p = 0.62$) (Table 4.5c). However the subsequent exposure influenced the longevity of females ($p < 0.001$) (Table 4. 5c). The fecundity of females subsequently treated with imidacloprid at 48 mg

Table 4.5a. Mean ± SE egg production and longevity of resistant females primed with imidacloprid at 0.0012 and 12 mg a.i. per pot as immatures, then exposed to different concentrations of imidacloprid as adults.

Subsequent exposure concentration (single drench, continuous leaf exposure) (mg a.i. per pot)	Resistant strain primed with 12 mg a.i per pot (single drench, leaf disc collected 72 h after application)			Resistant strain primed with 0.0012 mg a.i. per pot (single drench, leaf disc collected 72 h after application)		
	Adult female count	Eggs per female	Longevity (days until mortality)	Adult female count	Eggs per female	Longevity (days until mortality)
0	13	58.0 ± 7.3	22.3 ± 2.4	15	56.2 ± 9.0	20.2 ± 2.3
0.00012	13	57.3 ± 7.6	23.5 ± 2.5	12	63.5 ± 9.8	21.9 ±2.1
0.0003	13	62.7 ± 8.2	24.6 ± 2.8	15	73.6 ± 8.0	22.2 ± 2.1
0.0009	11	61.7 ± 7.5	23.3 ± 2.7	13	72.2 ± 6.5	25.0 ± 2.3
0.0012	13	72.8 ± 7.5	24.4 ± 2.1	15	94.1 ± 5.5	26.8 ± 1.3
0.003	11	58.9 ± 7.8	25.1 ± 1.6	14	78.1 ± 8.3	26.1 ± 2.3
0.006	8	70.6 ± 8.3	23.8 ± 1.8	10	90.5 ± 8.1	25.0 ± 1.7
0.009	12	58.0 ± 9.4	24.8 ± 2.7	14	81.4 ± 8.1	27.0 ± 2.5
0.012	9	38.4 ± 7.3	17.0 ± 2.1	9	53.3 ± 9.9	20.1 ± 2.2
12	14	28.8 ± 2.1	14.0 ± 0.5	12	36.3 ± 4.6	16.2 ± 1.6
48	12	19.9 ± 3.8	12.7 ± 1.4	12	17.4 ± 3.7	9.9 ± 1.2

Table 4.5b. Summary of the generalized linear mixed model for the data on fecundity in Table 4.5a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	245	6.59	0.01
Subsequent exposure	10	144	16.48	< 0.0001

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean fecundity	SE
0	54.4a	7.9	0	51.2abc	7.4
0.0012	46.0b	6.7	0.00012	52.8abc	7.7
			0.0003	63.5ab	9.2
			0.0009	65.6ab	9.7
			0.0012	79.3a	11.4
			0.003	65.4ab	9.6
			0.006	80.1a	12.4
			0.009	64.5ab	9.3
			0.012	40.0bc	6.3
			12	31.9c	4.7
			48	15.3d	2.4

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer .

Table 4.5c. Summary of the generalized linear mixed model for the data on longevity in Table 4.5a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	245	0.24	0.62
Subsequent exposure	10	144	10.97	<0.0001

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	21.0a	0.7	0	21.0ab	1.4
0.0012	20.4a	0.7	0.00012	22.1a	1.5
			0.0003	22.6a	1.5
			0.0009	24.4a	1.7
			0.0012	25.3a	1.7
			0.003	25.7a	1.8
			0.006	24.6a	1.9
			0.009	25.6a	1.7
			0.012	18.1ab	1.5
			12	14.9bc	1.1
			48	11.1c	0.9

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

a.i. per pot was also lower than that of females treated with low concentrations of imidacloprid from 0 to 0.009 mg a,i per pot (Table 4.5c). The longevity of females treated with imidacloprid at 12 mg a.i. per pot was also lower than that of the females treated with low concentrations from 0.00012 to 0.009 mg a.i per pot (Table 4.5c).

III-1-4 Study of the effect of priming of parents on F1 generation female offspring of the resistant strain.

The fecundity of resistant females of the F1 generation from primed parents was investigated (Table 4.6a). F1 females were not primed during their immature stage, only their parents were primed with imidacloprid. The analysis of their egg production indicates a significant difference among treatments ($p < 0.0001$) (Table 4.6 b). The follow-up Tukey-Kramer test indicates that egg production by $F1_{RP}$ females treated as adults with imidacloprid at 48 mg a.i. per pot (7.34 eggs/female) are significantly decreased compared with that of the untreated $F1_{RP}$ females as adults (42.6 eggs/female) ($p = 0.02$ and < 0.0001) (Table 4.6 c).The inhibitory effect was associated with the lethal effect of imidacloprid as suggested by the decrease in longevity at these high concentrations. Egg production peaked at a concentration of 0.0012 mg a.i. per pot (44.2.2 eggs/female), but the fecundity at this concentration is not significantly different from that of the untreated female adults (Table 4. 6b). The leaf disc used for the adult exposure was found to decay earlier in this treatment due to the low quality of the cotton leaves available. The analysis on longevity of female indicates a significant difference ($p < 0.0001$) (Table 4. 6c). The longevity of females treated as adults with imidacloprid at 24 and 48 mg a.i. per pot was lower than these of the other treatments (Table 4.6c).

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Table 4.6a. Mean ± SE egg production and longevity of the F1 generation females from resistant parents primed with imidacloprid at 0.0012 mg a.i. per pot exposed to different concentrations of imidacloprid as adults.

Imidacloprid concentration for adult exposure (mg a.i. per pot)	F1 resistant strain		
	Adult Female count	Eggs per female	Longevity (days until mortality)
0	12	42.6 ± 2.0	13.7 ± 0.4
0.00012	14	40.4 ± 3.5	13.2 ± 0.7
0.0012	12	46.2 ± 4.7	10.9 ± 0.8
0.012	14	37.5 ±2.7	13.7 ±0.2
12	14	40.7 ± 4.1	13.0 ± 1.0
24	15	25.6. ± 5.3	10.4 ± 1.3
48	15	10.8 ± 3.4	6.8 ± 1.4

Table 4.6b. Summary of the generalized linear mixed model for the data in Table 4.6a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	1	89	11.57	<0.0001
Longevity					
	Imidacloprid exposure	6	89	6.45	<0.0001

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	42.2ab	8.2	0	13.7a	1.7
0.00012	39.0ab	7.0	0.00012	13.2a	1.6
0.0012	44.3ab	8.6	0.0012	10.9a	1.4
0.012	36.6a	6.6	0.012	13.7a	1.7
12	38.5ab	6.9	12	13.0a	1.6
24	19.2b	3.4	24	10.4a	1.3
48	7.3c	1.4	48	6.8b	0.9

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

III-2 Hormesis in the reference strain (2009 - 2012).

The study on hormesis due to imidacloprid exposure as measured by fecundity was also conducted with the reference strain between 2009 and 2012. No fertilizer was used during this study.

III-2-1 Study of the effect of priming on the reference strain.

The response of the reference strain to exposure to different concentrations of imidacloprid was first determined when these female adults were not primed with imidacloprid as immatures (0 mg a.i per pot) and the results was shown in the Table 4.7a. The analysis of the results indicates a significant difference in the fecundity among treatments ($p = 0.01$) (Table 4.7b). The follow up pairwise comparison test indicates that egg production of females at 6 mg a.i per pot was significantly lower than that of untreated females (76.7 and 124.2 eggs/female, $p = 0.015$) (Table 4.7b). This indicates that a sublethal concentration of imidacloprid affects the fecundity of females of the reference strain. Imidacloprid exposure had no effect on the longevity of these reference females ($p = 0.64$) (Table 4.7b).

After observation of this adverse effect of imidacloprid on fecundity, the reference females were pre-exposed to various concentrations of imidacloprid as immatures, then exposed to the same concentration as adults (Table 4.8a). As opposed to the results without priming, no statistical difference was observed among treatments with these primed females of the reference strain ($p = 0.15$) (Table 4.8 b). The significant reduction in fecundity observed at higher concentration of imidacloprid (6 mg a.i per pot) in reference females without priming was not seen anymore. However, it was noteworthy that the effect of priming with a relatively high concentration of imidacloprid (6 mg a.i. per pot) on survivorship of the immatures was not assessed in this experiment. The egg production was observed to peak at the concentration of 0.00012 mg a.i. per

Table 4.7a. Mean ± SE egg production and longevity of females of *Bemisia tabaci* B biotype collected from the reference colony and exposed to different concentrations of imidacloprid as adults.

Imidacloprid treatment (mg a.i. per pot)		reference strain		
Priming (single drench, continuous leaf exposure)	Subsequent (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0	0	10	128.2 ± 10.3	23.9 ± 0.8
0	0.00012	14	115.9 ± 5.5	26.3 ± 1.0
0	0.0012	15	116.1 ± 8.0	25.9 ± 1.2
0	0.012	14	102.3 ± 8.7	24.5 ± 1.5
0	0.12	15	93.2 ±7.1	23.2 ± 1.2
0	1.2	15	97.6 ± 7.2	25.2 ± 1.7
0	6	14	81.7 ± 8.0	23.5 ± 1.5

Table 4.7b. Summary of the generalized linear mixed model for the data in Table 4.7a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	6	90	2.80	0.015
Longevity					
	Imidacloprid exposure	6	90	0.70	0.64

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	124.3a	13.9	0	23.8a	1.6
0.00012	114.1ab	10.8	0.00012	26.1a	1.4
0.0012	112.0ab	10.3	0.0012	25.9a	1.3
0.012	96.3ab	9.2	0.012	24.6a	1.3
0.12	89.5ab	8.3	0.12	23.2a	1.3
1.2	93.1ab	8.6	1.2	25.3a	1.3
6	76.7b	7.4	6	23.5a	1.3

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.8a. Mean ± SE total egg production and longevity of females of the reference strain of *Bemisia tabaci* B biotype primed with seven different concentrations of imidacloprid as immatures, then exposed to the same concentrations of imidacloprid as adults.

Imidacloprid exposure (mg a.i. per pot)		Reference strain		
Priming (single drench, continuous leaf exposure)	Subsequent (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0	0	14	72.1 ± 6.1	17.0 ± 1.0
0.00012	0.00012	14	90.4 ± 7.5	20.0 ± 1.4
0.0012	0.0012	14	84.4 ± 7.0	19.7 ± 1.2
0.012	0.012	12	77.7 ± 7.0	18.9 ± 1.0
0.12	0.12	10	67.0 ± 6.7	17.9 ± 1.2
1.2	1.2	13	66.6 ± 5.9	18.3 ± 1.4
6	6	14	71.1 ± 6.0	16.9 ± 1.2

Table 4.8b. Summary of the generalized linear mixed model for the data in Table 4.8a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	6	84	1.61	0.15
Longevity					
	Imidacloprid exposure	6	84	1.05	0.40

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	67.9a	6.8	0	16.9a	1.1
0.00012	88.3a	8.7	0.00012	20.0a	1.2
0.0012	81.7a	8.1	0.0012	19.7a	1.2
0.012	75.2a	8.1	0.012	18.9a	1.3
0.12	65.7a	7.8	0.12	17.8a	1.4
1.2	61.9a	6.4	1.2	18.3a	1.2
6	66.2a	6.6	6	16.9a	1.1

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.9a. Mean ± SE total egg production and longevity of females of the resistant strain *Bemisia tabaci* B biotype primed with imidacloprid at 0.0012 mg a.i. per pot as immatures, and then exposed to different concentrations of imidacloprid as adults.

Imidacloprid treatment (mg a.i. per pot)		Reference strain		
Priming (single drench, continuous leaf exposure)	Subsequent (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0.0012	0	10	36.7 ± 4.3	15.5 ± 1.2
0.0012	0.00012	15	44.3 ± 4.2	16.6 ± 1.0
0.0012	0.00012	14	51.3 ± 5.0	16.4 ± 1.0
0.0012	0.0012	15	46.2 ± 4.4	16.7 ± 1.0
0.0012	0.012	14	45.5 ± 4.5	15.0 ± 1.0
0.0012	0.12	8	35.3 ± 4.7	13.1 ± 1.2
0.0012	1.2	13	34.7 ± 3.6	16.1 ± 1.1
0.0012	6	13	40.5 ± 4.2	17.0 ± 1.1
0.0012	12	15	36.0 ± 3.5	15.9 ± 1.0

Table 4.9b. Summary of the generalized linear mixed model for the data in Table 4.9a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	8	108	1.84	0.07
Longevity					
	Imidacloprid exposure	8	108	0.88	0.53

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	36.6a	4.3	0	15.5a	1.2
0.00012	44.3a	4.2	0.00012	16.6a	1.0
0.00012	51.3a	5.0	0.00012	16.4a	1.0
0.0012	46.2a	4.4	0.0012	16.7a	1.0
0.012	45.5a	4.5	0.012	15.0a	1.0
0.12	35.3a	4.7	0.12	13.1a	1.2
1.2	34.7a	3.6	1.2	16.1a	1.1
6	40.5a	4.2	6	17.0a	1.1
12	35.9a	3.5	12	15.9a	1.0

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

pot (90.43 eggs/female) (Table 4.8a, b). No difference in longevity was observed among treatments (p = 0.40) (Table 4.8b).

The results of the previous experiments shows a non-significant increase of egg production when the reference strain was primed with concentrations of imidacloprid of 0.00012 and 0.0012 mg a.i. per pot. Also, the priming with imidacloprid at 0.0012 mg a.i. per pot causes a significant increase in the total egg production in the resistant strain (Table 4.2a). Thus this low concentration of imidacloprid (0.0012 mg a.i. per pot) was selected as the priming concentration for the next round of experiments. The females primed with this concentration 0.0012 mg a.i. perpot were subsequently exposed to a wide range of imidacloprid concentrations up to 12 mg a.i. per pot and the results are shown in the (Table 4.9a). The analysis of the results indicates an absence of significant difference in the fecundity of reference females (p = 0.07) (Table 4.9b). Thus, no significant difference was observed between the number of eggs deposited by the untreated female adults and the females subsequently exposed to imidacloprid at 12 mg a.i. per pot (36.70 and 36.00 eggs/female) (Table 4.9b). No difference in longevity was observed among treatments (p = 0.53) (Table 4.9 b).

III-2-2 Study of the effect of priming of parents on the F1 generation female progeny of the reference strain.

The fecundity and longevity of F1 reference female offspring of non-primed parents ($F1_{SN}$) and primed parents ($F1_{SP}$) was analyzed and the results are shown in the Table 4.10a. The effect of priming exposure of the parental generation on egg production of the F1 female adults indicates a significant difference (p = 0.0002) (Table 4.10b). The number of eggs laid by $F1_{SP}$ females from primed parents was higher than that of $F1_{SN}$ females from non-primed

Table 4.10a Mean ± SE egg production and longevity of the F1 generation of the reference females from the parents with or without priming with imidacloprid at 0.0012 mg a.i. per pot, then exposed to different concentrations of imidacloprid as adults.

Subsequent exposure concentration (single drench, continuous leaf exposure) (mg a.i. per pot)	Reference strain from non-primed parents			Reference strain from parents primed with 0.0012 mg a.i. per pot (single drench, leaf disc collected 72 h after application)		
	Female adult count	Eggs per female	Longevity (days until mortality)	Female adult count	Eggs per female	Longevity (days until mortality)
0	13	34.9 ± 3.2	12.0 ± 0.5	14	33.9 ± 3.2	11.5 ± 0.6
0.00012	14	28.2 ± 1.9	11.8 ± 0.7	13	39.0 ± 2.9	11.9 ± 0.9
0.0012	13	24.6 ± 2.2	9.6 ± 0.5	14	31.7 ± 3.1	10.8 ± 0.8
0.012	14	28.6 ± 2.9	12.0 ± 0.6	11	37.0 ± 2.3	13.9 ± 0.5
12	15	19.8 ±2.8	10.3 ± 1.1	13	36.7 ±4.3	11.9 ± 0.8
24	15	7.5 ± 1.7	7.6 ± 1.2	15	13.8 ± 3.2	8.6 ± 0.1.2
48	15	1.4 ± 0.6	2.8± 0.4	15	4.7 ± 2.5	5.1 ± 1.2

Table 4.10b. Summary of the generalized linear mixed model for the data on fecundity in Table 4.10a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	192	14.73	0.0002
Subsequent exposure	6	97	38.84	<0.0001

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE
0	13.6a	5.6	0	32.4a	6.5
0.0012	19.6b	8.0	0.00012	31.8a	6.4
			0.0012	26.7a	5.4
			0.012	30.6a	6.3
			12	26.1a	5.3
			24	9.1b	1.9
			48	1.9c	0.4

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.10c. Summary of the generalized linear mixed model for the data on longevity in Table 4.10a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	1	192	1.96	0.16
Subsequent exposure	6	97	22.69	<0.0001

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	11.7a	0.8	0	11.7a	0.8
0.0012	11.8a	0.8	0.00012	11.8a	0.8
			0.0012	10.2ab	0.8
			0.012	12.5a	0.9
			12	11.0a	0.8
			24	8.1b	0.6
			48	3.9c	0.4

*: means followed by different letters within the same column are statistically different according to Tukey-Kramer.

parents (19.6 and 13.6 eggs/female, p = 0.0002) (Table 4.10b), indicating an overall increase of 44 % in egg production of females that were generated from parents primed with imidacloprid during their immature stages. The subsequent exposure of female adults was also shown to induce a significant difference in fecundity of these females (p < 0.0001) (Table 4.10b). The fecundity of F1 reference females treated as adults with imidacloprid at 24 and 48 mg a.i. per pot was lower than that for females exposed to the lower treatment doses (Table 4.10b). The priming of the parental generation does not cause a statistical difference in the longevity of the F1 reference female offspring (p = 0.16) (Table 4.10c). However, the effect of subsequent exposure of F1 female adults to imidacloprid had a significant effect on longevity (p < 0.0001) (Table 4.10c). The F1 reference females treated as adults with imidacloprid at 48 mg a.i. pot had lower longevity than the F1 females treated with the other concentrations (Table 4.10c). Longevity of females treated with 24 mg a.i. per pot was also lower than that the other treatment except that of the treatment with imidacloprid at 0.0012 mg a.i. per pot (Table 4.10c).

III-3 Hormesis in the rebuilt reference strain (2013).

III-3-1 Study of the effect of the priming on females of the rebuilt reference strain.

The females of the rebuilt reference strain were exposed to various concentrations of imidacloprid from 0.00012 to 15 mg a.i. per pot. The results are shown in the Table 4.11a. Analysis of the data indicates no difference in fecundity among the treatments (p = 0.80) (Table 4.11b). There is also no significant difference in longevity among treatments (p = 0.57) (Table 4.11b, c).

As no significant decrease in fecundity was noticed, a second experiment was then conducted by using higher concentrations (up to 60 mg) as shown in Table 4.12a. The analysis of

Table 4.11a. Mean ± SE total egg production and longevity of female of the rebuilt reference strain exposed as adults to different concentrations of imidacloprid up to 15 mg a.i. per pot.

Imidacloprid treatment (mg a.i. per pot)		Rebuilt reference strain		
Priming (single drench, continuous leaf exposure)	Subsequent (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0	0	19	59.3 ± 2.5	16.0 ± 1.2
0	0.00012	18	62.0 ± 4.5	16.3 ± 1.2
0	0.0012	17	65.2 ± 3.5	16.9 ± 1.3
0	3	18	60.2 ± 2.5	18.1 ± 1.3
0	6	18	58.3 ± 4.1	16.5 ± 1.2
0	9	19	65.7 ± 3.0	16.7 ± 1.2
0	12	19	63.0 ± 2.3	18.5 ± 1.3
0	15	19	63.2 ± 4.3	17.2 ± 1.2

Table.4.11b. Summary of the generalized linear mixed model for the data in Table 4.11a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	7	139	0.54	0.80
Longevity					
	Imidacloprid exposure	7	139	0.81	0.57

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	58.7a	3.5	0	16.0a	0.9
0.00012	60.2a	3.7	0.00012	16.3a	0.9
0.0012	64.2a	4.0	0.0012	16.9a	0.9
3	59.6a	3.7	3	18.1a	1.0
6	56.8a	3.5	6	16.5a	0.9
9	65.0a	3.8	9	16.7a	0.9
12	62.5a	3.7	12	18.5a	0.9
15	61.4a	3.7	15	17.2a	0.9

*: means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.12a. Mean ± SE total egg production and longevity of female of the rebuilt reference strain exposed as adults to different concentrations of imidacloprid up to 60 mg mg a.i. per pot.

Imidacloprid treatment (mg a.i. per pot)		Rebuilt reference strain		
Priming (single drench, continuous leaf exposure)	Subsequent (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)
0	0	20	92.3 ± 5.9	15.6 ± 1.1
0	12	19	84.6 ± 4.3	17.4 ± 1.2
0	24	20	85.9 ± 5.7	16.3 ± 1.1
0	36	19	79.6 ± 5.4	18.7± 1.2
0	48	20	79.7 ± 3.9	18.2 ± 1.2
0	60	20	77.2 ± 7.2	16.7 ± 1.1

Table 4.12b. Summary of the generalized linear mixed model for the data in Table 4.12a.

Type III tests of fixed effects					
	Effect	Numerator df	Denominator df	F	P value
Fecundity					
	Imidacloprid exposure	5	112	1.06	0.38
Longevity					
	Imidacloprid exposure	5	112	1.61	0.16

Differences of means					
Fecundity			Longevity		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	92.3a	6.1	0	15.6a	0.8
12	87.6a	5.9	12	17.4	0.9
24	85.9a	5.7	24	16.3a	0.9
36	79.6a	5.4	36	18.7a	0.9
48	79.7a	5.3	48	18.2a	0.9
60	77.2a	5.1	60	16.7a	0.9

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

results indicates no significant difference in fecundity among treatments (p = 0.38) (Table 4.12b). However, a non-significantt decrease in egg production was observed for the relatively high concentrations of 36, 48 and 60 mg a.i. per pot when compared with the controls (Table 4.12b). It is noteworthy that the imidacloprid solution was poured at the base of the plants for these two experiments. The effect of priming of immatures with an exposure of 0, 0.000012 or 0.0012 mg a.i. imidacloprid per pot, on the fecundity of females of the rebuilt reference strain was tested by a subsequent exposure of females to imidacloprid at 36 or 72 mg imidacloprid a.i per pot. The results were shown in Table 4.13a. Results from this experiment indicates that the two subsequent exposure concentrations of 36 and 72 mg a.i per pot applied as drench over the entire soil surface were highly toxic. Many females showed symptoms of toxicity or died after the subsequent exposure for the three groups of females (non-primed and primed with 0.00012 and 0.0012 mg a.i. per pot) (Table A1). Apparently application of the drench over the entire soil surface increased uptake of imidacloprid. The effect of priming exposure on the fecundity of these females was not significantly different (p = 0.31) (4.13b). Thus, there is no difference between egg productions of non-primed females and females primed with 0.00012 and 0.0012 mg ai per pot. However, subsequent exposure was found to influence the fecundity of the females (p = 0.0005) (4.13b). The egg production of females treated as adults with imidacloprid at 72 mg a.i per pot was lower than that for females treated with 36 mg a.i per pot and for untreated female adults (38.6, 97.6, and 74.3, p = 0.0005, 0.01) (Table 4.13b). The priming exposure did not have a significant effect on the longevity of females (p = 0.42) (Table 4.13c). However, the subsequent exposure effected the longevity of females (p = 0.008) (Table 4.13c). The females treated as adults with imidacloprid at 72 mg a.i per pot had reduced longevity compared with females treated with imidacloprid at 36 mg a.i per pot and untreated female

Table 4.13a. Mean ± SE egg production and fecundity of females of the rebuilt reference strain when primed as immatures and then exposed as adults to imidacloprid at 0, 36 and 72 mg a.i. per pot.

Imidacloprid (mg a.i. per pot)	Priming exposure (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
Subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female	Longevity (until mortality)	Adult female count	Eggs per female	Longevity (until mortality)	Adult female count	Eggs per female	Longevity (until mortality
0	25	101.2 ± 4.8	14.8 ± 0.6	25	91.0 ± 5.0	14.0 ± 0.8	25	107.2 ± 7.0	14.6 ± 0.7
36	25	88.5 ± 8.0	15.2 ± 1.1	25	75.0 ± 10.2	12.9 ± 1.4	25	95.2 ± 9.9	15.1 ± 1.3
72	25	58.8 ± 10.0	12.6 ± 1.6	25	62.0 ± 10.5	11.9 ± 1.4	25	47.0 ± 11.4	10.0 ± 1.6

Table 4.13b. Summary of the generalized linear mixed model for the data on fecundity in Table 4.13a.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	2	222	1.15	0.31
Subsequent exposure	2	72	8.40	0.0005

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE
0	54.0a	26.8	0	97.6a	16.1
0.00012	37.7a	18.7	36	74.3a	12.3
0.0012	40.1a	19.9	72	38.6b	6.4

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.13c. Summary of the generalized linear mixed model for the data on longevity in Table 4.13a.

Type III tests of fixed effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	2	222	5.87	0.42
Subsequent exposure	2	72	5.07	0.008

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot))	Mean* longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	13.3a	0.8	0	14.4a	1.0
0.00012	11.9a	0.7	36	14.0a	1.0
0.0012	12.1a	0.7	72	10.8b	0.8

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.14a. Mean ± SE total egg production and longevity of females of the rebuilt reference strain exposed to three priming concentrations of imidacloprid (0, 0.00012 and 0.0012 mg a.i. per pot) as immatures and then exposed to plants systemically treated for 36 hours with imidacloprid at 0 and 12 mg a.i per pot.

Imidacloprid treatment (mg a.i per pot)	Priming exposure (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
Subsequent exposure (single drench, leaf disc collected 36 h after application)	Adult female count	Eggs per female	Longevity (days until mortality)	Adult female count	Eggs per female	Longevity (days until mortality)	Adult female count	Eggs per female	Longevity (days until mortality)
0	29	148.7 ± 8.0	16.3 ± 0.9	30	149.1 ± 8.3	15.8 ± 0.6	29	162.5 ± 8.3	16.5 ± 0.6
12	29	147.3 ± 9.5	16.0 ± 0.8	29	176.0 ± 7.8	17.1 ± 0.5	29	164.1 ± 8.7	16.5 ± 0.7

Table 4.14b. Summary of the generalized linear mixed model for the data on fecundity in Table 4.14a.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	2	172	2.05	0.13
Subsequent exposure	1	58	1.03	0.31

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE
0	140.2a	6.2	0	150.1a	5.8
0.00012	156.3a	6.8	12	158.7a	6.1
0.0012	156.0a	6.9			

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

Table 4.14c. Summary of the generalized linear mixed model for the data on longevity in Table 4.14a.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure	2	172	0.23	0.79
Subsequent exposure	1	58	1.03	0.31

Differences of means					
Priming exposure			Subsequent exposure		
Imidacloprid (mg a.i. per pot)	Mean* longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	15.7a	0.5	0	15.9a	0.4
0.00012	16.2a	0.5	12	16.1a	0.4
0.0012	16.1a	0.5			

*: Means followed by different letters within the same column are statistically different according to Tukey-Kramer.

adults (10.8, 14.4, 14.0 days, p = 0.01, 0.02) (Table 4.13c).

Another test was also conducted by using a shorter exposure time (36 hours) with a lower subsequent exposure concentration (12 mg a.i. per pot) as shown in the Table 4.14a. Analysis of the data shows that the effect of priming exposure on the fecundity of the female adults was not statistically significant (p= 0.13) (Table 4.14b). The overall egg production of females primed with imidacloprid at 0.00012 and 0.0012 mg a.i per pot was higher than that of non-primed females but not statistically different (p = 0.18, 0.19) (Table 4.15b). The effect of subsequent exposure on fecundity among the treatments was also not statistically significant (p = 0.31) (Table 4.14b) as well as the effect of priming exposure on longevity (p =0.79) (Table 4.14c). Similarly, the subsequent exposure did not affect the longevity of the rebuilt reference female (p =0.63) (Table 4.15c).

III-3-2 Study of the effect of priming on the parental generation of the rebuilt population.

At this point there was enough information to run an experiment on parental generation that could be repeated several times. The three parental generations are referred to as $P1_1$, $P1_2$ and $P1_3$. The fecundity and longevity of the females of these parental generation are shown in Table 4. 15 a,b,c. Analysis of the data indicates no interaction of the effects of priming exposure and subsequent exposure on the egg production of the females (p = 0.34) (Table 4. 15d). However, the effect of priming exposure on egg production of females shows a significant effect (p = 0.004) (Table 4.15d). The follow-up Tukey-Kramer test indicates that egg production of females primed with imidacloprid at 0.00012 and 0.0012 mg per pot was higher than that of the non-primed females (186.7, 191.1 and 169.9, eggs/female, p = 0.03, 0.004) (Table 4.15d). This suggests an increase in fecundity of the rebuilt reference females primed with low concentrations

Table 4. 15a. Mean ± SE total egg production and longevity of the rebuilt reference females of the parental generation $P1_1$ exposed to three priming concentrations of imidacloprid (0, 0.00012 and 0.0012 mg a.i. per pot) as immatures and then exposed to plants systemically treated for 72 hours with imidacloprid at 0, 6 and 12 mg a.i. per pot.

Imidacloprid concentration (mg a.i. per pot	Priming exposure (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female (P_{11})	Longevity (days until mortality)	Adult female count	Eggs per female (P_{11})	Longevity (days until mortality)	Adult female count	Eggs per female (P_{11})	Longevity (days until mortality)
0	27	114.0 ± 7.9	20.8 ± 1.2	30	101.1 ± 8.9	17.7 ± 1.4	29	129.0 ± 11.4	21.7 ± 1.4
6	30	126.7 ± 11.5	23.4 ± 1.8	30	163.2 ± 15.1	25.5± 1.8	28	163.4 ± 14.0	25.7 ± 1.8
12	30	116.6 ± 10.0	17.7 ± 1.4	30	140.4 ± 13.2	18.8 ± 1.6	28	121.9 ± 0.6	17.7 ± 1.4

Table 4.15b Mean ± SE total egg production and longevity of the rebuilt reference females of the parental generation $P1_2$ exposed to three priming concentrations of imidacloprid (0, 0.00012 and 0.0012 mg a.i. per pot) as immatures and then exposed to plants systemically treated for 72 hours with imidacloprid at 0 and 6 mg a.i. per pot.

Imidacloprid concentration (mg a.i. per pot	Priming exposure (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	eggs per female ($P1_2$)	Longevity (days until mortality)	Adult female count	eggs per female ($P1_2$)	Longevity (days until mortality)	Adult female count	eggs per female ($P1_2$)	Longevity (days until mortality)
0	27	119.6 ± 9.9	17.6 ± 1.0	30	127.3 ± 8.8	18.1 ± 1.2	29	140.3 ± 10.4	20.1 ± 1.0
6	29	133.9 ± 9.8	20.1 ± 1.6	27	153.6 ± 14.4	19.6± 1.4	28	166.6 ± 11.8	20.3 ± 1.4

Table 4. 15c. Mean ± SE total egg production and longevity of the rebuilt reference females of the parental generation $P1_3$ exposed to three priming concentrations of imidacloprid (0, 0.00012 and 0.0012 mg a.i. per pot) as immatures and then exposed to plants systemically treated for 72 hours with imidacloprid at 0, 6 and 12 mg a.i. per pot.

Imidacloprid (mg a.i. per pot)	Priming exposure (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female ($P1_3$)	Longevity (days until mortality)	Adult female count	Eggs per female ($P1_3$)	Longevity (days until mortality)	Adult female count	Eggs per female ($P1_3$)	Longevity (days until mortality)
0	28	312.7 ± 18.3	29.7 ± 1.8	28	342.2 ± 20.8	32.3 ± 2.0	29	316.0 ± 17.5	28.0 ± 1.9
6	29	304.7 ± 17.0	28.5 ± 1.8	28	325.6 ± 25.1	29.8 ± 2.2	30	351.3 ± 21.9	30.4 ± 2.1
12	30	344.0 ± 19.5	32.6 ± 1.7	29	349.0 ± 17.7	29.4 ± 1.5	29	365.5 ± 20.6	31.7 ± 1.7

Table 4.15d. Summary of the generalized linear mixed model for the data on fecundity in Table 4.15a, b, c.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure (P)	2	674.3	5.53	0.004
Subsequent exposure (S)	2	674.2	8.45	0.0002
P X S	4	674.6	1.13	0.34

Differences of means					
Priming exposure (P)			Subsequent exposure (S)		
Imidacloprid (mg a.i. per pot)	Mean * fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* fecundity	SE
0	169.3a	51.7	0	168.7a	51.4
0.00012	186.7b	56.9	6	195.2b	59.4
0.0012	191.8b	58.4	12	184.7ab	56.4

*Mean followed by the same letter are not statistically different according to Tukey-Kramer.

Table 4.15e. Summary of the generalized linear mixed model for the data on longevity in Table 4.15 a, b, c.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure (P)	2	672.1	0.31	0.73
Subsequent exposure (S)	2	667.6	6.25	0.002
P X S	4	672.5	0.20	0.93

Differences of means

Priming exposure (P)			Subsequent exposure (S)		
Imidacloprid (mg a.i. per pot)	Mean * longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	22.8a	3.3	0	22.4a	3.2
0.00012	22.7a	3.3	6	24.6b	3.6
0.0012	23.3a	3.4	12	21.8a	3.2

*: Mean followed by the same letter are not statistically different according to Tukey-Kramer.

of imidacloprid during immature stages. The effect of the subsequent exposure on the egg production of the females also indicates a significant difference among treatments ($p = 0.0002$) (Table 4.15d). The egg production of females treated as adults with imidacloprid at 6 mg a.i per pot was higher than that for untreated female adults (195.2 and 168.7 eggs/female, $p = 0.0001$) (Table 4.14d). The fecundity of the females of these three parental generations of the rebuilt reference strain was illustrated in the cumulative mean egg production graphs (Figure A3, A4, and A5). The graph of the cumulative fecundity of primed females was seen to be above of that of the non-primed females at the concentration of 6 mg a.i per pot. The analysis also indicates that there is no interaction of the effects of priming exposure and subsequent exposure on longevity of the females ($p = 0.93$) (Table 4.15e). Similarly, the effect of priming exposure on longevity was also not statistically significant ($p = 0.73$) (Table 4.15e). However, the effect of the subsequent exposure on longevity was statistically significant among the treatments ($p = 0.002$) (Table 4. 15e). Females treated as adults with imidacloprid at 6 mg a.i per pot have an increased longevity compared with females treated with imidacloprid at 0 and 12 mg a.i. per pot (24.6, 22.4 and 21.8 days, $p = 0.01, 0.005$) (Table 4.15e).

III- 3-3 Study of the effect of priming of parents on the first generation of the rebuilt reference strain.

The effect of priming was found to be transferred to the offspring of the first generation of the reference strain in the study of 2009-2012. Therefore, a test on the first generation of offspring was also conducted on the rebuilt reference strain to determine if a priming effect can be transferred to the offspring. The fecundity and longevity of the first generation of female offspring from three experiments ($F1_1$, $F1_2$, $F1_3$) are shown in the Table 4.16a, b, c. Data analysis indicates that the priming exposure of the parental generation did not cause a significant

Table 4.16a. Mean ± SE total egg production and longevity of the $F1_1$ female progeny of the rebuilt reference strain from parents with and without priming exposure to imidacloprid exposed as adults to imidacloprid at 0, 6 and 12 mg a,i. per pot.

Imidacloprid (mg a.i. per pot)	Priming exposure of parents (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female ($F1_1$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_1$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_1$)	Longevity (days until mortality)
0	27	222.5 ± 21.4	29.7 ± 3.1	29	255.1 ± 21.5	31.5 ± 2.6	27	259.8 ± 19.7	36.0 ± 2.8
6	28	273.3 ± 24.5	36.2 ± 2.8	28	258.7 ± 16.7	31.7± 2.0	26	295.5 ± 21.1	37.7 ± 2.7
12	25	263.4 ± 22.7	31.6 ± 2.5	26	244.2 ± 26.3	27.1 ± 2.6	26	304.3 ± 22.9	34.3 ± 2.5

Table 4.16b. Mean ± SE total egg production and longevity of the $F1_2$ female progeny of the rebuilt reference strain from parents with and without priming exposure to imidacloprid exposed as adults to imidacloprid at 0 and 6 mg a,i. per pot.

Imidacloprid (mg a.i. per pot)	Priming exposure of parents (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female ($F1_2$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_2$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_2$)	Longevity (days until mortality)
0	27	214.0 ± 16.6	33.3 ± 1.9	29	212.6 ± 16.4	31.0 ± 1.7	27	179.2 ± 21.2	27.5 ± 2.2
6	28	216.2 ± 20.6	32.9 ± 1.9	28	232.5 ± 16.4	32.0 ± 1.5	26	223.0 ± 19.6	30.3 ± 2.1

Table 4.16c. Mean ± SE total egg production and longevity of the $F1_3$ female progeny of the rebuilt reference strain from parents with and without priming exposure to imidacloprid exposed as adults to imidacloprid at 0, 6 and 12 mg a,i. per pot.

Imidacloprid (mg a.i. per pot)	Priming exposure of parents (single drench, continuous leaf exposure)								
	0			0.00012			0.0012		
subsequent exposure (single drench, leaf disc collected 72 h after application)	Adult female count	Eggs per female ($F1_3$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_3$)	Longevity (days until mortality)	Adult female count	Eggs per female ($F1_3$)	Longevity (days until mortality)
0	28	324.7 ± 18.7	37.2 ± 1.5	28	277.9 ± 21.1	34.6 ± 2.0	29	303.2 ± 22.4	34.8 ± 2.0
6	29	332.2 ± 16.6	37.8 ± 1.5	28	347.8 ± 20.4	42.1± 21.0	30	351.0 ± 26.8	38.7 ± 1.6
12	30	299.0 ± 19.6	36.8 ± 2.1	29	294.1 ± 27.4	34.3 ± 2.0	29	364.8 ± 19.1	38.9 ± 1.6

Table 4.16d. Summary of the generalized linear mixed model for the data on fecundity in Table 4.16a, b, c. .

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure (P)	2	643	2.05	0.125
Subsequent exposure (S)	2	643.9	5.01	0.006
P X S	4	643.2	1.89	0.11

Differences of means					
Priming exposure (P)			Subsequent exposure (S)		
Imidacloprid (mg a.i. per pot)	Mean * fecundity	SE	Imidacloprid (mg a.i. per pot)	Mean* Fecundity	SE
0	257.3a	31.4	0	248.0a	30.1
0.00012	257.0a	31.4	6	278.6b	33.9
0.0012	275.7a	33.7	12	263.8ab	32.6

*: Mean followed by the same letter are not statistically different according to Tukey-Kramer.

Table 4.16e. Summary of the generalized linear mixed model for the data on longevity in Table 4.16 a, b, c.

Type III tests of Effects				
Effect	Numerator df	Denominator df	F	P value
Priming exposure (P)	2	3.93	0.64	0.57
Subsequent exposure (S)	2	647	18.04	<0.0001
P X S	4	647	2.93	0.02

Differences of means					
Priming exposure (P)			Subsequent exposure (S)		
Imidacloprid (mg a.i. per pot)	Mean * longevity	SE	Imidacloprid (mg a.i. per pot)	Mean* longevity	SE
0	34.1a	2.2	0	32.8a	1.8
0.00012	32.2a	2.1	6	35.4b	2.0
0.0012	34.0a	2.2	12	32.2a	1.8

*: Mean followed by the same letter are not statistically different according to Tukey-Kramer.

difference on fecundity of F1 female offspring among the treatments ($p = 0.12$) (Table 4.16d). The fecundity of the F1 progeny from primed parents was higher than that of the F1 progeny from the non-primed parents (175 and 157 eggs/female), however this increase is not statistically different. The cumulative mean egg production of these F1 females offspring was also shown in Figure A6, A7 and A8. The graph of cumulative fecundity of F1 females from primed parents at 0.0012 mg a.i. per pot was seen to be above of that of the two others groups of females at 12 mg a.i. per pot. The effect of the subsequent exposure of the F1 progeny on their fecundity was statistically significant ($p = 0.006$). The fecundity of F1 rebuilt reference females treated as adults with imidacloprid at 6 mg a.i. per pot was higher than the untreated F1 female adults (0 mg a.i. per pot) (278. 6 and 248.0 eggs/female , $p = 0.004$) (Table 4.16d). The effect of the priming exposure of the parental generation on longevity of the F1 female progeny was not significantly different among the treatments ($p =0.57$) (Table 4.16e). However, the effect of the subsequent exposure on longevity was statistically significant ($p < 0.0001$). The longevity of rebuilt reference females treated as adults with imidacloprid at 6 mg a.i per pot was higher than for females treated with 0 and 12 mg a.i. per pot (35.4, 32.8 and 32.2 days, $p <0.0001$, <0.001). Analysis of longevity data for the F1 progeny females also shows an interaction of the effects of priming of the parent generation and the subsequent exposure of the F1 female progeny ($p = 0.02$).

IV Discussion

Low concentrations of toxicants can induce a stimulation of many biological processes in a variety of organisms including insects, and this phenomenon is known as hormesis (Calabrese and Blain, 2005). In hormesis, the stimulation can be revealed as an increased positive response

above the level of the control, or it can also be expressed as an adaptive response of the organism to a second and more severe exposure to the stressors (Calabrese and Baldwin, 2002; Stebbing, 2009). Several important factors can determine the occurrence of the stimulation, among them are the dose of the toxicants and the stage of the development of the organism. This study set a hypothesis that treatment of whitefly females with low doses of imidacloprid applied at an early stage in their development will induce an increase in fecundity of the same females when they become adults.

A wide range of concentrations was first tested to determine the priming concentration used to treat the immature stage. A stimulatory response in fecundity of adult females in the resistant strain was observed for individuals that were primed with imidacloprid at 0.0012 mg a.i. per pot during the immature stage then treated with the same concentration as adults (Table 4. 2b, c). This result suggests that priming of females at this concentration during immature stage might increase the fecundity of adult females. Indeed, in a following test, an overall increase in fecundity of 15% was noticed in resistant females primed with imidacloprid at 0.0012 mg a.i per pot when compared to females without priming and using a wide range of the subsequent exposure concentrations (Table 4.4b). An overall 18% increase in egg production was also observed for these resistant females primed with 0.0012 mg a.i per pot compared to that of females primed with 12 mg a.i per pot (Table 4.5b). This also suggests that only a low concentration of imidacloprid may elicit a stimulation in fecundity. In a related experiment, Ayyanath *et al.* (2013) exposed immatures of the aphid *M. persicae* to imidacloprid for several generations, and these authors found that only a continuous exposure to low concentrations of imidacloprid of 0.025 µg/l caused an increase in fecundity of the aphids whereas the continuous exposure to the higher concentrations (0.1 to 25 µg/L) did not cause a stimulation.

Preliminary tests were also conducted with the reference strain to determine appropriate subsequent and priming concentrations. A desirable subsequent exposure concentration is one that decreases fecundity without causing significant mortality. In an initial test, the fecundity of the non-primed reference females decreased after they were treated with imidacloprid at 6 mg a.i per pot, without causing significant mortality (Table 4.7b). This is consistent with a report on reduction of egg production in whitefly females exposed to sublethal dose of imidacloprid. He *et. al.* (2013) showed that females of *B. tabaci* treated with imidacloprid at a sublethal concentration of 5 ppm for 24h exhibited a reduction of egg production. Then a test was conducted to determine an appropriate priming concentration of imidacloprid for treating immatures. Two low concentrations, 0.0012 and 0.00012 mg a.i. per pot, were found to elicit a non-significant increase of the fecundity of adults (4.8a,b). The reference females were then primed with the concentration of 0.0012 mg a.i per pot in the following experiment; and as opposed to the previous results without priming, no decrease of fecundity was seen with the reference females even at a relatively high subsequent exposure concentration of 12 mg a.i. per pot (4.9b). However, since no females without priming were used as controls in that experiment, it is not known whether this disappearance of inhibition of egg production might be due to reduced uptake of imidacloprid by the plants. A test on the F1 generation was also conducted to assess the inheritance of a priming effect with the reference strain. The reference female progeny from primed parents exhibited 44% higher fecundity than the female progeny from non-primed parents when they were exposed to different concentrations of imidacloprid as adults (Table 4. 10b).

After observing a stimulation in fecundity of whitefly that were primed as immatures with a low dose of imidacloprid, the study was re-initiated with the rebuilt reference strain for a

set of experiments to confirm these results. It is noteworthy that for this new set of experiments fertilizer was used to grow cotton plants more rapidly, and that the MSU greenhouses had switched to a new growing medium that we used to grow our plants. The initial objective was to investigate if priming of whitefly females with a low concentration of imidacloprid could protect them against the adverse effect of a subsequent and more severe imidacloprid exposure, a phenomenon known as conditioning hormesis (Calabrese and Mattson, 2011). However, no decrease in fecundity was observed for females exposed to concentrations up to 15 mg a.i. per pot (Table 4.11b). Only a slight but not significant decrease of egg production was observed when the imidacloprid concentration was increased up to 60 mg a.i. per pot (4.12 a, b).

A test was conducted using different priming concentrations (0, 0.0012, 0.00012 mg a.i. per pot) and relatively high subsequent exposure concentrations (36 and 72 mg a.i per pot). In contrast to the results of the aforementioned experiment, these high concentrations exhibit toxicity in the females (Table 4.13b). It is noteworthy that in this experiment, the imidacloprid solution was applied uniformly over the soil surface instead of pouring the solution around the base of the plants, to ensure that the solution did not leak through the new potting soil. The uptake of imidacloprid by the plants may have been more efficient when the imidacloprid drench was applied in this way; however no experiment was conducted to confirm this. In brief, no difference in fecundity was observed between primed and non-primed females and the exposure of females to concentrations 72 mg a.i per pot caused a significant reduction in fecundity of all females in the experiment (Table 4.13b, c). A significant decrease in fecundity was also observed when primed females of the resistant and reference strain were exposed to concentrations of 24 and 48 mg a.i. per pot (Table 4. 4b, c). These results suggest that above a certain level of severity of subsequent exposures, the effect of priming on the stimulation of fecundity may no longer be

efficient. The treatment with the higher concentration of imidacloprid (72 mg a.i per pot) was then found to cause a decrease in fecundity and high mortality in this experiment whereas the treatment with 36 mg a.i per pot elicits various symptoms of toxicity in the females but did not cause a significant decrease of their fecundity (Table 4.13b, c). This lack of an adverse effect of high concentration of imidacloprid on fecundity on this rebuilt reference strain may be associated with some increase in resistance due to movement of the whiteflies between cages. But some differences in these results compared with earlier experiments may also be associated with the use of fertilizer. In fact, Bi *et al.* (2001) already showed that fertilizer can modulate the metabolism of sugar and amino acid in cottons, which consequently enhance the growth of adults and immatures of *B. tabaci* B biotype. He *et al*. (2013) showed that the adverse effect of sublethal dose of imidacloprid on reproduction of whitefly *B. tabaci* was associated with the antifeedant effect of this compound. Therefore, the use of fertilizer on the cotton plants might increase the nutrients available to the whitefly, which may consequently soften the antifeedant effect. This may partially explain the difficulty of finding a sublethal concentration that negatively affects fecundity without causing mortality of the females under the new conditions. Thus, the lower concentrations of 6 and 12 mg a.i per pot that did not elicit symptoms of toxicity and did not affect mortality under the conditions of this study were used in the next set of experiments to test the effect of priming on fecundity. The stimulatory effect of priming on fecundity was evaluated in these experiments but the protective effect of the priming exposure, thus the conditioning hormesis, could not be evaluated. In this new set of experiments with the rebuilt reference strain it was found that the fecundity of females primed as immatures with imidacloprid at 0.00012 and 0.0012 mg a.i. per pot, and then given a subsequent exposure as adults at concentrations of 0, 6 or 12 mg a.i per pot, was 9% and 12% greater than that of non-

primed females (Table 4.15d). It was also noticed that fecundity of females given subsequent exposures of 6 mg a.i. per pot as adults was higher than that for females given a subsequent exposure of 0 or 12 mg a.i. per pot (Table 4.15d). The test was also conducted on the F1 progeny. However, no difference in fecundity was observed between the F1 female offspring from primed and non-primed parents.

Hormesis is a phenomenon characterized by a stimulation of various biological processes by a low dose of toxicant and inhibition of these processes by high doses of the toxicant (Calabrese and Baldwin, 2002). Numerous studies have reported a stimulation of different biological endpoints by sublethal doses of insecticides in insects as cited in the review paper of Cutler (2013). The results of our study suggest that application of imidacloprid at an early stage elicits an increase in the fecundity of whitefly adults. Such hormesis in reproduction has already been observed in some insects such as aphids (*M. persicae)* exposed to a low dose of imidacloprid (Annyath *et al*, 2013), a parasitoid wasp (*Pimpla turionellae)* exposed to a low dose of malathion (Büyükgüzel, 2006), and in a predatory stinkbug, *Podisus distinctus*, exposed to a low dose of permethrin (Zanuncio *et al*., 2013). These studies are similar to ours when considering the fact that the insecticide exposure was carried out during immature stages of these species. The study of a parasitoid wasp and of a stink bug show that reproduction in these two species were stimulated by a single treatment of the immature stage (Büyükgüzel, 2006, Zanuncio *et al*., 2013). In the parasitoid study, the wax moth host, *Galleria mellonella,* was fed a diet with a very small amount of malathion (0.01 and 0.1 ppm). The parasitoid, *P. turionellae*, grew in the pupal stage of the moth, and after adult emergence from the host, exhibited an increase in fecundity (Büyükgüzel, 2006). Similarly, the third instar of a stinkbug was treated

once with low dose of permethrin applied as topical treatment, and the emerging female adult exhibited a hormesis, without a subsequent exposure of the adults. In our research with whiteflies, fecundity was also observed with the priming of the immature stage. However, it is noteworthy that a consistent trend throughout all sets of our hormesis experiments is that a subsequent exposure of adults seems to affect the observed level of increase in fecundity, while the fecundity of primed and non-primed females that were not treated as adults with imidacloprid are not different. The results of Ayyanath *et al*. (2013) also showed that fecundity increased when aphids were continuously exposed to a low concentration of imidacloprid (0.025 µg/L) and reproduction was not increased if the aphids were treated only once in the first instar with the same concentration 0.025 µg/L. However, these authors found an increase in fecundity of aphids when females were treated with imidacloprid once during the immature stage with a higher concentration of imidacloprid (0.1 and 10 µg/L) (Ayyanath *et al*. 2013). In fact, numerous authors already suggested that time is an important factor in the study of hormesis, and that the occurrence of hormesis might be determined by the pharmacodynamic and pharmacokinetic of the chemicals, thus the level of the dose of the chemicals and its related effect, their rate of absorption, degradation, and elimination in the organism over a course of time (Rozman and Doull, 2003). That suggests that a repeated exposure may be required for some chemicals or for some dose of the chemical depending of chemical's rate of absorption, distribution and excretion, thus numerous factors that were not investigated in the current study. A use of a wide range of priming doses at different times and with a different number of exposures would provide a better explanation of this phenomenon.

The occurrence of a wide variation in the fecundity of whiteflies used in our tests was also a limiting factor in our research. This variation was mainly associated with variation in the

nutritional quality of the cotton plants. Although our cotton plants were grown as uniformly as possible and leaves of the same age were used in each test, average fecundity levels still varied considerable from one experiment to another. Variation in the uptake of imidacloprid by cotton plants from one experiment to another is also suspected. However, variation of fecundity within each experiments was small, as indicated by the similarity of the fecundity of the untreated controls between the groups of females to be compared (Table 4.4a, 4.5a). In our experiments variation of fecundity caused by a plant effect was minimized by cutting the leaf discs of replicates of treatments to be compared (primed and non-primed for example) from the same plant. Therefore the leaf discs used in replicate of a primed and non-primed comparison were cut from the same leaves, and so on. This approach enabled us to detect even a 10% difference in fecundity of the female whiteflies.

Low concentrations of imidacloprid have been reported in soil and plants up to one year after its application in some cropping systems, and residual amounts have been detected in untreated plants the following crop season (Bonmatin *et al*, 2003). Thus the application of imidacloprid to one crop may contaminate the following crop that whitefly may use as a host. The effect of a residual amount of imidacloprid on the population growth of whitefly has not been determined for a cropping system, but results of recent hormesis studies, mostly conducted in the laboratory raise some interesting questions. Ayyanath *et al*. (2013) did report a significant increase of the population size of the aphids exposed to low doses of imidacloprid in their greenhouse experiments.

Longevity of the reference strain and the resistant strain was also analyzed in this study. The priming of the females with low concentration of imidacloprid during immature stage seems to not affect the longevity of these females. Ayyanath *et al.* (2013) also reported to not observing

a significant increase of longevity in green peach aphids that were treated with a low concentration of imidacloprid in the immature stage. Cutler *et al.* (2009) also found a significant increase in fecundity but not on longevity of the green peach aphids that they exposed to low dose of imidacloprid. However, this study showed that the treatment of females of the rebuilt reference strain with imidacloprid at 6 mg a.i. as adults increased their longevity (Table 4.15e, 4.16 d). An increase in fecundity was also noticed along with the increase in longevity at this concentration. This effect might be associated with the effects of imidacloprid on cotton plants. Indeed, imidacloprid has already been reported to have some effect on the physiology of treated plants. For example, Szczepaniec *et al*. (2011) found that elm trees treated with imidacloprid had larger leaves than the untreated trees, and the spider mites *Tetranychus schoenei* on these treated trees exhibited higher fecundity than on untreated controls. It is also a possibility that treatment with imidacloprid at a relatively high but not lethal doses might induce a stimulatory effect in the insects but with different mechanism.

V Conclusion

The present study shows that a low concentration of imidacloprid applied at an early stage of development of female whiteflies can induce a stimulation of their fecundity when they become adults. The priming of whitefly during their immature stage is then necessary for the stimulation to occur. Although the current study did not identify the range of doses that is most efficient for this priming, the results suggest that the effect can occur with a very low priming dose, while priming of immature whitefly with a high dose does not appear to induce a stimulation. It also appears that there is a certain level of subsequent exposure above which the stimulation induced by the priming effect could not be observed. Overall, the rate of increase of the fecundity due to

priming was modest, 10 to 20 % greater than the untreated control. This modest increase could be easily masked by variation of the endpoints. It also appears that the stimulatory effect induced by a single priming exposure can be transferred to the first generation of female offspring. A higher fecundity was observed in reference females generated from the parents primed with low concentration of imidacloprid in the current study. However, the stimulation was less consistent and less obvious. A significant increase in fecundity of the female progeny of the rebuilt reference strain was not observed. Overall, the current study suggests that treatment with a low dose of imidacloprid applied during an early immature stage of development has an effect on adult whitefly fecundity. Further molecular and biochemical studies are needed to elucidate this phenomenon.

Summary and future research direction.

Among the neonicotinoid insecticides, imidacloprid is one of the most successful as indicated by its volume of sales worldwide (Elbert *et al.*, 2008). Because imidacloprid is still widely used for many key pests, including whiteflies, it is important to determine the effects of this insecticide at range of concentrations to provide insight into long-term resistant management strategies.

In the research presented in this dissertation it was found that heavy use of imidacloprid could lead to the rapid development of resistance in populations of B biotype whitefly. However the resistance appears to be unstable as it was lost with a temporal refuge without exposure to imidacloprid. Also, heritability of the resistance was found to be relatively small (0.10) suggesting that only a small proportion of the resistance phenotype variation could be attributed to the additive genetic variation. However, resistance can rise again rapidly with a new selection regime. All of these findings could partially explain the wide variation in the level of imidacloprid resistance in B biotype whiteflies in space and time as reported in the literature. Thus, the frequency of use of imidacloprid is one of the key factors to be considered to modulate the development of the resistance in whitefly. Allowing enough time for resistance to decline before the re-use of imidacloprid would be then a good approach to manage resistance to this insecticide.

After the introduction of imidacloprid, more neonicotinoid products became available to growers to control whitefly including thiamethoxam, acetamiprid and dinotefuran. Therefore, the development of cross resistance between these neonicotinoids is a concern for growers in greenhouse industry. In previous research on cross resistance in B biotype whitefly, variability in the level of cross resistance was found among several published studies. In this research the

heavy use of imidacloprid caused a cross-resistance to thiamethoxam. The P450 detoxification system may be involved in the resistance as suggested by the synergistic effect of PBO, an inhibitor of P450. A low level of cross-resistance to acetamiprid was also found in the same resistant population. These results seem to be consistent with published studies on metabolism of neonicotinoids in whitefly and in plants. No significant cross resistance to dinotefuran was observed in the present study, however an occurrence of cross resistance to this neonicotinoid insecticide in a population having much higher resistance level to imidacloprid is not ruled out. This research also increases our understanding of the causes of variation in the levels of cross resistance to neonicotinoids found in populations of B biotype *Bemisia tabaci* in different regions. The results suggest that in addition to P450 detoxification, another mechanism of resistance is very likely involved in whitefly resistance to neonicotinoids, and that the level of the cross resistance among neonicotinoids depends on the mechanisms of how resistance developed in each population. Therefore, the results of this study do not support the use of neonicotinoids as alternative insecticides for control of imidacloprid-resistant populations of whitefly.

Determining the effect of low doses of imidacloprid on populations of whitefly may be important because of the persistence of imidacloprid after its application. Several studies have reported that sublethal doses of insecticide are either neutral or even helpful for pest management because low doses may reduce the rate of reproduction of the pests. Bonmatin *et al.* (2003) detected a residual amount of imidacloprid as low as 0.1−1 μg/kg in soils 1 to 2 years after application of this insecticide as seed-dressing. The imidacloprid concentration used in the current study was 1.2 μg a.i per pot, thus a concentration that may be encountered in field 2 years after imidacloprid application. The results of this study suggest that this residual amount of

imidacloprid can cause an increase in fecundity of whitely when applied at the immature. The current study provides evidence that exposure to a low dose of imidacloprid applied during an early stage of development has an effect on adult whitefly fecundity. .

Hormesis has drawn the attention of researchers studying aging and senescence, and more recently from those studying the effects of various pollutants on health. One contribution of the research in this dissertation is to alert other researchers that hormetic effects can be subtle and are easily overlooked. The underlying mechanism of the hormesis is not well determined yet. Calabrese and Baldwin (2002) argued that no single mechanism could explain hormesis given that this phenomenon was observed on many endpoints across several taxa with various hormesis -inducing agents. However, it was also often reported that conditioning hormesis involves the antioxidant defense system and reactive oxygen species (ROS) in various organisms such as paramecia, yeast, nematodes, insect and mammals (Smith *et al*., 1996; Davies *et al.* 1995; Zhao and Wang, 2012; Lebourg, 2007; Ramakrishnan *et al*., 2013). ROS such as peroxide hydrogen H_2O_2 and the antioxidant system was already found to play a role in mammalian reproduction through the egfr signaling pathway in mammal species (Shkolnik *et al.,* 2012). In insect, an increased activity of the superoxide dismustase SOD, one of the defense enzymes of the antioxidant system that protects organism against reactive oxygen species (ROS), was already found to be positively correlated with an increased fecundity. For example, Büyükgüzel (2006) reported a positive correlation between SOD and fecundity and longevity in the female of the parasitoid wasps *Pimpla turionellae* that emerged from the host wax moth treated with a low dose of malathion. It is noteworthy that exposure to imidacloprid was already known to induce an increase of SOD in whitefly *Bemisia tabaci.* (Gao *et al.,* 2013). In addition, in the present study the pre-exposure to low amount of imidacloprid was conducted when the whitefly

individuals were at immature stage, thus at an early stage of development when organism was susceptible to epigenetic change induced by environmental stimuli (Janish and Bird, 2003, Decluve *et al.,* 2009). Recent studies in mammals and yeast showed that hormesis was associated with epigenetic modification in these organism and this link was mediated by ROS and antioxidant system (Schroeder *et al.;* 2013, Bernal, 2013). In addition, the current study found that the stimulation in fecundity appear in the F1 generation of female offspring of the reference strain when fertilizer was used, This suggests an occurrence of transfer of the hormetic effect to the offspring females. Previous studies in mammals have already shown that the side effects of pesticides applied at an early stage of the individuals on reproductive system in mammals could be transferred to few generations of their offspring (Manikkam *et al.* 2012, Adamski, *et al.*, 2005; Anway *et al*., 2005). A transgeneration inheritance of the extension of longevity was also reported in the yeast and this inheritance was mediated by epigenetic phenomenon (Greer *et al*., 2011). This inheritance of the effect was not seen with F1 female progeny of the rebuilt population of the reference strain.

APPENDICES

APPENDIX A. Log probit dose plot.

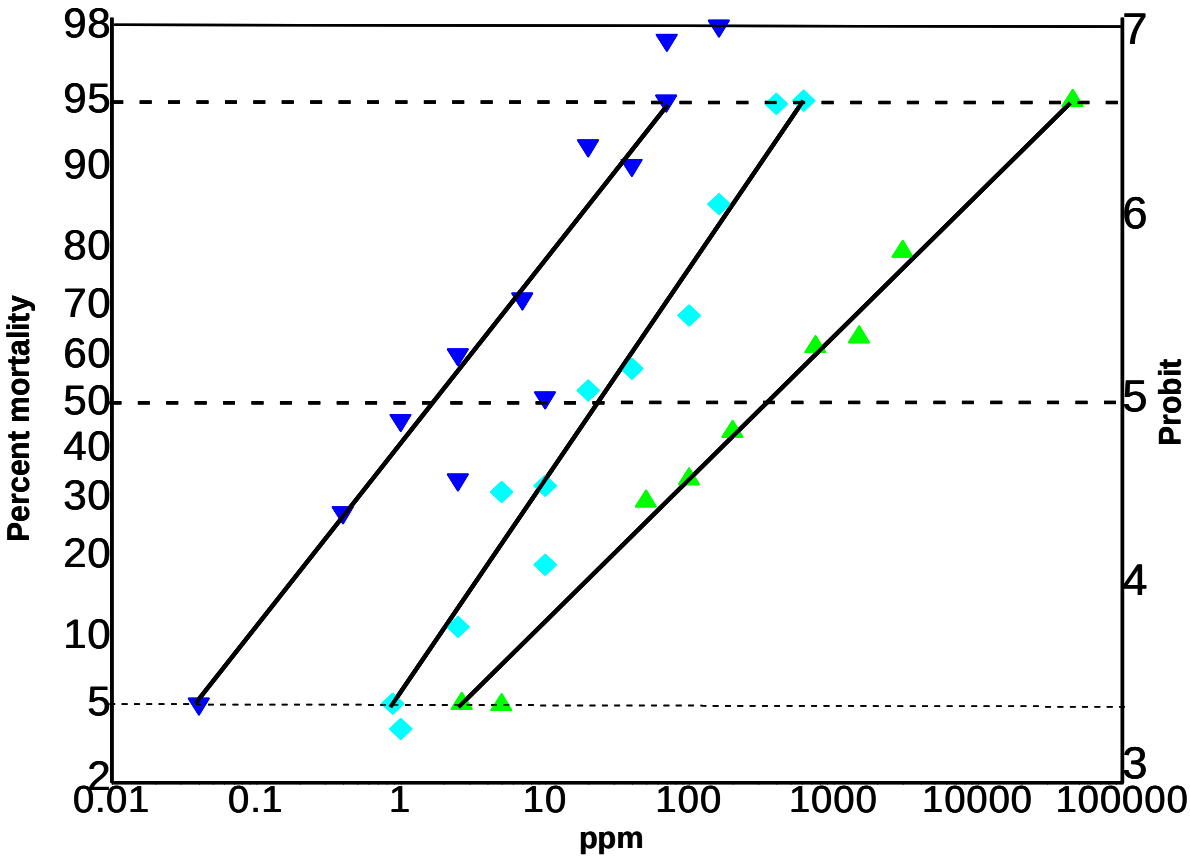


Figure A1. Log-probit dose plot for the toxicity of imidacloprid to the reference strain CA-s1 (dark blue triangles), the parental strain MI-R1 collected from a commercial greenhouse (turquoise diamonds), and the F5 (green triangles) generation of *Bemisia tabaci* B biotype after selection with imidacloprid**.**

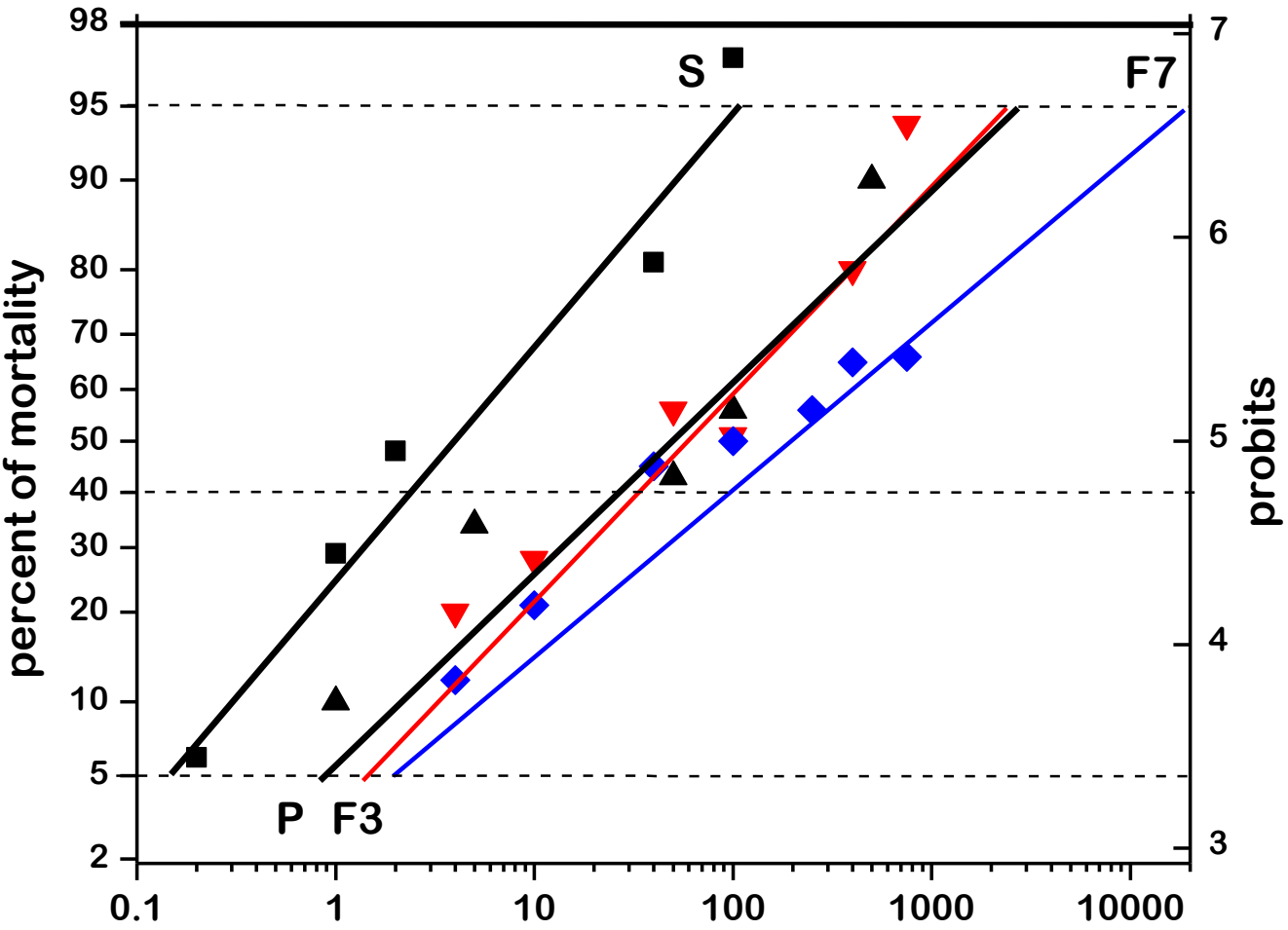
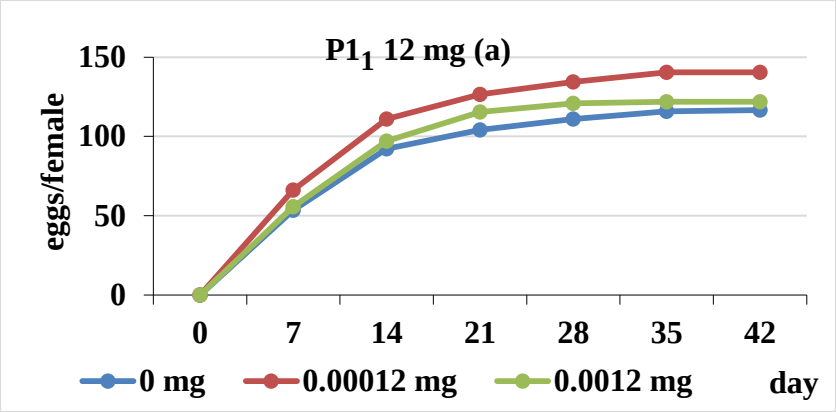


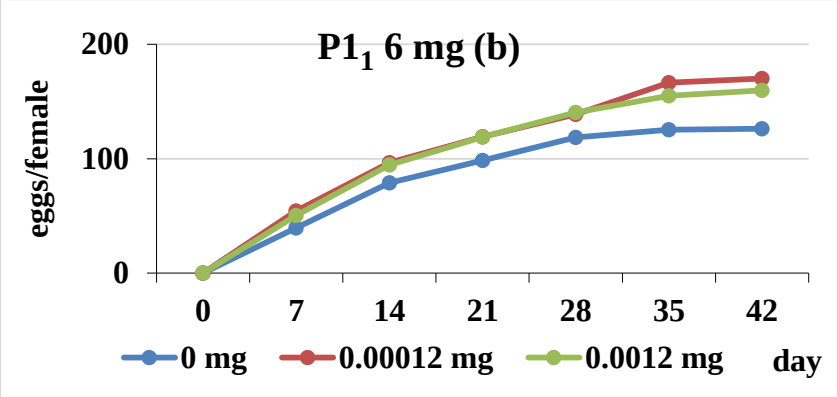
Figure A2. Log-probit dose plot for the toxicity of imidacloprid to the reference strain CA-s2 (black diamonds), P: the resistant parental of the resistant strain MI-R2 (black triangles) retrieved after the period of mite and thrips infestation, and the F'3 (red triangles) and the F'7 (blue diamonds) generation of the resistant strain MI-R2 after selection with imidacloprid.

APPENDIX B. Exposure of the rebuilt reference strain to imidacloprid.

Table A1. Proportion of affected female adults that exhibited various symptoms of toxicity after imidacloprid application. Some females recovered after a few days but some was died within five days.

Priming concentration (mg a.i. per pot)	0		0.00012		0.0012	
Subsequent exposure concentration (mg a.i. per pot)	female affected	female dead	female affected	female dead	female affected	female dead
0						
36	14/25	4/25	20/25	7/9	16/25	4/25
72	16/25	4/25	21/25	9/25	18/25	13/25





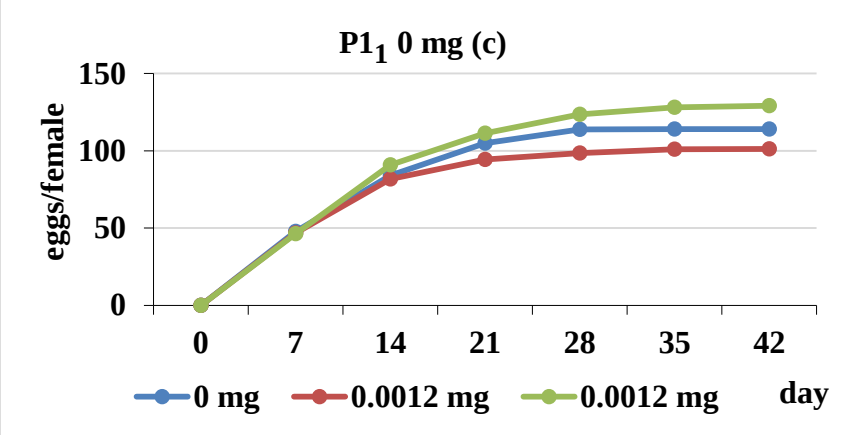
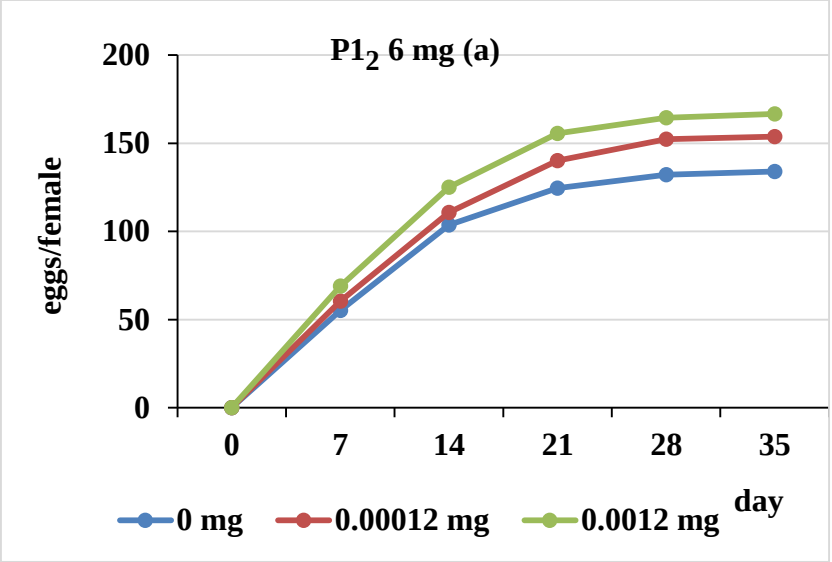


Figure A3. Cumulative mean total egg production per female of the first parental generation $P1_1$ of the rebuilt reference strain primed with different concentrations of imidacloprid (0, 0.0012, 0.0012 mg a.i per pot) and then subsequently exposed to imidacloprid at (a) 12 mg a.i per pot, (b) 6 mg a.i per pot, and (c) 0 mg a.i. per pot as adults.



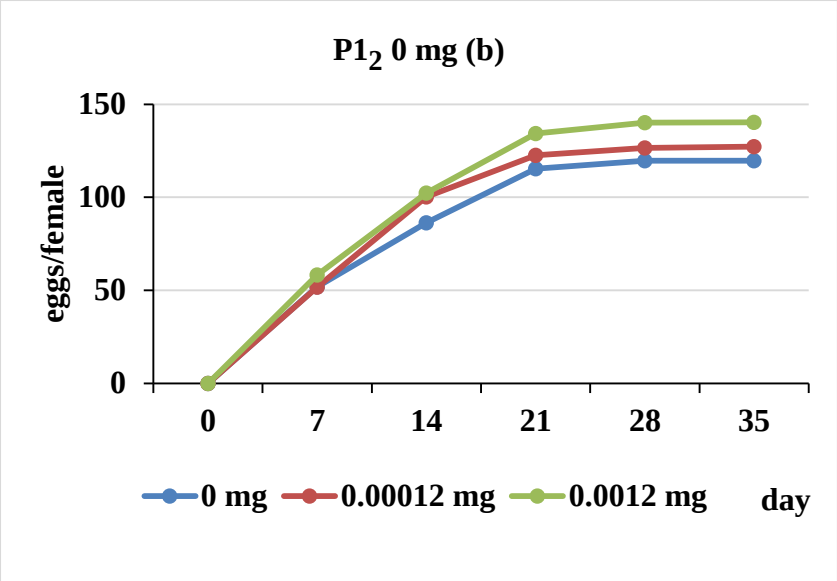


Figure A4. Cumulative men total egg production per female of the second parental generation $P1_2$ of the rebuilt reference strain primed with different concentrations of imidacloprid and then subsequently exposed to imidacloprid at (a) 6 mg a.i per pot, (b) 0 mg a.i. per pot as adults.

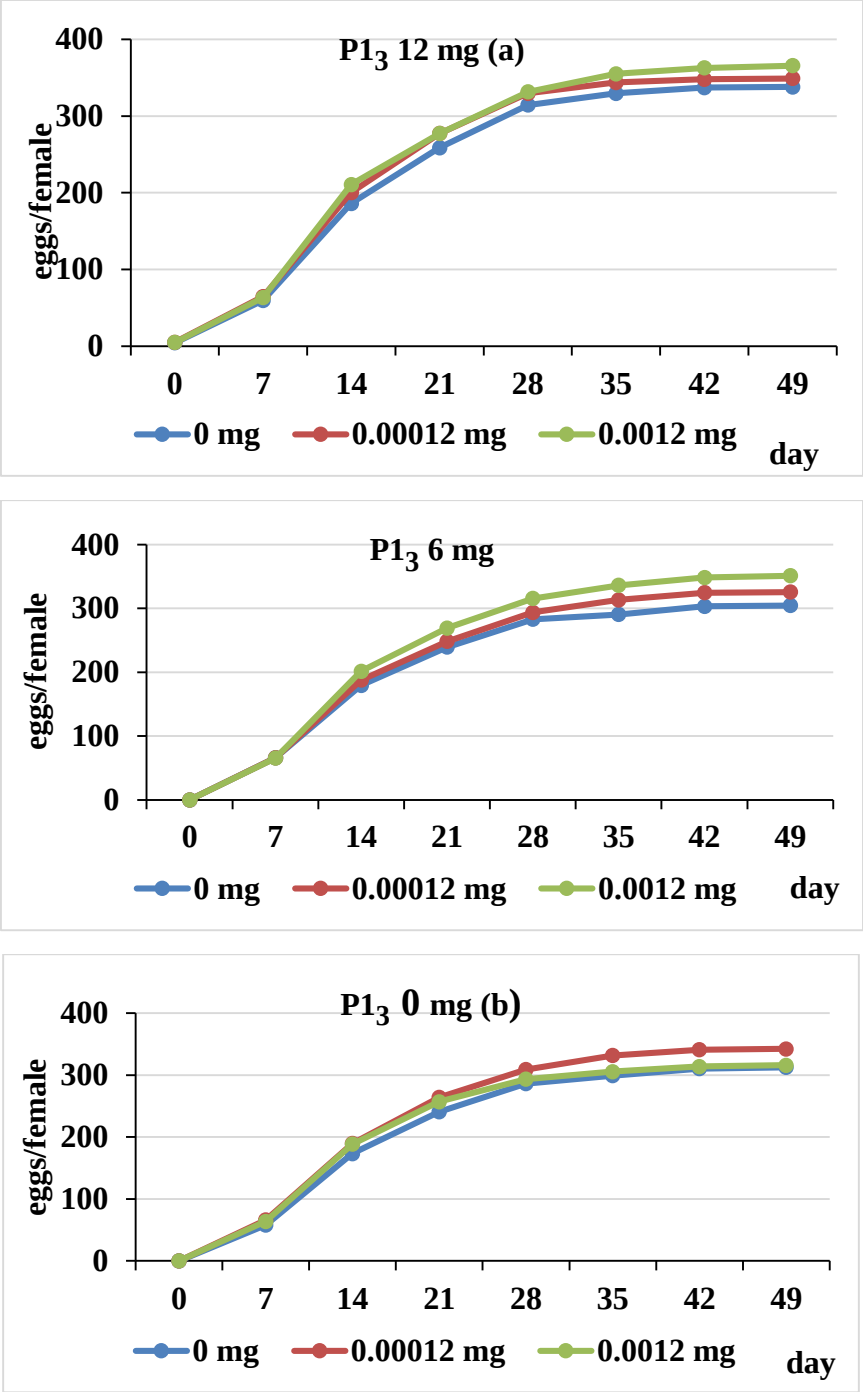


Figure A5. Cumulative mean total egg production per female of the third parental generation P_{13} of the rebuilt reference strain primed with different concentrations of imidacloprid and then subsequently exposed to imidacloprid at (a) 12 mg a.i per pot, (b) 6 mg a.i per pot, and (c) 0 mg a.i. per pot as adults.

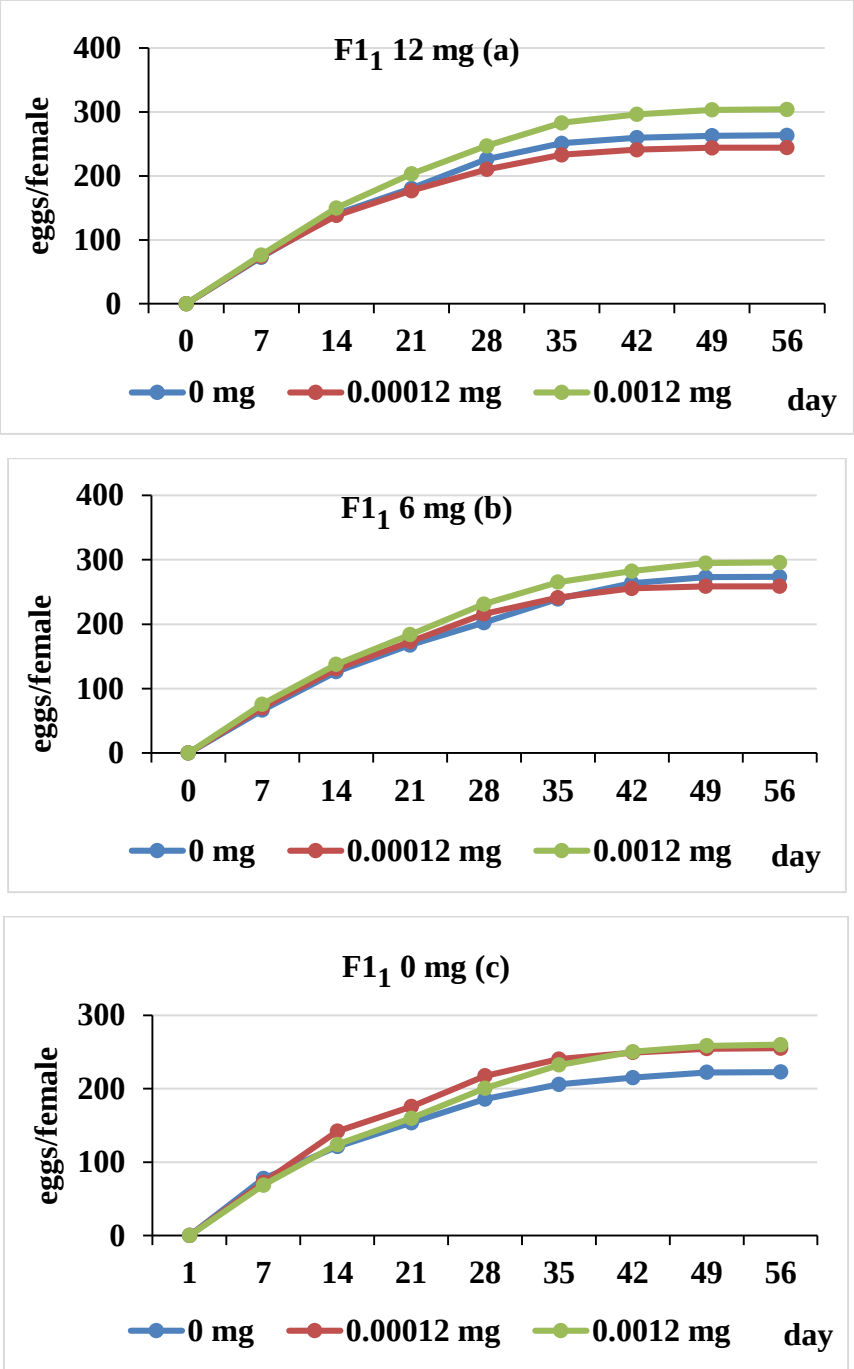


Figure A6. Cumulative mean ± SE total egg production per F1$_1$ female offspring of the rebuilt reference strain primed with different concentrations of imidacloprid and then subsequently exposed to imidacloprid at (a) 12 mg a.i per pot, (b) 6 mg a.i per pot, and (c) 0 mg a.i. per pot as adults

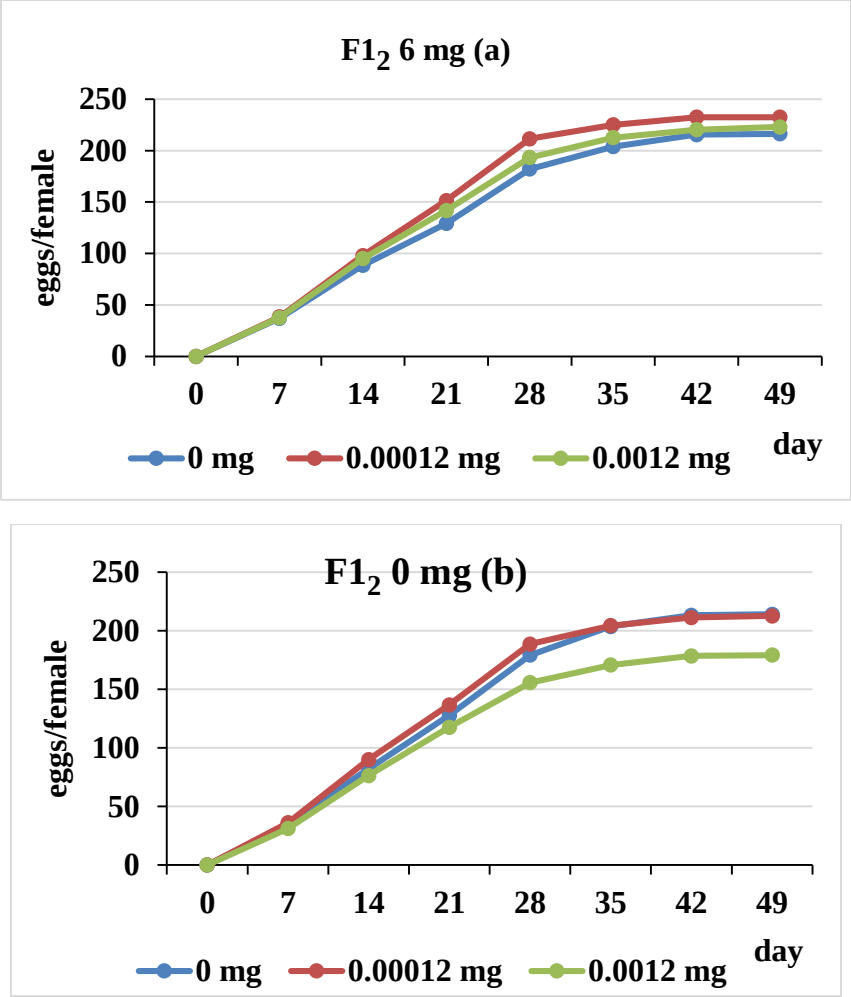


Figure A7. Cumulative mean total egg production per $F1_2$ female offspring of the rebuilt reference strain primed with different concentrations of imidacloprid and then subsequently exposed to imidacloprid at (a) 6 mg a.i per pot, (b) 0 mg a.i. per pot as adults

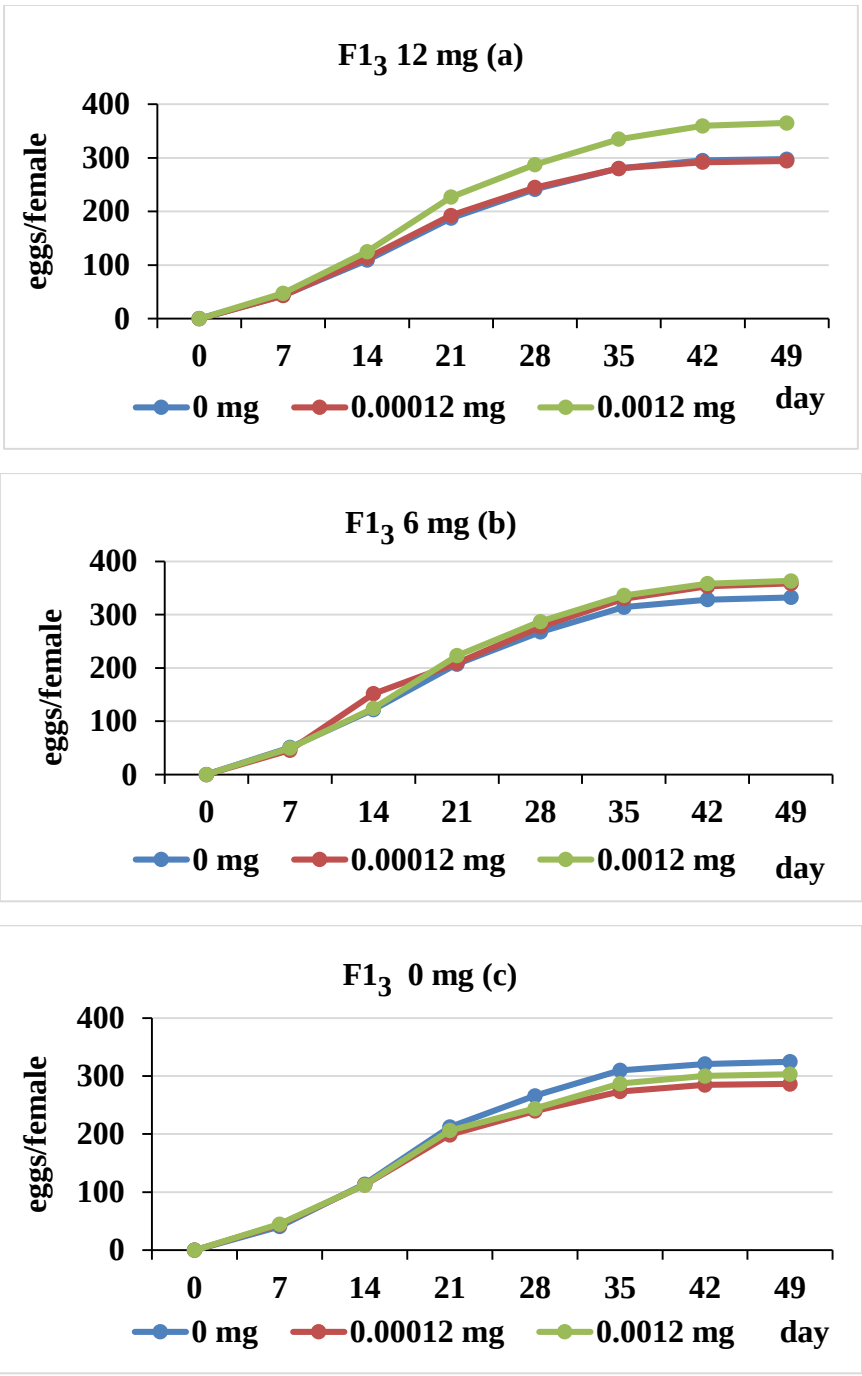


Figure A8. Cumulative mean total egg production per $F1_3$ female offspring of the rebuilt reference strain primed with different concentrations of imidacloprid and then subsequently exposed to imidacloprid at (a) 12 mg a.i per pot, (b) 6 mg a.i. per pot as adults and (c) 0 mg a.i per pot.

APPENDIX C. Record of deposition of voucher specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____#_2013-13_____

Author and Title of thesis: Response of the whitefly *Bemisia tabaci* B biotype to repeated exposures of imidacloprid

By Mamy L. Rakotondravelo

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Aleyrodidae	*Bemisia tabaci* (B biotype)	adult	10	alcohol
Aleyrodidae	*Bemisia tabaci* (B biotype)	larva	10	alcohol
Aleyrodidae	*Bemisia tabaci* (B biotype)	pupae	10	alcohol

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