

# Interaction between weaver ants, mango infesting fruit flies and their exotic parasitoids

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Interaction between weaver ants, mango infesting fruit  
flies and their exotic parasitoids

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Cover page: A weaver ant worker (*Oecophylla longinoda*) chasing *Diachasmimorpha longicaudata*

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In particular, for chapter 2 and 4, only the Abstract are presented as the content of these chapters has been already published. Indication on the scientific journals where to find the full content of these chapters is given at the beginning of the respective chapter.

## Table of contents

Summary .....	6
1. General Introduction .....	9
1.1. Trophic interactions and their role in structuring ecological communities .....	10
1.2. Individual foraging behaviour and population ecology .....	11
1.3. Trophic interactions predator-prey effect at the population level.....	12
1.4. Trophic interactions within agroecosystems.....	13
1.5. Using behavioural ecology to improve pest control.....	14
1.6. The study system: The IPM project to protect mangoes in Africa .....	16
1.7. Objectives of the study .....	21
1.1 References.....	22
2 Physiology vs. environment: what drives oviposition decisions in mango fruit flies ( <i>Bactrocera invadens</i> and <i>Ceratitidis cosyra</i> )?.....	29
2.1 Abstract.....	30
3 Exploitation of hetero- and conspecific oviposition punctures as an oviposition strategy in mango fruit flies	31
3.1 Abstract.....	32
3.2 Introduction .....	32
3.3 Materials and Methods .....	36
3.3.1 Rearing of fruit flies.....	36
3.3.2 Experimental Set-up .....	37
3.3.3 Behavioural observations .....	37
3.3.4 Statistical Analysis .....	38
3.4 Results.....	39
3.4.1 No-choice experiments.....	39
3.4.2 Choice experiments .....	44
3.5 Discussion.....	46

3.6	References.....	51
4	At lunch with a killer: the effect of weaver ant on host-parasitoid interactions in mango.....	53
4.1	Abstract.....	54
5	General discussion and Conclusion .....	55
5.1	Implication of oviposition strategies in fruit fly competitive interactions .....	56
5.2	Effects of predation pressure on fruit-fly parasitoid population dynamics .....	58
5.3	Conclusion .....	61
5.4	References.....	64
	Acknowledgements.....	68
	Erklärung .....	71

## Summary

A detailed understanding of competitive and trophic interactions is particularly important to elucidate population patterns that ultimately determine how ecological communities are structured. Competitive and trophic interactions within an ecological community can be very complex to disentangle. When two species within the same trophic position share the same resources, competition may occur with important consequences at population and species level. Competitive interactions can also be mediated by predation. Predation pressure can have both direct and indirect effects on the species involved, affecting ultimately the density of population. Moreover, the consequences of predation can cause trophic cascades, as the effects of a top predator can affect not only the prey, but also other species connected to the prey. As individuals are the units of populations, the investigation on individual behaviour is a key study approach to clarify how individuals interact with each other and respond to the presence of other organisms. Elucidating individual behavioural strategies can help clarifying the mechanisms which determine the dynamics of the populations, and ultimately the species involved within an ecological community. Agroecosystems can offer interesting scenarios to study competitive and trophic interactions, as agricultural management can bring together new assemblages of species. Behavioural studies can help understanding and predicting how the species involved in the new assemblages interact together. When the agricultural community involved pests of economic importance, understanding competitive and trophic interactions is helpful to improve agricultural management such as biological control plans to reduce pest damages. Thus, this research investigated competitive and trophic interactions in a predator-parasitoid-pest system involving two mango infesting fruit flies, *Bactrocera dorsalis* an invasive species in sub-Saharan Africa, and *Ceratitidis cosyra*, an indigenous species, two exotic parasitoids, *Fopius arisanus* and *Diachasmimorpha longicaudata*, a pre-pupal and larval parasitoid, respectively, used to control fruit fly populations, and the African weaver ant, *Oecophylla longinoda*, an indigenous generalist predator in Kenya.

In particular, in **chapter 2** the effects of fruit fly physiological status and fruit characteristics on host oviposition acceptance and clutch size for the two fruit fly species was studied. The egg load was the primary driver for oviposition in both *B. dorsalis* and *C. cosyra*. The two fruit fly species differed in their ability to mature eggs, *B. dorsalis* having larger egg loads than the native *C. cosyra*. When looking at the influence of fruit ripeness on the probability to oviposit, while *B. dorsalis* was not influence by the stage of ripeness of the mango, *C. cosyra* females preferred ovipositing in ripe and fully-ripe mangoes compared to unripe ones. However, *B. dorsalis*

females adjusted their egg clutches depending on mango variety, with Ngowe receiving the biggest clutches and Kent the smallest. The differences in ability to mature eggs and in the oviposition strategies adopted to successfully reproduced can be some of the factors explaining the competitive displacement between the two species, as *C. cosyra* disappeared from some areas in Kenya, being present only at highlands.

In **chapter 3**, particular focus was given to the usage of previously-drilled oviposition punctures established on the fruit by *C. cosyra* and *B. dorsalis* females. An important question here was whether the foraging females could oviposit in previously-made oviposition punctures on the fruit, and whether they would differently respond to conspecific- or heterospecific-made punctures. Both *B. dorsalis* and *C. cosyra* females laid their eggs in oviposition punctures made by other females on the fruit. By using already made punctures, the both fruit fly could lay a larger quantity of eggs, as they could have multiple ovipositions within a 30-minute observation timeframe. In fact, ovipositing in previously-drilled punctures reduce the fruit handling time, i.e. the time spent penetrating the fruit skin, thus reducing the overall oviposition time. While *B. dorsalis* did not respond differently to ovipositing in conspecific- and heterospecific-made punctures, the native *C. cosyra* preferred ovipositing in conspecific-made punctures, potentially enhancing intraspecific competition among developing larvae. As according to literature studies, *B. dorsalis* larvae develop faster than *C. cosyra* ones, the implications related to having eggs of both fruit fly species within the same punctures is discussed here in terms of competitive interactions between the native and the invasive fruit fly species. This oviposition strategy can be another factor contributing explaining competitive displacement between the two fruit fly species which is observed in the field. As exploiting oviposition punctures already present on the fruit reduce the overall oviposition time, the opportunity to exploit such oviposition strategy when foraging under increased risk pressure, such as in the presence of a predator, is discussed here.

As part of a biological control program aimed to reduce the fruit fly populations, and exotic parasitoids are released in order to achieve pest reduction, this study also examined the implication of having a generalist predator involved in the system. The effectiveness of multi-natural-enemy assemblages is often debated, with contrasting results depending on the analysed system.

Thus, in **chapter 4** the role of the African weaver ant on the two fruit fly species, as well as on the parasitoids was examined using both field cage experiments and behavioural bioassays. The weaver ants were reducing the number of eggs laid in mangoes by the foraging fruit flies being suitable biocontrol agent to control fruit fly population. However, the ants had also a negative effect on both *F. arisanus* and *D. longicaudata*, decreasing

the rate of parasitism by the parasitoid females. Even if the weaver ants were already shown capable to reduce fruit fly infestations in mango orchard, an important question here was identifying the mechanisms responsible to such effect. The weaver ants were not directly predating on the foraging fruit flies and parasitoids, rather their negative effect was due to indirect predation: in the presence of the generalist predator both fruit flies and parasitoids spent a shorter amount of time searching on the fruit, thus reducing the probability to lay eggs. The implications of indirect predation impact on the fruit fly and the parasitoid populations were discussed here. As *F. arisanus* was the most affected in terms of searching time reduction, the pros and the cons of having both the weaver ants and the parasitoids as biocontrol agents of the flies are argued as well.

In the concluding section (**chapter 5**), the importance of understanding competitive and trophic interactions is discussed as a crucial information to improve making predictions on population dynamics within ecological communities. Further, it is argued on how behavioural investigation at the individual level can contribute to the understanding of population fluctuations. As this study involves species of agricultural economic importance, the connection between behavioural studies and agricultural management is also stressed here, discussing the importance of investigating behaviour to improve pest management.

## Zusammenfassung

Ein Verständnis der Konkurrenzbeziehungen und trophischen Interaktionen innerhalb von Artengemeinschaften ist notwendig, um jene Muster auf Populationsebene verstehen zu können, die letztlich die Artenzusammensetzung innerhalb solcher Gemeinschaften bestimmen. Dabei können solche Beziehungen komplex und schwer zu entwirren sein. Wenn zwei Arten auf der gleichen trophischen Ebene die gleichen Ressourcenansprüche haben, können daraus Konkurrenzbeziehungen erwachsen, die deutliche Konsequenzen auf Populations- oder sogar Artniveau haben. Zusätzlich können solche Beziehungen durch Prädationsdruck verändert werden, wobei Prädation neben direkten auch indirekte Effekte auf die beteiligten Arten haben kann, welche die Populationsdichte beeinflussen. Darüberhinaus kann Prädation Dominoeffekte über trophische Stufen hinweg auslösen, von denen Arten, die mit der Beute interagieren, beeinflusst werden.

Da Populationen aus Individuen bestehen, sind es individuenbasierte Ansätze in wissenschaftlichen Untersuchungen, die aufklären können wie Individuen in Populationen miteinander interagieren, wie sie auf die Anwesenheit anderer Organismen reagieren und damit letztlich Populationsdynamik und Artenzusammensetzung beeinflussen. Agroökosysteme können dabei interessante Untersuchungsszenarien für das Studium von Konkurrenz und trophischen Beziehungen bilden, da diese Systeme oft neue Arten miteinander in Kontakt bringen. Verhaltensuntersuchungen können dabei helfen, Interaktionen aufzuklären und in diesen neuen Artengemeinschaften vorherzusagen. Besonders wenn sich Agrarschädlinge in diesen Artengemeinschaften befinden, kann eine Analyse der Interaktionen beim zielgerichteten Einsatz einer biologischen Schädlingsbekämpfung helfen die Schäden zu reduzieren. Die hier vorgelegte Arbeit untersucht Konkurrenz und trophische Interaktionen in einem System, das als Schädlinge zwei tropische Fruchtfliegen umfasst, als Parasitoide zwei nach Afrika für die biologische Schädlingsbekämpfung eingeführte Brackwespenarten sowie als Prädator die in Kenia einheimische Weberameisenart, *Oecophylla longinoda*. Bei den Fruchtfliegen handelt es sich um die afrikanische Mangofruchtfliege *Ceratitis cosyra* sowie die aus Asien ins äquatoriale Afrika eingeschleppte *Bactrocera dorsalis*. Unter den Brackwespen attackiert *Fopius arisanus* die Eier der Fruchtfliegen, *Diachasmimorpha longicaudata* die Altlarven und beide Arten schlüpfen aus den Puparien der Fliegen.

Im **Kapitel 2** der Arbeit wurde die Akzeptanz von Mangofrüchten und die Gelegegröße in Abhängigkeit des physiologischen Status der Fliegen und von Fruchtcharakteristika untersucht. Der Eivorrat der Fliegen war der wichtigste steuernde Parameter, wobei allerdings *B. dorsalis* eine höhere Eireifungsrate und einen höheren Eivorrat hatte. Während *B. dorsalis* eine deutliche Präferenz für bestimmte Mangosorten hatte, nicht jedoch einen bestimmten Reifegrad präferierte, zeigte *C. cosyra* eine deutliche Präferenz für reife und vollreife Früchte, zeigte jedoch keine Sortenpräferenz. Die Unterschiede in der Eireifung können ein Faktor sein, der die Verdrängung von *C. cosyra* durch *B. dorsalis* in weiten Teilen Kenias jenseits des Hochlandes vorantreibt.

**Kapitel 3** beschäftigt sich mit der Nutzung bereits vorhandener Eiablagebohrungen durch Weibchen der beiden Fruchtfliegenarten und einer möglichen Präferenz innerartlicher oder zwischenartlicher Bohrlöcher. Beide Arten nutzen bereits vorhandene Bohrlöcher und konnten dadurch innerhalb der Beobachtungszeit mehr Eier legen und mehrere Eiablageorte nutzen. Dieser Vorteil entstand durch verringerte Handhabungszeit zum Durchbohren der Fruchthaut für die Eiablagestelle. Während *B. dorsalis* nicht zwischen den Bohrlöchern von Artgenossen und jenen von *C. cosyra* diskriminierte, zeigte *C. cosyra* eine deutliche Präferenz für arteigene Bohrlöcher, was zu einer erhöhten innerartlichen Konkurrenz führen sollte. Da nach Literaturdaten *B. dorsalis* eine schnellere Larvalentwicklung hat als *C. cosyra*, werden die Befunde im Hinblick auf die Konkurrenzsituation zwischen der einheimischen und eingeschleppten invasiven Fliegenart diskutiert. Die hier beschriebene Eiablagestrategie könnte ein weiterer Faktor bei der Verdrängung der einheimischen Fruchtfliegenart sein. Da die Nutzung bereits vorhandener Bohrlöcher die für Eiablagen benötigte Zeit reduziert, wird hier eine solche Eiablagestrategie als adaptiv unter Prädationsdruck durch in Fruchtnähe befindliche Räuber diskutiert.

Diese Arbeit widmet sich auch der möglichen Rolle eines generalistischen Prädatoren im Mango-Fruchtfliegen-Parasitoiden-System, da zur biologischen Schädlingsbekämpfung zwei auf Fruchtfliegen spezialisierte Parasitoide in Kenia freigesetzt wurden. Immer wieder wird in der Wissenschaft anhand der ganz konträren Befunde debattiert, ob Systeme mit mehreren Arten in der biologischen Bekämpfung effektiv sein können.

Daher wird in **Kapitel 4** der Blick darauf geworfen, welche Effekte die afrikanischen Weberameisen auf die beiden Fruchtfliegenarten und die beiden Parasitoidenarten haben, wobei Feldkäfiguntersuchungen und Verhaltenstests durchgeführt wurden. Es konnte gezeigt werden, dass die Weberameisen deutlich

die Anzahl Eier, die von den Fruchtfliegen in Mangos gelegt werden, reduzieren und damit für eine biologische Bekämpfung geeignet sind. Doch zeigten die Ameisen auch negative Effekte gegenüber den Parasitoiden *F. arisanus* und *D. longicaudata*, indem sie deren Parasitierungserfolg verminderten. Auch wenn schon aus früheren Untersuchungen bekannt war, dass die Weberameisen zu einer erniedrigten Eiablage der Fliegen beitragen, war der Mechanismus hierfür nicht bekannt. Es konnte gezeigt werden, dass die Ameisen die Fliegen und Parasitoide nicht direkt erbeuteten, sondern indirekte Effekte des Prädatoren die Ergebnisse erzeugten: in der Gegenwart von Weberameisen reduzierten sowohl die Fruchtfliegen als auch die Parasitoide die Suchzeit auf den Mangos und hatten dadurch weniger Gelegenheit zur Eiablage. Die Bedeutung der Befunde auf Populationsebene von Fruchtfliegen und Parasitoiden werden hier diskutiert. Da die negativen Effekte auf den Parasitoiden *F. arisanus* stärker waren als auf die restlichen Arten wird diskutiert, inwiefern sich eine gemeinsame Nutzung von Weberameisen und Parasitoiden in der biologischen Bekämpfung der Mangofliegen lohnt.

In der zusammenfassenden Diskussion (**Kapitel 5**) wird noch einmal die Relevanz eines vertieften Verständnisses der Konkurrenz und trophischen Beziehungen für die Möglichkeit, Vorhersagen zur Populationsdynamik in Artengemeinschaften zu machen beleuchtet. Es wird argumentiert, dass Verhaltensuntersuchungen auf dem Niveau individuellen Verhaltens dazu beitragen können Populationsfluktuationen zu verstehen. Da es sich bei den hier untersuchten Arten um landwirtschaftlich bedeutsame Schädlinge handelt, wird auch betont, wie Verhaltensuntersuchungen dazu beitragen können, Ansätze der biologischen oder integrierten Schädlingsbekämpfung zu verbessern.

# 1. General Introduction

## **1.1. Trophic interactions and their role in structuring ecological communities**

A large part of the diversity we observe today in nature is a result of interactions taking place in species communities: it is difficult to think about a species living in complete isolation from other species. Major events in diversification have been due to the appearance of novel assemblages of species, which gave rise to new sets of interacting species (Thompson 1999). The way in which species that occupy the same environment relate to each other ultimately determines the structure of their ecological community (Chase *et al.* 2002). Often, species assemblages may fluctuate around stable states of interacting populations, revealing a certain organization among the species that share the same area (Hairston and Hairston 1997). Species co-existence is usually the product of an evolutionary process, which can involve several forces, positive and negative, based on the nature of the interactions taking place within a given community. Perturbations in the composition of species, such as the planned or accidental introduction of novel species, may interfere with the already structured web of interactions. As most species interact with a different number of species, the understanding of the mechanisms shaping natural communities remains a main challenge for evolutionary biology (Thompson 1999). Competitive and trophic interactions are considered as two of the key forces determining community structure, as they can influence the distribution and abundance of species (Gotelli and Ellison 2006). Species sharing the same limited resources will likely compete with each other, thus, depending on their ability in exploiting or monopolizing the resources, limiting one another in their potential resource acquisition. Competition can occur at three levels, either through (1) exploitative competition, which occurs when a competitor is better able to exploit the shared resource, through (2) interference, when competitors engage in fights, in which only the winner acquires access to the resource, or (3) apparent competition, when competition takes place via a shared natural enemy. Competitive interactions between different species can, at least in theory, lead to the extinction of the inferior competitor species. They may otherwise lead to coexistence, given the weaker competitor can still get sufficient resources to survive through traits that allow to reduce competition, for example, by occupying areas in which the better competitor cannot survive, or shifting activity patterns in order to forage when the better competitor is not active (Connell 1961; Tanner 2008).

Competition for food may easily occur also between species that occupy the same trophic level within a food chain. However, to understand how trophic interactions may influence species and population dynamics it is not sufficient to consider only competitive interactions within the same trophic level. A consumer/predator species can be in fact a resource for another one. Thus, trophic interactions can be quite complex, involving

different species, linked by predator-prey relationships as well as competition, when sharing the same prey. Both competitive and trophic interactions can have effects on the population density of the involved species, thus, elucidating the nature of interactions among species within a community can help making predictions on the dynamics influencing the structure of such community.

## **1.2. Individual foraging behaviour and population ecology**

Ecological communities are a result of the interplay of different species, which co-exist within the same area, and interact in different ways. Competitive and trophic interactions represent some of the modes of interaction, and the understanding of trophic interactions among species within a community can help making predictions on population fluctuations and equilibria a species can go through. Whilst population ecology deals with population density dynamics, it often lacks explaining the drivers causing and influencing such dynamics (Łomnicki 1999). Knowledge on the mechanisms responsible of these processes can help making prediction on the effects at population level. It has been discussed how individual behaviour and resource use contributes to understanding the processes underlying population dynamics (Roitberg 2007; Schoener 2011). Behavioural ecology approaches at the individual level can help elucidating the forces causing population fluctuations (Łomnicki 1999). By using an evolutionary approach based on fitness maximization (Davies *et al.* 2012), behavioural ecology investigates at the individual level to extrapolate for effects at population level. The past decades have seen an increasing interest in combining behavioural and population ecology in order to improve predicting power on population patterns (Grimm and Railsback 2013). As individuals can be considered as the basic units of populations, individual behavioural strategies determine an individual's success in surviving and reproducing, thus, its contribution to population density (Sutherland 1996). Ideally, foragers should adopt behaviours maximizing resource gain. However, physiological limitations, resource distribution and availability, and biotic interactions (i.e. presence of competitors, exposure to predators) among other factors can interfere with an individual's resource maximization gain. Thus, foragers have to balance between the costs and benefits of a given behaviour they might express. Individual decision making usually is the result of both internal (i.e. physiological) and external (i.e. environmental) stimuli, which help the forager making evaluations on the foraging strategy to adopt. Optimal foraging theory predicts the strategy a forager should adopt taking into account the trade-off between resource gain and costs of foraging. For example, when studying host searching behaviour in parasitic wasps, it might be important not only to consider whether egg availability has an effect on patch residence time on a host, but also whether predation risk or host availability can also influence the

duration of patch visits (Roitberg *et al.* 2010). As individuals do not live in isolation, competitive and trophic interactions may affect individual foraging strategies, influencing an individual's contribution to the future of the population. Thus, an inclusive consideration of the factors that may play a role on decision making and individual foraging tactics is crucial to improve our comprehension of population density fluctuations, and ultimately of the forces structuring ecological communities, both in natural and managed ecosystems.

### **1.3. Trophic interactions predator-prey effect at the population level**

Through their foraging decisions, foragers can affect the density, distribution, and abundance of their food species, with consequences on the dynamics of the population. As mentioned above, however, foraging individuals can also become at the same time a resource for other predators. Thus, to better understand the numerical outcome of these interactions and to make predictions at population level, it will be important to consider predator-prey interactions within a community. A predator species that consumes a prey has an influence on the prey species as well as the reverse is true. Traditionally, the consequences of predator-prey interactions have been identified as density-dependent effects of a predator consuming a target prey. However, as predator and prey species usually interact with other species, the impact of these interactions have broader consequences, which affect community and ecosystem levels. For example, the removal or introduction of a top predator may lead to ecosystem degradation, as result of a "trophic cascade", i.e. the progression of the effect of a top predator along lower trophic levels (Estes *et al.* 2001). When wolf density drastically decreased in North America, plant diversity was consequently reduced due to the increased number of herbivores (Ripple and Beschta 2014). In some cases, it is not difficult to identify that the causes of the observed effects are due to trophic interactions rather than to other mechanisms. For instance, density reduction of a species sympatric with a competitor for the same resources may be erroneously attributed to competitive interactions rather than to predation effect. However, this phenomenon known as apparent competition is the result of predation pressure, due to the preference of a predator for one of the two competing species (usually the more abundant one). As a consequence, predator density increases at the expenses of the lower abundant species. The consumption upon different seed species by two species of rodents favoured the abundance of some plant species over others, due to rodent preferences more than to competitive interactions among seedlings (Veech 2001). A more complex situation occurs when predators sharing the same prey engage in intraguild predation (Polis *et al.* 1989). Symmetric intraguild predation occurs when both competitors can feed on each other, which is common in aquatic communities, where organisms have different sizes at larval and adult stage (reviewed

in Polis *et al.* 1989). Contrarily, when only a competitor is feeding on the other one, we have asymmetrical intraguild predation, particularly common between generalist and specialist predators (Janssen *et al.* 1998). Intraguild predation is common in nature, affecting the abundance, distribution, and evolution of many species. Theoretical models predict that coexistence of intraguild predator and prey occurs only when the latter is a superior competitor for the shared prey. Whatever the nature of predator-prey interactions, lethal effects and consumption are not the only consequences of predation pressure. The physical presence of a predator and/or the presence of its cue in an area can also influence prey population, causing density-dependent effects (Bolker *et al.* 2003; Prasad and Snyder 2006; Laundré *et al.* 2010). In the attempt to avoid being predated, prey can modify their morphological (Peacor 2003; Montserrat *et al.* 2007) and behavioural (Pangle *et al.* 2007) traits, developing structures that can increase predation avoidance, or adopting elusive behaviours. These “indirect” predation effects can be as costly as direct consumption for the population of the target prey. Moreover, trophic cascades can occur as a result of modifications in foraging strategies of the target prey and its consequences indirectly affect other species linked to the prey or the predator. Peacor and Werner (2000) studied the effects of predation in a food web composed by four different consumer species, sharing the same resources, and a predator, which fed only on three of the considered consumers. They found that the mere predation risk due to the presence of a predator in the area reduced the foraging activity of one of the prey species (i.e. the focal species), causing a decrease in the density of the other prey species due to enhanced predation pressure. Indirect predation can, thus, alter foraging patterns within species, with consequences on other species, thus ultimately on community structure.

#### **1.4. Trophic interactions within agroecosystems**

It has been estimated that more than 38 percent of the terrestrial ice-free landscapes has been transformed for agricultural exploitation (FAO 2011), due to raising demand for food, feed, and energy by the human population (Godfray *et al.* 2010). In most cases, agricultural practices modify the natural vocation of the soil, changing land use, and sometimes threatening biodiversity, as very few plant species and varieties are of high economical interest for farmers and markets. However, the modified ecological conditions created by agricultural practices can bring together newly arranged assemblages of species, making agroecosystems interesting scenarios to study the forces shaping ecological communities (Duyck *et al.* 2006). Agriculture is particularly important in tropical areas, where it represents a main source of income, employment creation, and access to education and healthcare (Weinberger and Lumpkin 2007). Exchange with foreign markets

generate a valuable economic potential income for larger-scale farming. However, most of agricultural activity in the tropics are due to small-scale growers, who cannot access foreign exchange markets, and need sustainable techniques to manage their lands (World Bank 2008). Moreover, the increase threatens of introducing new pests, and the rise in health concern from importing countries have imposed stricter restrictions for pesticide use, thus, the need of alternative agricultural practices which guarantee enhanced crop production with low chemical residuals (USDA-APHIS 2008). The raising interest in agricultural practices to enhance crop production, and reduce pest damages, increased the request for research aimed at understanding which interactions between crop plants, herbivores and predators or parasitoids contribute to shape these heavily altered species communities. As the modification of natural areas for agriculture can bring together species that naturally would not occur in sympatry, agricultural areas can give the opportunity to study the evolution of interspecific competitive interactions in action (Duyck *et al.* 2004). Management of agro-ecosystems can include the use of natural enemies (i.e. pathogens and predators), i.e. biological control, to reduce the damages caused by pest species below economic thresholds. Apart from pathogens, specialist (i.e. parasitoids) and generalist predators are considered as suitable biological control agents, making knowledge on trophic interactions among the involved species an asset for biological control practices. Since trophic interactions within a biological control programme can be quite complex, agricultural landscapes can offer suitable scenarios to study trophic interactions from different angles like population ecology and foraging ecology.

### **1.5. Using behavioural ecology to improve pest control**

The knowledge on foraging interactions among natural enemies and a pest species can determine the success of biological control programs that rely on the suppression and control of the nuisance organism. Predation can have an important role within managed agro-ecosystems, as the foraging habits of parasitoids and generalist predators on a target pest can be exploited to reduce crop damages caused by such pests. Thus, understanding foraging strategies of a natural enemy can be important to predict its effect on the pest population. Biological control practices can be classified as (1) Augmentative biological control, which is based on the enhancement of natural enemies already present in the target areas. The population density of the natural enemy is reinforced by releasing additional individuals, especially during the period in which the density is low. (2) Conservative biological control aimed to preserve the environment such that biological agents of interest will be preserved. There is an on-going debate on this practice, which usually targets to the

preservation of natural enemy diversity. However, the outcomes from studies on conservative biological control range from positive, neutral, to negative results, depending on the investigated system (Straub *et al.* 2008).

(3) Classical biological control is based on massive release of exotic, introduced natural enemies to suppress pest population, and establish natural populations of the biocontrol agent in the field. As classical biological control relies on the introduction of new species to control the pest of interest, foraging interactions within the targeted ecological community can be altered, because the introduced species interacts with the native species. Even if the introduced species should target specifically the pest of interest, its presence might interfere with native parasitoids due to non-target effects. In turn, if generalist predators are present in the release areas, they may threaten the success of biological control agent establishment, because they may feed not only on the pest, but also on other natural enemies. In some cases, using an assemblage of multi-species natural enemies, including both generalists and specialists, can still result in an overall reduction of the target pest (Colfer and Rosenheim 2001; Chacón *et al.* 2008). However, the overall density reduction of the pest organism does not elucidate which and the way in which each predator contributes to the final effect. Studies on foraging behaviour strategies can help elucidating how natural enemies within a given assemblage might interact, and/or how they may affect one another, to better explain the final result on pest control. Thus, pest control management can be planned and improved in the light of such knowledge (Roitberg 2007). For example, a single natural enemy alone can be responsible of the overall pest density reduction, questioning usage of a multi-biocontrol agent assemblage (Colfer and Rosenheim 2001). Moreover, multi-species natural enemy systems involving both generalist and specialist (i.e. parasitoids) predators may cause intraguild predation to occur, due to the catholic habits of the generalist predator (Pell *et al.* 2007). Even if pest suppression might still be enhanced in the presence of both natural enemies, intraguild predation may reduce the population density of the specialist biocontrol agent. A typical example for this are biological control programmes aiming to reduce aphid populations. Here, parasitoids and ladybird beetles may be used as biological control agents: as ladybird beetles are generalist predators, they may feed on aphid mummies (i.e. aphids that have been already parasitized by parasitoids), being intraguild predators of the parasitoids. Although the aphid population is reduced, the parasitoids developing inside the aphids are also consumed, thus, not only reducing parasitoid fitness, but impacting host parasitoid population dynamics to the benefit of the pest species (Straub *et al.* 2008). Studies on foraging behaviour can help elucidating the way in which natural enemies can affect pest populations, but also how different species of biocontrol agents might interact

with each other. Together with the overall outcome of biological control on the target pest species, predator-prey interactions within multiple assemblage of biological control agents should be considered to better understand their effects on focal species of biological control, thus, improving biocontrol programs.

### **1.6. The study system: The IPM project to protect mangoes in Africa**

Tropical agricultural landscapes can offer interesting scenarios to improve biological control programmes, as agriculture is one of the most important opportunities for employment creation, affording access to education and healthcare (Weinberger and Lumpkin 2007). It provides not only for local and regional support, but also it contributes to create valuable foreign exchange through export trading. For example, in 2008 the horticulture sub-sector industry generated US\$1 billion in foreign exchange from exported commodities and over US\$ 650 million domestically to Kenya with directly and indirectly employment of over 4 million people (HCDA 2009). Across several of the African countries, a wide diversity of fruits and vegetables are grown both for domestic and export markets.

Among other cultivars, mango (*Mangifera indica* L., Anacardiaceae) is one of the most important ones. In fact, mango fruits are a primary source of vitamin A in Africa (Blum 1997; Meda *et al.* 2000). Moreover, these fruits constitute one of the main tropical trades, both locally and globally, and therefore a potential source of income and valuable foreign exchange. Primary destinations for the export market is Europe, with USA being an emerging one. However, growers of mangoes are facing constraints due to yield losses and quarantine restrictions on the export markets, because fruits are infested by several insect pests, such as mango seed weevil (*Sternochetus mangiferae*), termites (Isoptera: Termitidae), mealybugs (Hemiptera: Psococidae) and fruit flies (Diptera: Tephritidae) (Vanniere *et al.* 2004). Among them, none has garnered greater notoriety than tephritid fruit flies. These insects are recognized worldwide as the most important threat to the horticultural industry (reviewed in Aluja and Mangan 2008). Sub-Saharan Africa alone counts 915 fruit fly species, belonging mainly to four genera: *Ceratitis* Macleay, *Dacus* Fabricius, *Bactrocera* Macquart, and *Trirhithrum* Bezzi.

The genus *Ceratitis* include several native species attacking mango fruits and causing a direct damage of 40 to 80 percent depending on locality, variety and season (Lux *et al.* 2003; Vayssières *et al.* 2009). In particular, *C. cosyra* (Walker) was identified as primary pest of mango fruits (Ekesi *et al.* 2006). Commonly known as “mango fruit fly” or “marula fruit fly”, this species has been recorded in 23 sub-Saharan countries from the Eastern to the Western coast, including also South Africa (Steck 2000; EPPO 2005).

The situation became worse with the invasion of *Bactrocera dorsalis* (Hendel), an alien species with Asian origins, firstly recorded in Kenya in 2003 (Drew *et al.* 2005) and identified as a new species, with the name of *Bactrocera invadens*. A prompt request for the support of FAO was submitted by Kenya, Tanzania and Uganda to contain the damages and prevent the diffusion of the new pest. Unfortunately, the surveillance revealed soon that the pest was already widely distributed among these countries (Ekesi *et al.* 2006). Thanks to its highly mobility and the absence of natural parasitoids and predators, this species was able to disperse to all sub-Saharan countries, rapidly reaching the west coast (Drew *et al.* 2005; Vayssières 2009). It is currently reported in 28 African countries, including the Comoros Islands and Capo Verde (Vannierie *et al.* 2004; Ekesi *et al.* 2006; Khamis *et al.* 2009; Vayssières *et al.* 2008; De Meyer *et al.* 2012; José *et al.* 2014). As a polyphagous fruit fly, its host range numbers 44 plant species, belonging to 23 different families (Badii *et al.* 2015). In Kenya it has been recorded from more than 14 species that are used as hosts (Ekesi and Billah 2006). The Inter-African Phytosanitary Council of the AU has described it as a devastating quarantine pest (French 2005). As invasive species, *B. dorsalis* has been found to have a high competitive ability in interactions with native fruit flies. In several cases co-infestation by intergeneric species was observed and, in general, suitable hosts have produced a high number of *B. dorsalis* emergences compared with indigenous pests like *C. capitata* and *C. cosyra* (Mwatawala *et al.* 2006). Moreover, this species has been found to exert a high competitive dominance over *C. cosyra*, though it has not been able to drive the latter to extinction (Ekesi *et al.* 2009; Rwomushana *et al.* 2009). *C. cosyra* in fact, as aboriginal species, is more specialized in searching for mango hosts (Ekesi *et al.* 2009). Moreover, its resistance to lower temperature makes it to be restricted to highlands where the exotic competitor finds hostile conditions for development (Rwomushana *et al.* 2008; Ekesi *et al.* 2009).



**Figure 1-1:** A female fruit fly of *Bactrocera dorsalis* species.  
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Damages of the fruits are due to the direct effect of fruit fly larvae feeding activity, which cause the mangoes go rotten and so becoming unmarketable. Economical losses are also associated to quarantine restrictions (USDA-APHIS 2008; Ekesi *et al.* 2010). In fact, infestation and the mere presence of the flies in a country can limit the export of fresh horticultural products to large lucrative market abroad. For example, export of potential host species of *B. dorsalis* such as mango, avocado and cucurbit from Kenya, Tanzania and Uganda has been banned in Seyshelles, Mauritius and South Africa (Ekesi *et al.* 2010). Also USA blocked the importation of several cultivated fruits and vegetables from African countries where *B. dorsalis* has been reported (USDA-APHIS 2008). The European restrictions concerning the use of pesticides (Maximum Residue Levels) compound the problem and limit the trades (Ekesi *et al.* 2010). Restriction consequences are economically unsustainable for the exporting countries. For example, in 2008 Kenya lost US\$ 1.9 million due to the restrictions of exporting avocado in South Africa.

When the initial purpose of containing the pest could not be met, other effort has been spent to understand the biology and ecology of the pest and develop management tools useful to control its population and damages. Pesticide restrictions from importing countries and the risk of pest resistance development brought to the exploration of environmental friendly techniques for pest management. Among the others, natural enemies, native and exotic ones, has received a particular attention as a cheap and long lasting method to reduce fruit fly population.

The family Braconidae (Hymenoptera: Parasitica) counts several primary parasite species and therefore has attracted increasing interest as emphasis in pest control has shifted toward biotic agents. In particular, the subfamilies Opiinae and Alysiinae are almost entirely restricted to parasitize Diptera. The opine *Fopius arisanus* (Sonan) has been identified as a suitable parasitoid for *B. dorsalis*, as originally collected from puparia of this fruit fly species in the Indo-Pacific area (Rousse *et al.* 2005). Its importance raised when it was found able to attack at least seven species of tephritid fruit flies in the western hemisphere (Wharton and Gilstrap 1983). As a koinobiont endoparasitoid, it completes its development inside the host puparia and emerge as an adult, usually a few days after the emergence of fruit flies from unparasitized puparia. The parasitoid remains at its first larval instar until the host reaches the pre-pupal instar (when it leaves the feeding site to form its puparium). The parasitoid then moults for the first time and develop further devouring its host inside the puparium from which it will emerge. It has been observed though also parasitize 1st instar larvae (Rousse *et al.* 2005). As other fruit fly parasitoid, it has a piercing ovipositor and probing with or without actual egg-laying

may in itself result in significant mortality of the attacked insect (Calvitti *et al.* 2001; Rousse *et al.* 2005). *Fopius arisanus* parasitism rates vary depending on the fruit where the host eggs have been placed. In banana and mango fruits high rates of survival were observed. Moreover, fruit responsiveness increase with fruit ripeness (Rousse *et al.* 2005). Once landed on a fruit, a female spend more than 90 percent of its time in searching for host eggs and exhibiting egg-laying behaviour. Females show associative learning, preferring hosts previously visited. This lead to a viable progeny production for experienced females compared to the naive ones (Dukas and Duan 2000).



**Figure 1-2:** A female parasitoid of *Fopius arisanus* on mango. © Thomas S. Hoffmeister

*Diachasmimorpha longicaudata* (Ashmead) (Rousse *et al.* 2005) is a solitary larval-preupal endoparasitoid. Originally distributed in the Indo-Pacific area, it parasitizes 14 species of *Bactrocera* genus (Wharton and Gilstrap 1983). Because of its suitability for larval stages, it has been considered a valid enemy. In fact, its attitude of foraging more frequently on the over-ripe rotten fruits may enhance over all parasitism through attacks on late instar larvae which may have escaped parasitism by egg (Purcell *et al.* 1998).

Unfortunately, it showed poor performances on *B. dorsalis* in lab conditions. However, it was found effective in the control of *Ceratitis* spp. (Mohamed *et al.* 2008). Thanks to its long ovipositor, *D. longicaudata* would gain greater access to host larvae that are beyond the reach of the native parasitoid ones with shorter ovipositors.

Weaver ants (genus *Oecophylla* Smith) are one of the major inhabitants of the tropical canopy. Only two species belong to this genus: *O. smaragdina* (Fabricius), the green weaver ant, largely spread in Asia and *O. longinoda* (Latreille), the red weaver ant, present in the African tropics. They have been traditionally used as effective biological control agent in Asia (Needham 1986).

As generalist predators, weaver ants are known to protect 12 woody crops against more than 50 different arthropod pests (Way and Khoo 1992; Offenberg and Wiwatwitaya 2009). Recent studies showed that substituting conventional spraying programs with *O. smaragdina* biocontrol has led to increase net incomes for more than 70 percent in Australian cashew and Mango plantations (Peng and Christian 2005) and up to 40 percent in Vietnamese citrus (Offenberg and Wiwatwitaya 2010). Because naturally present in the environment and active all over the year, their use highly diminishes the costs of pest management (Peng and Christian 2005). Despite their wide use in Asia, only recently scientists are evaluating their potential effect (van Mele *et al.* 2007). For example, the control by *O. longinoda* has matched the use of chemicals pesticide formulations in cashew in Ghana (Dwomoh *et al.* 2009). In Benin, fruit growers participating to fruit fly control project noticed that the presence of *Oecophylla* ants improve the mango quality and yield by reducing the amount of rotten fruit, extending the mango shelf life, giving the fruit a healthy appearance and improving its juice quality, especially its sugar content (Singozaan *et al.* 2008). Mean fruit fly damage ranged from 1 to 24 percent with the weaver ant being either abundant or absent (van Mele *et al.* 2007). Moreover, the chemicals deposited by the workers as a track to the source of food act as a strong predator avoidance cues, preventing fruit fly from ovipositing in mangoes on which ants had patrolled (van Mele *et al.* 2009; Adandonon *et al.* 2009).



**Figure 1-3:** A nest with workers of the African weaver ant, *Oecophylla longinoda*.  
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## 1.7. Objectives of the study

In the attempt to better elucidate the effect of resource use, competitive, and foraging interactions, and their influence on population dynamics in this pest-parasitoid-generalist predator system, this study investigated several aspects of the foraging behaviour of the two fruit fly and parasitoid species, and the effect of a potential predator such as the weaver ants on their foraging activity.

In particular, **chapter 2** investigates the effects of fruit fly physiological status and fruit characteristics on host oviposition acceptance and clutch size, showing that the physiological status of the foraging females was a primary driver for oviposition probability. It is shown here the different response to fruit ripeness stage and mango variety shown by the fruit flies, discussing the implication how such differences could contribute to competitive displacement between the two species. **Chapter 3** focuses on the usage of previously-drilled oviposition punctures established on the fruit by *C. cosyra* and *B. dorsalis* females, observing whether the foraging females could oviposit in conspecific or heterospecific made punctures. It is discussed here the implications related to competitive interactions between the native and the invasive fruit fly species, arguing whether such behavioural strategy can be adopted by foraging females to successfully oviposit when foraging at increased risk situations, such as in the presence of a predator. Finally, **chapter 4** explores the role of the weaver ants as potential biocontrol agent of the two fruit fly species with semi-field experiment conditions and behavioural bioassays. It is also reported within this chapter the effects this ant species had on the exotic parasitoids used to control the fruit fly populations. The study approach adopted here allowed not only to quantify the impact this ant species has on the foraging fruit flies and parasitoids, but also the nature of the interactions between the generalist predator, the parasitoids, and the fruit flies. Potential pros and cons of having both the weaver ants and the parasitoids as biocontrol agents of the flies are discussed here, further underlying the importance of behavioural investigation at the individual level to improve biological control planning.

## 1.1 References

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## **2 Physiology vs. environment: what drives oviposition decisions in mango fruit flies (*Bactrocera invadens* and *Ceratitis cosyra*)?**

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## 2.1 Abstract

In frugivorous insects, the physiological state of the female (e.g. age, egg load) as well as environmental parameters like fruit availability and characteristics may affect oviposition decision, host choice and the resulting clutch size. We studied host acceptance and clutch size decision as a function of fly egg load as physiological state and mango variety and ripeness stage as the environmental parameter in two major mango pests in sub-Saharan Africa, *Bactrocera invadens* and *Ceratitidis cosyra*, under laboratory conditions. In addition, egg maturation dynamics were investigated because for both species, egg production had never been explored before. Female egg load was the most important factor influencing host acceptance in both species. With respect to fruit characteristics, ripeness stage was significant for oviposition decisions only in *C. cosyra*. In particular, ripe and fully ripe fruits had more probability of oviposition than unripe ones. In contrast, fruit variety affected clutch size in *B. invadens*, with, respectively, the local variety Ngowe receiving the biggest clutches and the export Kent the smallest. Moreover, the invasive species showed a significantly higher egg production compared with the native species. Implications in terms of competitive displacement between the two species are also discussed.

### **3 Exploitation of hetero- and conspecific oviposition punctures as an oviposition strategy in mango fruit flies**

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### 3.1 Abstract

In tephritid fruit flies, the larvae develop within the host structure the mother chooses to lay her eggs in. Thus, a female's decision on where to lay the eggs is crucial for both her own reproductive success and her offspring's fitness. However, the optimal oviposition decision for a female is not necessarily always the best option for the larvae. This occurs, e.g. when female fruit flies use oviposition punctures of other females on host fruits to save energy and time spent penetrating the fruit skin. The offspring, in this case, faces competition with offspring of other females that have previously infested the fruit. For a female a reduction in time spent drilling punctures may be advantageous because it offers her more opportunities to oviposit elsewhere, potentially increasing her reproductive success. Moreover, it may pay under predation pressure, where a reduction in host handling behaviour may reduce the exposure to predation on the fruit. Based on this rationale, we studied the usage of previous oviposition punctures in *Ceratitis cosyra* and *Bactrocera dorsalis* on mango fruits, with a choice and a no-choice experimental set up.

We found that while clutch size was not influenced by ovipositing in a previously drilled or a self-drilled puncture, for both species the number of eggs laid doubled within the 30-minutes observation time when exploiting previously drilled punctures, because the fruit flies were able to oviposit in both a previously drilled and a self-drilled puncture. The probability that the first oviposition occurred in a puncture already present on the mango increased with increasing number of punctures. However, *B. dorsalis* females showed a 25 percent higher propensity to use a previous oviposition puncture at first compared to *C. cosyra* females, that preferred ovipositing in conspecific punctures. Moreover, ovipositing in a previously drilled puncture reduced the time spent drilling on the fruit skin by 6 minutes for *B. dorsalis* and 4 minutes for *C. cosyra*.

### 3.2 Introduction

Reproductive strategies adopted by individuals are crucial in contributing to population survival and growth. In parasitic insects, females lay their eggs into a spatially delimited food source (i.e. host) in which the offspring live until completion of their development. As offspring survival depends on the available resources of the host, the "preference–performance" hypothesis predicts that a female should prefer to oviposit into hosts that maximize offspring survival and development (Jaenike 1978). However, resources are usually limited, thus, females have to balance between the unpredictability of finding suitable hosts and the reproductive success gained from those hosts. Consequently, oviposition behaviour is best seen as an optimal foraging problem where costs and benefits of reproductive behaviour are analysed, as they may influence oviposition decisions.

In particular, optimal foraging theory considers (1) when a female should leave a patch, where “patch” can be either an area containing several hosts or a single host itself, (2) which host a female should accept for oviposition and (3) how many eggs (i.e. clutch size) she should lay within a single host. Several factors may contribute to a female’s oviposition decision process, including environmental factors (host availability, mortality risk, presence of conspecifics), physiological parameters (egg production and egg load, age) and experience (Visser *et al.* 1992). If it seems likely to find only few suitable hosts to successfully lay eggs, females may accept already occupied host to lay their eggs. This is especially true for females with higher egg loads that show an increase tendency to accept already infested hosts (van Randen and Roitberg 1996; Minkenbergh *et al.* 1992; Lalonde and Mangel 1994; Papaj 2000). Superparasitism (i.e. the tendency to exploit hosts infested by conspecifics) has been considered as an adaptive reproductive strategy under resource limitation in nature (van Alphen and Visser 1990). If a female refrains from exploiting an already occupied host, she might miss an opportunity of successful oviposition, given suitable hosts are rare. Thus, if host availability is limited, a female might accept to exploit already occupied ones, potentially enhancing the number of offspring produced (Lalonde and Mangel 1994). The reduced likelihood of successful offspring development due to competition within the already exploited host might be ameliorated by reducing the number of eggs laid into such hosts. Exploiting infested hosts may also be advantageous to access resources otherwise inaccessible (Papaj and Messing 1996). Fruit-parasitic insects lay their eggs inside a fruit host, thus, females have to penetrate the fruit epicarp in order to successfully oviposit. Drilling the fruit skin can be energetically demanding, time consuming and can cause ovipositor damage, potentially, impairing a female’s reproductive lifespan (Jones and Kim 1994). Thus, females may reduce the energetic demand and ovipositor wear by exploiting punctures already established by other females (Lalonde and Mangel 1994). Ovipositing in punctures drilled by other females may also reduce the time spent to handle the host (Papaj 1992, 1993; Papaj and Alonso-Pimentel 1997), that can be advantageous to avoid or reduce predation risk, when foraging for hosts. In the presence of a predator, parasitoid females with shorter host handling time had more success in ovipositing than females with longer host handling duration (Weisser *et al.* 1994; Völkl and Kroupa 1997; Barzman and Daane 2001). However, in response to already exploited punctures, females might lay smaller clutches due to the competition of their offspring with other larvae in the same fruit (Papaj *et al.* 1992). This may also decrease the time spent ovipositing, since oviposition duration is often proportional to the clutch size (Papaj *et al.* 1989).

Besides superparasitising conspecifically exploited fruits, females may also exploit hosts occupied by the offspring of heterospecifics, a phenomenon known as multiparasitism (Smith 1916). In this case, however, the offspring from different species may differ in their competitive abilities to exploit the resources. If, for example, the eggs of one species hatch earlier than the one of the other, the larvae of this species may access the resources earlier, benefitting from a priority effect (Shorrocks and Bingley 1994). Thus, asymmetric competition may arise if the offspring of one species is better able to monopolize the resources available, potentially impairing the reproductive success of the other species. Moreover, if the superior competitor shows the ability to use hosts infested by heterospecifics, multiparasitism may become a factor contributing to the competitive displacement of the inferior competitor.

The species we investigate here with respect to their oviposition decisions belong to the family of true fruit flies (Tephritidae). They are plant-parasitic insects, with a number species using fruits as hosts (Prokopy and Roitberg 1984). As many species are infesting fruits of economic importance, they are known as one of the world's most damaging agricultural pest (Aluja and Mangan 2008). Therefore, there has been an extensive interest in studying their reproductive and oviposition strategies. Some species lay eggs in clutches inside the fruit pulp and adjust the size of the clutch depending on the fruit size as an indication of the resources available for the offspring (Mc Donald and Mc Innis 1985). Some fruits have sufficient resources to support the successful development of several clutches, even laid by different females, as evidenced by the number of adults emerging from a single host which exceeds the number of eggs that a single female can lay (Lalonde and Mangel 1994). Additionally, adults of different species can emerge from the same host (Ekesi *et al.* 2006), supporting the fact that super- and multi-parasitism can occur. In some cases, females are not only sharing the same host, but also using punctures drilled by conspecifics, interpreted as host handling duration saving strategy (Papaj and Alonso Pimentel 1997). However, there is still a lack of knowledge with respect to the usage of punctures by con- and/or heterospecific females, especially regarding differences in preferences, host-handling time and/or clutch size. If one species, for example, is able to better exploit punctures established by hetero- rather than conspecifics, this might affect the competitive interactions between the two species. Moreover, if the species also differ in the time needed to drill an oviposition hole into the fruit, the species with the longer host handling duration may gain higher advantage by exploiting previously established punctures, as the time saved can be further invested in attempting oviposition in new sites.

*Bactrocera dorsalis* (Hendel) and *Ceratitidis cosyra* (Walker) are two polyphagous fruit fly species, primarily infesting mango fruits in sub-Saharan African countries (Ekesi *et al.* 2006). Both species are able to continuously mature eggs during their lifespan (Migani *et al.* 2014), having egg loads that can exceed 100 eggs. While *C. cosyra* is a species native to Africa, *B. dorsalis* is an invasive species with Asian origins. Both species share common host plants and co-infestation has been recorded in several fruits with adults of both species emerging from a single host (Ekesi *et al.* 2006). Thus, it is reasonable to conclude that super- and multiparasitism occur between the two species. Despite the fact that *B. dorsalis* and *C. cosyra* offspring can co-exist within the same fruit host, the larvae of the invasive species are superior competitors to *C. cosyra* (Ekesi *et al.* 2009). In some areas, *B. dorsalis* has competitively displaced *C. cosyra* (Ekesi *et al.* 2009). *Bactrocera dorsalis* females are known to exploit fruit cracks and oviposition punctures established by heterospecific females, however, there is no indication on how and whether females adjust clutch size or time spent ovipositing when exploiting heterospecific vs. conspecific punctures. Moreover, the ability and occurrence of super- and multi-parasitism with respect of the usage of oviposition punctures has not been explored for *C. cosyra*. Here, we studied the exploitation of punctures established by hetero- and con-specific females in these two fruit fly species in no-choice and choice situations. We have particularly focused on clutch size, host-handling and oviposition duration in newly drilled and previously established oviposition punctures. We expected females to opportunistically exploit existing punctures to save time, but to adjust the clutch size laid to the competitive situation. However, the time saved may be invested to attempt further ovipositions, thus, increasing the number of eggs laid in total. Therefore, we also expect the number of oviposition events attempted within the same timeframe to increase, when the fruit flies are ovipositing in already-established punctures. Yet, we still expect a difference in the number of eggs laid in mangoes with and without previously established punctures. In particular, we investigated: (1) whether the number of eggs differs depending on ovipositions occurring in clean or already infested mangoes as well as when ovipositions occur in new and previously drilled punctures, (2) where the first oviposition occurs, when previously-established oviposition punctures are present on the mango; (3) the frequency at which previous oviposition punctures are accepted; (4) whether there is a preference to re-use self-made punctures compared to punctures drilled by other females; (5) whether the use of already-drilled punctures has an effect on the time spent probing and ovipositing, respectively, and (6) whether the fruit flies show a preference for a mango with or one without oviposition punctures, with respect to first landing, occurrence of oviposition events, and clutch size. We

discuss our findings with respect to advantages and costs of super- and multiparasitism, with particular emphasis on competitive interactions due to possible asymmetric preferences in oviposition strategies between the two fruit fly species.

### 3.3 Materials and Methods

All experiments were conducted in the behavioural laboratory at the Institute for African Insect Science for Food and Health (*icipe*), Duduville campus, Nairobi (Kenya). For rearing and experiments, we used ripe mangoes of the “Apple” variety, which is one of the most highly infested varieties under East African field conditions and for which *B. dorsalis* has been shown to produce large clutch sizes (Migani *et al.* 2014). The mangoes were collected from local farms that use pesticide-free management to prevent fruit fly infestations. They were stored at 4°C to stay fresh until use. Only newly ripened mangoes were selected for experiments. We used ‘mango domes’ both for making females experienced with oviposition and for the experiments. The domes were constructed by cutting off both sides of each mango around the seed and removing the pulp. The usage of mango domes also prevented the usage of mangoes that potentially had previous fruit fly infestation in the field.

#### 3.3.1 Rearing of fruit flies

*Bactrocera dorsalis* and *C. cosyra* puparia were collected from the main colonies reared at the ARCU unit at *icipe*, Duduville campus, Nairobi, Kenya and placed in a cage for adult emergence. The puparia were placed into new cages according to date of emergence in order to know exactly the age of the flies in the cage. Laboratory rearing conditions were maintained at environmental temperature and humidity (around 25±1°C and 50 ± 8% RH). Flies were reared with a photoperiod of 12:12 h (L:D), as in natural conditions. Experiments were conducted under the same conditions. Rearing was done using the methodology according to Ekesi and colleagues (2008). *B. dorsalis* and *C. cosyra* were kept in separate cages and males and females were kept together to facilitate breeding. All fruit fly females used for the experiment were previously experienced with oviposition: a mango dome was introduced in the cage at least twice before the flies were used in the experiments. Infested mangoes were collected from local mango farms and markets in Kenya to refresh the main colonies with wild flies every six months.

### 3.3.2 Experimental Set-up

#### 3.3.2.1 No-choice experiments

Each mango dome was placed in a Petri dish (8.7 cm in diameter) and sand was placed around the edge of the mango to prevent the female flies from ovipositing at the dome edges. Since host size can influence clutch size, we took the Petri dish diameter as standard diameter for the mango size, as the mango was cut in order to fit the Petri dish. We also determined the mango height as the highest point of the dome and took this variable as indicator of fruit size.

In order to obtain natural oviposition punctures on the mangoes, we placed two domes in a cage with either *C. cosyra* or *B. dorsalis*, respectively. They were monitored constantly for oviposition events. We checked the domes repeatedly under a stereomicroscope for oviposition punctures to obtain a minimum of 3 to a maximum of 14 natural oviposition wounds. Since the flies had the time to lay eggs, we carefully removed previous eggs by using a fine camel brush and we marked each oviposition site on the dome skin using a ballpoint pen. We recorded the total number of oviposition punctures for each dome and we used domes with the same or similar ( $\pm 1$  or 2 max.) oviposition wounds in order to have comparable numbers of trials for each replicate. We carried out a total of 27 replicates for *C. cosyra* and 29 replicates for *B. dorsalis* per treatment, respectively.

#### 3.3.3 Behavioural observations

For the experiment, we used three Perspex cages, measuring 20x20x20 cm. In each of them we placed a single mango dome with oviposition wounds either from *C. cosyra* or *B. dorsalis* and one dome without wounds (i.e. control). The mango was left inside each cage for 20 minutes before each trial started, to allow mango volatiles to fill the cage. We released a single female fruit fly, previously experienced with oviposition (see above), per cage. We selected female *B. dorsalis* flies between 18-22 days old, while female *C. cosyra* flies were chosen between 14-18 days of age, as at this stage the flies reach a high egg production (Migani *et al.* 2014). We use females of only one species for each trial and we released them in their three cages simultaneously. We observed female behaviour continuously for 30 minutes, recording number and frequency of fruit visits, probings, and ovipositions using The Observer software (Noldus Information Technology, Wageningen, The Netherlands). We used the recorded parameters to calculate the duration of fruit visit, probing and oviposition, respectively. We could differentiate between oviposition and probing since the fly would remain completely still while ovipositing, while it was visibly moving up and down the abdomen to drill

the hole during probing. For probing and oviposition events, we recorded if the female was using an existing puncture (self-made in the experiment or previously drilled by another female) or if she was drilling a new one. Given ovipositions occurred, we marked oviposition sites after each trial and counted the number of eggs laid. We conducted two replicates per day, one for *C. cosyra* and one for *B. dorsalis*. The experiments were carried out one after the other and each species was alternated each day for occurring first or second.

#### **3.3.3.1 Choice experiment**

For choice trials we used a Perspex cage measuring 40x20x20 cm. In this cage we placed two mango domes, equally distant apart to one another and to the wall of the cage. While one mango dome was free of oviposition punctures (i.e. control), the other mango dome was previously exposed to *B. dorsalis* and *C. cosyra* to receive punctures from both species. The same procedure as above was used for counting and marking previous oviposition sites, but we used different marking to differentiate for wounds made by *C. cosyra* (marked with a cross) and those made by *B. dorsalis* (marked with a dot). The number of previously drilled punctures on a mango ranged between 6 and 20, with equal numbers of punctures per species. For the experiment, we released one female fly of either *B. dorsalis* or *C. cosyra* inside the cage. In order to provide the flies with equal chances to visit one mango or the other, we placed the fly in a small Petri dish (1.5 cm diameter), positioned at equal distance between the two domes and then removed the lid to release the fly. We started recording the fly behaviour only once the fly left the Petri dish. Every day we ran a trial for both *B. dorsalis* and *C. cosyra*, switching every day the order of the species used in the first trial in order to avoid time effects. Moreover, we switched the position of control and wounded mango domes in the cage for each trial to avoid light effects. We observed the fly for 30 min and recorded the behaviour as for the “No-choice” experiment.

#### **3.3.4 Statistical Analysis**

##### **3.3.4.1 No-choice experiments**

We used R version 3.2.1 (R Core Team 2015) for data analysis and for graphical data presentation. We tested (1) the effect of oviposition occurring in mangoes with and without previously drilled punctures and the species tested in the experimental trial on the number of eggs laid, (2) the number of eggs laid as a function of using a previously non-punctured or a punctured fruit and whether using an existing puncture or not, (3) the effect of the fruit fly species, whether mangoes had been punctured either by con- or heterospecific females, and the number of punctures present on the dome on the site where first oviposition occurred, and (4) the frequency

of re-usage of self-made punctures as function of the tested species and self-made or hetero- and conspecifically drilled punctures using Generalized Linear Models (GLMs) with main terms and their interactions. For both (1) and (2), we implemented a model for count data with Poisson distributed error, while for (3) and (4) we used a binomial distributed error. In all cases, we checked for possible over- or underdispersion of the data. We tested the interactions among the explanatory variables and we conducted a stepwise backward deletion of the non-significant term to find the Minimum Adequate Model (MAM). For testing (5) the effect of oviposition punctures on the time spent probing and ovipositing and on (6) the frequency of acceptance of previously drilled punctures, we used Generalized Linear Mixed Models (GLMMs) (package “lme4” by Bates *et al.* 2015) with female fly identity as random term, since females could oviposit more than once on the same fruit within a single observation. We fitted the model using Gamma distributed error with log-link function for (5) and Poisson distribution for (6).

#### **3.3.4.2 Choice experiment**

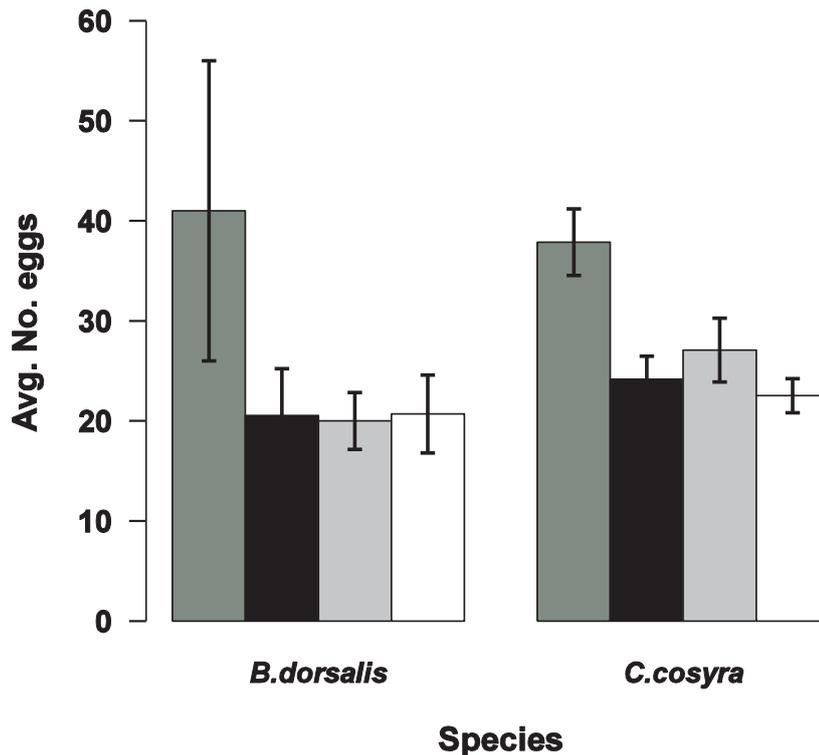
We used GLMs with a binomial distributed error to look at (1) the preference in alighting first on already punctured vs. non-punctured mangoes, and (2) the probability that, within the 30-minute observation frame, an oviposition occurred as function of mango domes with vs. without previously drilled punctures. To test the number of eggs as a function of the species, the mango (with/without punctures) used to oviposit and the usage of con-, heterospecific or newly drilled punctures, we used a GLMM with Poisson distributed error and we tested two-way interactions among the explanatory variables. We then conducted a stepwise backward deletion of the non-significant terms in order to find the MAM. We also tested the same variables as above for each species separately.

### **3.4 Results**

#### **3.4.1 No-choice experiments**

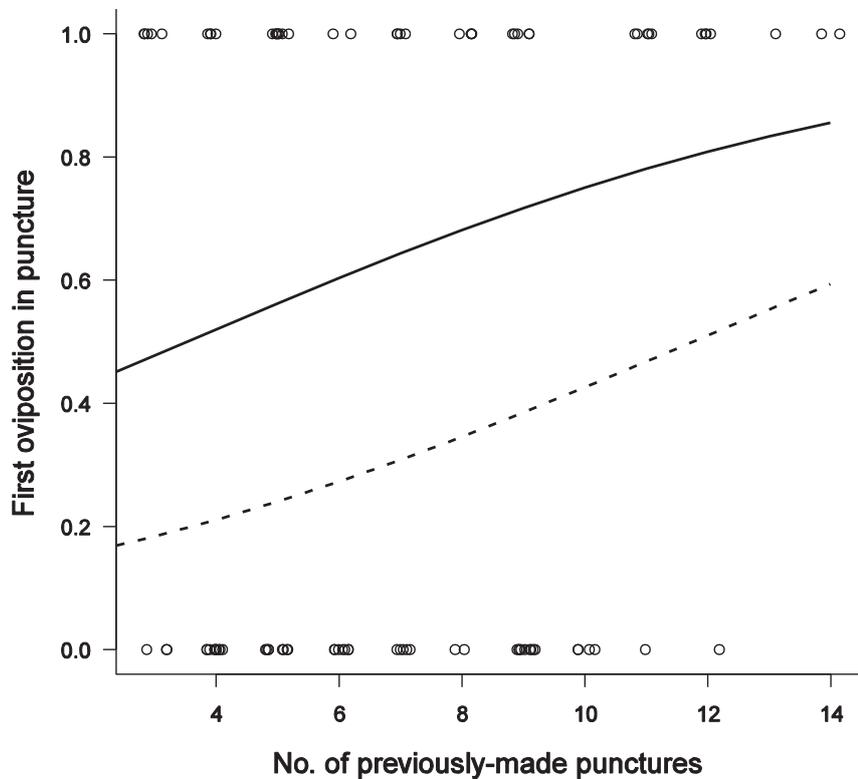
We did not find any difference in clutch sizes laid in mangoes with or without previous oviposition punctures, neither for *B. dorsalis* nor for *C. cosyra* (mangoes with/without punctures:  $F_{1,121}=0.73$ ;  $p=0.4$ ; species:  $F_{1,121}=3.9$ ;  $p=0.06$ ; punctured/unpunctured mango x species:  $F_{1,121}=0.45$ ;  $p=0.5$ ). Similarly, the clutch size did not differ among the two fruit fly species ( $F_{1,121}=1.85$ ;  $p=0.17$ ) when ovipositing only once during the 30-minute observation time, but doubled when the fruit flies had the opportunity to oviposit twice, exploiting two different

oviposition sites, i.e. a newly-drilled and a previously drilled puncture, compared to when just drilling their own puncture or using a previously-drilled one ( $F_{1,121}=1.89$ ;  $p= 0.02$ ; Fig. 1).



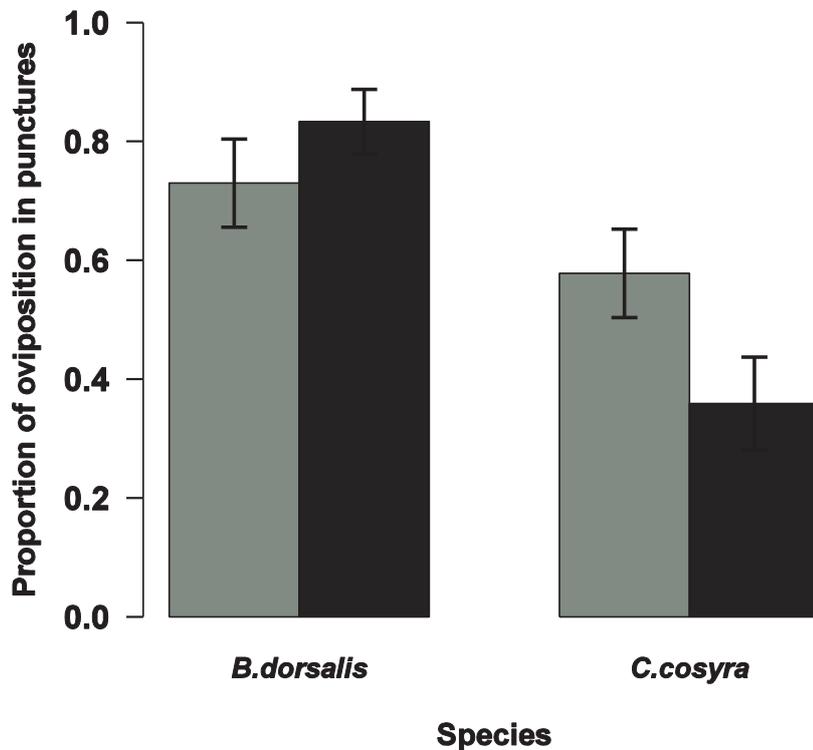
**Figure 3-1:** Number of eggs (averages  $\pm$  1 S.E.) laid by *Bactrocera dorsalis* and *Ceratitits cosyra* as function of ovipositions taking place on punctured mangoes in both newly- and previously-drilled punctures (dark grey bars), in newly-drilled punctures only (black bars), in already-drilled (grey bars) punctures only, and in newly drilled sites on mangoes that were not punctured before (white bars).

When punctures were available on the mango, the probability that the first oviposition occurred in an already drilled puncture was a function of the number of previously established punctures on the fruit ( $\chi^2=4.66$ ;  $df=1$ ;  $p=0.03$ ; Fig. 2): at increasing number of punctures, the proportion of oviposition occurring in already drilled punctures increased. The chance that the first oviposition occurred in an already drilled puncture was 25 percent higher for *B. dorsalis* compared to *C. cosyra* females ( $\chi^2=10.02$ ;  $df=1$ ;  $p=0.002$ ; Fig. 2).



**Figure 3-2:** Effect of the number of punctures present on the fruit on the probability of having a first oviposition occurring in a previously existing puncture for *Bactrocera dorsalis* (solid line) and *Ceratitis cosyra* (dotted line).

A two-way interaction between the tested species and whether the previous puncture had been made by a heterospecific or conspecific female influenced the tendency that a fly accepted a puncture for oviposition ( $\chi^2=4.71$ ;  $df=5$ ;  $p=0.03$ ; Fig. 3). While *B. dorsalis* females showed an equal tendency to use punctures established by con- or heterospecific females, in *C. cosyra* the frequency of oviposition occurring in punctures established by conspecific females was almost twice as high compared with the usage of heterospecifically drilled punctures, showing the potential that larvae may experience higher intra- than interspecific competition.

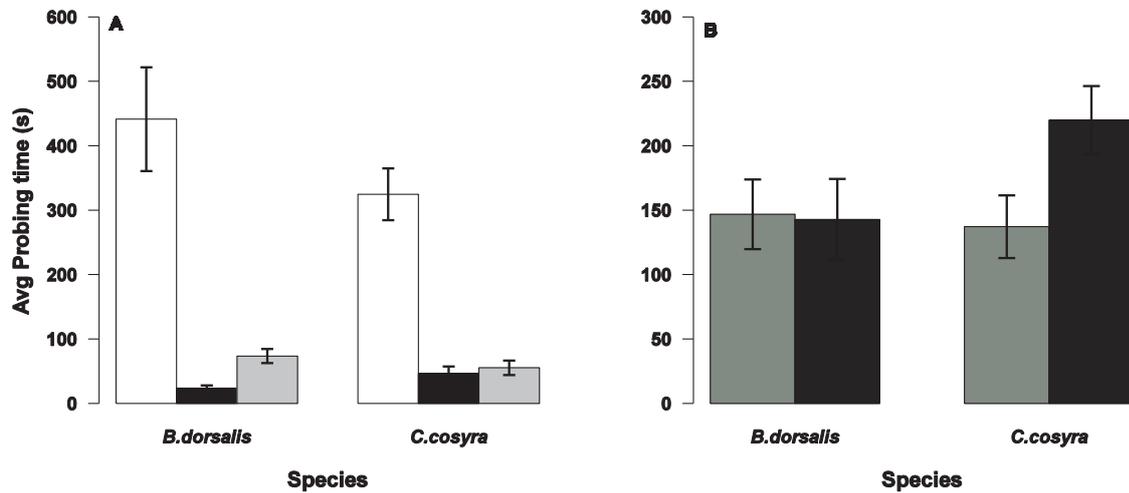


**Figure 3-3:** The influence of conspecifically (grey bars) and heterospecifically (black bars) made-punctures on the probability to oviposit in a puncture already present on the mango dome in *Bactrocera dorsalis* and *Ceratitidis cosyra* (averages  $\pm$  1 S.E.).

In *B. dorsalis* the frequency to re-use oviposition sites where a female had already laid eggs within the same observation was approximately twice as high compared with *C. cosyra* females ( $\chi^2=7.84$ ;  $df=1$ ;  $p=0.005$ ).

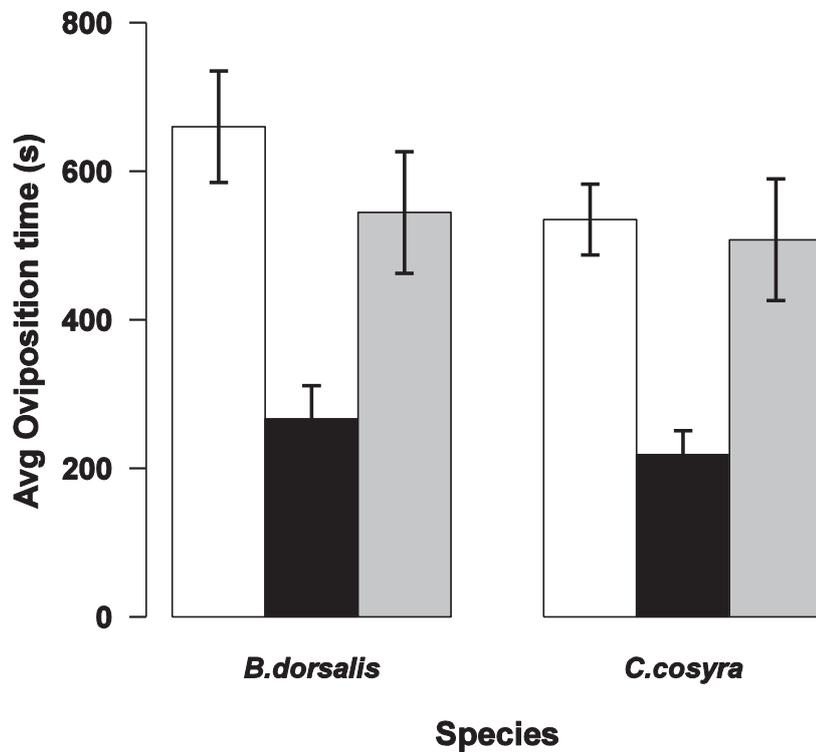
As the usage of previously drilled punctures may save time to penetrate the fruit epicarp, we investigated the effect of ovipositing in a previously established puncture on the time a fly spent drilling on the fruit skin. The statistical interaction between the fly species under investigation and the type of puncture used for oviposition (i.e., previously-established, newly-drilled punctures, and the re-use of self-exploited oviposition sites) was significant with respect to the duration of probing the fruit skin (species x oviposition site:  $\chi^2=10.16$ ;  $p=0.006$ , Fig. 4A). When producing new punctures, *B. dorsalis* females needed roughly 7.5 minutes and *C. cosyra* 5.5 minutes., and thus, *C. cosyra* flies were 2 minutes faster to penetrate the epicarp. By either using punctures already present on the fruit or laying eggs more than once in the same puncture, *B. dorsalis* females saved 6 minutes of time probing on the fruit epicarp, while *C. cosyra* females spent 4 minutes less to penetrate the fruit skin. There was also a difference between the two fruit fly species in the duration of probing in punctures

established by conspecific or heterospecific females (species x con-/heterospecific-established punctures:  $F_{2,169}=4.10$ ;  $p=0.04$ , Fig. 4B): *C. cosyra* females spent around 33 percent more time when probing in a puncture made by *B. dorsalis* than when probing on a puncture previously drilled by conspecifics.



**Figure 3-4:** Duration of time (averages  $\pm 1$  S.E.) that *Bactrocera dorsalis* and *Ceratitits cosyra* females spent probing on the fruit epicarp. In (A) the duration of probing is a function of drilling in a new (white bars), in a previously-established puncture (grey bars) and returning to oviposit where the female had already laid eggs, while the effect of probing in a puncture established by con- (dark grey bars) or heterospecific females (black bars) is shown in (B).

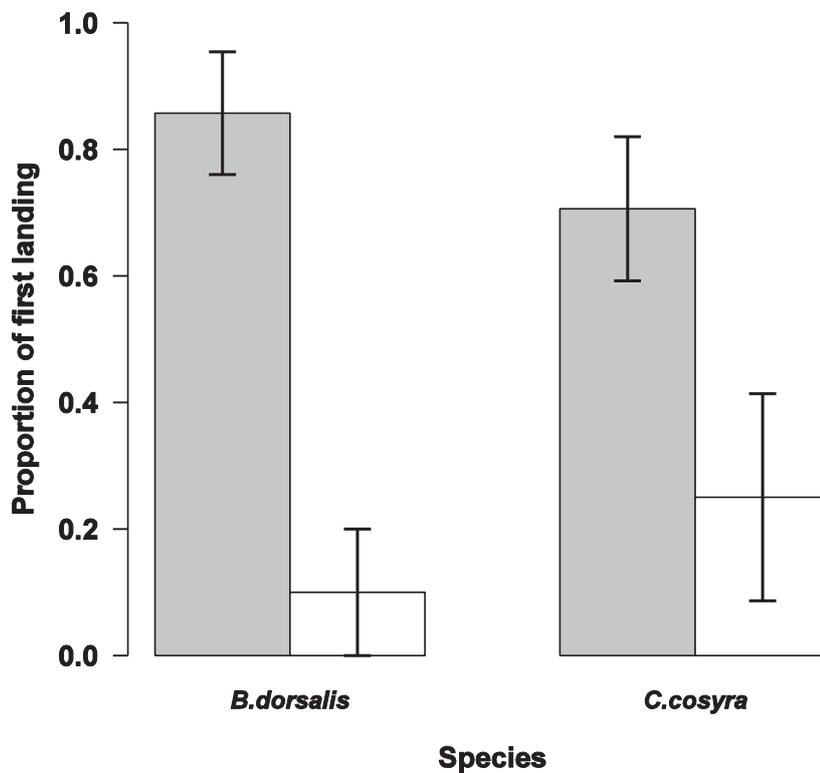
A similar, but more pronounced pattern exists when taking into account ovipositions rather than probing. By returning to oviposit in a puncture (either newly established or already present on the fruit), females of both fruit fly species reduced the time spent laying eggs by 47 percent in comparison to the time they used for their first laying both in a previously-drilled or a newly-drilled puncture ( $F_{2,206}=16.94$ ;  $p < 0.001$ ; Fig. 5). In contrast, ovipositions into newly probed sites and heterospecifically probed sites did not differ significantly.



**Figure 3-5:** Duration of time spent ovipositing (averages  $\pm 1$  S.E.) as function of oviposition occurring in newly drilled punctures (white bars), in a puncture previously drilled by another female (grey bars), and in a puncture self-made or where the female had already laid eggs (black bars) in *Bactrocera dorsalis* and *Ceratitis cosyra*, respectively. Data of observations on mangoes that were previously punctured are shown.

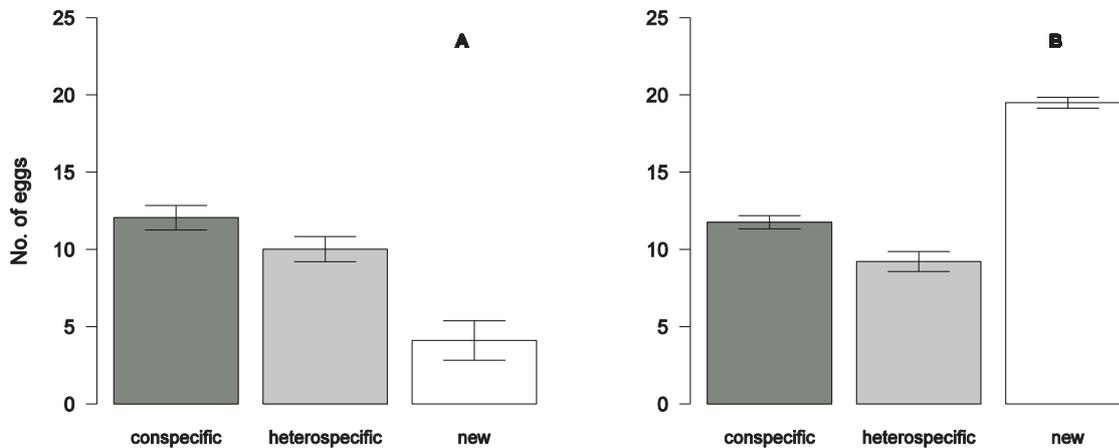
### 3.4.2 Choice experiments

For both fruit fly species, the likelihood to land at first on a mango harbouring previous oviposition punctures was higher than landing on domes without previous oviposition punctures ( $\chi^2=18.08$ ;  $df=1$ ;  $p<0.001$ ; Fig. 6).



**Figure 3-6:** Probability to land on a mango with (grey bars) or without (white bars) previously drilled punctures for *Bactrocera dorsalis* and *Ceratitis cosyra* females when both mangoes are available in the same cage.

While for *C. cosyra* the occurrence of ovipositions was not different between a mango with and without previously drilled punctures, the probability that an oviposition occurred within the 30-minute time frame of an observation was 38 percent higher for *B. dorsalis* when females were on a previously punctured mango than on one with no previously-drilled punctures (species:  $\chi^2=11.01$ ;  $df=1$ ;  $p=0.0009$ ; mangoes with/without punctures:  $\chi^2=7.68$ ;  $df=1$ ;  $p=0.005$ ). The number of eggs laid was only influenced by the usage of heterospecific, conspecific or newly drilled punctures ( $\chi^2=10.03$ ;  $df=1$ ;  $p=0.02$ ; Fig. 7), with newly drilled punctures receiving 39 percent more eggs than conspecifically-drilled and 52 percent more eggs than heterospecifically-drilled punctures, respectively. To elucidate these results, we considered each species separately. While we found no difference for *B. dorsalis* females in clutch sizes laid in conspecific, heterospecific or newly drilled punctures ( $\chi^2=3.16$ ;  $df=2$ ;  $p=0.21$ ; Fig.7A), *C. cosyra* females showed to lay twice as many eggs in newly drilled punctures compared to hetero- or conspecific punctures ( $\chi^2=9.35$ ;  $df=2$ ;  $p=0.009$ ; Fig.7B).



**Figure 3-7:** Number of eggs laid during an oviposition (average  $\pm$  1 S.E.) as function of oviposition occurring in conspecific (dark grey bars), heterospecific- established (grey bars) and self-drilled punctures (white bars) in *Bactrocera dorsalis* (A) and *Ceratitis cosyra* (B), when both mangoes with and without punctures are available in the same cage.

### 3.5 Discussion

In fruit-parasitic insects, reproductive success is linked to the strategy a female chose to oviposit successfully in a host. As the availability of fruits may be limited, females have to balance between the environmental limitation of finding suitable hosts and successful offspring production under competition. In this perspective, super- and multiparasitism are reproductive strategies that can be adopted in nature. Our experiments showed that both *B. dorsalis* and *C. cosyra* accepted to oviposit in punctures drilled by other females, thus to super- and multiparasitise mango hosts. Interestingly, landing probability was higher for mangoes carrying previous oviposition punctures than for uninfested ones, as seen in the choice experiments. This suggests that flies can tell apart previously drilled and undrilled mangoes from a distance. As the fruit skin is damaged by already drilled punctures, these fruits may emit a higher amount of volatiles from the fruit pulp. Different composition in chemical cues released by the hosts is one of the factor influencing host choice by foraging females (Städler 2002). Thus, the difference in chemical volatiles emitted by mangoes with and without punctures may drive females to choose mangoes with punctures rather than those with no-previously drilled punctures. Moreover, oviposition probability increased at increasing number of punctures present on the fruit, probably due to an increased chance to find a puncture while females were foraging on previously punctured fruits. Exploiting previous oviposition punctures enhanced the number of successful oviposition attempts a female could

achieve within the same time frame, doubling also the number of eggs laid. The increase in successful oviposition events also correlated with a reduction in the time spent probing on the fruit, when females oviposited in already drilled punctures. While host handling duration was reduced when females used punctures already established rather than drilling theirs, we found no difference either in the time a female spent laying eggs or in the clutch size laid in no choice experiments. Interestingly, in choice experiments, this pattern was found again for *B. dorsalis*, but not in *C. cosyra* females that reduced their clutch sizes when exploiting punctures previously drilled by other females. When females oviposit in a previously drilled puncture, they will add eggs to a clutch laid by another female. Thus, competition may occur among offspring. Optimal foraging theory predicts a reduction in the clutch size when females lay their eggs in pre-existing punctures, as a measure to reduce the competitive pressure among offspring (Papaj *et al.* 1989). The reduction observed for *C. cosyra*, but not for *B. dorsalis* in choice experiments might be due to a difference in the ability to identify con- and heterospecific drilled punctures: while members of the genus *Ceratitidis* release chemical cues (i.e. host marking pheromone) to signal that the host is already occupied with eggs, *B. dorsalis* does not show this behaviour (reviewed in Silva *et al.* 2012). Thus, *C. cosyra* females may be able to distinguish among heterospecific and conspecific punctures, adjusting their clutch size accordingly. The difference in the results obtained in no-choice and choice experimental set up might be due to the fact that in the latter, mangoes had a combination of both conspecific and heterospecific drilled punctures, while in no-choice experiments punctured mangoes had only punctures from one of the species. The combination of con- and heterospecific punctures might have induced a stronger response from *C. cosyra* females to reduce competitive interactions for the offspring.

*Bactrocera dorsalis* females showed a higher tendency for self-superparasitism compared to *C. cosyra* ones. However, females spent a shorter time ovipositing when re-using a puncture exploited before, thus potentially reducing the number of eggs laid, as oviposition duration is proportional to the clutch size (Papaj *et al.* 1989). Self-superparasitism is considered costlier than multi- and superparasitism as adding eggs to an already self-exploited puncture may increase the competitive pressure among siblings, with a potential cost for female's fitness (van Alphen and Visser 1990). As clutch size is not reduced when super- or multiparasitism occurs in *B. dorsalis* and *C. cosyra*, exploiting previously drilled punctures might really be advantageous to increase the number of offspring laid. Both *B. dorsalis* and *C. cosyra* are able to produce eggs continuously and do not re-absorb mature eggs, when no host is available (Migani *et al.* 2014). As host selectivity is often related to the

amount of mature eggs available in the ovaries (van Randen and Roitberg 1996), females of both species may enhance their fitness by exploiting already infested hosts, rather than missing an opportunity to oviposit, thus, potentially wasting their eggs. Using punctures drilled by other females may also reduce ovipositor wear (Jones and Kim 1994), enhancing a female's reproductive lifespan. However, *B. dorsalis* females showed a higher tendency to oviposit in punctures rather than in a new site compared to *C. cosyra* females. From our experiments, *B. dorsalis* was also the species with the longer host-handling duration, when females established their own puncture: in fact, *C. cosyra* females were 2 minutes faster when establishing a new puncture on the fruit skin compared to the invasive species. By ovipositing in punctures already established on the fruit, *B. dorsalis* females can save 30 percent more time to penetrate the mango epicarp in comparison to *C. cosyra* females. A reduction in host handling duration may reduce a female's exposure to danger, such as predators (Weisser *et al.* 1994; Völkl and Kroupa 1997; Barzman and Daane 2001). When ovipositing on a host, fruit flies are known to be highly vulnerable to predation risk (Papaj 1993). The African weaver ant *Oecophylla longinoda* (Latreille) is a natural inhabitant of fruit trees and a generalist predator of insects. When weaver ant workers are present on the mango fruit, the time that both *B. dorsalis* and *C. cosyra* females spend foraging on the fruit is reduced, negatively affecting the number of eggs that these fruit flies lay (Migani *et al.*, in press). As drilling into an already established puncture reduce the host-handling duration for both fruit fly species, adopting this strategy may enhance the likelihood of successful oviposition, when the weaver ants are present. Furthermore, as *B. dorsalis* is the most affected species by the presence of the weaver ants (Migani *et al.* submitted), the usage of already established punctures may weaken the effect of the weaver ants on the invasive species, at the advantage of the invasive species over *C. cosyra*. While *B. dorsalis* did not show any preference for super- vs. multiparasitism, *C. cosyra* females used conspecifically made punctures with higher frequency than heterospecific ones. Possibly, a difference in puncture accessibility rather than asymmetric interspecific competition among offspring can explain this result. In support to this hypothesis, there is the finding that *C. cosyra* females showed to be faster when probing on conspecific rather than on heterospecific-established punctures. We think that between the two species there might be a difference in ovipositor size (i.e. diameter), with *B. dorsalis* having a smaller ovipositor diameter in comparison to *C. cosyra*. This may also explain the longer time *C. cosyra* female spent probing in *B. dorsalis* punctures, as the smaller diameter of the puncture may limit *C. cosyra* females to easily access these punctures. In order to evaluate if *C. cosyra* preference for superparasitism may increase the intraspecific competition, with a cost for developing

offspring, we should consider the carrying capacity of a single mango fruit, and thus the costs to exploit a puncture or to lay eggs in an already occupied fruit. Acceptance of already infested fruit should depend on the survival probability of the offspring: if the fitness return from rejecting an already occupied site is lower than the chances of offspring survival, a female should super- or multiparasitise. There is evidence that fruit fly females may oviposit in a fruit already occupied by con- or heterospecific larvae, however, females then oviposit on the opposite side of the fruit to where the larvae are located (Fitt 1984). Mango fruits collected in the field supported the development of both *B. dorsalis* and *C. cosyra* adults within a single fruit. However, the number of adults emerging per fruit varied with a range between 1 to 3 adults for *C. cosyra* and 1 to 60 adults for *B. dorsalis*, respectively, depending on the location (i.e. altitude) from which the fruits were collected (adapted from data published by Ekesi *et al.* 2006). Moreover, from semi-field experiments, the average clutch size laid on mango domes exposed to 50 females was around 60 eggs for *B. dorsalis* and 38 for *C. cosyra*, with mangoes exposed to *C. cosyra* having more punctures than those exposed to *B. dorsalis*, as evidence that despite using the same fruit to oviposit, *C. cosyra* has the tendency to drill new punctures (Migani, unpublished data). As co-infestation is occurring in fruits collected from the field, we suppose that a single fruit can be sufficient to sustain successful development of several clutches, whose number should vary accordingly to the size of the fruit. In this view, drilling a self-made puncture or using a pre-existing one might not affect the competitive interactions among offspring, as they will still have the resources available from the same fruit. We have to consider, in fact, that *B. dorsalis* does not show any preference for con- or heterospecific punctures and, overall, showed a tendency to exploit previously-made oviposition punctures more than the native species. Furthermore, there is evidence of asymmetric competition between *B. dorsalis* and *C. cosyra* offspring, when occurring into the same host. In mangoes artificially co-infested with the same number of *B. dorsalis* and *C. cosyra* larvae, the amount of offspring adults emerging was higher for *B. dorsalis* than for *C. cosyra*, unless *C. cosyra* larvae were 2 days older than *B. dorsalis* ones (Rwomushana *et al.* 2009). Thus, considering competitive interactions and the higher tendency of *B. dorsalis* to exploit already previously drilled punctures, the invasive species may benefit from the usage of punctures more than *C. cosyra*. The higher tendency for super- and multiparasitism shown by the invasive species may be one of the factors contributing to the competitive displacement of the native *C. cosyra* from some areas. Our findings may also be important from an applied point of view. Biological control programs promote the usage of generalist predators and

parasitoids to control infestation levels in the field. However, through super- and multi-parasitism, *B. dorsalis* may be able to reduce the effect of predators, such as weaver ants, impairing the efficiency of the programs. In conclusion, our study shows that super- and multiparasitism can be adopted as successful oviposition strategy by both *B. dorsalis* and *C. cosyra* to enhance the chances of successful reproduction, but that asymmetries found in competition between the species are mirrored in the usage of previously existing oviposition punctures by the two species.

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## **4 At lunch with a killer: the effect of weaver ant on host-parasitoid interactions in mango**

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## 4.1 Abstract

Predator-prey interactions can affect the behaviour of the species involved, with consequences for population distribution and competitive interactions. Under predation pressure, potential prey may adopt evasive strategies. These responses can be costly and could impact population growth. As some prey species may be more affected than others, predation pressure could also alter the dynamics among species within communities. In field cages and small observation cages, we studied the interactions between a generalist predator, the African weaver ant, *Oecophylla longinoda*, two species of fruit flies that are primary pests of mango fruits, *Ceratitis cosyra* and *Bactrocera dorsalis*, and their two exotic parasitoids, *Fopius arisanus* and *Diachasmimorpha longicaudata*. In all experiments, either a single individual (observation cage experiments) or groups of individuals (field cage experiments) of a single species were exposed to foraging in the presence or absence of weaver ants. Weaver ant presence reduced the number of eggs laid by 75 and 50 percent in *B. dorsalis* and *C. cosyra* respectively. Similarly, parasitoid reproductive success was negatively affected by ant presence, with success of parasitism reduced by around 50 percent for both *F. arisanus* and *D. longicaudata*. The negative effect of weaver ants on both flies and parasitoids was mainly due to indirect predation effects. Encounters with weaver ant workers increased the leaving tendency in flies and parasitoids, thus reduced the time spent foraging on mango fruits. Parasitoids were impacted more strongly than fruit flies. We discuss how weaver ant predation pressure may affect the population dynamics of the fruit flies, and, in turn, how the alteration of host dynamics could impact parasitoid foraging behaviour and success.

## **5 General discussion and Conclusion**

## 5.1 Implication of oviposition strategies in fruit fly competitive interactions

Competitive and trophic interactions can determine density-dependent processes within populations, in turn influencing abundance and distribution of the species within an ecological community. As populations of a given species are made up by different individuals, the understanding of individual behaviour can cast light on the mechanisms influencing population, and ultimately species, dynamics. Based on both their physiological and environmental conditions, individuals need to make decisions on the foraging strategies to adopt in order to gain the resources needed to survive and successfully reproduce. This decision making process is important to determine their foraging success, thus, their contribution to future generations. Individual foraging strategies can be influenced by both physiological and environmental factors, including resource characteristics, distribution, and availability, which may determine the ability of a species to exploit the resources. In tephritid fruit flies, the oviposition strategy is a very important part of the foraging behaviour, as most of the species allocate their eggs into a fruit host, in which the offspring is forced to complete the development. The number of eggs available in the ovaries, and the characteristics of the fruits such as species, ripeness stage and fruit variety may influence a female's choice where to allocate her eggs. Species that share the same fruit hosts may be differently affected by such factors, thus, showing different resource exploitation strategies. My experiments on *Bactrocera dorsalis* and *Ceratitis cosyra* oviposition behaviour in Africa have shown that, despite egg load being the primary driver for fruit acceptance in both species, females of the two fruit flies differ in their oviposition strategies. The ability to mature larger quantities of eggs, the lower selectivity for fruit ripeness stages, and the higher tendency to exploit oviposition punctures established on the fruit by other females may confer the invasive *B. dorsalis* a competitive advantage in comparison to the native *C. cosyra*. My findings are in line with evidence from the field showing that the invasive species is competitively displacing the native *C. cosyra* in several areas in Kenya, with *B. dorsalis* being more successful to reproduce in lowlands, restricting *C. cosyra* at highlands (Ekesi *et al.* 2009). *Ceratitis cosyra* females showed a tendency to preferentially exploit oviposition punctures drilled by conspecific females compared with those drilled by *B. dorsalis* females. Despite the fact that there is still a lack of knowledge on how many larvae can successfully develop within a single mango fruit, there is evidence that *B. dorsalis* larvae develop faster than *C. cosyra*: from fruits co-infested with equal number of *B. dorsalis* and *C. cosyra* larvae, the invasive species had higher adult emergence/survival compared with the native species, confirming the higher competitive ability of *B. dorsalis* (Ekesi *et al.* 2009). Several studies showed that *B. dorsalis* is a successful invader: once introduced

in Hawaii, *B. dorsalis* competitively displaced *C. capitata* from coastal zones, restricting it to cooler regions (reviewed in Duyck *et al.* 2004). As introduction of a new species in an area can alter the competitive interactions among the new and the pre-existing species, invasion is an interesting opportunity to identify the factors that contribute to species competition. Interspecific competition can cause extinction of the lower competitor, or a stable coexistence, if the lower competitor reduces its resource usage, exploiting only resources that the dominant competitor is not able to use, e.g. occupying areas in which the better competitor cannot successfully live (Duyck *et al.* 2004). Documented cases of invasion by *Bactrocera* spp. in areas where *Ceratitis* spp. had been previously dominant competitors showed that the new invader reduced the niche of the previous occupants, but did not completely drive them to extinction (reviewed in Duyck *et al.* 2006). Even though there is evidence that invaders can be competitively superior to a resident species, all the factors contributing to the successful establishment of invading species in an area are often not clear yet. The absence of natural enemies (i.e. pathogens or predators) in the introduced areas is one of the factors known to facilitate successful invasions (Simberloff and Wilson 1970; Sakai *et al.* 2001). However, other factors, such as individual foraging strategies, may play an important role as well. Thus, understanding individual foraging behavioural will contribute to predicting the processes triggering competitive interactions between invaders and resident species, and in turn improving the possibility to make predictions on how an invader can be successful in establishing in such communities. The consequences of competitive interactions between native and invading species can directly affect the structure of the local community. In Kenyan coastal areas, where the population of *C. cosyra* have been significantly reduced, the native parasitoids species, which used *C. cosyra* as suitable host, and which were found unable to successfully parasitize *B. dorsalis* (Mohamed *et al.* 2006), have also experienced a significant reduction in population density (Ekesi, pers. communication). The partial displacement of the native fruit fly *C. cosyra* by the invasive *B. dorsalis* therefore is likely driven by both, a release of the invasive species from natural enemies as reported earlier by Simberloff and Wilson (1970) and the competitive advantage *B. dorsalis* gains from the oviposition strategies reported in this thesis, namely that the invasive species benefits from higher egg loads and strong usage of interspecific oviposition punctures whereas the native *C. cosyra* suffers from intraspecific competition brought about by their preference to use conspecific oviposition punctures.

Besides direct competition and differential mortality due to parasitoids, predation by generalist predators may also have an influence on competing species, as apparent competition may occur when a shared predator

mediates effects resembling competition. I showed here that the presence of weaver ants strongly reduces the number of eggs laid by both *C. cosyra* and *B. dorsalis* females. However, *C. cosyra* being the weaker competitor in resource exploitation, weaver ants may further affect the reproductive success of the native species. If we consider that the weaver ants are present on the coastal region in Kenya, where *C. cosyra* populations faced significant reduction and local extinctions, apparent competition may contribute or explain such effect.

## 5.2 Effects of predation pressure on fruit-fly parasitoid population dynamics

Predation can negatively affect prey population densities, both through lethal predation effects (i.e. when the predator kills the prey), or through indirect effects (i.e. when a predator is simply present in the area). In order to reduce and avoid predation, prey may change behavioural, life-history and morphological traits (Cortez 2011), which in turn can negatively affect prey population densities (Werner and Peacor 2003). Moreover, the consequences of predation pressure can generate indirect cascading effects on the species, which interact with the target prey, such as other predators and competitors (Ripple and Beschta 2004).

As generalist predators inhabiting mango trees, weaver ants were already known to reduce mango infestations by fruit flies (van Mele *et al.* 2007). However, the mechanisms triggering such an effect as yet were not fully understood. Moreover, when considering the use of both, predators and introduced parasitoids for the biological control of the fruit fly, it becomes crucial to understand the effect such generalist predator could have on the exotic parasitoids released in the field. The results obtained in this thesis clearly show that the weaver ants negatively impact the oviposition behaviour of both fruit fly and parasitoid species by threatening the foraging females.

The mere presence of the weaver ants reduced the time fruit fly and parasitoid females spent searching on the fruit, causing a consequent reduction in the number of eggs laid in mangoes by fruit flies, and in the rate of parasitism achieved by the parasitoids. Foraging individuals are usually exposed to increased predation risk, when searching for food (Anholt and Werner 1995). If the predation pressure increases, potential prey may modify their behaviour by seeking refuge (Preisser *et al.* 2005), increasing vigilance (Eccard *et al.* 2015), and/or reducing foraging activity in order to decrease the risk of being caught (Brown *et al.* 1992; Cresswell 2008; Eccard and Liesenjohann 2014). Even if not directly killing adult foraging fruit flies, the weaver ants reduced by half the time *B. dorsalis* and *C. cosyra* spent searching on the mango, interfering with their oviposition behaviour. Such changes, e.g. behavioural, induced by predation pressure can be as detrimental

as lethal effects on the prey population, negatively affecting prey density (Nelson *et al.* 2004). The effects of disturbance by a cohort of different generalist predators investigated in a pea aphid biocontrol system showed that predator disturbance reduced the pest population density at the same magnitude as did direct consumption (Nelson *et al.* 2004). As the interference by the weaver ants on the foraging fruit fly activity resulted in an egg laying reduction of 75 percent for *B. dorsalis* and 50 percent for *C. cosyra*, it is conceivable that the weaver ants can cause a reduction on the fruit fly population density. Thus, the weaver ants can be considered as a suitable biological control agent to reduce fruit fly infestation levels. Due to their lack of specificity which makes them less able to respond functionally to population density fluctuations of the target pest, as well as making them consuming alternative prey, generalist predators have seldom been considered as suitable biocontrol agents (Sabelis 1992). This opinion has been widely debated, as in some cases, generalist predators are suitable biological control agents, as, e.g., they can be available at low pest densities, when specialist predators, which respond functionally to the target prey may be at low population densities (Symondson *et al.* 2002). Although generalist predators can be suitable natural enemies to reduce pest populations, in multiple natural enemy assemblages, their catholic feeding habits may disrupt biological control, e.g., by parasitoids, as generalists can feed also on parasitoids (Snyder and Ives 2001). The weaver ants did negatively affect foraging parasitoids, reducing the parasitism rate by half in *F. arisanus*, and by 30 percent in *D. longicaudata*, potentially hampering their efficiency as fruit fly biocontrol agents. Intraguild predation is considered a widespread phenomenon in managed ecosystems where generalist predators and parasitoids are present (Vance-Chalcraft *et al.* 2007). Often, the predation effect is through direct mortality, but as shown above, indirect effects of intraguild predators can have the same consequences at the population level. In general, the consequences of having an intraguild predator are variable, depending on the ecological characteristics of both the intraguild predator and prey as well as on external factors, such as environmental conditions (Straub *et al.* 2008). There is an-going debate on how many species, i.e. natural enemies, should be included within an agricultural community in order to achieve successful pest reduction (Pell *et al.* 2008; Messelink *et al.* 2012). As natural enemies can regulate lower and/or the same trophic level within an agricultural community, their composition influences the structure of the whole community (Vandermeer *et al.* 2010). In their meta-analysis, Rosenheim and Harmon (2006) reported that, overall, intraguild predation does not disrupt pest suppression. However, most of the studies on intraguild predation in biological control systems focus only on the overall effect of a generalist-parasitoid biocontrol system on pest population reduction, often

neglecting to look specifically at the effects each species involved in the assemblage could have on the population of another natural enemy within the same assemblage (Pell *et al.* 2008). Despite the fact that having both a parasitoid and a ladybird beetle to control an aphid population had an additive effect in pest suppression, the parasitoid reproductive success was reduced due to intraguild predation by the predator preying upon the aphid mummies, thus, killing developing parasitoids (Colfer *et al.* 2001). Interference by native generalist predators with parasitoids can be even more detrimental in case of classical biological control, when releases of exotic natural enemies are conducted in order to establish natural populations in the field. If the presence of generalist predators negatively affects the reproductive success of the released parasitoids, it might hamper the successful establishment of natural parasitoid populations. Both *F. arisanus* and *D. longicaudata* are exotic species, and massive parasitoid releases are conducted in areas of interest in order to establish natural populations in the field. Even if having both parasitoids and weaver ants may still result in achieved pest suppression, the presence of weaver ants in areas of parasitoid releases can threaten the establishment of natural populations in the field. Though the weaver ants do not directly kill foraging parasitoids, by reducing the rate of parasitism, weaver ants can negatively interfere with the parasitoid reproductive success. Taylor and colleagues (1998) showed how the presence of cues left by *Coccinella septempunctata* Linnaeus decreased by half the time the parasitoid *Aphidius ervi* spent searching for hosts, reducing the number of offspring produced. Considering the results shown within this study, it must be asked whether *F. arisanus* and *D. longicaudata* should be released at all in areas where the weaver ants are present.

As non-lethal effects of predation may also involve the prey to change behavioural strategies in order to avoid being killed (Blake and Gabor 2014; Liesenjohann *et al.* 2015), we have also to consider fruit fly alternative foraging strategies shown within this study. By exploiting oviposition punctures already established on the fruit, fruit fly females reduce fruit handling time, thus, decreasing the overall oviposition duration. A reduction in handling time duration can be advantageous to enhance foraging success under predation pressure (Völkl and Kroupa 1997). Thus, the presence of the weaver ants may favour the foraging fruit flies to adopt such opportunistic behaviour. However, the addition of eggs to already established oviposition punctures may reduce successful parasitism by the egg-prepupal parasitoid *F. arisanus* due to a refuge effect (Merkel *et al.*, submitted). Thus, the parasitoid population may be further negatively affected by the presence of weaver ants, due to both a reduction of foraging activity by searching females, and an increased probability of refuge effects, if the presence of ants increases the exploitation of previously drilled oviposition punctures. It would be

interesting to further investigate which foraging behaviour is adopted under field conditions by the fruit fly species when the weaver ants are present.

This study shows how investigation on individual foraging behaviour gives crucial information on the mechanisms and on the factors, which can influence the adoption of a certain strategy. Such knowledge is important to make predictions on the dynamics of populations, thus to understand the forces that structure ecological communities. From an applied point of view, biological control planning and management of agroecosystems significantly benefit from knowing which factors influence individual behaviour, and which impact the adoption of behavioural strategy can have on different species involved in pest management (Roitberg 2007; Merkel *et al.* 2016).

### 5.3 Conclusion

This study investigated individual behavioural strategies in order to elucidate what drives competitive and foraging interactions in a predator-parasitoid-pest system involving two tropical fruit fly species, two parasitoid species attacking eggs and larvae of the fruit flies, respectively, and a weaver ant species interfering with both foraging flies and parasitoids. Understanding how individuals respond to physiological patterns and environmental stimuli to successfully forage is important to scale up from behavioural patterns to interactions that ultimately determine the structure of populations and ecological communities. This study shows that a female egg load is a primary driver for oviposition in *B. dorsalis* and *C. cosyra* fruit flies. This finding is in line with literature, as the number of mature eggs available in a female's ovaries is considered one of the main factors influencing oviposition decisions in foraging females (Minkenberg *et al.* 1992). It shows also the importance of fruit characteristics, specifically fruit ripeness and fruit variety, on determining oviposition behaviour of the two fruit fly species. In particular, it was found that while *Ceratitis cosyra* females are more prone to oviposit in fully ripe and ripe fruits compared to unripe ones, *B. dorsalis* allocates different clutch sizes depending on mango variety. Ripeness stage and variety can be connected to host quality (i.e. nutritional value), an important characteristic for developing fruit fly larvae, as well as to fruit skin penetrability, important for fruit accessibility via ovipositor of the foraging female (Jones and Kim 1994). The ability to exploit a broader range of fruits at different ripeness stages shown by *B. dorsalis* may reduce the interspecific competition among developing larvae within fruits conferring a competitive advantage to the invasive species (Diaz-Fleischer and Aluja 2003). It is known that host selectivity is inversely proportional to egg availability (Papaj

2000): *B. dorsalis* is able to mature a larger quantity of eggs in comparison to *C. cosyra*, and this can also explain the lower selectivity for fruit hosts shown by the invading species. In this study it was also explored fruit fly oviposition exploitation of oviposition punctures already drilled on the fruit, showing here that both fruit fly species are able to exploit for oviposition. The usage of punctures reduced the time spent handling the fruit to penetrate the host, which can be an advantage to reduce the overall time spent foraging, thus, for example, reducing the risk of being predated (Völkl 1994). However, the two fruit fly species show a different tendency towards ovipositing in punctures, *B. dorsalis* females having a higher oviposition success when exploiting already-made punctures on the fruit in comparison to *C. cosyra*. In the invasive species, the usage of punctures increased the number of successful oviposition events, thus, of eggs laid in the fruit in 30 minute-observation bouts. Moreover, while *B. dorsalis* do not respond differently to punctures established by conspecific or heterospecific females, *C. cosyra* prefers to oviposit in conspecific-made punctures. The superior competitive abilities shown by an invading species is usually due to different factors, including physiological, life-history traits, behavioural, environmental factors (e.g. predation), which often contribute to the displacement of previously established species from the invaded area (Sakai *et al.* 2001).

The asymmetric usage of oviposition punctures by the two fruit fly species, the differences in physiological traits, and in oviposition behaviour found within this study can also help further explaining the competitive dynamics establishing between the native *C. cosyra* and the invasive *B. dorsalis*, helping further identifying crucial aspects of the processes involved in the displacement at lowland areas observed for these two species. As an ecological community usually includes an array of species connected by trophic interactions, this study also investigated the influence of predation pressure on individual foraging behaviour. The presence of a generalist predator such as weaver ants negatively interfere with both fruit fly and parasitoid foraging ability. The weaver ants are reducing both the number of eggs laid by the fruit flies as well as the parasitism rate of both *F. arisanus* and *D. longicaudata*. One important information here is that the observed reduction is mainly due to indirect predation effects rather than to direct consumption, as the weaver ants interfere with fruit fly and parasitoid foraging behaviour, reducing the time that females spent on the fruit. Indirect predation effects can have the same or higher impact at prey population level as direct consumption of the prey (). The reduction of 50 percent or more for number of eggs laid by the fruit flies, and rate of parasitism by the foraging parasitoids can significantly reduce the reproductive success of these species. As the study system included species of agricultural economic importance, we should consider these results also in the light of pest control

management. In this perspective, the weaver ants should be used as biological control agents for the fruit fly, in areas where the ants are present, as this predator alone can reduce fruit infestation levels due to the fruit fly pests. van Mele and colleagues showed how mango orchards would benefit from the presence of weaver ant colonies on trees to reduce fruit infestation. However, the negative effect of the weaver ants on *F. arisanus* and *D. longicaudata* should prevent from releasing the parasitoids in areas where these ants are present. Thus, *F. arisanus* and *D. longicaudata* releases should target weaver-ant-free areas, where the parasitoids should have higher probability to successfully reproduce, and establish in the field. The use of multiple natural agents to improve pest control has given different, contrasting results, going from negative, neutral, and positive output. However, most of the studies poorly address the effect on the single species involved within the assemblage, mainly focusing on the effects on the pest species. The research conducted here shows how behavioural studies and investigation at individual level can help clarifying the processes involved in the observed effects, improving understanding the processes that ultimately affects population dynamics within ecological communities.

## 5.4 References

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# Erklärung



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