University of Hohenheim

Institute of Phytomedicine

Department of Applied Entomology (360 c)

Prof. Dr. Dr. Claus P. W. Zebitz

Development and evaluation of attract-and-kill to control the tomato leafminer *Tuta absoluta* (Meyrick)

Master Thesis

Crop Sciences

Ayse Gül Ünlü

Äxtlestrasse 4

70599 Stuttgart

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1 Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest of economically important cash and food crops, especially of tomato (*Solanum esculentum* L.), potato (*Solanum tuberosum* L.) and various other solanaceous crops (URL 1). The species originates from South America and was unintentionally introduced as an invasive species to Southern Europe, including the Mediterranean Basin, North Africa and the Middle East (Desneux et al. 2010, 2011). Still more regions with similar environmental conditions of South American regions are highly threatened by the high invasion potential of *T. absoluta* (URL 1). Where field conditions do not match the environmental demands, *T. absoluta* may establish in protected crops with adequate climatic conditions and a year-round availability of food plants (Desneux et al., 2010)

The preference for tomato as host plant is reflected by tremendous yield loss of 80-100% in greenhouse and field tomatoes in newly invaded areas with lacking control measures (Desneux et al. 2010). The economic impact of *T. absoluta* in tomato production in South America is immense, since it is the main factor limiting tomato yields (Ferrara et al. 2001).

Under favorable conditions, *T. absoluta* can produce up to 12 generations per year (EPPO 2005). After mating the females preferentially oviposit on leaves as well as on the remaining aerial plant surface (Torres et al. 2001). When the larvae hatch, they penetrate the surface to feed on the mesophyll. Hence they damage the whole tomato plant (leaves, stems and fruits) by mine- and gallery formations, resulting in a loss of photosynthetic active leaf area, reduced plant growth and major fruit damages and losses (Vargas et. al 1970, Urbaneja et al. 2013). Although secondary infections by pathogens, causing fruit rot, might be induced by the feeding activity of *T. absoluta* in the fruit (EPPO 2005). In potato it infests leaves, stems and tubers and might become a serious pest under favorable environmental conditions (Pereyra and Sánchez 2006).

Several control strategies, aiming to decrease the risk of yield reduction, were developed and continuously improved (Desneux et al. 2010, Urbaneja et al. 2012)The use of chemical insecticides is a common management practice in South America (Lietti et al. 2005), which induced insecticide resistance (Siqueira et al. 2000, 2001, Lietti et al. 2005).

Additionally, the abundance of natural enemies of *T. absoluta* might be reduced by insecticide applications (Vacas et al. 2013) and thus interfere with their role as biological control agents. To overcome these problems, integrated pest management (IPM) strategies could be a more sustainable and effective alternative, in the long term. The attract-and-kill strategy has proven to be a promising control method to control lepidopteran pest species, such as the potato tuber moths *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) (Kroschel and Zegarra 2010, 2013).

An insecticide-pheromone coformulation is the basis of this strategy. The species-specific sexual pheromone acts as attractant for the male moths, which are killed, after coming into contact with the insecticide compound. Thus, successful reproduction and consequently plant damage is effectively prevented. Showing a high efficiency and a protective effect on non-target organisms, the attract-and-kill strategy could be a cost effective solution for small scale farmers, especially in developing countries of the tropics and subtropics (Kroschel and Zegarra 2013).

1.1. The tomato leafminer, *Tuta absoluta*

1.1.1 Origin and distribution

T. absoluta (syn. *Gnorimoschema absoluta* (Clarke), *Scrobipalpula absoluta (*Povolny) and *Scrobipalpuloides absoluta (*Povolny), the South American tomato pinworm is an oligophagous micro lepidopteran species originally described and discovered in the Peruvian highlands by Meyrick in 1917 (Barrientos et al. 1998, USDA 2011).

The species originated in South America (URL 1), particularly in the Andes (Peru, Bolivia, and Chile) (Sannino and Espinosa 2010), where it was considered a pest species in the 1960s in tomato and potato production (Cisneros 1966, Souza et al. 1983, Larraín 1986). In the 1970s, a massive range expansion of *T. absoluta* was recorded in South America and at the beginning of the 1980 it was a key pest in tomato cultivation in Argentina, Bolivia, Brazil, Chile, Colombia Ecuador, Paraguay, Peru, Uruguay, and Venezuela (Barrientos et al. 1998, Urbaneja et al. 2013). Today the whole South American sub-continent is affected by *T. absoluta* (Viggiani et al. 2009); primary through international trade of tomato fruits (Cáceres 1992). Before the European invasion, besides South America, also the Easter Islands (Ripa et al. 1995) were affected by the presence of *T. absoluta.* In 2004 the European and Mediterranean Plant Protection Organization (EPPO) listed *T. absoluta* on the A1 action list no. 321, declaring this species as a quarantine pest, being absent from the European and Mediterranean region (EPPO 2005). Nevertheless, *T. absoluta* was unintentionally introduced at the end of 2006 to eastern Spain, possibly by the import of infested fruits (Urbaneja et al. 2007, Desneux et al. 2010). In 2007, serious damages were recorded in tomato production systems of Spain’s Mediterranean region. In 2008 and 2009 further tomato producing countries of the Mediterranean region were invaded. Moreover countries in temperate regions such as the UK, Switzerland, Germany and the Netherlands reported *T. absoluta* infestations under protected conditions. (Desneux et al. 2010). In 2010, *T. absoluta* was detected in Sudan, where it continued to disperse to Ethiopia and Kenya, reaching Tanzania in 2014 (URL 2, URL 3). The pest is characterized by its immense invasion potential and continues to rapidly spread across Europe, Africa, and Asia (Desneux et al. 2010, 2011), where its sudden invasion tremendously damaged field tomato and greenhouse production (Desneux et al. 2010). The introduction of *T.absoluta* into Spain was probably initiated through trade of infested tomato fruits (Desneux et al. 2010).

Similar environmental conditions compared to South America and the intensity of tomato production might have fostered the rapid invasion and increased the abundance of *T. absoluta* in the Mediterranean region. Since biological control agents are missing in the exotic environments, there is a lack of natural balance among *T. absoluta* and natural enemies, leading to increasing pest densities and higher yield losses. (Desneux et al. 2011).

1.1.2 Biology

T. absoluta is a multivoltine species, producing several generations per year depending on environmental conditions. Under favorable conditions the pest species might develop 12 generations per annum. Overwintering may occur in almost all developmental stages (eggs, pupae and adults) (EPPO 2005). *T. absoluta* has the population characteristics of an r-strategist species (Pereyra and Sánchez 2006).

T. absoluta is a holometabolous insect, characterized by the four stages, egg, larva, pupa and adult (Fig.1) as complete metamorphosis and by the differing appearance of each stage.

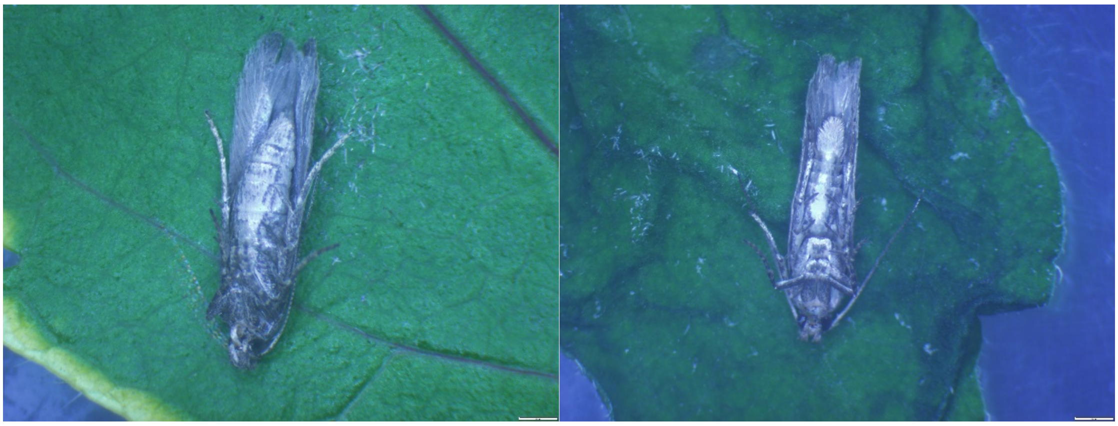

Figure 1. Ventral view of adult female (left) and male (right) *T. absoluta*

The cylindrical eggs have a length of 0.4 mm and a diameter of 0.2 mm. The egg color changes with time from creamy-white, after being laid to yellow-orange, before hatching. After hatching, which takes 4-5 days (EPPO 2005), the larvae undergo four larval instar stages, until they reach maturity and develop to imagines. The first instar is of creamy color with a dark head capsule and a length of 1.6 mm. While it penetrates aboveground plant organs and starts feeding and developing on the plant mesophyll, it changes its color to green. The second and third instars measure about 2.8 mm and 4.7 mm in length. The last instar is characterized by a pinkish coloration of the dorsal body parts and can develop a length of up to 8 mm. *T. absoluta* remains 13-15 days in the larval stage (EPPO 2005), before it stops feeding activity and starts pupating (Urbaneja et al. 2013). For pupation larvae can stay on the plant tissue, but prefer to move to the ground by a silky thread, which is used to form a white cocoon (Uchoa-Fernandes et al. 1995). The cylindrical pupa has a length of about 4.3 mm and 1.1 mm in diameter. During the 10-13 days of development time (URL 1), the pupa changes its color from green to brown (EPPO 2005, Urbaneja et al. 2013). While females have a pupal development time of 10-11 days, males need 11-13 days to emerge (URL 1), However, under laboratory conditions at a temperature of 25±1°C, pupal development lasted 9.48±0.38 days for females and 9.57± 0.36 days for males, respectively (Erdogan and Babaroglu 2014). The body length of adults measures 7-10 mm. The scales of the wings are silverish-grey colored, with black spots on the forewings. Furthermore the moths are characterized by filiform antennae and recurved labial pulps (Imenes et al. 1990). The abdomen of female and male *T. absoluta* moths varies in color and shape (EPPO 2005, Urbaneja et al. 2013) (Fig.2).

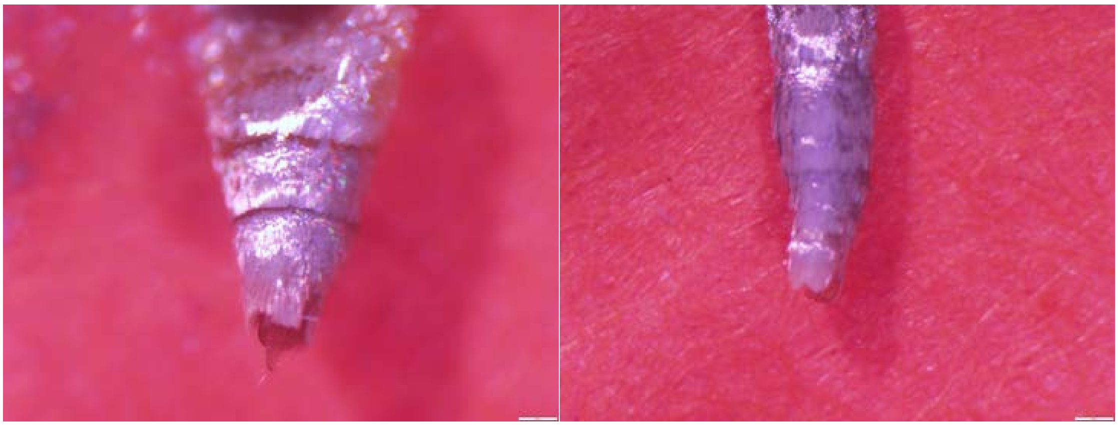

Figure 2. Abdomen of female (left) and male (right) *T. absoluta* moths

Most of the studies on the lyfe cycle of T.absoluta were conducted on tomato, as main host plant and very few on alternative host plants, such as potato.

The average development time of *T. absoluta* to complete its life cycle are 76.3, 39.8 and 23.8 days at temperatures of 14, 19.7 and 27.1 °C (Barrientos et al. 1998, Urbaneja et al. 2013), while under 25±1°C it was reported to be 30.18 days (Erdogan and Babaroglu 2014). Shorter average development times have been reported by Cuthbertson et al. (2013) with 58, 37, and 23 days at temperatures of 13, 19, and 25 °C, respectively. *T. absoluta* larvae are for several weeks biologically active at extreme temperatures of 4 °C (Vercher et al. 2010). However, Barrientos et al. (1998) determined the minimum temperature threshold for the development of eggs, larvae and pupae at 8.1±0.2 °C. Additionally the total thermal constant was estimated with a value of 453.6±3.9 degree-days (Barrientos et al. 1998). Cuthbertson et al. (2013) observed that under glasshouse conditions and temperatures of 10 °C eggs hatched, but did not complete their life cycle. The longevity of adults varied, depending on the sex, mating behavior, as well as environmental conditions. Erdogan and Babaroglu (2014) observed longevity of males and females of 15.8 and 18.16 days under laboratory conditions (25±1°C, 65±5% rel. humidity, photoperiod 16:8 h L:D). In addition, adult longevity of up to 40 days were reported under laboratory conditions of 10 °C and good food supply (honey/water) (Cuthbertson et al. 2013, URL 1).

The development time and longevity of *T. absoluta* under laboratory conditions of 24±1 °C (65±5% relative humidity, and 16 L : 8 D photoperiod) on four different potato varieties (Bintje, Charlotte, Spunta and Nicola) was studied by Caparros Megido et al. (2013) and subsequently compared to one tomato variety (Moneymaker). On potato, the time for larvae development was between 10.8-14.02 and the development of pupae ranged between 7.2-10.23 days. Adult longevity varied between 8.22-14.19days. Compared to the tomato variety (larval development time of 13.72 days, pupal development time of 8.51 days and adult longevity of 12.47 days), three of four potato varieties (Charlotte, Spunta and Nicola) showed a shorter egg to pupa development (Caparros Megido et al. 2013). Further studies, comparing the developmental parameters of *T. absoluta* of fed on potato and tomato plants, under laboratory conditions of 25±1 °C (65±5 % relative humidity, and 16 L : 8D photoperiod) reported significantly shorter larval developmental time on tomato (12.14 days) than on potato (14.00 days) by Pereyra and Sánchez (2006).

1.1.3. Mating behavior

Mating in *T. absoluta* occurs in less than 24 hours after emergence of adults mainly during the morning when males actively localize calling females (Mirranda-Ibarra 1999). In laboratory trials the majority of female moths called for males between 5.30 and 7.30 a.m. (Attygalle et al. 1996). However, searching behavior of males was studied by pheromone trap catches, delimiting the time span of the mating activity between 7-11 a.m. (Miranda-Ibarra 1999). Females calling behavior is characterized by unfolded antennae (Lee et al. 2014) and exposure of the ovipositor through moving the abdomen above the wings (Hickel and Vilela 1991). While remaining in that position, their pheromone gland, an intersegmental glandular membrane, localized on the abdominal tip produces the sexual pheromone to attract males (Attygalle et al. 1996). The sequence of *T. absoluta* male mating behavior consists of the long-range female location and the short-range courtship. The former is driven by the searching behavior of males, including localization of females by antennae, induced wing fanning and approaching by oriented flight while the latter consists of contact to female moth and copulation (Hickel and Vilela 1991). Experiments in wind-tunnels showed that after release, males perceived rapidly the sexual pheromone, fanned their wings and localized the female by oriented flight, as instantaneous response (Attygalle et al. 1996). Mating of *T. absoluta* can last more than six hours (Lee et al. 2014) and at least 30 minutes to guarantee successful spermatophore transfer (Ouye et al. 1965, Seth et al. 2002). During 24 hours, *T. absoluta* females were observed to mate once (Hickel and Vilela, 1991, Lee et al. 2014). However, during the lifetime of male and female *T. absoluta* moths, frequent rematings and subsequently polyandry has been observed (Wedell et al. 2002, Lee et al. 2014). Mating with different male *T. absoluta* moths showed a higher fecundity (total number of eggs), fertility (% viable eggs) and an increase in the longevity of *T. absoluta* female moths in laboratory studies (Lee et al. 2014). In the same study the mating males were observed to have the same longevity as mating females; instead, Fernández and Montagne (1990) observed that unmated males have a significantly higher longevity than mating males and females. Average female mating frequency was 10.4 times in laboratory trials, resulting in about 260 eggs per female (Imenes et al. 1990, Uchoa-Fernandes et al. 1995, URL 1). Oviposition was observed to be highest during the first 2-3 days after the first mating (Lee et al. 2014), while more than 70% of eggs are laid during the first week (Uchoa-Fernandes et al. 1995, Lee et al. 2014). *T. absoluta* females prefer to lay eggs on leaves (73%), followed by leaf veins and stem margins (21%), while on sepals (5%) and green fruits (1%) oviposition is less (Estay 2001, Desneux et. al. 2010). Ripe tomatoes are not used for *oviposition* (Monserrat 2009). Under laboratory trials Caparros Medigo et al. (2012) observed the asexual reproduction of *T.absoluta*. Since viable eggs of both sexes were produced to equal parts deuterotokous parthenogenesis occured (Portier 1949, Caparros Medigo et al. 2012). However, the occurrence of asexual reproduction must be investigated under natural conditions (Caparros Medigo et al. 2012)

1.2. Host plants of *T. absoluta*

1.2.1. Tomato and potato as host plants

T. absoluta prefers to feed and complete its whole life cycle on tomato as primary host plant. The above-ground plant organs are damaged by gallery- and mining-formations due to feeding activities of larvae (Fig 3.). Leaves, stems, flowers buds and fruits are affected. Larvae attack the photosynthetically active leaf area and subsequently reduce plant growth. The damage of flowers prevents the production of fruits, while gallery formations on stems might negatively influence plant development (EPPO 2005, Urbaneja et al. 2013). The feeding-damages on fruits lead to yield losses, marketing problems due to unaesthetic appearances of the fruit and secondary infections by pathogens, causing fruit rot (Urbaneja et al. 2013). Without control yield losses of up to 100% are reported (Desneux et al. 2010).

Figure 3. Leaf-mining damages caused by feeding activity of *T. absoluta* larvae

In addition, various other solanaceaous crops serve as food source and for reproduction and development of *T. absoluta*, including aubergine (*Solanum melongena* L.), pepper (*Capsicum annum L.*) (URL 1) and potato (Galarza 1984, Desneux et al. 2010). Since the range of host plant species of *T. absoluta* increased after invading Europe (Desneux et al. 2010), the invasion of new regions might be a thread to alternative and new host plants of high economic and nutritional value, primarily potato (Caparros Medigo et al. 2013), ranked as third most important food crop in the world (URL 4).

T. absoluta was known as serious leaf- and stem-mining pest in potato production of the Peruvian costal region (Sarmiento and Razuri 1976). In the 1970s and 1980s several studies on chemical control of *T. absoluta* were conducted (Cisneros 1966, Torres and Zapata 1966). In Peru, as well as in Chile Columbia and Argentina the pest was reported to attack aerial plant parts and tubers (Pastrana 1967, Campos 1976, Garcia and Espul 1982, Pereyra and Sánchez 2006).The damage of mining and gallery formations on aboveground plant material caused a reduction in the photosynthetic capacity of the plant resulting in yield losses. Further damages through mining activity, might occure on the tubers after the vegetative cycle of the potato plant, which might cause tuber rot (Pastrana 1967).

Recent studies in South America and in the newly invaded regions predict an economically significant pest potential of *T. absoluta* on potato under favorable climate conditions and the absence of natural enemies (Pereyra and Sánchez 2006; Unlu 2012). After two years of pest invasion in Turkey, *T. absoluta* was observed in regions without tomato production, causing potential economical threats to potato (Kilic 2010, Unlu 2012).

Compared to potato leaves, *T. absoluta* has a significantly shorter larval development time and higher pupal weight when fed with tomato leaves (Pereyra and Sánchez 2006). One indicator of better food quality as well as suitability of a host plant in herbivorous insects is a rapid development time (Awmack et al. 2002, Greenberg et al. 2002). Hence, tomato has a better nutritional quality for *T. absoluta* than potato and is hence the preferable host plant (Pereyra and Sánchez 2006).

However, Medigo et al. (2013) found that egg to pupa development was significantly shorter in three of four potato varieties (Charlotte, Spunta and Nicola) compared to the tomato variety Moneymaker. Thus, concluding that these potato varieties are qualitatively more suitable host plants compared to the studied tomato variety.

1.2.2. Global importance of tomato as vegetable crop

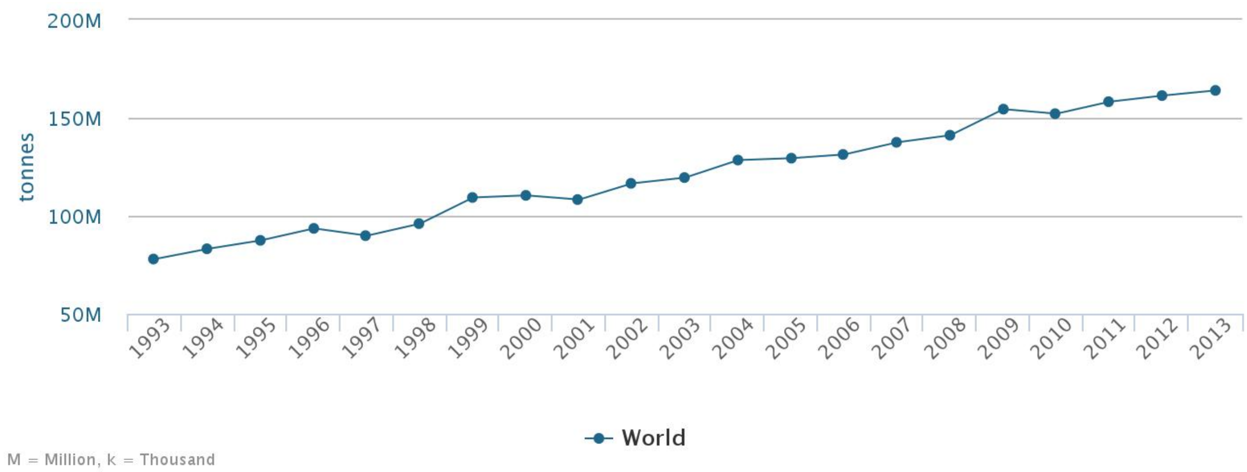

Figure 4. World tomato production in tons 1993-2013 (URL 5)

Tomato originated in the Andean region of Chile, Colombia, Ecuador, Bolivia, and Peru (Sims, 1980). While the ancestor of the tomato, most probably a wild form of the cherry tomato (*Lycopersicum esculentum* L. var. *cerasiforme* (Dunal)) grew in these regions, domestication is assumed has been initiated in Mexico (Jenkins 1948, Sims 1980, Harvey et al. 2002). Nevertheless, another hypothesis states that the domestication of tomato started in Peru (Muller

1940, Luckwill 1943). At the beginning of the 16th century, tomato was shipped into Europe by the Spanish, however, first cultivations for consumption started in the middle of the 16th century in the South of Europe, when fruits were accepted to be edible, despite the close relationship to other poisonous Solanaceae (Harvey et al. 2002). Until the end of the 18th century, it had spread to the rest of Europe: The introduction to China, southern and south-eastern Asia occurred in the 17th century in the 18th century to Japan and the USA (Siemonsma and Piluek 1993).

Today, tomato is consumed almost everywhere, due to its importance as vegetable crop and its high conservation potential in processed form. The quantity of consumption makes a significant contribution to human nutrition, since the tomato fruit contains less macronutrients but providing energy and calories. However, the tomato fruit is a rich source of minerals and vitamins (Ensminger et al. 1995) especially of folic acid, potassium, vitamin C, vitamin E, carotenoids and flavonoids playing a significant role in human nutrition (Shi et al. 2004). Some carotenoids in the tomato fruit are Provitamine A compounds, which are metabolized by humans to Vitamin A. An insufficient supply of Vitamin A causes blindness, compromise the immune system and enhances child mortality. Primarily children in developing countries suffer from these physical impairments due to a lack of Vitamin A in their diets (Underwood 2004).

Due to its high nutritional value, the tomato fruit has a steadily increasing economic importance. Within twenty years the tomato world production increased by >100% from 78 million tons in 1993 to 164 million tons in 2013, mainly by expanding the production areas (Fig 4). While in 2013 the leading tomato producing countries were China, India, USA, Turkey and Egypt, more than half of the global tomato production occurred in Asia (60.5%), followed by Americas (15%), Europe (12.8%), Africa (11.4%) and Oceania (0.3%) (URL 5). However, highest yields are reported from Belgium and the Netherlands indicating a higher global yield potential of tomato within the leading producers (URL 5). The differences in yields between tomato producing countries are explained by unfavorable environmental conditions, technological gaps and lack of investments in the tomato industry (Nicola et al. 2009).

Tomato prefers relatively dry and cool climatic conditions for the development of high yields and good quality fruits (Nicola et al. 2009). The perennial plant is frost-sensitive and is subsequently cultivated in warm seasons as field crop or under protected conditions e.g. in greenhouses. The adaptation potential of tomato plants allow the cultivation under various climatic conditions (Naika et al. 2005).

1.2.3. Tomato production in the Tropics

The potential of tomato production for tropical countries was described by Villareal in 1980, pointing out, with examples illustrating the current situation of his time, that tomato as vegetable crop, has an increasing production potential in the Tropics (Villareal 1980). Villareal (1980) concluded, that a rising tomato production enhances rural and urban employment, being more labor intensive than other crops and creating new business opportunities, in the field of processing and cultivation improvement. With new cultivation areas and higher production capacities, it could be possible to tap new market potentials and foster export trade, especially to developed countries. Moreover the value of tomato as a cash crop, could improve the income situation of farmers by being more profitable than alternative crops. The nutritional value of tomatoes in human diet might contribute to health benefits for people suffering from Vitamin A and C insufficiency in tropical developing countries. The versatility of the crop, its potential uses and the suitability as cash crop and for own consumption were additional benefits of tomato producers and consumers. However, there are potential constrains of tomato production, which might negatively influence the cultivation and expansion of tomato crops in the Tropics. First, inefficient markets might decrease the crops actual value, due to the power of intermediaries, setting low prices and benefitting from the poverty and monetary dependence of small-scale farmers. Moreover tomato prices are not stable and depend on supply and cultivation season. Since the main production season in the Tropics, might provoke a reduction of prices, independent from individual yields and off-season production is profitable, but risky, due to harsh environmental conditions, small scale farmers might be less willing to produce tomatoes. Additionally, a lack of funds and research dealing with the improvement of vegetable crops, having less contribution to food security than staples, high postharvest losses of 5-50 % and low yields due to imported varieties, not being adapted to the tropical environment, hinder tomato cultivation. The cultivation of unadapted varieties caused an increased susceptibility to pests and diseases, physiological development problems and low quality (Villareal 1980).

Nowadays, the risk potential of biotic and abiotic factors in the Tropics is reduced, but still the potential damaging factors are relatively high, compared to production sides in non-tropical environments. To overcome the major risk factors of tomato cultivation, especially, unfavorable climate conditions, as well as, pests and diseases, a successful crop management program must be performed. This program includes the selection of adapted varieties, technology orientation, agricultural planning and harvest and post-harvest management (Nicola et al. 2009)

Improved tomato varieties, being resistant against major pathogens, are used and protected cultivation, in automated greenhouses is carried out, to prevent unwanted environmental influences and guarantee controlled growth conditions. Harvest and post-harvest losses might be reduced by refrigeration technology and cooling systems. These installations are costly and not always reliable, as climate conditions are not easy to control, however, higher quality might increase the market price of tomato fruits. The whole process of tomato production must be accompanied by an economic strategy, including, sale and marketing, as well as the economical purchase of inputs (Nicola et al. 2009).

1.2.4. Economic impact of *T.absoluta* in tomato production

The global invasion of *T.absoluta* has negative impacts on environment and economics (Desneux 2010, 2011) Until 2006, T. absoluta only threatened tomato production in South American and subsequently 5 % of global tomato production, being 3.1 % of surface tomato cultivation, were damaged by *T.absoluta* (Desneux et al. 2011). Since the invasion of *T.absoluta* started spreading to other continents than South America, the damage on global tomato production has immensely increased. In 2011, 27.2% of global tomato production, being 21.5 % of surface tomato cultivation, were affected by *T.absoluta* infestations. However, 87.4 % of total tomato production in the world, representing 84.9 % of global tomato cultivated area, could be damaged by *T.absoluta* infestations in the future, due to presence of the pest species (Desneux et al. 2011). After 2011, the global dispersal of *T.absoluta* continued, as well as tomato production, indicating an increasing infestation potential (URL 1). The FAO listed *T.absoluta* outbreaks in African countries in its Quarterly Early Warning Bulletin for Food and Agriculture of January to March 2015, classifying it as a moderate to high thread to food chain in Kenya, Rwanda, Tanzania, Uganda, and the Sudan (URL 6).

Besides production losses in tomato cultivation, an increase in insecticide use was registered, leading to higher production costs and adverse effects for biodiversity and human health (Panuwet et al. 2008, Plianbangchang et al. 2009, Desneux et al. 2011). Desneux et al. (2011) estimated the increase in global pest management costs for the future control of *T.absoluta*, resulting in 240-420 M € additional costs.

1.3. Control strategies of *T. absoluta*

1.3.1. Chemical Control

By the time *T. absoluta* developed to be a serious pest species on crops, chemical control mainly by using organophosphates was considered to be the most efficient control measure in South American countries (Cisneros 1966, Torres and Zapata 1966, Sarmiento and Razuri 1976, Galarza and Larroque 1984, Lietti et al. 2005). In the 1970s a successful control of the pest was realized by the use of pyrethroids, and in the 1980s cartap and thiocyclam were introduced to manage *T. absoluta* outbreaks (Moore 1983, Larraín 1986; Lietti et al. 2005). In the 1990s insecticides with new mode of actions appeared for controlling *T. absoluta* (Galarza and Larroque 1984, Cáceres 2000).

From the 1980s onwards a reduction in insecticide efficiency of some insecticidal compounds was reported from Argentina, Bolivia, Chile, and Brazil (Moore 1983, Larraín 1986, Salazar and Araya 1997, 2001; Siqueira et al. 2000, 2001; Lietti et al. 2005). Several studies supported the development of resistances of South American *T. absoluta* populations to several insecticides with differing mode of actions due to overuse (Salazar and Araya 1997, 2001; Siqueira et al. 2000, 2001; Lietti et al. 2005, Bielza 2010, Silva et al. 2011; Reyes et al. 2012).

Moreover, the use of unselective insecticides controlling a wide range of insect species reduced the abundance of beneficial species and biocontrol as important contribution to a successfully and sustainably pest management (Desneux et al. 2006, 2007, Barros et al. 2015). In addition, the feeding habit of *T. absoluta* larvae in the plant's mesophyll or in fruits increased the inefficiency of conventional synthetic insecticides (Lietti et al. 2005). Furthermore, the incorrect application and storage of insecticides causes health risks to farmers, coming in touch with hazardous substances. A lack of protective equipment and poor knowledge of correct application techniques cause serious health issues (Panuwet et al. 2008; Plianbangchang et al. 2009).

Thus, considering the negative side effects of the use of insecticides, a pure chemical control is not sustainable in the management of *T. absoluta* (Desneux et al. 2010).

1.3.2. Biological control

An implementation of biological control to control *T.absoluta* is considered as a significant control measure of integrated pest management programs (Haji et al. 2002) and might be a sustainable pest management strategy in the long term (Urbaneja et al. 2013). A wide range of biocontrol agents (parasitoids, predators and entomopathogens) of *T.absoluta* were identified in South America and even in invaded areas of the Mediterranean regions several indigenous natural enemies were observed to control *T. absoluta* (Miranda et al. 1998, Mollá et al. 2008, Desneux et al. 2010, Gabarra and Arnó 2010, Urbaneja et al. 2013).

Several parasitoids have been successfully used in biological and integrated pest management programs of *T. absoluta* (Desneux et al. 2010, Urbaneja et al. 2013). While mass releases of egg and larval parasitoids were reported to be successful in several South American countries (Haji et al. 2002; Polack 2007; Desneux et al. 2010), pupal parasitoids are rare (Polack 2007, Desneux et al. 2010). The presence of parasitoids of *T. absoluta* outside of South America has been recorded in the Mediterranean region (Mollá et al. 2008, Gabarra and Arnó 2010).The number of described local parasitoids attacking *T. absoluta* steadily increases reflecting the adaptation potential of indigenous species of controlling an exotic pest species. However, research on the potential of indigenous parasitoids must be intensified, before large scale biocontrol programs can be initiated (Desneux et al. 2010).

In addition to parasitoids, predators significantly contribute to a reduction of the *T. absoluta* population. Among the predators reported from in South America are various species of *Coleoptera, Hemiptera, Hymenoptera and Thysanoptera.* Still, the potential of arthropod predators, which could cause approximately 80% of larval mortality is underestimated in South American countries due to a lack of research and the successful use of parasitoids (Miranda et al. 1998, Desneux et al. 2010). Instead, in the Mediterranean region, the biocontrol through indigenous predators is known to be of great significance. Especially, predatory mirid bugs (Hemiptera: Miridae) were observed to be highly efficient in preying on *T. absoluta and are* included in IPM programs (Arnó et al. 2009, Urbaneja et al. 2012). A combination of egg parasitoids and specific mirid bugs in *T. absoluta* control programs are recommended by Urbaneja et al. (2012) to control outbreaks. Still, the mismanagement of the use of natural enemies can have negative impacts on crop production and must be well planned before implementation (Urbaneja et al. 2012).

The use of entomopathogens in *T. absoluta* control is dominated by *Bacillus thuringiensis* (*Bt*) strains in applicable formulations (Giustolin et al. 2001, Desneux et al. 2010) . Moreover, a Brazilian granulovirus isolate proved to cause high mortalities in *T. absoluta* (Mascarin et al. 2010). Laboratory and greenhouse trials with entomopathogenic nematodes showed high infection levels and larval mortality even inside the plant tissue (Batalla-Carrera et al. 2010, Garcia-del-Pino et al. 2011). Still, further research on the potential use and application of entomopathogenic fungi, nematodes and viruses must be conducted before recommendations can be made of its integration in *T. absoluta* IPM programs (Desneux et al. 2010).

Despite the occurrence of indigenous biocontrol agents in newly *T. absoluta* invaded regions, the introduction of non-indigenous, highly specific natural enemies from regions in which *T. absoluta* has been originated might have a potential to reduce pest densities of *T. absoluta* more effectively

(Desneux et al. 2010). However, the introduction of exotic species in new environments might harbor unpredictable risks, especially for the long-term (van Lenteren et al. 2006, Desneux et al. 2010) and is therefore subject to the FAO Code of Conduct for the introduction and release of exotic biocontrol agents (Schulten 1997).

1.3.3. Integrated Pest management

Integrated Pest Management systems (IPM) consist of various pest management tactics, aiming to sustainably reduce pest abundance on crops and subsequently severe economic and environmental losses. An economical threshold reflects the pest level, when pest management actions should be considered to prevent these losses. Significant for a successful IPM strategy is the use of alternating cost-efficient management methods, reducing the use of pesticides to obtain a sustainable agro-ecological system on the cropping site (URL 7)

The establishment of IPM requires multiple actions to prevent and control a pest species. Initially, preventive and suppressive measures, including crop rotation, pest-resistant crop varieties and the promotion of natural enemies, should be considered (URL 8) In the case of suspicion of the presence of a pest species, it must be properly identified in the area of cultivation, which is realized by surveillance (Jones 1998) For the precise assessment of presence, location and abundance of a pest species, monitoring devices, such as traps, are placed in the potentially affected (Jones 1998, URL 8).

Before control measures are implemented, a control threshold is set, which particularly decides about the timing of action, informs about the level of risk and might indicate trends in population development and pest distribution (Jones 1998). The economic thresholds of pest species, represent the density of the pest species, which might cause severe economic losses, if no control measures are applied. After exceeding this threshold, the pest density will increase, until the economic injury threshold is reached, which means that the management costs will exceed the losses caused by the uncontrolled pest density (Stern et al. 1959). The Economic Injury Level concept entails reductions in pesticide use and its negative side-effects (Alston 1996a), including the development of resistances to frequently applied pesticides, pesticide residues on crops and the decline of beneficial species, due to nonselective pesticide applications. The prevention of economic losses in IPM requires the use of cultural, biological, and mechanical control measures and justifies chemical control, including a rational and selective pesticide application to rapidly reduce the pest density (Alston 1996b, URL 8) and to maintain the control action of natural enemies. Environmental conditions of the previous years and their effects on the pest species, as well as pest-related biological information, are essential to develop forecasts and subsequently to achieve higher control management efficiency (Jones 1998).

1.3.4. Attract-and-kill strategy

Attract-and-kill is a pest management strategy, mainly consisting of an attractant luring a target species and a killing agent eliminating the attracted individual (Jones1998). Visual cues, like colors or objects, acoustical cues, odors and combinations are used to attract a pest species (Lanier 1990). In particular, the scent attractant is a semiochemical, released by insects for inter- or intraspecific communication. While allelochemicals are involved in interspecific communication, the chemical communication within a species is based on pheromones (Howse 1998). Especially insects have sensitive receptors, efficiently detecting low-concentrated messenger chemicals. These compounds induce a specific response by the recipient and subsequently influence its behavior or physiology (Howse 1998, Heuskin et al. 2011). Synthetic semiochemicals, such as synthetic sex-pheromones, being used in insect pest management are volatile compounds, dispersing over long distances in the air to cover wide areas of infestation and targeting flying insect pest species (Heuskin et al. 2011). However, crawling insects are attracted by crude baits (El-Sayed 2009). After the target species is attracted to a luring source, it must come in contact with the killing agent, which can be a synthetic insecticide, sterilizing agent or entomopathogens. Attract-and-kill as part of an integrated pest management system is used to sustainably reduce and keep the pest density below the economic damage. This concept is applied in pest control of Lepidoptera species, but nevertheless, eradication programs for Dipteran and Coleopteran pests based on attract-and-kill have been also developed (El-Sayed et al. 2009).

A significant approach to control Lepidoptera species is the use of synthetic sex pheromones for male annihilation through either mass-trapping or attract-and-kill. Since the species specificity of sex pheromones attracts only the pest species, the attract-and-kill strategy is a highly selective control approach. Since very small quantities of pheromones are needed (Witzgall et al. 2010), no pheromone residues in crops exposed to pheromones were reported (Tinsworth, 1990) and no toxicity effects on other species were observed (in particular natural enemies). The use of synthetic sex-pheromones is a sustainable pest management approach (Witzgall et al. 2010).

The development of an Attract-and-kill approach for Lepidopteran species, using a combination of a synthetic sex pheromone and an insecticide, requires several attributes to cause a significant pest reduction. The main compounds within the formulation must be adjusted to the biology of the target species. Especially, the identification and the synthetization of main sex pheromone compounds is key prerequisite to develop a successful pheromone-based attracticide (Lösel et al 2000, El-Sayed et al. 2009). Since pheromones are highly volatile, a constant release of the sex pheromone within the Attract-and-kill formulation should be maintained, providing a steady male attraction throughout the day, whereas the natural sex pheromone elicitation by female moths is restricted to certain time periods (Lösel et al. 2000, Krupke et al. 2002). Moreover the eliminating substance, must be an insecticide, with a sufficient and rapid control activity. Common insecticides used in attract-and-kill formulations are pyrethroids with rapid action (El-Sayed et al. 2009), primary targeting the voltage-gated sodium channels in the nervous system (Goldin 2003).

The formulation of the blend, as well as the dose of its components, is another challenge, as interactions between the sex pheromone, insecticides and additives might cause a repellent effect on the pest species (El-Sayed et al. 2009). Additionally, before practical implementation, the blend must be tested for competition with mated and unmated females (Krupke et al. 2002) and subsequently longevity and stability under field and protected conditions (El-Sayed et al. 2009).

The successful practical application of sex pheromone based attract-and-kill, strongly depends on several factors, regarding the pest biology, distribution and development. Several studies state that a successful control of the target pest was achieved in low-density populations of Lepidopteran pest species (Angeli et al. 2000, Charmillot et al. 2000, Lösel et al. 2000, Ebbinghaus et al. 2001). Moreover, an isolated pest population is more efficiently controlled, since an increase in pest density through migration may reduce the effect of the approach (Downham et al. 1995, Angeli et al. 2000, Charmillot et al. 2000). In addition the synthetic sex pheromone compound in the formulation must be more attractive than calling females (Angeli et al. 2000, Charmillot et al. 2000, Ebbinghaus et al. 2001). Besides target-pest related factors, the implementation of the lures in the target area must be properly planned. Especially, the amount of lures and their placement in the infested area, strongly influence the efficacy of the lures. Furthermore, the attract-and-kill strategy should be applied during the whole flight period, before male moths emerge (Charmillot et al. 2000, Ebbinghaus et al. 2001).

1.4. Aim of the study

The aim of the Master thesis is to adapt the attract-and-kill strategy for controlling the tomato leafminer *T. absoluta* by studying and optimizing the pheromone and insecticide concentration and testing its stability under controlled laboratory and natural field conditions. These are first important steps prior of its field evaluation, successful application and use under farmers' field conditions.

2 Material and methods

2.1. Experimental site

The experiments were performed at the experimental station of the International Potato Center in Lima, Peru. Tomato plant production and mass-rearing of *T. absoluta* were carried out under protected conditions in greenhouses under an average temperature of 22.61 °C (16.20- 36.91 °C). The experiments were conducted under controlled conditions in the laboratory.

2.2. Tomato plant production

In weekly intervals tomato seeds of variety "Rio Grande" were sown into a plastic tray (1 gr seeds/tray) filled with soil (SOGEMIX SM-2, Growing mix substrate de culture). After four weeks the seedlings (Fig.5) were separated and replanted in pots (10 x 10 x 9.5 cm) (3 seedlings /pot).

Figure 5. Tomato seedlings of variety "Rio Grande" in plastic tray

After 7 weeks, when plants reached an appropriate size and biomass, they were considered as food material for *T. absoluta* rearing. Additionally, tomato leaves were used in the experiments, to simulate natural conditions. The tomato plants were daily watered. During plant development fertilizer was applied to increase plant-growth. Since natural infestation by *T. absoluta* were observed in the greenhouses, sex-pheromone and light based water traps (Fig), were placed between the plants to monitor and reduce the *T. absoluta* population and early infestation of seedlings. Tomato plants were daily visually checked and infested plant parts removed.

2.3. Rearing of *T. absoluta moths* for experiments

The laboratory colony of *T. absoluta* derived from a collection of moths in La Molina field station (300 m.a.s.l) (12°4´39'' latitude S and 76°56´53'' longitude W) on tomato plants, in 2013. *T. absoluta moths* were mass-reared in wooden cages covered by a metal mesh (120 x 60 x 100 cm).The cages were filled with 7-week old tomato plants and subsequently either directly infested with *T. absoluta* larvae or by releasing mixtures of female and male adult moths. The cages were regularly checked for a successful infestation and plants regularly watered. After two weeks, plastic trays (45 x 30 x 5 cm) were filled with a thin layer of sand on the bottom, as pupation site for larvae. A grid was subsequently placed on the trays, to separate the pupation site (Fig. 6)

Figure 6. Infested tomato plants in the rearing cages, containing plastic tray with pupation substrate, covered with a grid.

Infested aboveground material of the tomato plants was cut and placed on the grid. After 1-2 weeks the sand in the trays was sieved and the pupae were collected.

Additionally, *T. absoluta* was reared under laboratory conditions in plastic containers (30 x 20 x 7.5 cm).The bottom of the containers were covered with a wet cloth, providing humidity, and subsequently a grid was placed inside. Fresh tomato branches and leaves were put on the grid until the containers were half full. Thereafter the plant material was infested with first instar larvae of *T. absoluta* (Fig. 7). Each second day the larvae were supplied with fresh plant material and the cloth on the bottom was moistened. The containers were incubated at 25°C (65 % relative humidity (RH) and a photoperiod of 12L: 12 D) for 12 days, until fourth instar larvae developed. The cloth on the bottom was subsequently replaced by a thin layer of sand, as pupation site for larvae. After approximately six days, the sand on the bottom of the box was sieved and the pupae were collected.

Figure 7. Plastic container infested with first instar larvea of *T. absoluta*

The pupae were stored under a temperature of 10 °C in, until a sufficient amount was collected to conduct the experiments. The pupae were subsequently put one by one in plastic boxes (individualized). After emergence of adult moths, color and shape of the abdomen was visually checked to separate the sexes (Fig. 8). The male/female ratio under the given mass-rearing conditions was 1:3.

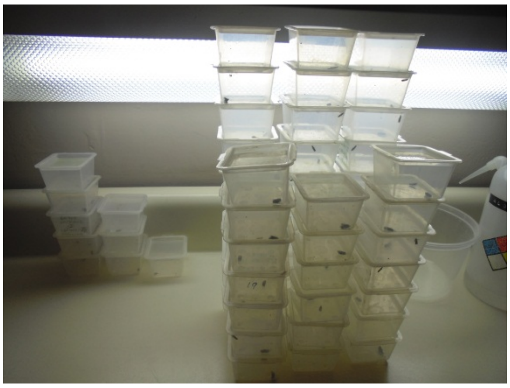

Figure 8. *T. absoluta* adults in plastic boxes

2.4 Chemical composition of attract-and-kill formulation

2.4.1 Pheromone compounds

The synthetical sex pheromone of *T. absoluta* females was one of the main components of the *T. absoluta* attract-and-kill formulation. Primarily, it consists of two compounds, (3E, 8Z, 11Z)-3,8,11-tetradecatrien-l-yl acetate (TDTA) as major compound (Attygalle et al. 1996) and (3E, 8Z)-3,8- tetradecadien-l-yl acetate or TDDA (Svatoš et al. 1996) as minor compound (>97% isomerically purity), synthesized by Pherobank (Netherlands).

2.4.2 Insecticide

The insecticide used in the attract-and-kill formulation was Beta-Baythroid 125 SC (125 gr/l beta-cyfluthrin) produced by Bayer Crop Science. The active compound, beta-Cyluthrin (cyano-4-fluoro-3-phenoxyphenil)-methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) is a nonsytemic pyrethroid (Type II) (Chauhan et al. 2013)..

2.4.3 Additives

The attract-and-kill formulation contained additives including U.V. absorbers (Uvinul M40 and Uvinul N539T, BASF, Germany) (each 75 g/L), the emulsifying agent Cremophore 40 (BASF, Germany)(75 g/L) and hydrophilic silica (Aerosil 200 V, Degussa Pharma, Germany) (70 g/L) guaranteeing the fluidity of the product. These compounds were dissolved in Castor (*Ricinus communis* L.) oil (664, 5 g/L). The additives of the attract-and-kill formulation were homogeneously mixed and put into a water bath (60 °C) for 10 minutes. Thereafter the active ingredients were added. The protocol for the attract-and-kill formulation was prepared according to Lösel et al. (2000) and Kroschel and Zegarra (2010).

2.5 Testing the optimum beta-cyfluthrin and pheromone concentration in the Attract-and-kill formulation under laboratory conditions

2.5.1 Testing the optimum beta-cyfluthrin concentration

The assess the optimum insecticide concentration to kill *T. absoluta* males over time three different doses of beta-cyfluthrin (2.34, 5.0 and 6.5 g/L) and one control without insecticide (water) under two exposure times (5 and 10 seconds) of *T. absoluta* male moths of the same age were tested, applying the tarsal dip method. The insecticide used, was Beta-Baythroid 125 SC, containing 125 gr/L beta-cyfluthrin, as active ingredient. The experiment was performed in petri-dishes (8.5 cm diameter), in which one ml of each concentration was applied. Ten *T. absoluta* males of the same age were subsequently dipped with their tarsi into one concentration. After the exposure time, *T. absoluta* males were transferred into a petri-dish containing a filter paper, which was covered with a plastic vessel (12 cm diameter x 6 cm depth) and fixed on the bottom with tape (Fig. 9). The petri-dishes were stored at constant temperatures of 25°C, 65 relative humidity and a photoperiod of 12 L : 12 D. After 24 hours the first evaluation was performed. The number of dead T. absoluta males was daily assessed, until 100 % mortality was observed. In total, there were three repetitions per treatment and two replicates.

Figure 9. Petri dishes, covered with plastic vessel containing *T. absoluta* males exposed to insecticide treatment

2.5.2 Testing the optimum pheromone concentration

In cage experiments, three different sex pheromone concentrations (0.05%, 0.025%, and 0.0125%) in the attract-and-kill formulation and one control without active ingredient were tested on male *T. absoluta* moths to assess the pheromone concentration with the highest attractiveness. Beta-cyfluthrin was used at 5.0 g/L in the all formulations.

The experiment was conducted in the laboratory in wooden cages (0.80 x 1.20 x 0.60 m), which were covered by a nylon gauze and/or glass under constant temperatures of 25±1°C, 65% relative humidity (Fig 10). The cages were arranged in shelves and grouped into blocks within the different shelve layers to account for potential differences in temperature.

Tomato leaves were put in a plastic vial and placed in the center of the cage to stimulate natural conditions. The viscous attract-and-kill formulation was used in volumes of 100 µL droplets, dropped on an open petri dish and placed in the cages next to the tomato leaves. In each cage 15 newly hatched *T. absoluta* male moths were released. The experiment was evaluated daily by assessing the mortality rate of the male moths inside the cages. A tweezer and a petri dish were inserted through the sleeves into the cages and the death moths were collected and counted. The experiments ended, when no moth could be observed in the cages. In total, four repetitions per treatment were performed and the whole experiment was conducted twice.

Figure 10. Wooden cages to test optimum pheromone concentration in Attract-and-kill formulation

2.6 Testing the preference and competence of virgin female *T. absoluta* moths against the attract-and-kill formulation in olfactometer bioassays

The effectiveness of three different pheromone concentrations (0.05%, 0.025%, and 0.0125%) in the Attract-and-kill formulation against three different numbers of virgin female moths (two, four and eight) was assessed in a six-armed olfactometer bioassay. The amount of beta-cyfluthrin as insecticide compound was constant (5.0 g/L).

The six-armed circular olfactometer consisted of 6 outer cups connected to a central cup by tubes (Fig.11). While the cups were 15 cm high and had a diameter of 12 (top) narrowing to 6 cm on the bottom, the tubes had a diameter of 3 cm and a length of 20 cm. The different numbers of T. *absoluta* females were released in petri-dishes (9.2 cm diameter) with wholes on the lid, covered with a nylon gauze, for ventilation. One drop of 100 µl of the attracticide treatments was pipetted on an open petri-dish. Thereafter, the treatments were placed into the outer cups by alternation of attracticide treatment and *T.absoluta* females. The outer cups were subsequently covered with a textile-gauze and sealed with an elastic band. Tomato leaves were put in a small vail and placed in the middle of the central cup and sealed with a plastic cover. A group of 50 unmated male moths were released into the middle cup and after 24 hours the amount of male moths in each cup and the particular tube was assessed. The experiment was conducted under laboratory conditions (temperature of 25 °C, 65 % RH and photoperiod of 12 L: 12 D) and was repeated three times.

Figure 11. Six-armed olfactometer bioassay used to test preference of *T. absoluta* males between attract-and-kill formulations, differing in sex-pheromone concentrations and different numbers of virgine *T. absoluta* females

2.7 Stability and efficacy of Attract-and-kill formulation under natural environmental conditions

Figure 12. Attract-and-kill application on potted tomato plants to test stability of formulation

The Attract-and-kill formulation, containing 5.0 g/L beta-cyfluthrin and 0.05 % sex pheromone of *T. absoluta*, was randomly applied in droplets (100 µl) on leaves of potted tomato plants, exposed to outdoor conditions at CIP's experimental station La Molina (240 m a.s.l.) (Fig.12). In one-week intervals (7, 14, 21, 28 and 35 days post application), three tomato leaves, containing the attract-and-kill droplet were collected and placed in a single open petri dishes. The petri-dishes were transferred into wooden cages (0.80 x 1.20 x 0.60 m), and subsequently 15 freshly emerged *T.* absoluta moths were released. The number of dead moths was daily assessed until no living moth could be observed in the cages. The untreated control experiment, did not contain active ingredients (insecticide and pheromone) in the Attract-and-kill formulation and was not exposed to outdoor conditions. The treatment and control experiments had four repetitions and two replicates and were conducted under laboratory conditions of 22°C, 65 % relative humidity.

2.8 Statistical analysis

The total number of dead *T. absoluta* males, assessed at the end of all experiments was used as initial number of moths, since males escaped during the experiment and thus could not be considered dead. Death of males caused by experimental errors was excluded from the statistical analysis. The total number of moths was converted into mean cumulative mortality (%) and Schneider-Orelli's (1947) formula was used to assess efficacies. The results obtained in the experiments were statistical analyzed by JMP® 11.0.1 software (SAS 2007). The particular statistical methods are specified in the legends of the tables.

3 Results

3.1 Testing the optimum beta-cyfluthrin and pheromone concentration in the attract-and-kill formulation under laboratory conditions

3.1.1 Testing the optimum beta-cyfluthrin concentration

To assess the efficacy of treatments in the insecticide experiments, each day the number of dead *T. absoluta* males in the Petri-dishes were recorded. The absolute numbers of dead moths were converted into percentage mortality. In total, the cumulative mortality was analyzed until all treated moths were dead (Fig. 13).

Since mortality in the untreated control exceeded 50 % after four days post start of experiments (Fig. 14), the relative efficacy as calculated following the formula of Schneider-Orelli (1947) became less reliable and significant. Thus, the efficacy will be compared and discussed in detail only for the first three days post start of experiments (Tab. 1).

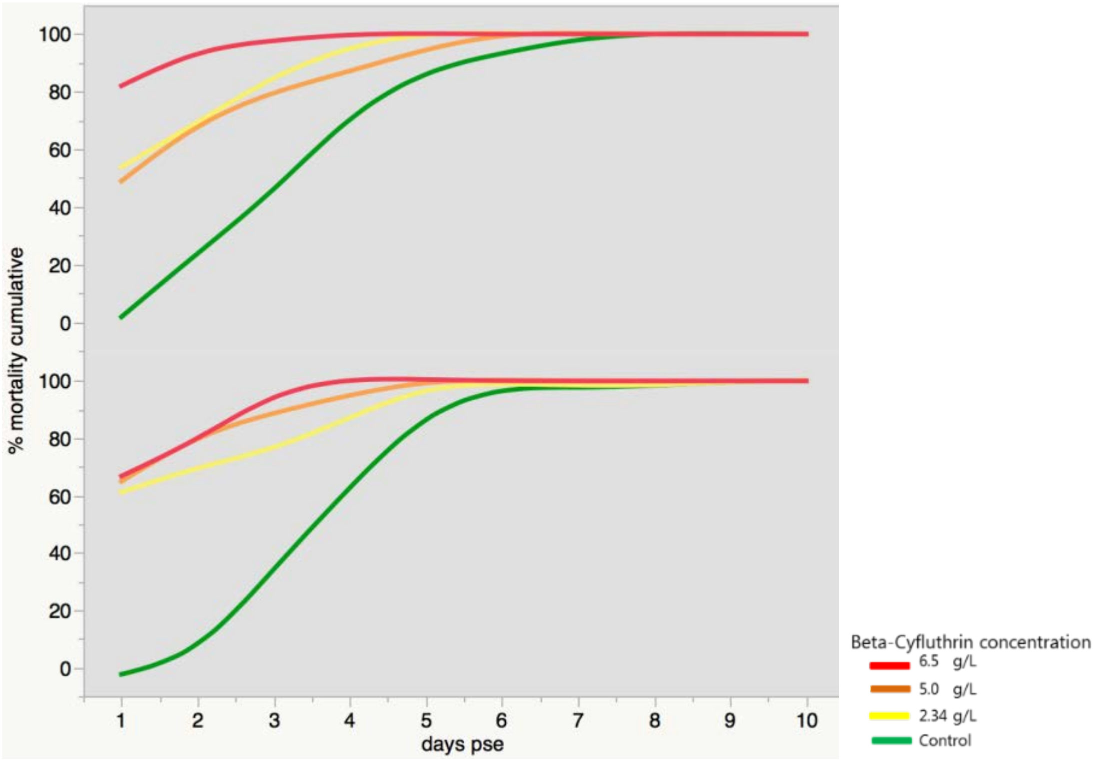

Figure 13. Mean cumulative mortality (%) of *T. absoluta* males over time (days post start experiment (pse)) after 5 and 10 second exposure (upper and lower figure, respectively) experiments to beta-cyfluthrin solution by tarsal dip method

In the 5-second exposure experiments, the highest concentration of 6.5 g/L beta-cyfluthrin caused the highest cumulative mortality rate of *ca.* 81 %, one day after treatment. Three days post start of experiment (dpse), 96.67% mortality was recorded. On the first day post start of experiment the formulation containing 2.34-g/L beta-cyfluthrin caused a mortality of 53.98 % which exceeded the mortality of the formulation containing 5.0 g/L beta-cyfluthrin (47.96 %). On the third day post start of experiment the concentration of 2.34 g/L caused a cumulative mortality of 85.45 %, whereas in the trials containing 5.0 g/L a cumulative mortality of 79.44% was observed. The control mortality showed a cumulative mortality of 43.67 % on the third day post start of experiment (Fig. 13). In the 10 second exposure experiments, the highest concentration of 6.5 g/L beta-cyfluthrin caused a mortality of more than 67.22 % on the first day post start of experiment whereas the mortality caused by the medium concentration of 5.0 g/L was 64.07 %. However, the differences in the cumulative mortality on day three post start of experiment were observed to be substantial i.e. 96.48 % in the trials when treated with the highest concentration of beta-cyfluthrin whereas it was 87.96 % in the trials when exposed to the medium concentration. The treatments, containing 2.34 g/L beta-cyfluthrin exhibited a lower mortality on the first day post start of experiment around 60.91 % and on day three post start of experiment it was 75.45%. The cumulative control mortality increased from zero to 36.37 % within three days post start of experiment (Fig. 13). The comparison of both exposure times showed in total, that the highest concentration of 6.5-g/L beta-cyfluthrin caused the highest mortality.

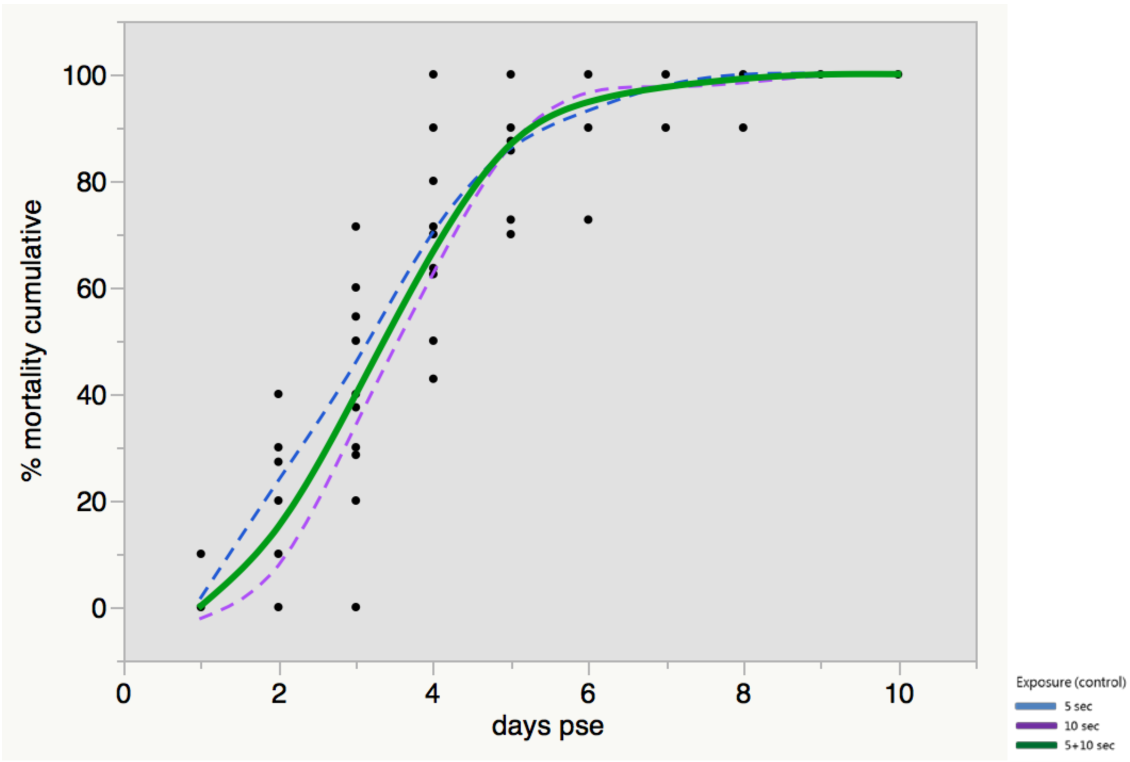

Figure 14. Mean cumulative mortality (%) *T. absoluta* males in Control after 5 and 10 second exposure to control substance of insecticide trials over time (days post start of experiment (pse))

The cumulative mortality of both Control experiments (5 and 10 second exposure) showed a similar development. Still, the cumulative mortality of male moths, being exposed to the control substance for 5 second increased faster until the fifth day post start of experiment where both cumulative mortalities exceeded 80 %. The mortality in both exposure trials reached almost

100 % on the seventh day post start of experiment. The cumulative combined mortality of the control experiments were observed to be approximately 40 % on day three and increased to 60% on day four. On day five the combined mortality was observed to be higher than 90% (Fig. 14).

The mean efficacies of the differing beta-cyfluthrin solutions on *T. absoluta* males were statistically analyzed by a one-way ANOVA, followed by a LSD-test, to determine significant differences (Tab. 1.).

Table 1. Cumulative mean efficacy (±SD) of beta-cyfluthrin concentrations on *T. absoluta* male moths over time (days post start experiment (dpse))

Beta-cyfluthrin concentration (g/L)	**Mean efficacy (%)**					
	5 second exposure			**10 second exposure**		
	dpse1	dpse 2	dpse 3	dpse 1	dpse 2	dpse 3
2.34	53.46 ±20.4 **b**	60.36 ±26.2 **b**	75.14 ±28.68 **ab**	60.91 ±32.2 **a**	68.86 ±27.07 **a**	64.51 ±25.93 **b**
5.0	46.77 ±26.7 **b**	57.88 ±35.6 **b**	63.30 ±23.99 **b**	64.07 ±17.5 **a**	80.40 ±12.10 **a**	83.14 ±15.72 **ab**
6.5	80.65 ±14.7 **a**	93.53 ±10.4 **a**	94.04 ±9.27 **a**	67.22 ±21.5 **a**	77.71 ±17.17 **a**	95.41 ±7.12 **a**
F	4.2943	3.4459	4.6468*	0.0988	0.5586	4.6236
P	0.0335	0.0587	0.0456*	0.9065	0.5835	0.0456
d.f.	2, 15	2, 15	2, 8*	2, 15	2,15	2, 15

Means within a column followed by the same letter do not differ significantly (one-way ANOVA followed by LSD-test: $P < 0.05$)

* F, df, and P values were corrected by Welch-ANOVA-test because of variance inhomogeneity after Levine test

Under 5 second exposure experiment, the mean efficacy of the beta-cyfluthrin concentration of 6.5 g/L, exceeded 80 % on the first day and 90 % on the second day post start of experiment respectively and was notably higher than beta-cyfluthrin concentrations of 2.34 and 5.0 g/L. The beta-cyfluthrin concentration of 2.34 did not differ significantly from the beta-cyfluthrin concentrations of 6.5 g/L on day three post start of experiment.

The comparison of mean efficacies of beta-cyfluthrin concentration of 2.34, 5.0 and 6.0 g/L, of 10 second exposure experiment, proved not to be remarkably different on the first and second day post start of experiment. The highest efficacy on day two post start of experiment has been observed in the beta-cyfluthrin concentration of 5.0 g/L. On the third day post start of experiment no significant differences could be observed between the 6.5 g/L and 5.0-g/L concentration, whereas the 2.34 g/L concentration were particularly lower than the 6.5 g/L concentration respectively (Tab. 1).

3.1.2 Testing optimum pheromone concentration

To determine the optimum pheromone concentration in the attract-and-kill formulation, containing 5.0 g/L beta-cyfluthrin, the number of dead *T.absoluta* male moths in the cages were recorded and subsequently converted into percentage mortality, until all moths were dead (Fig. 15). Some moths were observed to be dead in the viscous attract-and-kill droplet (Fig.16), while others were found dead on the cage floor. The assessment of cumulative mortality was followed by the calculation of the relative efficacy, corrected by Schneider- Orelli (1947).

Since high cumulative control mortality on day four post start of experiment (ca. 50%) (Fig 15) resulted in less reliability of the efficacy, a detailed comparison and discussion of the efficacy was limited to the first three days post start of experiment (Tab. 2).

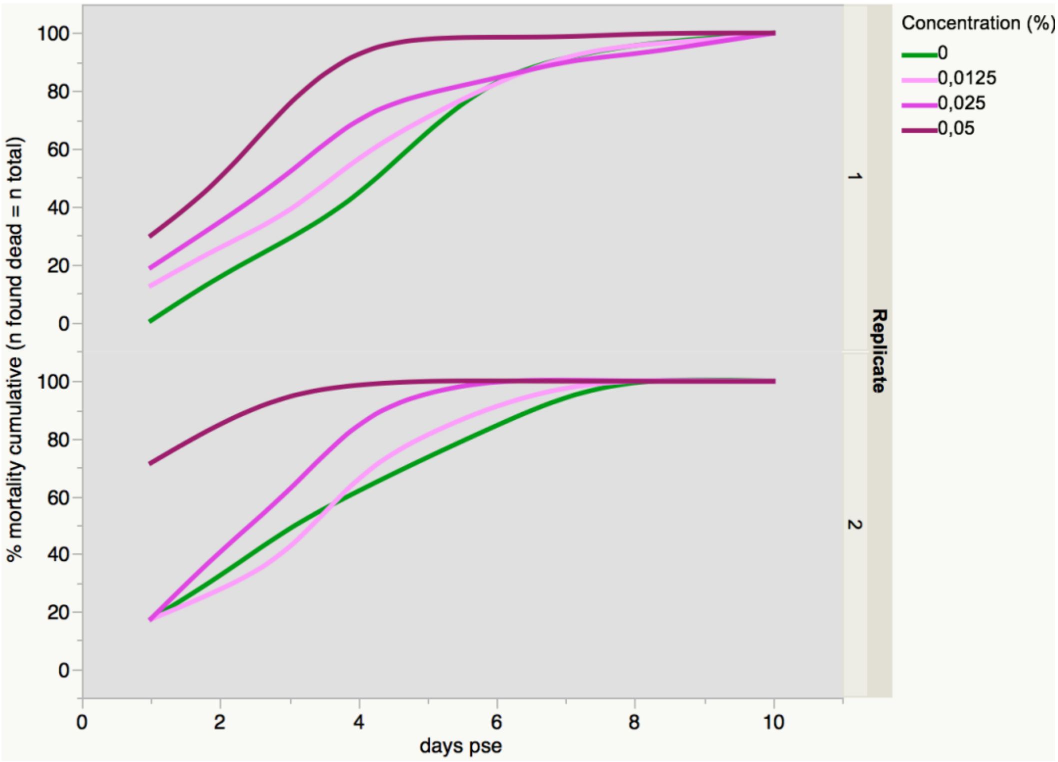

Figure 15. Mean mortality cumulative (%) (n found dead = n total) of replicate 1 and 2 of *T. absoluta* male moths exposed to Attract-and-kill formulation contained 5 g/L beta-cyfluthrin and varying pheromone concentration (0.05%, 0.025% and 0,0125 % respectively) and one control (without pheromone) over time (day post start of experiment (pse)) in cage experiment.

In first replicate the highest concentration caused 31, 66% mortality on the first day and 78,33 % on the third day post start of experiment. The other attract-and-kill formulation led to lower mortality; the formulation of the medium pheromone concentration caused 19.06 % on the first day and 48. 38 % on the third day post start of experiment, whereas the formulation with the least pheromone concentration caused 12.25 % on the first day and 34.47 % on the third day post start of experiment. The control mortality was observed at 0 % on the first day and 29.11 % on the third day post start of experiment. Mortality on day 4 was observed at 95% in the highest concentration, 74.46 % in the medium and 59.72 % in the least concentration, whereas the control mortality stood at 41.42 %.In total the highest concentration of the synthetic sex-pheromone caused the highest cumulative mortality (%) compared to the other concentrations, followed by the medium and the least concentrations: The second replicate shows a mortality rate of 71.00 % on day one and exceeded to 95 % on the third day post start of experiment caused by the formulation, containing the highest synthetic pheromone concentration. On day 1 the medium concentration was 16.89 %, and the least concentration was 17.60 %: It caused less than 20 % mortality on day one, whereas on day three post start of experiment the cumulative mortality was observed to be 57.27% in the medium and 35.09% in the least concentration. The control mortality increased almost steadily, starting at 17.74 % on day one and exceeding 50 % on day three post start of experiment, being higher than in the least concentration. In total the highest concentration of the synthetic-sex pheromone caused the highest mortality % followed by the medium concentration. The control mortality was higher than the formulation, containing the least pheromone amount until day 4 post start of experiment (Fig. 15).

To assess significant differences between the mean efficacies, differing in pheromone concentrations, a one-way ANOVA was performed, followed by a LSD-test (Tab 2.).

Table 2. Cumulative mean efficacy (±SD) of Attract-and-kill formulation differing in pheromone concentration on *T. absoluta* male moths over time (days post start experiment (dpse))

Pheromone Concentration (%)	**Mean efficacy (%)**		
	dpse 1	**dpse 2**	**dpse 3**
0.0125	6.04 ±13.56 **b**	5.65 ±16.45 **b**	-11.70 ±26.87 **c**
0.025	9.02 ±14.97 **b**	19.48 ±20.23 **b**	20.48 ±25.36 **b**
0.05	48.21 ±21.52 **a**	56.16 ±25.62 **a**	80.49 ±15.53 **a**
F	15.2628	12.2325	32.7094
P	<.0001	0.0003	<.0001
d.f.	2, 21	2, 21	2, 21

Means within a column followed by the same letter do not differ significantly (one-way ANOVA followed by LSD-test: $P < 0.05$)

The attract-and-kill formulation, containing the highest pheromone concentration, had a strikingly higher mean efficacy over the first three days post start of experiment, than formulation containing lower pheromone concentration. On the other hand, the formulation, containing the medium pheromone concentration, showed a significantly higher efficacy on day three post start of experiment than formulation with the least pheromone concentration (Tab 2).

Figure 16. Dead *T.absoluta* males in Attract-and-kill droplet containing 0.05 % pheromone concentration

In both replicates the mortality post start of experiment of the male moths increased with the increasing amount of synthetic sex pheromone concentration within the attract-and-kill formulation.

3.2 Testing the preference and competence of virgin female *T. absoluta* moths against the attract-and-kill formulation in olfactometer bioassays

The preference and reaction of unmated *T.absoluta* male moths was tested in olfactometer bioassays. The number of males in the cups, containing different treatments and the middle cup, without treatment, were counted.

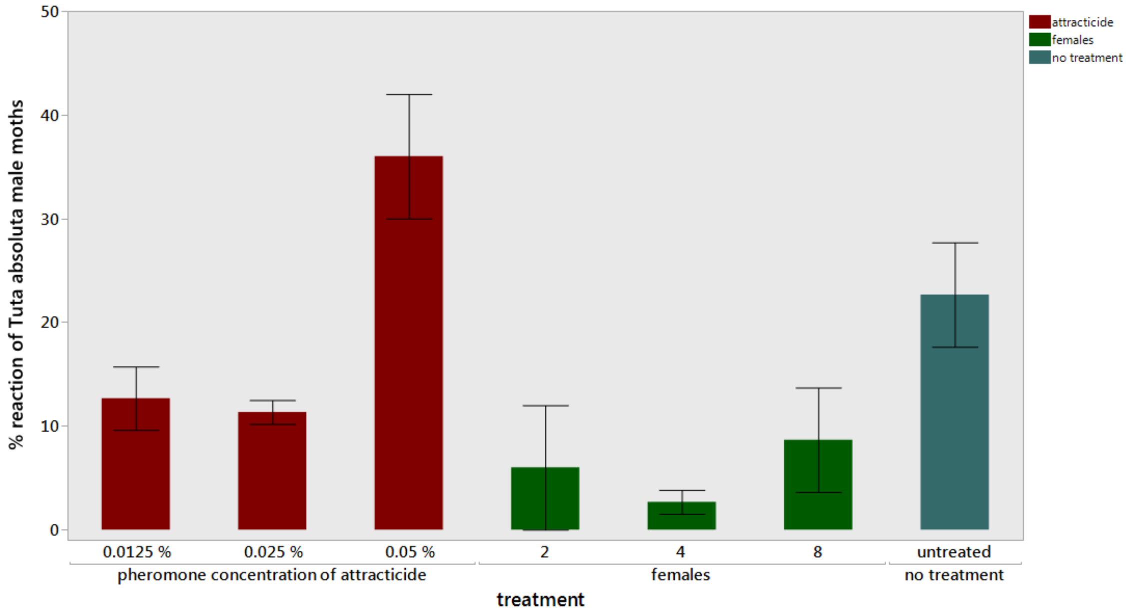

Figure 17. Mean reaction (%) (± SD) of *T. absoluta* male moths after 24 hours in circular olfactometer bioassay containing Attract-and-Kill formulation with varying pheromone concentrations (0.0125, 0.025 and 0.05 %) or groups of virgin *T. absoluta* moths (2, 4 and 8) and one middle cup, containing no treatment

In total the pheromone concentrations attracted more male moths than the groups of females. The highest mean reaction to female *T. absoluta* moths was observed in the cups, containing 8 virgin females with 8.67 %, followed by 2 females with 6.00 %. The cup containing 4 *T. absoluta* virgin female moths showed the least attraction (2.67 %). The attract-and-kill formulation containing a pheromone concentration of 0.0125 % induced 12.7% reaction, followed by the 0.025 % pheromone concentration with 11.3 % reaction. The attract-and-kill formulation containing the highest pheromone concentration (0.05%) showed the highest reaction of *T. absoluta* male moths with 36.00 %. The male moths, which were in the middle cup containing no treatment and showing no reaction neither to the varying pheromone concentrations nor to the female *T. absoluta* moths, were observed to be 22.7 %(Fig 17.) .

The mean reaction of *T.absoluta* to the pheromone concentrations or varying number of female moths was subsequently analyzed for significant differences in a one-way ANOVA, followed by a Tukey–Kramer HSD-test (Tab. 3).

Table 3. Mean reaction of *T.absoluta* moths in (%) (n=116) to Attract-and-kill formulations differing in pheromone concentration (0.0125 %, 0.025 % and 0.05 %) and varying number of virgin females (2, 4 and 8)

Treatment	Reaction of male *T. absoluta* moths (%)
pheromone concentration of **0.05 %**	46.90±10.27 **a**
pheromone concentration of **0.025 %**	14.71±1.92 **b**
pheromone concentration of **0.0125 %**	16.33±3.36 **b**
8 females	11.09±6.30 **b**
4 females	3.41±1.28 **b**
2 females	7.57±7.70 **b**
F	19.6717
P	<0.0001
d.f.	5; 12

Means within a column followed by the same letter do not differ significantly (one-way ANOVA followed by Tukey–Kramer HSD-test: $P < 0.05$)

The Attract-and-kill formulation containing a pheromone concentration of 0.05%, resulted in a significantly higher reaction of T. absoluta male moths than all other treatments. Between the attract-and-kill formulations, containing pheromone concentrations of 0.025 % and 0.0125 %, and the virgine females, no significant differences could be observed (Tab. 3).

3.3 Stability and efficacy of Attract-and-kill formulation under natural environmental conditions

The efficacy of the attract-and-kill formulation, containing a concentration of 0.05% synthetic sex-pheromone and a concentration of 5.0 g/L beta-cyfluthrin, was tested on *T. absoluta* male moths in cage experiments after exposure to outdoor conditions (1 , 7 , 14 , 21 , 28 and 35 days respectively). The mortality of the male moths was evaluated and converted into mean cumulative mortality (Fig.18).

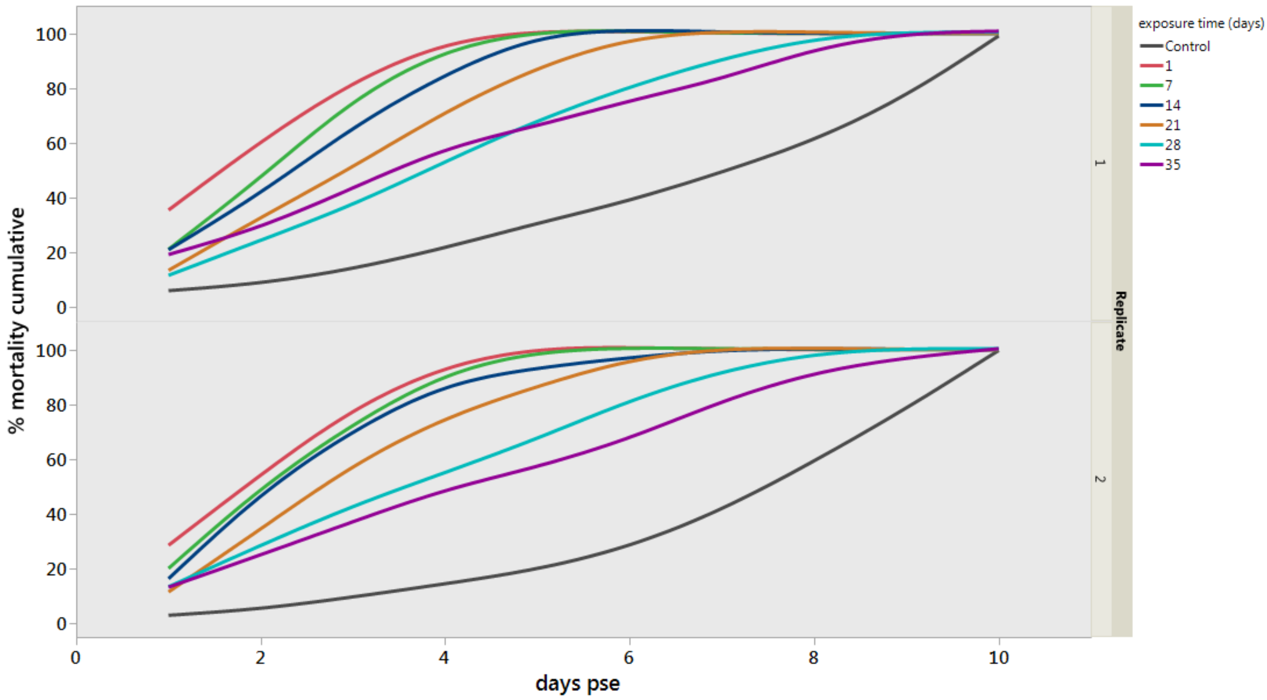

Figure 18. Mean mortality cumulative (%)(n found dead = n total) of replicate 1 and 2 of *T. absoluta* male moths, exposed to Attract-and-kill formulation (containing 5 g/L beta-cyfluthrin and 0.05% sex-pheromone) with different exposure times to outdoor conditions (1, 7 , 14 , 21 , 28 and 35 days respectively) and one control without active ingredients and outdoor exposure.

The cumulative mortality decreases with increasing exposure time of the attract-and-kill formulation to environmental conditions.

In replicate 1 the highest cumulative mortality was observed in the attract-and-kill formulation being exposed first day to outdoor conditions, showing 35 % mortality, followed by 7, 14 and 35 days exposure with ca. 20 % mortality on the first day post start of experiment. The attract-and-kill formulation, being exposed for 21 and 28 days to outdoor conditions showed a mortality of 13.33 and 11.67 % on the first day post start of experiment, whereas the control mortality was observed to be 6.5 %. On day three post start of experiment the attract-and-kill formulation with first day outdoor exposure caused a cumulative mortality of 81.67 %, after 7 days of exposure it was observed at 76.67%, after 14 days of exposure it was 66.67% and. after exposure of 21 days a cumulative mortality of 50% was observed. The attract-and-kill formulation exposed to outdoor conditions of 28 and 35 days caused mortalities less than 50% on day 3 post start of experiment. The control mortality was observed to be 14.21 % on the third day post start of experiment (Fig. 18).

In total, both replicates showed a similar development in cumulative mortality over time.

The highest mortality on day one post start of experiment were caused by the attract-and-kill formulation with the least exposure time of one day with 28.33% mortality, followed by 7 days exposure at 19.10% and 14 days exposure at 15.00%, in replicate 2. All the other Attract-and-kill formulations (outdoor exposure of 21 , 28 and 35 day) showed a mortality less than 15% on day 1 post start of experiment. The control mortality on day one was observed to be 3.33 %.

On the third day post start of experiment the attract-and-kill formulation exposed first day to outdoor conditions exceeded cumulative mortality of all other treatments with 78.33 %, on 7 days the exposed to outdoor conditions (70.90 %) and the one of 14 day outdoor exposure (68.33%). The attract-and-kill formulation, being exposed 21 days to the environment showed a mortality of 58.33%, whereas all other attract-and-kill formulations (exposure 21, 28 and 35 day) showed mortality less than 50 %. The control mortality was 10 % on day three exceeded to 20 % on day four and 60% on day eight post start of experiment.

A one-way ANOVA, followed by a Tukey–Kramer HSD-test was performed to assess significant differences between the efficacies of the Attract-and-kill formulation, differing in exposure time (days) to environmental conditions (Tab. 4).

Table 4. Cumulative mean efficacy (±SD) of attract-and-kill formulation, differing in exposure time (1, 7, 14, 21, 28, 35 days) to environmental conditions, on *T. absoluta* male moths over time (days post start experiment (dpse))

	Mean efficacy (%)								
Exposure time (days)	**dpse1**	**dpse 2**	**dpse 3**	**dpse 4**	**dpse 5**	**dpse 6**	**dpse 7**	**dpse 8**	**dpse 9**
1	28.17 ±10.70 **a**	53.56 ±12.34 **a**	77.28 ±7.94 **a**	93.95 ±5.62 **a**	100.00 ±0 **a**	100.00 ±0 **a**	100.00 ±0 **a**	100.00 ±0 **a**	100.00 ±0 **a**
7	16.26 ±6.43**ab**	43.67 ±7.78**a**	70.23 ±6.87**a**	90.84 ±6.74**a**	98.96 ±2.95**a**	100.00 ±0**a**	100.00 ±0**a**	100.00 ±0**a**	100.00 ±0**a**
14	14.14 ±7.68**b**	39.99 ±11.37**ab**	62.98 ±6.53**a**	82.49 ±10.18**ab**	94.79 ±8.84**ab**	97.67 ±4.31**a**	100.00 ±0**a**	100.00 ±0**a**	100.00 ±0**a**
21	7.96 ±5.89**b**	28.45 ±6.91**bc**	47.71 ±8.53**b**	67.19 ±8.12**b**	80.91 ±10.67**b**	96.32 ±5.10**a**	100.00 ±0**a**	100.00 ±0**a**	100.00 ±0**a**
28	7.93 ±9.00**b**	21.26 ±9.12**c**	31.60 ±12.95**c**	43.71 ±13.68**c**	56.09 ±17.43**c**	70.94 ±17.03**b**	82.81 ±11.74**b**	95.7 ±7.83**a**	100.00 ±0**a**
35	12.40 ±8.67**b**	21.29 ±6.17**c**	31.70 ±13.61**c**	44.80 ±18.66**c**	47.17 ±18.05**c**	57.62 ±16.93**b**	65.82 ±16.09**c**	83.28 ±15.59**b**	92.31 ±14.24**a**
F	6.63	16.23	31.91	30.65	30.38	25.65	24.78	7.07	2.33
P	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0588
d.f.	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42	5, 42

Means within a column followed by the same letter do not differ significantly (one-way ANOVA followed by Tukey–Kramer HSD-test: $P < 0.05$)

The efficacy of the Attract-and-kill formulation decreased with increasing exposure time to environmental conditions. The Attract-and-kill formulation, which was exposed for one day to outdoor conditions showed the highest efficacy over time. In total the Attract-and-kill formulation on 1st, 7th and 14th day outdoor exposure with efficacies of 77.28%, 70.23% and 62.98 % respectively after 72 hours, did not show any significant differences after the second day post start of experiment. The attract-and-kill formulation on the 21st day outdoor exposure showed significantly higher efficacies i.e. 47.71 % on the third day post start of experiment than the attract-and-kill formulation with 28 and 35 days outdoor exposure after the second day post start of experiment observed at 31.6% and 31.70 % after the third day post start of experiment (Tab. 4).

A probit analysis was performed to determine the LT_{50} and LT_{99}- values of the tested *T. absoluta* males exposed to the Attract-and-kill formulation, varying in exposure time to environmental conditions, (Tab 5).

Table 5. Mean lethal time (LT_{50} and LT_{99} with 95 % confidential limit (CL)) of *T. absoluta* male moths after exposure to attract-and-kill formulation, varying in exposure time (1, 7, 14, 21, 28, 35 days) to outdoor conditions over time (days post start of experiment (dpse))

Exposure time (days)	n[1]	Concentration - Probit efficacy response	r^2	LT_{50} (dpse)	95 % CL for LT_{50}	LT_{99} (dpse)	95 % CL for LT_{99}
1	120	3.7756488 + 0.6493118*days pse	0.865883	1.89	1.57 - 2.25	5.47	4.91 - 6.13
7	118	3.3947972 + 0.6928331*days pse	0.924728	2.32	2.06 - 2.60	5.68	5.26 - 6.15
14	120	3.5732483 + 0.5380907*days pse	0.858439	2.65	2.27 - 3.09	6.98	6.32 - 7.74
21	120	3.1652046 + 0.5494215*days pse	0.871988	3.34	2.95 - 3.78	7.58	6.95 - 8.30
28	120	3.3526507 + 0.3650755*days pse	0.766292	4.51	3.87 - 5.25	10.89	9.81 - 12.16
35	120	3.7151722 + 0.2448774*days pse	0.652008	5.25	4.37 - 6.31	14.76	13.07 - 16.81

n[1] number of tested *T. absoluta* male moths

The attract-and-kill formulations on 1, 7 and 14 days outdoor exposure, showed a LT_{50} of 1.89, 2.32 and 2.65 days post start of experiment. The LT_{50} of the attract-and-kill formulation with 21, 28 and 35 days outdoor exposure were observed to be 3.34, 4.51 and 5.25 days post start of experiment.

For an exposure time of 1, 7 and 14 days respectively the LT_{99} of 5.47, 5.68 and 6.98 days post start of experiment were assessed. The attract-and-kill formulation on 21, 28 and 35 days outdoor exposure showed a LT_{99} of 7.58, 10.89 and 14.76 days post start of experiment.

In total, the LT_{50} and LT_{99} increased with longer exposure to outdoor conditions (Tab.5). I

4 Discussion

4.1 Discussion of methodology

The adult longevity for T. absoluta male moths, was observed to be 15.8 days under laboratory conditions of 25±1°C (Erdogan and Babaroglu 2014). However the natural mortality in the control experiments to assess the optimum pheromone and insecticide concentration was observed to be high, which might be due to temperature conditions of 25°C. The high temperatures might have affected the reliability of the efficacies. Thus all experiments were subsequently performed under temperature conditions of 22°C, where mortality in the control treatments were observed to be lower (results not shown).

To assess the optimum pheromone concentration, the attract-and-kill formulation was applied in 100 µm droplets in cages, filled with 15 *T. absoluta* males. All cages were repaired and properly cleaned before start of the experiments. Still, the assessment of dead moths showed missing *T. absoluta* individuals. One explanation that is possible, might be, that some of the moths might have been escaped or died in cracks or sleeves. Since the disappearance of moths, could not be evaluated as death caused by the treatment, they were excluded from the statistical analysis. Thus, the initial number of moths was equated with the total number of recorded dead moths.

The cages had been blocked and arranged in shelves, to overcome potential temperature differences. However, the close arrangement of the cages, containing different pheromone concentrations, might have influenced the olfactory perception of *T. absoluta* males in the cages.

In Pre-experiments, to assess the optimum insecticide concentration, T. absoluta moths have been exposed to differing beta-cyfluthrin concentrations on filter-papers through tarsal contact (results not shown).The methodology was changed, since the exposure to filter-papers containing beta-cyfluthrin in petri-dishes, did not guarantee an equal exposure-time for all moths, since some moths might be repelled, resting on the lid of the petri-dish.

The tarsal dip method is more precise in assessing insecticide susceptibility, since each individual is exposed to the treatment for the same quantity of time. Thus, alternatively, the tarsal dip method, performed. Further insecticides might be tested to determine higher susceptibilities.

Before development of the attract-and-kill formulation, the susceptibility to the insecticide should be tested and resistances excluded (Silva et al. 2015).

The olfactometer bioassays assessed the preference of *T. absoluta* males to different attractants sources after 24 hours. Further experiments on the behavior of *T. absoluta* males, directly after exposure to the Olfactometer might be conducted, to find out, if preference changes within the observation time of 24 hours.

Sex-pheromone based control of *T. absoluta*, aims to reduce male density in the target population to prevent mating. However, female and male *T. absoluta* moths were observed to remate frequently (Lee et al. 2014). Thus, males might mate several times before being controlled through the Attract-and-kill application, which might still result in relatively high population densities. Moreover, *T. absoluta* was observed to reproduce asexually under laboratory conditions (Caparros Medigo et al. 2012), which might affect pheromone-based control measures of *T. absoluta*. However, further research should assess the required conditions for the occurrence of deuterotokous parthenogenesis and subsequently natural occurrence, must be proved (Caparros Medigo et al. 2012). Despite these observations, which might negatively affect pheromone-based control measures, these strategies, including mass trapping (Witzgall et. al 2010) and Attract-and-kill (Al-Zaidi 2010) proved to be successful and effective in the control of *T.absoluta.*

4.2. Discussion of results

4.2.1 Testing the optimum beta-cyfluthrin and pheromone concentration in the Attract-and-kill formulation under laboratory conditions

4.2.1.1 Testing the optimum beta-cyfluthrin concentration

Insecticides used in Attract-and-kill formulation are mainly pyrethroids with a rapid action, including cyfluthrin, cypermethrin and permethrin. A potential problem of the insecticide compound is a repellent effect (El-Sayed, 2009), which was solved through research on attract-and-kill formulations (De Souza et al. 1992, Brockerhoff and Suckling 1999, Poullot et al. 2001, Lösel et al. 2000). The active compound beta-cyluthrin (cyano-4-fluoro-3-phenoxyphenil)-methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate), is a nonsytemic pyrethroid (Type II), with a neurotoxic effect after oral and/or dermal uptake, causing contact and stomach poisoning (Chauhan et al. 2013). Since it has a rapid action, it is used against a wide range of Lepidoptera, Coleoptera and Hemiptera pests in rice (Naini et al., 2003), cotton, corn, sunflower, soybean (Addy-Orduna, 2011) and Solanaceaous crops (Sinha and Gopal, 2002) including tomato (Dikshit et al. 2003, Dharumarajan et al. 2009).

Since the use of pyrethroids is included in a common conventional management program of *T. absoluta*, resistances against pyrethroids have been reported and must be considered in the development of the attract-and-kill formulation (Barros et al. 2015, Silva et al. 2015).

After coming in touch with the viscous substance, containing a fast acting pyrethroid, *T. absoluta* males might soon be killed within a few minutes through lethal doses (Suckling and Brockerhoff 1999). A sublethal doses might induce resistance development in male moths (El-Sayed 2009). However, mating activity has been observed to be restricted in surviving males (Suckling and Brockerhoff 1999) and a higher susceptibility to natural enemies, (due to high sublethal effects of the insecticide) might keep the risk of resistance development very low (El-Sayed 2009).

The concentration of 6.5 g/L beta-cyfluthrin proofed to have higher efficacies, than concentrations of 5.0 g/L and 2.34 g/L, in both exposure times (5 and 10 second). The 5 second exposure experiments indicate a relatively high susceptibility of the *T. absoluta* moths, exposed to a concentration of 6.5 g/L beta-cyfluthrin with a mean efficacy of 93.53 % and 94.04 % on the second and third day post start of experiment.

Since no significant differences between the 6.5 g/L and the 5.0 g/L concentration were found, the efficacies of the 10-second exposure indicated, that a concentration of 5.0 g/L might be sufficient for the attract-and-kill formulation. Further Attract-and-kill formulations on other Lepidoptera species, containing a beta-cyfluthrin concentration of 5.0 g/L, proved to be highly effective as killing agent (Kroschel and Zegarra 2010; Lösel et al. 2000). Moreover, the attract-and-kill formulation should be compatible with the principles of an IPM, promoting the reduction of pesticide use. Thus, the concentration of 5.0 g/L was considered as optimum beta-cyfluthrin concentration of the *T. absoluta* attract-and-kill formulation. Further studies, investigating the efficacy of attract-and-kill formulations, containing 6.5 g/L beta-cyfluthrin might be performed.

The Attract-and-kill formulation TAC-37, which was reported to be effective in the control of *T. absoluta,* contains the active ingredient cypermethrin (3 %), which could be considered as alternative insecticide (Al-Zaidi, 2010). However, the concentration 30g/L cypermethrin is relatively high, compared to a concentration of 5.0 g/L beta-cyfluthrin. Furthermore, resistances to cypermethrin were reported in Brazilian *T. absoluta* populations (Silva et al. 2015), which might reduce the efficacy of the Attract-and-kill formulation.

4.2.1.2 Testing optimum pheromone concentration

Males searching for calling females, can localize the pheromone source even from long distances and start approaching the attract-and-kill droplet (Hickel and Vilela 1991, Heuskin et al. 2011). Pheromone elicitation by female moths is restricted to certain time periods, while the sex pheromone within the attracticide formulation might provide a steady male attraction throughout the day (Lösel et al. 2000, Krupke et al. 2002). Since, the pheromone is species-specific, natural enemies are not attracted by the attract-and-kill droplet (Witzgall et al. 2010) and might be combined with the attract-and-kill approach.

The sexual pheromone of *T. absoluta* females consists primary of two compounds,(3E, 8Z, 11Z)-3,8,11-tetradecatrien-l-yl acetate (TDTA) as major compound of approximately 90 % (Attygalle et al. 1996) and (3E, 8Z)-3,8- tetradecadien-l-yl acetate or TDDA (Svatoš et al., 1996) as minor compound of about 10 %.

To determine the optimum concentration of synthetic sex-pheromone of T. *absoluta* for the development of an attract-and-kill formulation, a range of 0.05, 0.025 and 0.0125 % pheromone was tested. In total, the attract-and-kill formulation containing a pheromone concentration of 0.05 % proved to be statistically the most effective concentration in killing *T. absoluta* males. On the first day 48.21 % efficacy has been observed, exceeding 80 % on day three post start of experiment.

TAC-37, an attract-and-kill formulation to control *T. absoluta* males, used a concentration of 0.3 % *T. absoluta* sex-pheromone, for field experiments in greenhouses (Al-Zaidi, 2010). However, a less synthetic sex-pheromone concentration of 0.065 % (Codlemone) was reported to be successful in the development of an attract-and-kill formulation in Codling moth (*Cydia pomonella* Lepidoptera: *Tortricidae*) (Lösel et al. 2000). For the potato tuber moths *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) (both Lepidoptera: *Gelechiidae*), a synthetic sex-pheromone concentration of 0.05%, within the attract-and-kill formulation, was reported to be highly effective in attracting male moths (Kroschel and Zegarra 2010). Thus, the pheromone concentration of 0.05 % based on experiences of previous attract-and-kill formulations of Lepidoptera pest species and on the obtained results, was the optimum pheromone concentration for the *T. absoluta* attratcticide formulation.

4.2.2 Testing the preference and competence of virgin female *T. absoluta* moths against the attract-and-kill formulation in olfactometer bioassays

The synthetic female sex-pheromone compound of the attract-and-kill formulation competes with the natural sex-pheromone produced by *T. absoluta* females, calling for male moths (Lösel et al. 2000).Moreover composition of the synthetical pheromone compound and its release rate are an essential basis for a successful attract-and-kill formulation (Jones 1998).

The statistical comparison of the mean reaction of the male moths to all treatments, showed a strong preference for the Attract-and-kill formulation, containing a pheromone concentration of 0.05 %, since it was significantly higher than the other pheromone concentrations, as well as the varying number of females. Moreover, the mean reaction of male moths to the varying pheromone concentrations in the Attract-and-kill formulation, was higher than to all groups of females, showing the higher competence of the synthetic pheromone compared to the varying number of females. Thus, the attract-and-kill formulation 0.05 % pheromone concentration might successfully compete with females, under natural conditions. Potential repellent effects, due to the beta-cyfluthrin concentration of 5.0 g/L, within the attract-and-kill formulation, could not be observed.

4.2.3 Stability and efficacy of Attract-and-kill formulation under natural environmental conditions

The attract-and-kill formulation was exposed to outdoor conditions for 1, 7, 14, 21, 28 and 35 days respectively to test its stability. An outdoor exposure of 1, 7 and 14 days did not remarkably reduced its efficacy. Thus the formulation did not significantly decreased in stability during an outdoor exposure of 14 days.

The efficacy on the 21 day outdoor exposure, however, showed a value of 47.7 % after three days post start of experiment, which was specially higher after 28 (31.60 %) and 35 (31.70 % day respectively of outdoor exposure. Thus the efficacies of the attract-and-kill formulations after more than 21 days outdoor exposure decreased even faster over time. The probit analysis showed, that with increasing exposure time the LT_{50} and LT_{99} (in days post start of experiment) of the tested *T. absoluta* moths increased, indicating decreasing efficacies of the attract-and-kill formulation over time. The LT_{50} and LT_{99} between 21 and 35 days exposure time showed an even larger increase. Thus, the results confirmed, that after more than 21 days of exposure time a remarkable reduction in stability was observed. Since the volatile pheromone compound has an unstable structure, it was highly prone to the high UV-radiation and temperatures of the exposure site (Kroschel and Zegarra 2010, Witzgall et al.2010, Heuskin et al. 2011). Moreover, UV-radiation leads to a rapid degradation of pyrethroid insecticides, such as cyfluthrin (6 hours) (Hussein et. al 1990). The UV-protectors detained the effect of photo degradation and are subsequently crucial compounds of the attract-and-kill formulation (Hussein et al. 1990, Lösel et al. 2000, Kroschel and Zegarra 2010).

5 Conclusion

The most effective Attract-and-kill formulation to control the tomato leafminer (*Tuta absoluta*) was developed and evaluated in laboratory experiments. Thereby, the concentration of the main ingredients, the synthetic sex-pheromone of *T. absoluta* and the contact-insecticide beta-cyfluthrin were optimized. The stability and efficacy of the optimum Attract-and-kill formulation were further evaluated after exposure to environmental conditions.

The optimum insecticide concentration was determined by the “tarsal dip-method” conducted on T*. absoluta* males. The highest efficacy, exceeded 90%, was caused by the beta-cyfluthrin concentration of 6.5 g/L, on the 3rd day post start of experiment. The beta-cyfluthrin concentration of 5 g/L however did not show any significant differences, causing efficacies of over 80 % on the 2nd day post start of experiment in the 10 second exposure. Consequently, the optimum beta-cyfluthrin concentration was determined to be 5 g/L. Furthermore, investigations on the efficacy of Attract-and-kill formulations, containing 6.5 g/L beta-cyfluthrin and alternative contact insecticides, might be conducted.

The optimum pheromone concentration was analyzed in cage experiments and in olfactometer-bioassays. The concentration of 0.05 % *T. absoluta* sex-pheromone in the Attract-and-kill formulation showed the highest efficacy with over 80 % on the 3rd day post start of experiment. The Attract-and-kill formulation, containing a pheromone concentration of 0.05 %, resulted in the significantly highest attractiveness in the olfactometer-bioassays and accordingly was more competitive compared to virgin *T. absoluta* females.

The optimum attract-and-kill formulation (containing 5.0 g/L beta-cyfluthrin and 0.05 % of the synthetic sex-pheromone) exhibited after 21 days of outdoor exposure, efficacies of approximately 50% on the 3rd day post start of experiment in cage experiments and thus, still, proved to be relatively stable.

Under laboratory conditions the Attract-and-kill strategy to control T. *absoluta*, showed favourable results and high efficacies. Further studies should be conducted on the field performance of the optimum Attract-and-kill formulation to determine application density and frequency (Kroschel and Zegarra 2010).

The conventional management of *T.absoluta* through frequent applications of insecticides did not reduce pest densities for the long-term. Thus, a sustainable control of *T. absoluta* requires an IPM program (Urbaneja et. al. 2013). Since the attract-and-kill approach is conform to IPM requirements (Kroschel and Zegarra 2013), it might be an effective, cost-efficient and sustainable solution in the integrated control of *T. absoluta*, especially for small scale farmers in the tropics and subtropics (Kroschel and Zegarra 2013).

6 Zusammenfassung

Eine “Attract-and-kill” Formulierung zur Bekämpfung der Tomatenminiermotte (*Tuta absoluta*), wurde in Laborexperimenten ermittelt. Dabei wurde die Konzentration des synthetischen Sexualpheromons von *T. absoluta* Weibchen und des Kontakt-Insektizids Beta-Cyfluthrin optimiert. Anschließend wurde die “Attract-and-kill” Formulierung auf Stabilität unter Umweltbedingungen getestet.

Die Ergebnisse der Laborexperimente zeigten, dass ein Beta-Cyfluthrin Konzentration von 6.5 g/L in der “Attract-and-kill” Formulierung die höchste Mortalität verursachte. Die Beta-Cyfluthrin Konzentration von 5.0 g/L in der 10 Sekunden Behandlung jedoch, wies keine signifikanten Unterschiede zur 6.5 g/L Konzentration auf und erzielte einen Wirkungsgrad von über 80 % zwei Tage nach Beginn der Experimente. Folglich wurde die optimale Beta-Cyfluthrin Konzentration, auf 5.0 g/L festgelegt. Zusätzliche Experimente könnten Aufschluss darüber geben, ob bei Erhöhung der Beta-Cyfluthrin Konzentration auf 6.5 g/L der Wirkungsgrad erhöht wird und ob alternative Kontaktinsektizide effektiver sind.

Die optimale Pheromone-Konzentration wurde in Käfigexperimenten und in Olfactometer-Bioassays getestet. In den Käfigexperimenten erzielte die “Attract-and-kill” Formulierung mit einer Konzentration von 0.05 % des Sexualpheromons und einer Beta-Cyfluthrin Konzentration von 5.0 g/L den höchsten Wirkungsgrad von über 80 %, drei Tage nach Beginn der Experimente. Die Ergebnisse der Olfaktometer- Bioassays zeigten, dass 0.05 % des synthetischen Sexual-Pheromones in der “Attract-and-kill” Formulierung, die signifikant höchste Lockwirkung auf *T. absoluta* Männchen ausübte und somit konkurrenzfähiger als das natürliche Sexualpheromone der jungfräulichen *T. absoluta* Weibchen war.

Die “Attract-and-kill” Formulation (5.0 g/L Beta-Cyfluthrin und 0.05 % des synthetischen Sexual-Pheromones enthaltend) zeigte nach 21 Tagen im Freien einen Wirkungsgrad von ca. 50% nach drei Tagen (nach Beginn der Experimente) in den Käfigversuchen und war somit relativ stabil.

Im Anschluss sollte die Ausbringung der “Attract-and-kill” Formulierung auf Versuchsflächen (Gewächshaus oder Freiland) getestet werden um die Applikationsdichte und die Applikationsfrequenz zu bestimmen (Kroschel und Zegarra 2010).

Die nachhaltige Bekämpfung von *T. absoluta* erfordert ein integriertes Pflanzenschutzprogramm (Urbaneja et al. 2013). Die “Attract-and-kill” Strategie ist eine kostengünstige Pflanzenschutzmaßnahme, die somit Kleinbauern der Tropen und Subtropen von Nutzen wären und die im Rahmen eines integrierten Pflanzenschutzprogrammes langfristig zu einer Abnahme der Schädlingsdichte von *T. absoluta* führen könnte (Kroschel und Zegarra 2013).

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Declaration

I,

Name, First name Ünlü, Ayse Gül

Matriculation number , 494195

hereby declare on my honor that the attached declaration,

□ Homework/Presentation
□ Bachelor Thesis
x Master Thesis
□ Diplom Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

1. Supervisor: Prof. Dr. Dr. Claus P. W. Zebitz, Institute of Phytomedicine (360), Applied Entomology (360c)

2. Supervisor: apl. Prof. Dr. Jürgen Kroschel, Agroecology/IPM, International Potato Center (CIP), Lima, Peru

Thesis topic: Development and evaluation of attract-and-kill to control the tomato leafminer *Tuta absoluta* (Meyrick)

Semester: 5

I declare, here within, that I have transferred the final digital text document (in the format doc, docx, odt, pdf, or rtf) to my mentoring supervisor and that the content and wording is entirely my own work. I am aware that the digital version of my document can and/or will be checked for plagiarism with the help of an analyses software program.

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