Sveriges lantbruksuniversitet
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The invasive Mediterranean Fruit Fly *Ceratitis capitata* Wied. (Diptera; Tephritidae): Life history, ecology, behaviour and its implication in ethological management.

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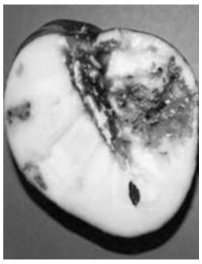

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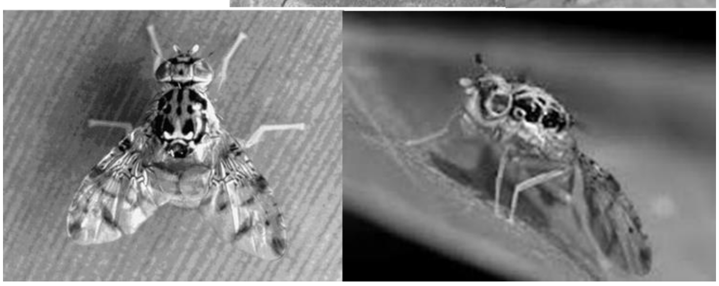

The invasive Mediterranean Fruit Fly *Ceratitis capitata* Wied. (Diptera; Tephritidae): Life history, ecology, behaviour and its implication in ethological management.

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Keywords: *Ceratitis capitata*, Mediterranean fruit fly, attractant, repellent, push-pull, trap-crop

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata* is considered a major pest of fruit production worldwide. It is a highly polyphagous species with big capability to adapt and spread into several different environments, giving it the status of a threating invasive species for fruit production in many countries. *C. capitata*'s high adaptability is mainly related to its life history, behavior and host location ecology, in which semiochemicals play a major role. Several control strategies have been developed using sexual and feeding attractants in monitoring and mass trapping programs, but little is known about the potential of plant-based compounds. These products, such botanical extracts and essential oils, could affect the behavior of pests like *C. capitata*. By studying and understanding adults' host location behavior when exposed to olfactory signals (attractant and repellents), the aim of this research is to explore the potential of odour sources for use in *C. capitata* control strategies for small and medium scale fruit production in Bolivian valleys. This research includes evaluation of locally available proteinbased attractants, screening and evaluation of native plant extracts and essential oils as spatial repellents, investigation of the *C. capitata* interaction with a local apple variety with potential as a trap-crop, and finally the study of the possibilities of the combined use of those components for use in the field.

Key words: *Ceratitis capitata*, Mediterranean fruit fly, attractant, repellent, push-pull, trap-crop

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1. Introduction

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann 1824 (Dipetera; Tephritidae) is considered a higher pest of fruit production worldwide (Malacrida, Gomulski *et al*. 2007) Female adults of this species oviposit into ripening fresh fruit, causing internal damage by maggots when fruits are still in tree close to harvesting. *C. capitata* has the ability to adapting to many different environments and is highly polyphagous, with a diverse host range including nearly 300 fruits and vegetables, both cropped and wild (Liquido *et al*., 1991). The species' adaptability and global fruit trade has allowed *C. capitata* to become a hazardous invasive species, with a large destructive capacity that threatens commercial fruit production in every area where it is present (CABI 2014), currently is considered the most economically important fruit fly species.

From its centre of origin in east-central Africa, *C. capitata* spread throughout the continent to the south and the Mediterranean basin in the north, including the European side (Gasparich, Silva *et al*. 1997). From this site, the global invasion progressed to Central and South America, Western Australia, the Middle East and the Hawaiian islands (Malacrida, *et al*. 2007). The species is responsible for high expenditures by countries confronted with its invasion such as Mexico, Guatemala, Chile, Peru and some states of USA (California, Florida), which put a lot of public effort to avoid and/or eradicate any presence and prevent for further invasions through elaborated monitoring systems (CABI 2014).

The Mediterranean Fruit Fly is present and widespread in Bolivia (Senasag, 2010, CABI 2014) The first report of its presence in the southern Bolivian valleys is from the 1940's reporting damage into peach and pears (Mendoza 1996), suggesting that this invasion originated in Argentina and moved throughout the country until reaching most of fruit productive zones in a wide range of fruit productive regions (Senasag 2010). Besides direct damage, *C. capitata* presence makes the country unable to export fresh fruits due to international trade regulations. Fruit growers and some isolated institutions have developed control strategies in rural productive orchards, including cultural management, mass trapping and chemical application. However, fruit production remains heavily affected in highly fruit productive areas such Cochabamba valleys (Figueroa 2005). Local strategies for Mediterranean fruit fly control include the use of home-made traps fabricated from plastic bottles and baited with kitchen fermented ingredients, but their potential for use as mass trapping tool in an integrated management program has not been properly evaluated.

For an effective control against *C. capitate*, any strategy should start prior fruits ripening. (Demirel 2007). Some botanical products may be able to prevent female oviposition when applied on the orchard acting as repellents or oviposition deterrent (Faraone *et al*., 2012). By studying the behavior of adults females when exposed with these natural products, the aim of this study is to research the potential of behavioural-based control components such odorant stimuli (plant-based repellents, attractants) and a possible trap-crop (local apple variety). Here, I discuss the possible use of these components into a combined strategy such the stimulo-deterrent diversion (push-pull) against medfly attack in Bolivian fruit orchards.

2. *Ceratitis capitata* (Wiedeman): Life history

2.1. Morphological description: Adults C. capitate are small flies of about 6 to 8 mm long, yellowish in general color, it has a tinge of brown especially in abdomen, legs and the wing markings (Carroll L.E., *et al*. 2002). Thorax surface is convex, of creamy-shite to yellow colour spotted with black blotches. The oval shaped abdomen is clothed with a fine, scattered black bristles, it has two narrow, transverse light coloured bands on the basal half. Female can be distinguished by is long ovipositor at the abdomen end, which contains a hard sclerotized organ called *Aculeus* (CABI 2014), this organ is used to insert eggs through fruit skin during oviposition. Male heads bears two long, black bristles with flattened diamond shaped tip, arising between both eyes near the antennae (Carroll L.E., *et al*. 2002). (Figure 1)

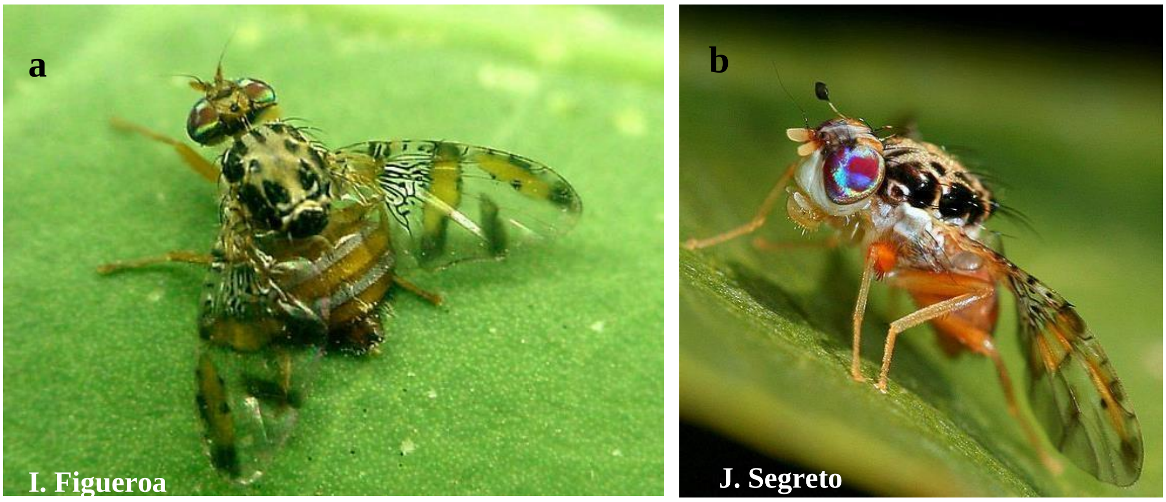

Figure 1: (a) Adult female *C. capitata* dorsal view. (Picture by I. Figueroa) (b) Adult male *C. capitata* side view with the black bristles in the head (Picture by Jari Segreto, www.flickr.com)

2.2. Distribution worldwide*:* *C. capitata* originated in sub-Saharan Africa. Since the 1880's, it has spread trhougthout the Mediterranean region, southern Europe, the Middle Est, Wetern Australia and eventually the Hawaiian islands. In the western hemisphere, *C. capitata* invasion started in Brasil and Argentina, and then spread throughout all South American countries with the sole exception of Chile (Gasparich, *et al*.1997). From Central America (Panamá, Costa Rica, Nicaragua, Honduras, and Guatemala) *C. capitata* is threatening Mexico and North America, where detections and often outbreaks carried large economical and logistical efforts to block any further spreading in those countries (CABI 2014) (figure 2).

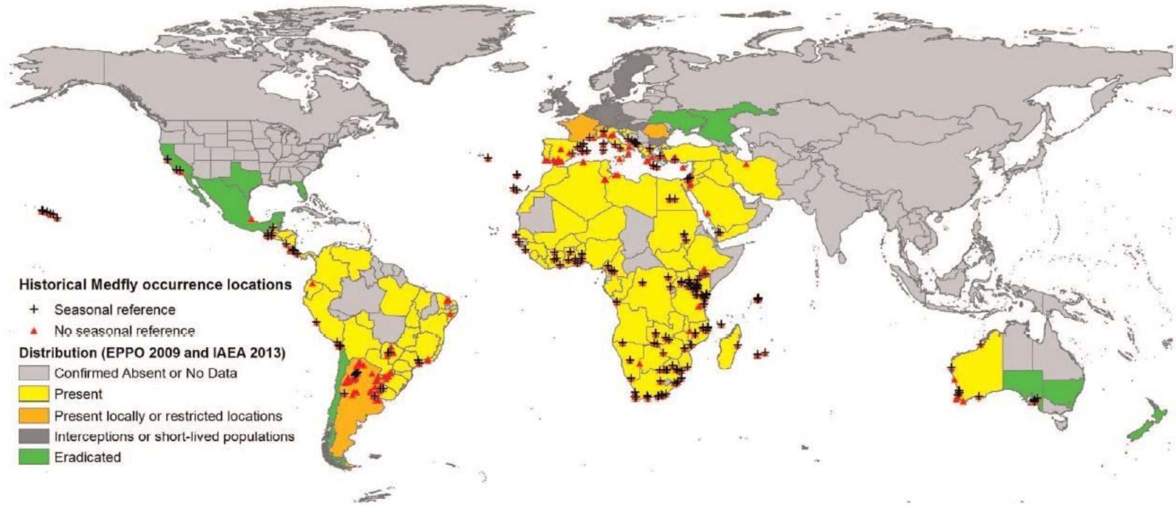

Figure 2: *Ceratitis capitata* distribution map (EPPO 2009, IAEA 2013) taken from (Szyniszewska and Tatem 2014)

2.3. Life cycle: The life cycle of *C. capitata* starts when a gravid female lays eggs inside a ripening fruit. When maggots emerge they start to eat on the fruit flesh (mesocarp and endocarp) causing the characteristic damage of fruit flies (Demirel 2007). Larvae tend to occur close to seeds or pits in the case of stone-fruits, and try to feed mostly in the matured part of the flesh (Yuval and Hendrichs 2000). When maggots complete their growth, they find their way out of the fruit, often with an exit hole and drop on the soil surface. Mature larvae dig into the soil and pupate at a few centimetres depth. Pupae are elliptical and brown coloured, and if the environmental conditions are good (high temperature and humidity), adult flies emerge from puparium out of the soil (CABI 2014) In temperate environment where winter or dry season is marked, pupae can overwinter several months until optimal climate conditions achieves (Malacrida, 2007). Adult males show a complex aggregation behavior and form small groups called leks, where males perform courting to potential female couples (Eberhard 2000, Yuval and Hendrichs 2000). Male courting behavior at close range includes pheromone releasing, visual displays and acoustic signals toward receptive female, and if she accept his performance, then mating occurs (Demirel 2007).

2.4. Host range: C. *capitata* is one of the world most destructive fruit pest, it has high ability to tolerate cooler climates and is able to spread over a wide range of tropical, template, humid or dry ecosystems. This flexibility is mainly because of its huge range of hosts in which can develop (Szyniszewska and Tatem 2014). *Ceratitis capitata* attacks more than 260 different species of fruits, flowers, vegetables and nuts (Liquido *et al*. 1991). Usually, thin-skinned, ripe, succulent fruits are preferred, such as peach, apricot, guava and mango. Host preferences vary in different regions and some fruits such as several cucurbit species has been recorded as host only under laboratory conditions and may not be attacked in the field (CABI, 2014). Knowledge of host preference in one region often help to predict the most likely host to be infested in a newly invaded area, but what may be a preferred host in one particular zone may be a poor host in another (Yuval and Hendrichs 2000).

3. Mating behaviour of *C. capitata*

3.1. Lekking or aggregation by males: Adults males show a complex aggregation behavior and form small groups called lek, a males group on tree foliage where they show themselves to choosier females (Eberhard 2000, Yuval and Hendrichs 2000). The definition of "lek" is a non-resource based male aggregation, visited by females only for copulation (Yuval and Hendrichs 2000). Sexual mature male starts stablishing a territory, usually foliage or underside leaves, and then he perform pheromone releasing by turning up the abdomen tip. He also displays acoustical and visual signals in order to attract females (Demirel 2007). This activity often attract males which form the aggregation group (lek) where they interact themselves with short encounters or combats. Although lekking males seems to be more successful in terms of courting and reproduction, there was no evidence of significant difference between them and a single resting male (Demirel 2007). But lekking males showed more robust, heavier and with more content of sugar and proteins related with resting males (Yuval, Kaspi *et al*. 1998).

Pheromone blend released by males are integrated by no less than 12 semiochemicals, including Methyl (E)-6-nonenoate, Ethyl (E)-3-octenoate, (E)-2-Hexenoic acid and Ethil acetate among others but currently is still unclear which compounds and/or blends are fully related with female attraction (Benelli, *et al*. 2014),. However, the responsible pheromone could be very close to the synthetic formulation of Trimedlure (Shelly 2000), which is very attractive to males, and improves matting success of exposed males resembling orientation toward lek sites, this is why Trimedlure is widely used in monitoring programs and mass trapping (Shelly *et al*., 1996)

3.2. Courtship showed by males: Once a male has established a territory, he starts releasing pheromones, visual displays and acoustic signals in order to attract a receptive female for performing courtship behavior and achieve mating (Demirel 2007). When a female is approaching, usually she walk with wings in down position toward the chosen male and faces him, then the male put his abdomen under his body and begin to vibrate his wings in a perpendicular position of his body. If the female still faces the male, he began to a second type of wing movement buzzing rapidly forward and backward toward the female. Additionally the head is moving rhythmically side to side. Finally, if the female is still motionless, the male leaps onto the female back and attempt to mate (Yuval and Hendrichs 2000).

4. Host location and host selection behaviour

Females forage for food immediately after emergence, and search for carbohydrates and protein sources, including fruit juices, nectar, yeast from rotten fruits and even bird feces (Yuval and Hendrichs 2000). This proteinaceus feeding is very important for enhancing egg production (Placido-Silva *et al*., 2005). Virgin females are also able to disperse more than males in searching for food, and sometimes also colonize to new locations (Yuval and Hendrichs 2000). When successful mating occurs, females switch from food foragers to host foragers for oviposition, thus they start to search for fruits (Levinson *et al*., 1990).

4.1. Olfactory and visual cues in Medfly: Once ovaries are matured, females tend to stay on or close to host plants where ripening fruits are available for egg laying. They also can disperse rapidly once fruit became scarce or when high competition with other females already copped most of available fruits for oviposition (Hendrichs and Hendrichs 1990). Major stimuli that mediate orientation in *C. capitata* females searching for oviposition sites include volatiles emitted from ripening fruits, green leaf volatiles and fermenting fruit in the ground surrounding fruit trees (Yuval and Hendrichs 2000). Visual cues play also an important role, since females tend to locate individual fruits based in fruit features such shape, size and color; preferring spheres to other shapes and larger to smaller fruits (Katsoyannos 1989). This behaviour plasticity during host location and oviposition, gives to this species this extraordinary adaptability to several host species and then new environments where they are able to colonize new niches and accept poor suitable host for egg and larval development (Yuval and Hendrichs 2000).

4.2. Oviposition behaviour: Once a fruit is located and gravid Medfly landed, she evaluates the suitability for eggs acceptance based on physical characteristics like surface structure and quality conditions (Katsoyannos, 1989). Then she inserts her "aculeus" and injects a clutch of eggs a few millimeters under fruit skin. Fruit assessment is critical for the future offspring development, since bigger fruits and close to ripening will give them the possibility to grow with less competitors (intra and interspecific) (Demirel, 2007 . It is also known that female *C. capitata* can deposit together with the oviposition, a pheromone which is detected by and averted by conspecifics, therefore, a gravid female is able to detect and discriminate between already infested and uninfested fruit (Yuval and Hendrichs 2000). Medfly behavior makes that any control strategy design must be addressed toward adults and oviposition avoidance; because once eggs are laid and maggots start damaging fruits, it is almost impossible to conduct any action against larvae, due to the well protected environment within the fruit (Malacrida, *et al*. Malacrida, Gomulski *et al*. 2007).

5. Pest management strategies against Medfly

There are many control methods for *C. capitata*: Cultural, mechanical, physical, ethological, biological, chemical, and legal. All can be included in Integrated Pest Management (IPM) programs against Medfly:

5.1. Cultural control: Is related to activities within the field or fruit orchard, this includes tillage, weeding, pruning, damaged fruit removing and disposal, irrigation, fertilization, etc. It is mainly developed and recommended by research centers and fruit growers organizations (SENASAG 2010), but has the disadvantage of high labor intensity which is usually in shortage in countryside reality. Nevertheless it is the basic fruit fly control activity that many growers are able to do.

From all this cultural practices, damaged fruit collection and adequate disposal of decomposing material seems to be the most effective cultural method. The activity alone may disrupt fruit fly cycle and then reduce populations and avoid further sources of infestation (Chueca P., *et al*. 2013). Elimination of damaged fruits also contributes to population declining by eliminating food sources for adult male and females. This collection can be made direct from trees and fallen fruits on the ground under trees, and the subsequent activity is usually to bury all the collected material. In some countries, recommendation for burying include the use of ashes or hydrated lime (Calcium oxide) spreading over buried fruits (SENASAG 2010). Damaged fruit collection and burring demand high labor, but can be done with mechanical approach for fruits chopping and grinding then spreading the remains for desiccation and or composting, this also contribute to larvae fitness reduction, (Chueca P., *et al*. 2013).

5.2. Regulatory control: Is the mandatory regulation of control activities to be made by stakeholders involved in fruit production, trade and transportation. These regulations may be consensual among growers of certain productive areas in small scale or a full-country laws promulgated by sanitary authorities that affect entire countries and their entrance ports (Enkerlin W., *et al*. 2015). Regulatory control include the mandatory application of control strategies in orchards, house backyards, gardens, and public areas where fruit trees are present. Restriction to fruit movement from one region to another, trading and consumption. Establishment of quarantine zones where fruit fly outbreak is detected, and so on. All this measures are designed to prevent further invasions and outbreaks by creating legal barriers, where violating stakeholders to those regulations may carry severe punishments (Suckling, *et al*. 2014). Mandatory regulation is very important in regions and countries where huge control and eradication programs are being executed, this allows country authorities to achieve good results when the whole population are committed with the program by law (Enkerlin W., *et al*., 2015).

5.3. Sterile Insect Technique (SIT): Also knows as "autocide control" because of the use of mass reared sterile insects to suppress population growth. SIT against Medfly population is widely used despite its cost and long term maintenance, and demonstrated to be a major and successful way to control and eradicate Medfly populations from certain areas under quarantine measures. This technique is also considered the most non disruptive pest control method where it is pest species-specific and with no introduction of toxic products or new genetic material into existing populations of target insects (Hendrichs, *et al*. 2002) .

SIT requires advanced facilities and equipment in order to produce very large quantities (on millions of individuals) of sterile *Ceratitis capitata* males by irradiation of pupae with Gamma rays and other source of radiation, then these pupae are released in an inundative way in places with target populations for suppression (Enkerlin, *et al*., 2015) . Sterile male in such quantities must compete with (if not overwhelm) wild males and copulate wild females; the resulting female eggs remains infertile and over the time, Medfly population declines toward eradication. (Suckling, *et al*. 2014) This technique is compatible with the courting and mating behavior of both males and females, but the successful key of this technique is an overabundance of sterile males relative to wild males, which have most chance of mating all available wild females (Hendrichs, *et al*. 2002) .

Currently this type of control is used in countries which face Medfly invasion from border countries (México, Chile, USA-California, Florida) and/or want to eradicate already stablished populations (Guatemala, Argentina, Perú, Australia, USA-Hawaii) (CABI 2014). This means a huge expenditure, but results worth many times the investment, since a location or even a whole country could be declared as Medfly-free after some years of SIT application, and the consequences are higher profitable fruit production and export (Enkerlin, *et al*. 2015)

5.4. Ethological control: Referred to techniques that modify the target pest behavior with semiochemicals in order to minimize species reproduction and/or targeting the plant/crop. Such semiochemicals may be insect-pheromones, food attractants, repellents, etc. (Aluja and Rull 2009) Although there is not yet a well-developed synthetic sex pheromone for *C. capitata*, ethological control is widely used against this pest by using feeding attractants like proteinaceous-based baits into monitoring and mass trapping programs (Epsky, *et al*. 2014). Some strategies, also use attractants combined with insecticides in broad application in order to lure-and-kill the more adults possible (Suckling, *et al*. 2014). There is also a synthetic male attractants like Trimedlure, which is used in monitoring and early detection systems due to its powerful male attractiveness (Tan, *et al*. 2014). Mass trapping and the development of fruit fly traps is also a matter or continuous search for a good trap design, especially for commercial companies dedicated to pest management (NavarroLlopis and Vacas 2014). While mass trapping with male lures and food attractants has been used by several years throughout the world, little research has been done in using repellents, deterrents or the combination of them in systems like push-pull.

Attract-and-kill: The addition of insecticides to food attractants has showed being effective in population suppression of tephritid fruit flies. (Epsky, *et al*. 2014). The principle behind this strategy is luring adult flies, primary females, to ingest the attractant and then intoxicate and ultimately die. Historically, proteinaceous food attractants were combined with organophosphate toxicants (Revis, *et al*. 2004). Newly approach of this technique is given by the widely use of the commercial product Hydrolysed protein + Spinosad, manufactured by Dow AgroScience. Spinosad is a mixture of spinsyns A and D, both metabolites of the soil microorganism *Saccharopolyspora spinosa*. This compounds are actively toxic to insects at low application rates, and are used widely in pest control for several species including mosquitoes and tephritid fruit flies with less impact on natural enemies (Stark, *et al*. 2004). This product is traded with several names including "GF-120", "Flipper", "Synéis-appât", among others. It contain 99.9% of hydrolysed protein and less than 0.1% of spinosad (Vargas, *et al*. 2009).

GF-120 is used as a powerful tool into integrated programs for fruit fly eradication, as a primary suppressor of new outbreaks in areas free of Medflies like Mexico, California, Argentina, Perú and Chile (Enkerlin, *et al*. 2015). One disadvantage of GF-120 is its low aging effectiveness after application. In studies for control of the Melon Fly *Bactrocera cucurbitae* in Hawaii, a difference of 2 hours after application resulted in 11 times less attractive and 50% ineffective when exposed to rain (Revis, *et al*. 2004).

Another approach of the attract-and-kill principle is the mixing of male attractants with spinosad. This technique were evaluated with Methyl Eugenol, a well-known male lure with a strong attraction effect over males of the genus *Bactrocera*. A combination of Methyl eugenol together with spinosad into a base matrix formulation of inert materials colled Specialized pheromone and lure application technology (SPLAT) (Vargas, *et al*. 2009). This base for the attractant resulted in longer time interval due to the slower releasing rate, up to 4 weeks and gives many possibilities of application due to the wide range of viscosity. This product resulted in a good candidate to be used massively in widearea of control, particularly *Bactrocera dorsalis* in Hawaii (Vargas, *et al*. 2009).

The stimulo-deterrent diversion strategy (push-pull): Control of a target pest can be more effective when repellents are combined with attractants in the same environment. This combination strategy is known as push-pull and is related to the behavioral manipulation of insect pest, making the crop unattractive (push) while lured them to attractive sources (pull) using non-toxic components such repellents, attractants, attractive plants, crop-traps, etc. (Cook, Khan *et al*. 2007). The effectiveness of this system is based in the chemical ecology of the target pest insects. The behavioral modification is achieved when an attraction stimuli interact at the same time with a repellent source in one environment (Khan *et al*., 2008).

When push-pull is used with natural stimuli sources like plants or crops, for instance repellent plants and trap-crops, this also increase natural enemies' activity and soil fauna (Midega, *et al*. 2009), the result is a decreasing pest population and little attack to the main crop.

This strategy has been developed most successfully in protecting maize crop from stemborer moths (*Busseola fusca*) in Western Africa. By using repellent intercropping in maize fields and trap-crop around, adult moths are repelled from the main field and then attracted to the trap-crop (Khan and Pickett 2004). From there, several other control systems are being developed using this principle (Cook, *et al*. 2007). Trap crops were an important component in the development of several push-pull strategies. A trap-crop is a plants species which are not primary crops, but they have the potential of been attractive for a target pest in the same environment, thus diverting the attack toward them instead of the main crop. (Shelton and Badenes-Perez 2006). Trap-crops also play an important role by releasing induced volatiles when attacked by herbivores. These volatiles could be attractive to the pest's natural enemies, the so called herbivore-induced plant volatiles (HIPV) (Khan, *et al*. 2008).

The push pull strategy against a fruit flies species was researched in olive orchards against the olive fruit fly *Bactrocera oleae* using kaolin as oviposition deterrent and bait traps at the same time in olive orchards from Sardinia, with a comparable efficiency of orchards treated with pesticides (Delrio *et al*. 2010). Some other experiments with other species of fruit flies such *Bactrocera* and *Anastrepha* included the use of fly-faeces extracts as oviposition deterrent combined with feeding-bait traps (Aluja and Rull 2009).

A good understanding of the related semichemicals to any pest species and their interaction with the environment is essential if we want to develop any strategy involving behavioral modification (Aluja and Rull 2009). For instance, in a study of *Rhagoletis* fruit fly species, lower risk of oviposition is achieved with the association of an efficient food attractant together with early application of host marking pheromones (Sarles, *et al*. 2015). *C. capitata* feaces also contain oviposition deterrent and may be used to reduce infestation. In n study of applying a concentration of Medfly faeces on coffee bushes, they achieved an infestation reduction up to 80% in treated coffee berries and 56% in adjacent non-treated bushes, showing an effect not only in the target plants but also in those nearby. (Arredondo and Diaz-Fleischer 2006). Fruit-fly semiochemicals shows great potential in integrated management programs. However, usually one infochemical is not enough to achieve optimal results in pest suppression, i.e. attraction in mass trapping. Therefore, the use of more than one infochemical in combined strategies must be developed for more optimal results in fruit flies control (Silva, *et al*. 2012)

In a very elegant study, there were a successful usage of several so called agroecological strategies to control cucurbit infestation by *Bactrocera* and *Dacus* fruit fly species in the island of La Réunion. This approach included trap crops, attractants trapping, fruit sanitation, enhancing parasitoids development and insecticide baits (Deguine, *et al*. 2015). Good results were achieved due to some fundamental factors: (i) A well-known pest status in the island's agroecology based on several years of research, and a good knowledge of the life cycle, seasonal occurrence, adults ecology and behavior out of mating and oviposition. This gave researchers a good picture over where and when apply strategies. (ii) A well-coordinated action involving all stakeholders in the cucurbit production sector of La Réunion. This coordination, allows them to introduce, socialize and adopt most of the strategies among growers, and then reduce pest population and the consequent reduction in pesticide application (Deguine, *et al*. 2015). From the elements of this integrated system against cucurbit fruit fly species, sanitation and the assisted push-pull strategy stand out: Sanitation through "augmentorium" is a technique where a tent-like cage is used for store infested fruits but with a little mesh window which avoid flies escaping but allow parasitoids exit. This augmentorium were used around cucurbit field and tested to be very effective in supress further infestations and meanwhile enhancing natural enemy's activity. The assisted push-pull consisted on insertion of trap plants in the system. After some trials with two poaceae species, maize were selected as the attractive trap crop for flies by planting around cucurbit crop. The effect of the trap-crop were enhanced by application of an attract-and-kill product: Hydrolysed protein and spinosad (GF-120). The bait were applied only in the surrounded maize crop and proved very effective in reducing populations and fruit infestation in the crop (Deguine, *et al*. 2015).

Although there were some control experiences reported in literature using semiochemicals for the Mediterranean fruit fly, there is not yet a combined strategy tested against *C. capitata*. But, after understanding the behavior ecology of this pest, a suitable push pull strategy should include the combination of repellent, attractant and/or trap-crops.

6. The use of plant-based products that affect *C. capitata* behaviour

Certain plant compounds, such fruit and leaf volatiles, are particularly attractive for medfly females searching for hosts (Levinson, *et al*.1990), but the role of repellent plant compounds is little studied. Plant secondary metabolites are produced by plants as a direct defense against herbivory (Elsayed 2011). Such compounds may be anti-feeding, oviposition deterrent, or spatial repellent for some groups of insects (Levinson *et al*., 2003). For instance, some extracts from the fern *Elaphoglossum piloselloides* were oviposition deterrents for *C. capitata* (Socolsky, *et al*. 2008), whereas there is toxic and repellent activity in extracts from some *Tagetes* plant species against male and female

C. capitata (Lopez *et al*. 2011). Essential oils from two cultivars of *Citrus limon* repelled adult females, even though *Citrus spp.* is a host of *C. capitata*. (Faraone, *et al*.2012). These examples show that plant secondary compounds have potential for control of *C. capitata* through behavioral management.

6.1. Secondary metabolites of plants against insect feeding activity: Plant secondary metabolites are a group of plant-produced complex compound biosynthesized from primary compounds such of carbohydrates, amino acids and vegetal oils (Mithofer and Boland 2012). There are very few concentration of those compounds in the vegetal tissue, they have restricted distribution among botanical kingdom and poses high specificity in presence (species specific) and biological activity (Schoonhoven, *et al*. 2005). Secondary metabolites has a broad spectrum of usages by plants, including the use as direct and indirect defense against herbivory (Elsayed 2011). This plant-based compounds revealed diverse degree of responses into multidisciplinary research about insect's physiology and chemical ecology. They can act as feeding inhibitors or induce some perturbations in growing, development, reproduction and in general behavior (Schoonhoven, *et al*. 2005). Traditionally, plant extract were tested against insect activity primary by toxicity in an attempt of developing "natural" insecticides. Several examples of successful experiences arose the use of bio-pesticides such the Neem extract (*Azadarachtina indica*), Nicotiana extract (*Nicotiana* sp.) and Pyretrum extract (*Chrisantemum* sp) and Capsicin (from *Capsicum* spp.), which are widely used against many insect species mainly lepidopteran pests (Isman 2006). Essential oils obtained from aromatic plants, are being used as lowrisk insecticides in recent years, they had an increasing popularity with organic grower and environmentally concerning consumers (Regnault-Roger, *et al*. 2012).

Botanical insecticides have long been used as alternative to synthetic chemical insecticides, mainly due to lower risk for human health, easy availability and environmental friendly usage. This features drove the use of botanical insecticides to best suited to be use into organic farming (Isman 2006). However, the effectiveness as insecticide usually are lower than synthetics and legal barriers for registration and trade are difficult to apply since they are treated as common chemical products.

6.2. Plant-based repellents: Nerio, *et al*. (2010) defined repellent as substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin (or surface in general). Different from toxic plant-based pesticides which primary goal is killing a target insect population, repellents in general act generating a spatial barrier avoiding the target insect from coming toward a protected surface, such a human skin, spaced place or even crop plots (Regnault-Roger, *et al*. 2012). This feature of spatial repellent, differs of the so called "deterrent", which is a compound which presence avoid or interrupt certain behavior, like oviposition, feeding, predation and so on. Deterrence exert such behavior change when the insect is in direct contact with the compound (Arredondo and Diaz-Fleischer 2006). It is reported that female fruit flies release substances together with eggs depositions when fruit infestation (Yuval and Hendrichs 2000). Some of these deliberately excreted compounds during oviposition also generate rejection for con-specific competitors such other gravid females. This kind of rejection to an already infested host is known as "oviposition deterrent", because is not considered spatial repellent but a strategy to avoid con-specific competition especially when suitable resources are scarce. Oviposition deterrent in fruit flies may came not only from egg deposition compounds but also from faeces. (Debboun, *et al*. 2014)

Emitted odours by non-host plants, may exert also repellence in those insects searching for suitable hosts (Khan, *et al*. 2008). For instance, when a gravid fruit fly is searching for hosts, she will fly toward primarily to those odor sources exerting ripening fruits instead of those with non-ripe fruits and/or non-suitable host (fruits with toxic compounds, antifedants substances, etc.) (Demirel 2007). In other cases, herbivore induced plant volatiles (HIOV) emitted by the host-plant, can act as repellent for the adult stage of the herbivore. This is evident in the cotton moth *Spodoptera litoralis*, when host-plant derived HIPV interfere the sensory system in the search and host location activity (Hatano, *et al*. 2015)

Botanical products has compounds with potential repellence against pestiferous insects (Moore, *et al*. 2014). Lemon grass oil (*Cingopogon citratus*) showed strong stimulant for the olfactory receptor of the stable fly *Stomoxys calcitrans*, and then repellent effect in behavioral experiments under laboratory conditions (Baldacchino, *et al*. 2013). Lemon grass oil and its main active components Geranial and Neral were tested for repellence activity against different species of disease-transmitting mosquitoes (Deng, 2014) and the common house fly (*Musca domestica*) (Kumar, *et al* 2011; Morey 2012), making this particular plant species, interesting for prospective research in dipteran repellence.

There is potential of certain botanical product such essential oils to be used as spatial repellents against herbivorous insects in crop protection (Isman 2006). However, most of research effort in repellence has focused on blood sucking insects such mosquito species related with public health and livestock dipteran pest (Nerio, *et al*. 2010). Research over natural essential oils for anti-insect purposes are gaining interest in an attempt to generate natural alternatives to synthetic products (Isman 2006).

Although most of chemical ecology techniques for semiochemical's research are well developed, they are used mainly for attractants and other infochemicals (sex pheromones, para-pheromones, attractant volatiles and so on). Adaptation of such techniques for repellence assessment usually is hard, and evaluation approaches must be designed according for the behavior and ecology of the target insect species (Moore, *et al*. 2014). Nevertheless, general approach to detect and evaluate repellent compounds follows: (i) attention of plant-based product to be tested. (ii) General screening by behavioral bioassays (iii) Chromatography techniques for key compounds identification, such GC-MS (Gas Chromatography and Mass Spectrometry) and GC-EAD (Gas Chromatography and Electro Anemography). (iii) Compound assessment by behavioral bioassay programs (Epsky, *et al*. 2014). GC-EAD is a very sensitive tool which allows to detect the precise compound that exert an olfactory reception in insects. This technique were useful to identify some mosquito repellent compounds from fresh crushed plants of Ocimun forskolei, which is used as repellent plant in some African countries (Dekker *et al*. 2011). Disadvantage of GC-EAD is evident because a sensory response detected by the technique is not possible to relate directly with repellence, it's always need a confirmation of behavioral assays. Some compounds causing repulsion might block or supress the sensory system, and this is also a disadvantage since a no response might or might not mean a repellence effect.

Mosquito repellents are the most researched products due to their highly effectiveness in preventing biting and blood-borne disease transmission (Deng, *et al*. 2014). Then, invitro techniques were developed as behavioral bioassays in order to evaluate repellence activity instead of using human voluntaries for biting. Some include using landing arenas with and without repellence treatment, adaptation of "y"- tube olfactometers, membranes, etc. Key in any assessment is the use of a proper attractant or stimuli in every test, and the outcome should be the successful blocking of such attractiveness by the repellent (Deng, *et al*. 2014). Other techniques for the analysis of behavior include the video tracking of treated and untreated arena and the subsequent time and/or surface of subjects staying in one or other arena. This methodology were used to determine the repellence of lemon grass oil (*C. citratus*) against the stable fly *Stomoxys calcitrans* (Baldacchino, *et al*. 2013).

7. Attraction

Chemical signals play an important role in any ecosystem, they influence behavior, physiology and ecology of insects in a variable ways, including plant-insect interactions, insect-insect interaction and insects-microbe interaction (Tan K.H *et al*, 2014). The sense of olfaction is key for most of flying insects (mainly in adult stage), and they have mostly a high developed olfaction sensory. Chemical signals that denote odour attractiveness are usually related to behavior activity such food searching, sexual communication, host location, host suitability assessment, etc. Insects are involved in a great odours environment that rule any aspects of their lives (Schoonhoven, *et al*. 2005)

A close definition of "attractant", may be a substance or blend of substances that exerts in an exposed insect a behavior of movement toward the source of such substance. In general is the opposite of repellence (Debboun, *et al*. 2014). Attractiveness of certain product may vary according of the chemical signal significance that express the target insect when detects the source of odour, for instance, in the case of tephritid flies, a food attractant denoted movement effect by a hungry insect toward the source of attraction (like proteinaceous and fermentation product) (Epsky, *et al*. 2014). Ssex pheromone is attractive only for a con-specific potential couple for copulation purposes (or other type of sex communication), and a suitable host volatiles are attractive to those insects that are ready to oviposit (like ripening fruits) (Yuval and Hendrichs 2000).

Attractants have been largely used in control programs against tephritid fruit flies (Epsky N., *et al*., 2014), this is an important tool in ethological control, where attractants are involved in capture, elimination and/or modification behavior and of economical important pest species (food attractant and para-pheromones). Monitoring and trapping system using attractants are used also in early detection programs in those countries or areas where fruit fly outbreaks and invasion is subject of control (Tan, *et al*. 2014).

7.1. Food attractants: Food attractants are those that are or mimic a suitable feeding source. Early control strategies of tephritid trapping used products such fermenting sugar baits, yeast and sub-products of liquor industry such brewery yeast and related, modified protein by hydrolysis (hydrolyzed proteins) and ammonia solutions among others (Epsky *et al*. 2014). Synthetic products and protein-based lures have been used as attractants in monitoring and control programs (CABI 2014). This attractants include hydrolyzed protein (from soybean, maize, whey and others), and torula yeast used as components in single or blended baits, for attraction of both males and females in mass trapping programs, or combined with insecticides into attract-and-kill systems (Benelli, Daane *et al*. 2014). Proteinaceous lures are highly attractive for females searching for food and also oviposition hosts (Placido-Silva *et al*., 2005).

Efforts to identify active compounds from those food attractants, resulted in ammonia (the primary product of protein hydrolysis) usually the key compound responsible for attraction (Mazor, 2009). Other compounds were also used for enhance attractiveness, like combined formulation of ammonium acetate and putrescine (Heath, *et al*. 1997). Most of compounds related to food attractance for tephritid fruit flies now are available as commercial products for monitoring and mass trapping including “Buminal ®”, TYB, BioLure, AFF lure and othes (Epsky, *et al* 2014). Although food attractant are a powerful tool for capturing target fruit flies, one disadvantage is the high level of non-target captured insects including benefit like bees, wasps and lacewing.

Food attractants usage in trapping, involve a great variety of traps design in order to (i) hold and preserve as long as possible the attractiveness power of the mainly liquid bait. (ii) Allow the insect entry and capture in the trap avoiding its escaping and (iii) avoid the capture of other non-target insects especially the benefits (Tan, *et al*. 2014). The most widely used trap for liquid food attractant is the “McPhail” trap with its beneath funnel principle, in which the attracted fly is lured through an inverted funnel of its base inside trap, the colorless top part of the trap allow fly not to escape from beneath by keeping it upper because of positive phototropism. Eventually the captured fly will die mainly by drowning in the liquid bait (Navarro-Llopis and Vacas 2014). Some variants of this trap were developed, and eventually the "Thephritrap" is now considered the most successful trap in catching tephritid fruit with liquid baits. The design include the same inverted funnel principle with the addition of four small circular windows in every direction in the middle part, reinforced with tubes (funnel like) inside (Miranda, *et al*. 2001). These windows allow better release of attractive volatiles and are also other entrance point for flies.

7.2. Male lures: Other kind of attractants that are used in monitoring and mass trapping are the male lures. This compounds (naturally or synthetic) may be responsible by ingestion for the synthesis of male aggregation pheromones (Tan, *et al*. 2014), which is important for the chemical communication among males for lekking and therefore for successful matting (Yuval and Hendrichs 2000). The most used discovered male lures are methyl eugenol, Raspberry ketone and Cue-lure which attract males of most of *Bactrocera* and *Dacus* species.

Trimedlure and Ceralure are synthetic male lures that attract *Ceratitis* species including the Mediterranea Fruit Fly *C. capitata*. (Avery *et al*., 1994). Trimedlure (t-Butyl-2-methyl4-chlorocyclohexanecarboxylate, various manufacturers) is a synthetic compound which has been adopted as chief male lure for *C. capitata* in detection and surveillance programs (Jang el al. 2001). Trimedlure has a powerful effect in attracting *C. capitata* males. It was used in liquid presentation, or included in releasing solid dispensers like rubber septa and polymer plugs. Releasing duration may vary from 4 to 30 weeks depending of the concentration and dispenser quality (Tan, *et al*. 2014).

Trimedlure and other male lures are used mainly in sticky traps (like Jackson traps) which are prismatic-shaped traps made of plastic or carton. Inside the base of this prismatic trap are located the sticky surface with special product which maintain its feature in open environment (Navarro-Llopis and Vacas 2014).

7.3. Evaluation approaches for attractants: Field test with traps is the primary method for evaluating food attractants and determine preference of wild tephritids (Epsky, *et al* 2014), in which baited traps with the attractants are deployed at a minimum distance of 3 m each other in a choice experiment, or more than 5 m of distance in non-choice experiments (Epsky, *et al* 2014) This also may include studies of different fruit species, orchard pest management and even geographical dispersion. Field assessment is largely dependent of wild population availability. Sometimes this is not always the case and therefore laboratory studies are also important to determine the effectiveness of certain food attractant (Tan, *et al*. 2014).

Laboratory bioassays for testing attraction has the additional advantage of factor management that are not possible to control in the field. This includes fly source (wild or reared), population level, sex, and physiological stage Laboratory behavioral approaches include olfactometers ("Y-tube", "T-maze", 4,4 arms tube, etc), wind tunnels, and traps/landing setup inside cages for choice and non-choice experiments (Epsky, *et al* 2014).

Compounds identification by Chromatography techniques, such GC-MS (Gas Chromatography and Mass Spectrometry) and GC-EAD (Gas Chromatography and Electro Anemography) are also powerful tools for screening and test those compound that are related with chemoreception in insect antennae detected by GC-EAD.

8. Aim and research pathway of this study

The main objective of this PhD thesis is the development and discussion of well-studied ethological components and their inclusion within a combined strategy. These components will include odorant stimuli (repellents and attractants) and a possible trap-crop. Understanding adult fruit flies interaction with these components will allow us to discuss the potential integration of those components into a combined push-pull strategy against medfly attack in Bolivian fruit orchards. At the end of this project I expect to obtain both efficient attractant and repellent, and a well-studied potential trap-crop which can be used together as an easy and practical control strategy for fruit growers to reduce the infestation of peach fruits by *C. capitata*.

8.1. Research project objectives: This research project has four objectives: (1)To evaluate local protein-based products as potential baits for *C. capitata* trapping system. (2) To screen and assess botanical extracts from native Bolivian plants as *C. capitata* spatial repellents. (3) To study the interaction of adult *C. capitata* and apple fruits of the local variety "Camueza" as potential trap-crop. (4) To evaluate control efficiency of the evaluated components (repellent, attractant and trap-crop) in a combined stimulo-deterrent diversion strategy against adults *C. capitata*.

8.2. Attractants evaluation: Some fruit growers use trapping in their *C. capitata* control strategy, but the lure that is thought to be most effective is imported and expensive, and usually unavailable for small growers, there are also references from San Benito research station in "Valle Alto", a very important peach production zone, where they promote to use "chicha" waste, a local fermented beverage as food bait. An effective attractant product for *C. capitata* control should be very attractive for females searching for both food and hosts (after mating), also inexpensive and available for fruit growers. In order to find such product, the following hypotheses are being testing:

<u>Hypothesis</u>: Local protein-based products show the same level of attraction for adult *C. capitata* as commercial/imported baits in Bolivian peach orchards.

A field trial in peach orchards around Cochabamba Valleys (Central Bolivia) has been undertaken to test the level of attractiveness of locally produced and inexpensive products, mostly based on protein 1.) Biofrut® (PROBIOTEC, trade-mark of fly attractant based in hydrolyzed protein) 2.) Baking yeast diluted in water (4%) and sugar. 3.) chicha, a local beverage made from fermented maize and 4.) Pellet of protein and borax, commercial hydrolyzed protein bait imported for specific frui-fly trapping system. The four treatments were used as baits in tephri-traps® (Pherobank). Number, sex, mating status and gonotrophic status of trapped adult *C. capitata* were scored during 5 weekly evaluations in a non-choice test, where traps were deployed in a distance of 16m each other. Then a second part of choice experiment where thraps were deployed at a minimum distance of 4

m. each other, trying to mach the subsequent 6 weekly evaluation with the top population fluctuation. Traps were deployed in 16 locations within peach orchards, during fruit season 2015 (March to May).

Mayor compounds identification from the most effective food attractant: GC-MS will be performed in order to determine the volatile profile of the most effective food attractant. GC-EAD running will be performed with both, males and females of wild population of *C. capitata* in order to determine and eventually identify those key active compounds that are responsible for antennae chemoreception.

When key compounds of the best food attractant were identifies, behavioral experiments for attraction will be performed with gravid females (reared into laboratory conditions). This assays may be designed in a choice or 2-choice experiments in landing arenas within cages. Eventually a field trial will evaluate it in peach orchards during fruiting season. In order to test synthetic compound attractiveness toward gravid females compared with the original bait. In addition, tests to determine the compound profile of the attractant when storage by freezing will be performed using GC-MS. Lab experiments will be held in mid2016 and field test in early 2017.

The expected outcome of this objective is a protein-based attractant locally available and suitable for using in traps for monitoring, mass trapping systems and combined control strategies. This product should be effective to attract gravid females and with potential to be used combined with other components into a push-pull strategy.

8.3. Evaluation of botanical products for spatial repellence: Hypothesis: At least one selected extract from a plant shows spatial repellence against *C. capitata.*

Essential oils are easily produced by steam distillation of plant material, they are complex mixtures of volatile organic compounds and contain many volatile, low-molecular-weight terpenes, sesquiterpenes and oxigenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) (Regnault-Roger, *et al*. 2012). They are frequently responsible for the distinctive odor of aromatic plants, however, their composition may vary between species, varieties and within the same species from different geographic areas, with different environments (Nerio, *et al*. 2010).

Since middle Andean valleys contain high botanical diversity, some local plants were identified as candidate repellents based from local knowledge as insect repellents or other features of anti-insect activity. This selection was done after a review of some available references from etno-botanical studies about Andean plants and their local uses ((Vidarurre, *et al*., 2006); (Bentley 2003). But almost all described plants pointed out in traditional medicine purposes for humans and animals, very little in other usages, some of them mention toxic plants that are harmful for animals and sometimes are used as insecticides in agriculture. I also talked with people involved (professionals and farmers). Botanical extracts or essential oils have been obtained by steam distillation and they are going to be tested for spatial repellence against *C. capitata* adults. The candidate's species are:

- **Lantana** (*Lantana camara*): Native bush which leaves are used as moth repellent in potato storage in Perú and Bolivia.
- **Muña** (*Hedeoma mandoniana*): Native bush common in the highlands and interAndean valleys, known as repellents of several insects including flies, mosquitos and moths.
- **Molle** (*Schinus molle*): The Peruvian pepper tree. South American tree, widespread in Cochabamba Valleys. Fruits and leaves are used as repellents (flies, mice, etc.)
- **Pampa anís** (*Tagetes pusilla*): Wild tagetes are small plants; well known by locals as "lice cleaners". Essential oil is very similar in smell to anise.
- **Andres Waylla** (*Cestrum parqui*): Native Solanaceus bush, known by its toxicity for house pests. In a lower concentration it could be repellent because of its strong smell.
- **Cedrón** (*Aloysia citriodora*): Small tree and/or bush native of South America with a smell close to citrus. It was selected because of its similar odor to citronella.
- **Lemon grass** (*Cingopogon citratus*): medium size grass widely crop and used for medicinal purposes. Its essential oil is also used in food industry. There are evidence of repellence effect in mosquito and other dipterans.
- **Eucaliptus** (*Eucaliptus globulus*): Australian native tree but widespread in valleys and high Andean places. Tender leaves are used as natural medicine and moth repellent.
- **Lime (***Citrus aurantifolium***) and grapefruit (***Citrus paradisii***):** Essential oils from peels. Congeners of the known *C. capitata* repellent *C. limon*

In order to determine the level of repellence of these compounds Behavioural bioassays in a "T-maze" approach will be performed in the presence of a stimulant (food or oviposition site) to *C. capitata* adults. Mated females (10-15 days after emergence) will be used in all the experiments, because we are targeting oviposition behavior due to its direct threat to fruits. Repellence is more difficult to evaluate than attraction. Therefore, a second experiment will be carried out evaluating actual fruit infestation in a semi-field setup, using fruits close to maturation and exposing them with the selected repellents and infesting with adult *C. capitata* in cages.

Additionally, GC-MS will be performed in those extracts which shows better repellence effect in order to determine the volatile profile and eventually, by GC-EAD running with reared population (the same used for the behavioural experiment) , key compounds related to antennal chemoreception will be identified.

Olfactometer experiments will be performed in the behavioural laboratory of Alnarp and the infestation experiment in Bolivian UMSS Entomology lab. The expected outcome of this experiment will be at least one plant-based extracts, and possibly compound(s), that shows medfly repellence activity and is subject to be used like broad spatial repellent within peach orchard.

8.4. "Camueza apple, a local variety with potential feature as trap-crop: Hypothesis: Local apple variety Camueza has the features of trap-crop against *C. capitata*, by being more attractive than commercially grown varieties.

The local apple known as "Camueza" is an old group of cultivated varieties in Cochabamba Valleys (particularly in Vinto), currently there is still not defined whether there is one or several varieties (Rojas J. personal communication). After observation of these apples in the field, it was evident that most of them were attacked by *C. capitata*. Several punctures all around the skin and surface malformation were present; there were traces of initial larval activity, but no development. This field observation shows the potential of this variety as trap crop. This variety has little commercial purposes anymore and most of the remained trees are big, aged and located in gardens and or abandoned orchards.

A set of experiments are proposed for this objective: (a) Behavioral choice experiments between this and commercial varieties, in order to show preference for oviposition and also replicate in laboratory the field observation. (b) Test for larval development in fruits compared with other apple varieties (c) A field experiment about preference of wild *C. capitata* population toward peach fruits (branches with ripening fruits) located close to Camueza apple trees, compared with peach branches in places with no apple trees nearby. These experiments are expected to carry out in fruit seasons 2016 and 2017. Expected outcome of these experiments would be a better understanding of the host-insect interactions between this apple variety and adult's *C. capitata*. If there this is a good trapcrop candidate, we can perform further field experiments and consider re-introduction of this variety in the fruit production as a possible trap-crop inside or around valuable fruit orchards.

8.5. The use of the best evaluated components in a combined strategy: Hypothesis: The combined use of an attractant and a repellent offers perspectives of use in *C. capitata* control into peach orchards in the Cochabamba valleys.

After identifying an efficient protein-based attractant and plant-based repellent, we will combine both under field conditions with a set up experiment that will be designed based on the outcome of the previous experiments.

The outcome of this field trial should be a low rate of fruit infestation, compared with control orchards with no pest suppression actions and as much as the same level of efficiency compared with orchards with traditional pesticide use. Field trials with the combined strategy could be performed in several orchards of peach production in Cochabamba valleys. This activity is expected to be held during the fruiting season (November 2017 and April 2018).

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