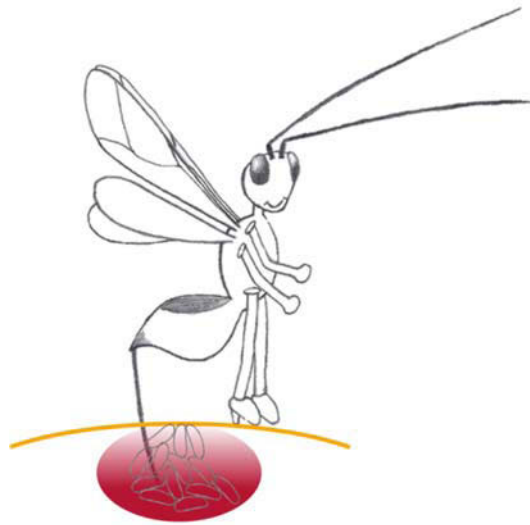


**Patch exploitation behaviour of the tephritid
parasitoid *Fopius arisanus*,**
a candidate for the biological control of mango flies



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Katharina Merkel

Front cover: A female of *Fopius arisanus* (from the laboratory colony at *icipe*, Kenya) that parasitizes eggs of *Bactrocera invadens* in a mango.

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a candidate for the biological control of mango flies

Dissertation
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Summary

The rationale behind successful pest control by natural enemies is based on theory developed for population dynamics. Thus, it is also not surprising that many empirical data on the interactions in host-parasitoid systems stem from pest species and their biological control agents. An important feature of host-parasitoid dynamics is the density dependence in a parasitoid's attack rate. To describe the dynamics one can study how the attack rate depends on the host's (functional response) and on the parasitoid's density. Although often considered separately the two processes are strongly interconnected.

The underlying causes of both, the host density and parasitoid density dependence of the parasitoid response, provide additional information that may help to understand what drives host-parasitoid dynamics. Among them, the proximate mechanisms that lead to foraging decisions in parasitoid females can be studied by behavioural experiments. Thus, studies on the functional response and mutual interference complemented with detailed behavioural analysis may provide new insight into population dynamics.

In this study I analysed the functional response of the parasitoid, *Fopius arisanus* that was introduced into Kenya, as a biological control candidate for the tephritid pest *Bactrocera invadens*. I further estimated the effect of mutual interference among parasitoids exploiting one patch at the same time, by measuring the reduction of the search rate with increased parasitoid density. The presented study thus aimed at linking individual traits of the parasitoid *Fopius arisanus* with population phenomena.

After providing a brief introduction into biological control and the biological system I worked with in chapter one and two, chapter three addresses the functional response of *F. arisanus* and the potential effect of a spatial host refuge on this response. The shape of the functional response displayed by *F. arisanus* followed a type II response, characterized by an increasing number of hosts parasitized at a decelerating rate with increasing host density. Interestingly, the influence of the handling time was negligible. Adding parameters to the model accounting for a host-density dependent spatial host refuge seemed to better explain the data. The results highlight that the outcome of biological control efforts with *F. arisanus* may depend on the targeted crop-system. Mangos are comparatively large and therefore attract large clutches, and hence generate larger host refuges, than for example coffee berries or guava.

Chapter four covers the effect of mutual interference among females simultaneously searching host patches on the search rate. Following expectations the search rate decreased with increasing parasitoid density, demonstrating parasitoid interference. The results further suggest that the level of interference depends on the host distribution and that it will be highest in habitats with clumped host distributions. This indicates that mutual interference may be severe in control efforts with mass releases that create locally high parasitoid densities in mango systems that allow for large host refuges.

The results in both, chapter three and four, show how *F. arisanus* uses information on host encounters in order to adapt the time spent searching on a patch. The parasitoids showed a decreased tendency to leave a patch after experiencing successful host encounters. This is in line with predictions from foraging theory that increases in the searching time upon successful host encounters lead to fitness maximization in habitats with aggregated hosts. Surprisingly, *F. arisanus* seemed to also employ an incremental mechanism when rejecting hosts. Such a response has rarely been described before. The observations of females facing competition also showed that their behavioural response mechanism towards encounters with conspecifics depended on the earlier investment on the patch. Females that started to exploit the host patch before their competitors were likely to have the highest reproductive return from such patches.

Despite the inverse density dependence in the attack rate, *Fopius arisanus* still parasitized more than 30 % at the highest host density tested. Together with the parasitism reported from field surveys in Hawaii and French Polynesia this indicates that *F. arisanus* poses a high potential as biological control candidate against *B. invadens*. The results reveal that the occurrence of spatial host refuges and mutual interference in the field should be considered in the development of biological control strategies. In addition, the observations suggest that mutual interference may affect the dispersal behaviour of *F. arisanus*, which requires further studies to attain an understanding of the mechanism.

I conclude that *F. arisanus* represents an exceptional candidate for biological control of tephritid flies. However, further studies can still provide knowledge to significantly improve establishment efforts and control strategies.

Zusammenfassung

Ansätze zur biologischen Schädlingsbekämpfung mit natürlichen Gegenspielern nutzen theoretische Modelle zur Beschreibung von Populationsdynamiken, um Vorhersagen über den Effekt des Einsatzes natürlicher Feinde zu machen. Ein wichtiges Merkmal für die Beschreibung von Wirt-Parasitoid Interaktionen ist die Abhängigkeit der Angriffsrate von der Wirtsdichte (funktionelle Reaktion) sowie der Dichte der Parasitoiden. Häufig werden Untersuchungen zur Abhängigkeit der Angriffsrate getrennt von der Wirts- und Parasitoidendichte betrachtet, wobei zu beachten ist, dass beide Prozesse eng miteinander verknüpft sind. Um Strategien zur biologischen Schädlingsbekämpfung zu entwickeln, ist es nicht ausreichend, dass man die Dichteabhängigkeit der Wirt-Parasitoid sowie der Parasitoid-Parasitoid-Interaktionen beschreibt und die damit verbundenen Parameter bestimmt. Vielmehr bedarf es einer gründlichen Analyse jener Prozesse und Mechanismen, die der Abhängigkeit zugrunde liegend. Um ihre Fitness zu maximieren, müssen Parasitoide bei der Wirtssuche entscheiden, wie lange sie einen Wirtspatch absuchen und wann sie den Patch verlassen, um einen Alternativpatch aufzusuchen. Die Bestimmung der proximatoren Mechanismen, die zu diesen Entscheidungen führen, kann Hinweise darüber geben, wie sich das Verhalten eines Individuums auf die Population auswirken kann. Daher bieten Beobachtungen zum Wirtssuchverhalten von Parasitoiden eine sinnvolle Ergänzung zu Untersuchungen zur Dichteabhängigkeit der Angriffsrate.

In dieser Studie sollte die funktionelle Reaktion und die innerartliche Konkurrenz des zur Kontrolle der Bohrfliege, *Bactrocera invadens*, eingeführten Parasitoiden, *Fopius arisanus*, untersucht werden. Ein weiteres Ziel dieser Studie war es, die Vernetzung der individuellen Eigenschaften des Parasitoiden, *F. arisanus*, mit den dichteabhängigen Prozessen der Parasitoid-Wirt Interaktion, aufzuzeigen.

Nach einer Einführung in die Fragestellung sowie in den Stand der Forschung im Bereich der biologischen Schädlingsbekämpfung (Kapitel 1) und einer Beschreibung des biologischen Systems mit dem sich diese Arbeit befasst (Kapitel 2), folgen in Kapitel drei die Ergebnisse meiner Studie zur funktionellen Reaktion von *F. arisanus*, die auf den möglichen Effekt eines räumlichen Wirtsrefugiums hinweisen. Die Anzahl der parasitierten Wirtseier nahm mit zunehmender Wirtsdichte zu, wobei die Rate der Zunahme mit zunehmender Wirtsdichte abnahm. Diese negativ dichteabhängige Angriffsrate für *F. arisanus* beschreibt eine Typ II Reaktion. Interessanterweise schien die

Handhabungszeit, also jene Zeit, die ein Parasitoid benötigt, um einen Wirt zu parasitieren und bereit für den nächsten Wirtsangriff zu sein, für die Beschreibung der Reaktion vernachlässigbar. Ein Modell, das statt der Handhabungszeit ein von der Wirtsdichte abhängiges, räumliches Wirtsrefugium berücksichtigt, ergab eine bessere Beschreibung der beobachteten Daten. Daraus lässt sich schließen, dass der Erfolg von *F. arisanus* maßgeblich von dem Zielsystem abhängig sein könnte. Im Gegensatz zu Mangos, könnte eine geringere Futtermenge in kleineren Früchten, wie Guaven und Kaffeebeeren, die Entstehung räumlicher Wirtsrefugien vermindern und damit den Erfolg von *F. arisanus* begünstigen.

Der Einfluss innerartlicher Konkurrenz auf die Effizienz von *F. arisanus* wird anhand der im vierten Kapitel dargestellten Ergebnisse diskutiert. Wie erwartet, führte eine höhere Parasitoidendichte zu einer Reduzierung der Suchrate von *F. arisanus*. Die Betrachtung von zwei verschiedenen Verteilungen der Wirte innerhalb des Patches ergab signifikante Unterschiede in der Stärke der innerartlichen Konkurrenz. Während Parasitoide, die in Patches mit einer uniformen Wirtsverteilung (gleichmäßige Verteilung von 80 Wirtseiern auf vier Teilpatches) nach Wirten suchten, nur gering beeinträchtigt waren, zeigten Parasitoide, die in Patches mit einer aggregierten Wirtsverteilung (Aggregation von 80 Wirtseiern auf einem von vier Teilpatches) suchten, eine fast zehnfache Verminderung ihrer Suchrate. Mangofrüchte bieten eine reichhaltige Futterquelle für die Larven von Bohrfiegen, wodurch sie eine Anhäufung von Eigelegen begünstigen. Diese Anhäufung von Wirtseiern kann zu starker innerartlicher Konkurrenz führen und damit die Effizienz von *F. arisanus* stark vermindern.

Wie oben erwähnt wurden die Versuche zur Untersuchung der funktionellen Reaktion und der innerartlichen Konkurrenz durch Verhaltensbeobachtungen ergänzt, um die Mechanismen zu bestimmen, die maßgeblich zum Entscheidungsverhalten bei *F. arisanus* beitragen. Das Parasitieren eines Wirtes führte zu einer verringerten Tendenz der Weibchen den Wirtspatch zu verlassen, dadurch resultierten Wirtsparasitierungen in einer verlängerten Suchzeit auf dem Patch. Dies bestätigt theoretische Vorhersagen zum optimalen Wirtssuchverhalten, welche besagen, dass Parasitoide, deren Wirte aggregiert vorkommen, mit einer Verlängerung ihrer Suchzeit auf Wirtsparasitierungen reagieren sollten, um eine Maximierung ihrer Fitness zu erlangen. Überraschend ist es, dass

Weibchen von *F. arisanus* ihre Suchzeit auch dann verlängerten, wenn sie einen Wirt abgelehnt haben. Eine solche Antwort auf Wirtsablehnungen ist bisher nur selten beschrieben. Aus den Ergebnissen in Kapitel vier geht weiter hervor, dass *F. arisanus* Mechanismen besitzt, welche zu einer Maximierung der Fitness unter Konkurrenz führten. Weibchen die, im Vergleich zu ihren Artgenossinnen, zeitiger mit der Parasitierung der Wirte eines Patches/ Teilpatches begonnen hatten, hatten mit hoher Wahrscheinlichkeit auch einen höheren Fortpflanzungserfolg für diesen Patch/ Teilpatch.

Obwohl die funktionelle Reaktion negativ dichteabhängig war, parasitierte *Fopius arisanus* bei der höchsten getesteten Wirtsdichte immerhin noch mehr als 30 % der Wirte. Dieses Ergebnis und die im Feld beobachteten Parasitierungsraten von *F. arisanus* auf Hawaii, Französisch-Polynesien, und den Fiji Inseln weisen auf das hohe Potential von *F. arisanus* für die Bekämpfung von Bohrfliegen hin. Diese Arbeit zeigt, dass das Vorkommen von räumlichen Wirtsrefugien und innerartlicher Konkurrenz die Effizienz von *F. arisanus* stark beeinflussen kann, weswegen diese Effekte bei der Entwicklung von Protokollen für den Einsatz von *F. arisanus* berücksichtigt werden sollten. Des Weiteren reagierte der Teil der Weibchen, der auf eine Konkurrentin traf ohne vorher Eiablageerfolg auf dem Patch gehabt zu haben, mit einer erhöhten Wahrscheinlichkeit, den Patch zu verlassen. Dieses Verhalten könnte im Freiland die Verbreitung von *F. arisanus* beeinflussen, welche einen wichtigen Prozess in der Etablierung eines Parasitoiden und seiner Effizienz am Zielort darstellt. Aus den dargestellten Ergebnissen folgere ich, dass *F. arisanus* ein exzellenter Kandidat für die biologische Kontrolle von Bohrfliegen darstellt. Allerdings, können weitere Untersuchungen, u.a. an den aufgezeigten Prozessen, zu einer erheblichen Verbesserung der Strategien für den Einsatz führen.

1

General Introduction

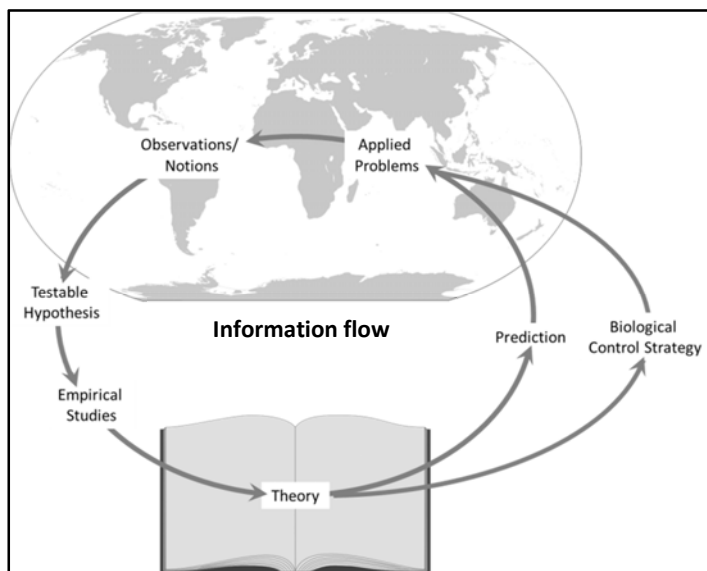


Figure 1-1: Information flow between ecological theory and applied problems.

In the field of ecology theory and practice are closely connected. Figure 1-1 demonstrates that link on the example of biological control. Species that impact human economy have been given special focus and studies on biological traits of agricultural pests and beneficial organisms are widely published. Many studies aim

to understand the population dynamics of economically important species, including the influence of varying biotic and abiotic conditions that impact population growth and spread. Therefore, it is not surprising that linkages exist between biological control and the theory of several ecological fields among, which population dynamics, invasion biology and coevolution are popular examples.

Many ideas contributing to ecological theory are based on observations of applied problems (Kareiva 1996). The myxoma virus introduced in Australia to control the European rabbit, *Oryctolagus cuniculus*, resulted in a unique opportunity to study the co-evolution of host and virus (Fenner 1983). Shortly after introduction of the myxoma virus into rabbits in Australia rabbits suffered a mortality rate close to 100%. This led to a strong selective pressure towards reduced virulence in the myxoma virus (Massad 1987). The data obtained on the virulence reduction of the myxoma virus, in European rabbits over time, considerably contributed to the theory of “evolution of reduced pathogenicity” in parasite-host systems (Anderson & May 1981; Massad 1987). The harlequin ladybird, *Harmonia axyridis*, has been repeatedly introduced to control aphid populations in North America (Gordon 1985; Koch 2003), Europe (Brown *et al.* 2008), South America (Gordon 1985; Koch 2003; Brown *et al.* 2008) and Africa (El-Arnaouty *et al.* 2000). Following a long period of unsuccessful introductions the species suddenly established and received the status of an invasive alien species (IAS) in North America (Koch & Galvan 2008), Europe

(Brown *et al.* 2008) and Africa (Stals & Prinsloo 2007; Stals 2010). The historical data on introductions of *H. axyridis*, and reports on its establishment and invasion, provided one of the first detectable examples for an invasive bridgehead effect (Estoup & Guillemaud 2010; Lombaert *et al.* 2010).

In turn applied approaches build upon, and greatly benefit from, theoretical models and investigations. Fagan *et al.* (2002) reviewed several examples of invasion theories, i.e. rates of spatial spread, and their potential to provide viable information for the practice of biological control. Hastings (2000) used records of parasitoid spread to verify the validity of applying simplified models to predict the rate of spread. The impact of *Pseudacteon tricuspis*, as potential biological control agent of the red imported fire ant, *Solenopsis invicta*, in the southeastern parts of the USA was estimated using simple diffusion models to predict the rates of spread in the release areas (Henne, Johnson & Cronin 2007). The example of the codling moth, *Cydia pomonella*, in western US demonstrates how stage-structured models can assist in identifying potential biological control agents (Mills 2005). Aiming at a more holistic approach, whereby only the most promising natural enemies are introduced for biological control, Mills (2005) used a stage-structured model to detect, which life stages of the codling moth were most vulnerable to parasitism and through that identified three candidate species for the control of codling moth in the western US.

A substantial part of classical biological control systems is based on host-parasitoid interactions and thus many parasitoids are studied in their capacity as natural enemies. To estimate the efficiency of potential biological control agents, host-parasitoid models have been used extensively (Hassell 1978; Murdoch, Chesson & Chesson 1985; Mills & Getz 1996; Murdoch & Briggs 1996; Mills & Kean 2010). Therefore, host-parasitoid models display a unique example of the flow of information between theory and practice in both directions. The application of population models, in order to predict the impact of biological control agents, revealed weaknesses, which led to an evolution of some “basic” models into some more complex ones (Hassell 1978; Mills & Getz 1996; Hassell 2000a). The Nicholson-Bailey model (Nicholson & Bailey 1935) presents a basic model that has been commonly used to describe the dynamic behaviour of insect populations. The model may describe population dynamics rather well, but it does not allow further inferences

about underlying behavioural mechanisms (Kidd & Jervis 2005). However, the model does provide a sound basis to integrate complex mechanisms (May *et al.* 1981). Rogers (1972) first introduced the random parasite equation by incorporating Holling's disc equation (Holling 1959b; Holling & Buckingham 1976) into the Nicholson-Bailey model. The modified model describes a parasitoid's response to increased host density that rises at a decelerating rate due to the proportion of time spend in handling hosts (Rogers 1972). Furthermore, the Nicholson-Bailey model has been extended to incorporate predator dependent processes in the host-parasitoid interaction, e.g. mutual interference among searching parasitoids (Hassell & Varley 1969; Beddington 1975; Xu & Boyce 2005), and aggregated responses of parasitoids to patches with higher host densities (Hassell & May 1973; May 1978). The aforementioned models make different assumptions about the processes that shape parasitoid responses towards host density. Hence, the inference about the underlying mechanisms, that impact the effect of a parasitoid on a host population, will be influenced by the model chosen to investigate the relationship. Therefore, the selection of a model to describe the attack rate of a parasitoid should be based not only on the statistical approximations of model fit but on the biological characteristics of the investigated system as well.

Despite the ongoing debate, and the vast amount of papers on how to evaluate potential biological control agents (Murdoch & Briggs 1996; Bigler *et al.* 2005), the problem remains pressing (Cock *et al.* 2010; van Lenteren 2012). The casual handling of species introduction in the past led to strong restrictions on biological control projects (Simberloff & Stiling 1996; Barratt *et al.* 2010). Thus today the development of sophisticated pre-release guidelines is more important than ever. Most systems display a high level of complexity, so that a combination of different approaches, which investigate different mechanisms, may provide the best results in studying system characteristics.

1.1. Density dependent processes in host-parasitoid systems

Naturally, populations are not static but dynamic. The per capita growth rate presents a measurement of the change in populations over time and it is subjected to several density dependent processes (Malthus 1798; Gompertz 1825; Getz 1996). In host-parasitoid systems the attack rate and hence the per capita growth rate of a parasitoid

often changes with both host and parasitoid density (Hassell, Lawton & Beddington 1977; Lessells 1985; Liljeström, Cingolani & Rabinovich 2013). The dependence of a parasitoid's attack rate on host and parasitoid density can be described by the functional and numerical response, respectively. It should be noted that the response on either host (functional response) or parasitoid density represents the extreme cases (Abrams & Ginzburg 2000). Generally, it can be assumed that the response will shift from a primarily host dependent response, at low parasitoid densities, to a more parasitoid dependent response at higher parasitoid densities (Arditi & Akçakaya 1990; Abrams & Ginzburg 2000).

Parasitoids, which induce density-dependent effects on their hosts, may achieve a suppression of the host population (Grasman *et al.* 2001; Kidd & Jervis 2005). However, the mechanisms resulting in substantial host suppression seem to be highly inconsistent between varying systems (Hassell & May 1973; Reeve & Murdoch 1985; Reeve & Murdoch 1986; Murdoch *et al.* 1992; Murdoch *et al.* 1996; Murdoch, Briggs & Swarbrick 2005). One aspect, considered a key feature of successful biological control, is the ability of a parasitoid to maintain pest populations at a very low equilibrium point (Hassell 1978). A parasitoid's high search rate and an aggregated response to patches of higher host densities are believed to contribute to low equilibrium points (Hassell & May 1973; Hassell 1978). On the other hand, refuge effects and mutual interference among searching parasitoids have been shown to reduce the efficiency of parasitoids (Hawkins, Thomas & Hochberg 1993). Therefore, behavioural experiments can provide insight about processes that affect a parasitoid's response and thus, offer a valuable tool to identify the primary factors that shape host-parasitoid interactions. Furthermore, detailed observations on foraging behaviour may help in identifying the underlying proximate mechanisms and to understand how they affect population dynamics.

1.1.1. The functional response

The functional response of a parasitoid describes the per capita parasitism as a function of host density (Solomon 1949). Habitats with higher host densities should intuitively lead to higher per capita parasitism of hosts, at least up to some limit (Hassell 1978).

Box 1-1 | The functional response types by Holling (Holling 1959a). The Figures 1-2, 1-3 and 1-4 have been modified after Fellowes et al. (2005)

The type I functional response (Figure 1-2) displays a linear relationship. This response is also regarded as density independent as the proportion of hosts attacked is constant until no more hosts are attacked. In parasitoids an abrupt stop of the attack may for example be caused by egg limitation. In general, however, a type I response can be considered unlikely in host-parasitoid systems as it requires the search time to be constant over all host densities. Parasitoids encountering hosts usually utilize some time to handle the host, thus reduce the time searching with increasing host encounters.

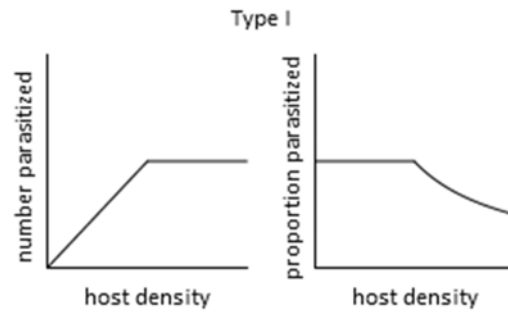


Figure 1-2: Functional response Type I

The type II functional response (Figure 1-3) takes into account that parasitoids spend time handling their hosts and thereby spend proportionally less time searching, with increasing host encounters. The response is characterized by a decelerating increase that approximates an asymptote at higher host densities. The type II response is also referred to as inversely density dependent since the proportion of parasitized hosts decreases with increased host densities. The type II response has been reported for many parasitoid species especially under laboratory conditions. However, it has been pointed out that the high number of reports on type II responses in parasitoids may be an artefact of fixed time experiments.

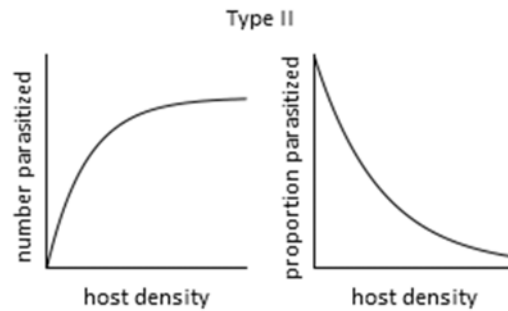


Figure 1-3: Functional response Type II

The sigmoid (type-III) functional response (Figure 1-4) often results from a higher foraging activity in parasitoids at higher host densities. Thus, the most intuitive explanation for the sigmoid response is an increase of the search time and/ or an increase in the search rate at higher host densities. In the field this response is often related to host switching behaviour.

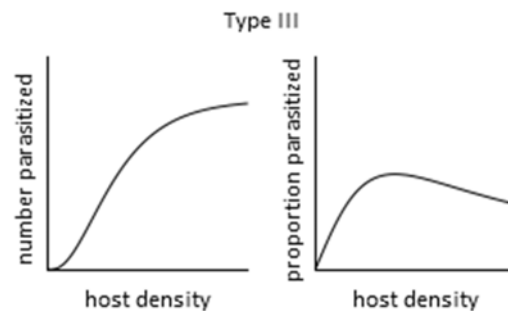


Figure 1-4: Functional response Type III

It is important to recognize the functional response as a habitat dependent response of a parasitoid rather than as an innate species characteristic.

Box 1-1 shows three types of responses proposed by Holling (1959a). To find the functional response type best describing the response of a parasitoid to host density is crucial in order to understand the population dynamics. However, it is evident that a species' type of response may vary, depending on the experimental design and the considered level of complexity.

To identify a parasitoid's functional response many studies have been conducted with a fixed time approach (Irvin, Suarez-Espinoza & Hoddle 2009; Farrokhi *et al.* 2010; Kidd & Amarasekare 2012) to estimate the rate of parasitism per unit time. The disadvantages of fixed time experiments, e.g. overexploitation of host patches, have been discussed in detail (van Lenteren & Bakker 1976; van Lenteren & Bakker 1978). In contrast, variable time experiments offer essential information about the individual's decision on patch exploitation and about the effects of these decisions on the functional response (van Lenteren & Bakker 1978; Abrams 1982; Fellowes, van Alphen & Jervis 2005). In time limited parasitoids, the time females spend on patches affects their fitness gain. In order to adjust the time spent searching a host patch female parasitoids may use past experiences, current experiences within a patch and their physiological state (van Alphen, Bernstein & Driessen 2003; Thiel & Hoffmeister 2009). The fact that parasitoids use several kinds of information to adjust their behaviour has been shown in several studies (Roitberg *et al.* 1993; Thiel & Hoffmeister 2006).

Studies on the type of the functional response have been shown to provide valuable tools to describe existing population dynamics, but they have shortcomings when the aim is to develop biological control programs (Fernandez-Arhex & Corley 2003). The mechanisms that drive host-parasitoid interactions are the key to understanding patterns in population dynamics. On the basis of Holling's work (Holling 1959b) on the functional response, many researchers attributed the type II response in parasitoids to the handling time. However, there are several potential factors that can drive the density dependent response in host-parasitoid systems, e.g. abiotic factors that impact life history, inter- and intraspecific competition, refuges or host defence mechanisms. To identify the processes or parameters that limit the functional response may help to draw conclusions about biological control scenarios.

The parasitoid *Fopius arisanus* has been repeatedly used for the biological control of tephritid flies. The outcome of such control attempts is diverse and ranges from great successes to failed attempts (see Rousse, Harris & Quilici 2005). Therefore, a deeper understanding of the mechanisms and processes that drive the host-parasitoid interaction could provide the basis for future decisions on biological control projects. The experiment presented in chapter three investigated the link between individual traits of the parasitoid *F. arisanus* and host characteristics with the density dependence of the functional response. Therefore, the response of *F. arisanus* towards different host densities was determined complemented by continuous observations of the behaviour (see below, subchapter 1.1.3.). Despite a description of the functional response the results show possible behavioural mechanisms that drive the decision making in *F. arisanus*. Furthermore, the results are discussed in the light of spatial host refuges, which offer an alternative explanation to the handling time in the observed functional response.

1.1.2. Mutual interference

Mutual interference results from encounters with conspecifics while searching for hosts and, is usually manifested by a reduced searching rate. The effects of mutual interference are more severe at high parasitoid densities. Female parasitoids that encounter conspecifics while foraging for hosts behave in various ways. Many parasitoid species tested in laboratory settings responded with an increased tendency to emigrate from the experimental host patch, which presents a mechanism that may lead to dispersal (Hassell 1978; Wajnberg, Curty & Colazza 2004). Parasitoids have been shown to defend a host resource against other foragers (Lawrence 1981; Hardy & Blackburn 1991; Field & Calbert 1998; Goubault *et al.* 2005; Humphries *et al.* 2006). In general, mutual interference results in a reduction of the time available for searching (Hassell 1978). Although, an aggregation of parasitoids commonly leads to a negative effect on the parasitoid population (Hassell 1978) it might be positive, e.g. if superparasitism helps to overcome host-defence mechanisms.

Often the aggregation of hosts also entails an aggregation of parasitoids, which in turn leads to mutual interference. Thus, the functional response and mutual interference are

closely linked and are hard to disentangle. However, the attempt to understand population dynamics in the field requires knowledge on the strength of the two processes. In order to make decisions on biological control programs it is favourable to gather information on both, the strength of the processes as well as their causes. While laboratory study cannot provide reliable estimates on the strength, experiments in confined space can provide deeper insights in the causes. For example, behavioural experiments may give valuable information on the mechanisms that drive foraging decisions. Information on the strength of both processes (functional response and mutual interference) in the field can be obtained through studies in the native range and/ or in regions where the species has been introduced.

Biological control programs are often based on mass releases of control agents and thereby an artificially high parasitoid density is created. Thus, mutual interference is most probably one key component of the population dynamics in such systems. Chapter four describes the experiment that was conducted to confirm that *Fopius arisanus* may suffer from interference effects by measuring the decrease of the search rate with increasing parasitoid density. The behaviour of searching females was analysed to answer how females adjust their behaviour in the face of competition (see below, subchapter 1.1.3). The simulation of a homogeneous or a highly aggregated host distribution further allowed concluding on the effect of host distribution.

1.1.3. Foraging behaviour

Intuitively, the foraging of one species on another species affects the growth rates of the involved populations. The foraging decisions of a parasitoid attacking its host influence the birth rate of the parasitoid and the host's death rate and hence, link foraging behaviour and population dynamics. Holt and Kimbrell (2007) provide a detailed overview about how foraging and population dynamics are intertwined. They discuss the advantages and disadvantages of population-based vs. individual-based models and highlight that hybrid approaches may offer a tool that balances between oversimplification and complication. While "basic" models are appropriate to describe existing population dynamics more complex models may provide tools to predict the outcome of species introductions. Applied problems like biological control generate a

growing demand for predictive tools. However, more complex models also require a more sophisticated understanding of the underlying mechanisms that drive the population dynamics. Thus, the accuracy of modern attempts to model population dynamics depends on the available information on the underlying mechanisms. This highlights the need for studies on foraging decisions in the context of population dynamics (Abrams & Kawecki 1999).

Parasitoids exploiting patchily distributed hosts, have to decide when to leave a patch. To make this decision parasitoids are known to process various kinds of information and adapt their behaviour accordingly. Waage (1979) developed a behavioural model to demonstrate the impact of oviposition events on the tendency to leave in the parasitic wasp *Venturia canescens*. Later, the Cox regression model, commonly used in medical and social sciences, was recognized as a tool to analyse patch leaving mechanisms (Haccou & Hemerik 1985; Haccou *et al.* 1991). Waage's model presents a deterministic model and estimates the time a parasitoid spends on a patch. In contrast, the Cox regression model presents a stochastic model and estimates the effect of events or parameters on the tendency to leave. Therefore, the Cox regression model allows identifying factors that considerably affect the leaving tendency of a parasitoid. The aim of this study was to identify the mechanisms leading to changes in the leaving tendency so that I used the Cox proportional hazards model to analyse my data.

Both, the study of the functional response and of the mutual interference were combined with behavioural observations to learn about the mechanisms that drive decision making in *F. arisanus*. Wang and Messing (2003a) studied the patch time allocation by *F. arisanus* attacking *Ceratitis capitata* on coffee berries. *Fopius arisanus* spends more time on patches of higher quality and decreases the time allocated to patches in the presence of traces from conspecifics. In contrast to the analysis of the patch residence time, I studied the effect of several factors regarding the leaving tendency. However, the effects can be discussed with respect to the patch residence time. Based on previous studies on *F. arisanus* and foraging theory I expected that an oviposition would lead to a decreasing patch leaving tendency and hence, an increased patch residence time. In contrast, host rejections are indicators of patch depletion, as encounters with previously parasitized hosts increase with progressing time and therefore should result in increased patch

leaving tendencies (Kolss, Hoffmeister & Hemerik 2006). Chapter three and four present the results on the effect of host encounters on the patch leaving tendency. It has been shown that *F. arisanus* allocates less time to patches that have been previously visited by conspecifics (Wang & Messing 2003a). For the study presented in chapter four, addressing the foraging decisions in females searching a patch simultaneously, I therefore, expected that females adapt their decision according to their current investment and information state. While, females that have already laid eggs are expected to show an increased tendency to stay and defend the patch, females without an oviposition are expected to leave.

1.2. Classical biological control

Population processes and their underlying mechanisms are not only of interest for population ecologists and theoreticians but find a justification in applied problems. Classical biological control refers to the introduction of a natural enemy into a new area for the control of a pest organism. Reviewing classical biological control shows how strategies on pest management improved by the integration of ecological knowledge. Classical biological control of animal pests builds upon the fact that especially populations of herbivorous species may frequently be limited by natural enemies.

The conducted study was part of the *African Fruit Fly Program*, an initiative to reduce the damage of fruit flies in Eastern Africa. Thus the presented study was done within a biological control context and aimed at the contribution to the development of a knowledge based management strategy.

1.2.1. Classical biological control in the past

The history of biological control demonstrates that natural enemies are powerful agents to combat pest species but at the same time, may pose the ability to become pests themselves. Any introduction of alien species, accidentally or intentionally, has consequences for the existent ecosystem.

The concept of biological control provoked an utterly controversial debate with strong supporters on the one end and absolute disputants on the other (Barratt *et al.* 2010). The use of biological control, i.e. the release of natural enemies to control organisms detrimental to agriculture, dates back to ancient times. The first reports originate from 304 A.D. when the Chinese used the predatory ant *Oecophylla smaragdina* to control pests of citrus (Huang & Yang 1987). However, biological control did not arouse a wide interest until much later when the vedalia lady beetle (*Rodolia cardinalis*) was introduced into California in 1889 (Howarth 1983). The beetle achieved an extraordinary success in the control of the cottony-cushion scale insect (*Icerya purchasi*) (Caltagirone 1981; Howarth 1991). Animated by this event and promoted by the popular assumption at the time that the introduction of natural enemies is “environmentally safe” followed a period

of trial and error (De Clercq, Mason & Babendreier 2011). The active exchange of potential natural enemies across the globe resulted in the introduction of thousands of arthropod species as control agents for agricultural pest insects worldwide (van Lenteren *et al.* 2006).

Reviews on successful biological control agents quickly revealed the strengths and benefits of this tool (Bartlett *et al.* 1978; Caltagirone 1981). One major goal of biological control practitioners is to reduce economic losses through pest insects and plants. The control program of cassava mealybug in Africa is an exceptional example, with a long follow up period, on the economic impact of the introduced parasitoid *Apoanagyrus lopezi* (Neuenschwander 2001). The estimated benefits exceeded the estimated costs by a factor of 200 and above (Zeddies *et al.* 2001). Natural enemies have also been successfully applied for nature conservation purposes (van Driesche *et al.* 2010; Simberloff 2012). The release of *Torymus sinensis* succeeded in lowering populations of the chestnut gall wasp, *Dryocosmus kuriphilus*, to non-destructive levels in Japan (Moriya, Shiga & Adachi 2002) and the USA (Cooper & Rieske 2007). The gall maker induced high mortality in chestnut trees (*Castanea spec*) and hence, threatened the biodiversity in the invasion range (van Driesche *et al.* 2010). In addition, the concern about adverse effects of pesticides on the environment, the economy and, on human and animal health (Konradsen *et al.* 2003; Pimentel 2005) further encouraged the application of biological control agents (Barratt *et al.* 2010).

The fallacy that no harm to nature emanates from the release of exotic natural enemies led to many carelessly planned introductions (Howarth 1991). Many species intentionally released for beneficial purposes have established with undesirable effects on the existing ecosystems (Simberloff & Stiling 1996; De Clercq, Mason & Babendreier 2011; Simberloff 2012). The predatory snail *Euglandina rosea* was introduced in several islands in the Pacific Ocean to control populations of the invasive giant African snail, *Achatina fulica*, (Davis & Butler 1964). The establishment of the snail has been linked to the extinction of several endemic snail species (Clarke, Murray & Johnson 1984; Coote & Loève 2003; Holland *et al.* 2012). The ladybird *Harmonia axyridis* had been widely released as biological control agent but is now regarded as an invasive alien species (Roy & Wajnberg 2008). The beetle has been associated with the decline in the number of native coccinellid

beetles (Koch & Galvan 2008). Its high abundance in some urban areas makes the beetle an annoying housemate and has been reported to cause allergic reactions in sensitive people (Goetz 2009). Despite the extensive reports on negative impacts of biological control agents the number of unreported cases is likely to be much higher. Indirect impacts of biological control agents are mostly vague reports of incidental observations or remain undetected. A prominent example of indirect effects is given by the ant *Pheidole megacephala*. The ant promotes the growth of scale populations by tending the colonies and preying on the natural enemies of the scale insects (Bach 1991; Gaigher *et al.* 2011). The mutual interaction between the ant and the scale insects has been associated with scale outbreaks that caused a decline in the number of the host plants (Gaigher & Samways 2013).

1.2.2. Classical biological control today

Since the potential impact of alien species has been recognized, modern science tries to unveil the mechanisms of biological invasions (Kenis *et al.* 2009). Several characteristics, e.g. phenotypic plasticity (Chown *et al.* 2007; Davidson, Jennions & Nicotra 2011) and life-history traits that favour future reproductive success (Sol *et al.* 2012), have been associated with exceptional invaders. The research in biological invasion includes many projects of former biological control agents, since the spread of organisms for biological control can be better traced than accidental introductions. The term “planned invasion” for biological control (van Driesche 2012) also elucidates that species invasion and species introduction are just a different perspectives of similar biological processes. One challenge of biological control projects is to isolate agent species that establish well without spreading further than the target region and hence endangering non-target populations. Both, establishment and spread are closely connected and thus to achieve the one but not the other requires a better understanding of the mechanisms that cause dispersal. An enemy with a very low dispersal rate may not be able to catch up with the pest population and an enemy with a very high dispersal rate may not establish or migrate from the target area. Behavioural experiments may give some indication of the mechanisms, which lead to migration that are employed by natural enemies.

The trust in the safety of biological control strategies has suffered from the past mistakes (Messing & Wright 2006) and the future prospect of it greatly depends on the shift to knowledge based introductions. There is a great need for guidelines to assess risk categories for potential agents that are ecologically meaningful (Messing & Wright 2006; Barratt *et al.* 2010). Given the diversity and complexity of possible relationships among species within an ecosystem, there will not be one universal approach to assess the risks of all biological agents for all biogeographic regions. Research questions should be specially geared to the characteristics of the agent as well as the affected ecosystem (Louda *et al.* 2003b). Despite a reduction of non-target effects, candidate biological control agents should pose a high potential to suppress pest populations.

1.2.3. Contribution of functional response studies and behavioural experiments to biological control

Two major properties need consideration, when identifying promising biological control agents. First, the agents should pose little or no adverse effects on the existing ecosystem (Louda *et al.* 2003a; Louda *et al.* 2003b). However, since biological control is never 100 % safe, the estimated risk should be low compared to the anticipated benefits. This leads to the second aspect, that the favourable natural enemy should exhibit the potential to considerably reduce the target pest population. The ideal candidate for biological control decreases a pest population to an acceptable level and then preserves a constantly low pest density to prevent future outbreaks (Mills & Getz 1996). Unfortunately, the prediction of population dynamics within new ecosystems is not straight forward. The most reliable way is still to gather a broad knowledge of the ecological characteristics of a natural enemy and the species community affected by the introduction using different methods (Mills & Kean 2010).

To study the density dependent response of a parasitoid towards host and parasitoid density is one method to attain knowledge on the ecological characteristics of the parasitoid. Density dependence in the interactions of host-parasitoid systems results in fluctuations in host suppression. Some studies focused on the importance of transient fluctuations (Kidd & Amarasekare 2012), stability and equilibrium points of populations (Murdoch & Briggs 1996) in order to identify potential biological control agents. They

investigated model behaviour with respect to the mathematical properties. Other studies estimated parameters like search rate, parasitoid density and handling time, which are important for the level of suppression that can be achieved by a natural enemy (Murdoch 1973; Hassell 1978). The identification of the functional response type is crucial for the estimation of the response parameters. However, the response has shown to be highly sensitive to the experimental conditions and level of the system complexity (van Lenteren & Bakker 1976; van Lenteren & Bakker 1978; Hassell 2000a). Therefore, the transferability of such studies to field situations is low. Laboratory studies may widely fail to quantitatively predict population dynamics in the field but they may reveal crucial information on the factors that affect host-parasitoid populations. Identifying processes and mechanism (e.g. mutual interference, refuges, defence mechanisms etc.) that cause density dependent responses may help to advice on the characteristics that should be studied in the field.

1.3. Fruit fly pests – a global problem

The family Tephritidae encompasses more than 4000 species worldwide, including some of the globally most important crop pests (Christenson & Foote 1960). Tephritid fruit flies are not only globally spread but also inhabit a wide range of environments (Bateman 1972), which is especially true for the pest species (Robinson & Hooper 1989). Economic losses due to fruit infestations by fruit flies are a persisting and global problem (Vargas *et al.* 2010; Oliveira *et al.* 2013). There are various causes for their economic importance, e.g. fruit losses through infestation, worldwide invasions through pest species and therefore, quarantine restrictions and regulations (White & Elson-Harris 1992). While the larvae of species widely recognized as crop pests are frugivorous, most larvae of the Tephritinae feed on the reproductive organs of flower heads and seeds (Christenson & Foote 1960). Many of the notoriously destructive pests are within the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis* (Christenson & Foote 1960; Fletcher 1987). Thus, the biology and characteristics of these genera have been extensively studied (Christenson & Foote 1960; Fletcher 1987; Aluja & Norrbom 2000). With the exception of the temperate species of the genus *Rhagoletis*, most pest species are polyphagous and thus feed on many different plant species and even families (Fletcher 1987). Another, characteristic of many pest species is a short generation time with more than one generation per year (Fletcher 1987). Furthermore, members of the *Bactrocera dorsalis* complex are known to show a high mobility and ability of range expansionexpanding ability (Ekesi, Nderitu & Chang 2007).

1.3.1. The *Bactrocera dorsalis* complex

The *Bactrocera dorsalis* complex represents one among 20 complexes within the genus *Bactrocera* (Clarke *et al.* 2005). The *B. dorsalis* complex arouses great interest since it includes some species of major economic importance. The latest, published status on this complex reported 75 closely related species (Clarke *et al.* 2005) not including *Bactrocera invadens*. It has been suggested that the species diversity in the *B. dorsalis* complex results from a rather recent and eventually still ongoing radiation event (Clarke *et al.* 2005). The origin of the complex is Southeast Asia, a region that displays unique geological features (Michaux 1996). Small islands and the occurrence of diverse habitats

combined with the richness on tropical fruits may have led to speciation events with adaptations to new host plants (Clarke *et al.* 2005). The biological plasticity of the *B. dorsalis* complex renders the management a difficult task. Thus, pest management programs need to cover a wide range of suitable habitats.

Bactrocera invadens belongs to the *Bactrocera dorsalis* (Hendel) complex within the subfamily Dacinae (Diptera: Tephritidae) (Drew, Tsuruta & White 2005). Recent evidence from inbreeding experiments (Bo *et al.* 2014), comparison of the male sex pheromone and molecular analyses suggests that *B. invadens* and *B. dorsalis* are the same biological species (Tan *et al.* 2011). Yet, the on-going dispute if *B. dorsalis* and *B. invadens* are two closely related species or form one biological species still needs to be solved conclusively. To prevent further confusion the presented study refers to *B. invadens* as distinct but closely related species to *B. dorsalis*.

1.3.2. Biological control of pest species within the genus *Bactrocera*

Given the biological traits of tephritid pests the control of these species by natural enemies presents an ambitious task. The rapid speciation process created new species that are able to inhabit a wide range of geographic regions and that utilize several host plants from different families. Past biological control efforts of tephritid flies have been, at least, partially successful. Among the greatest successes in the biological control of tephritid flies, ranks the suppression of *Ceratitis capitata* and *Bactrocera dorsalis* on guava and coffee berries in Hawaii (Clausen, Clancy & Chock 1965; Haramoto & Bess 1970). Less clear are the effects of the introduction of *Psytalia fletcheri* into Hawaii to control *Bactrocera cucurbitae* (Purcell 1998). Apart from some endemic parasitoids parasitism of the Queensland fruit fly, *Bactrocera tryoni*, in Australia, is attributed to the introduced *Fopius arisanus* and *Diachasmimorpha longicaudata* (Purcell 1998). In olive growing regions of Europe several attempts were made to establish *Psytalia concolor* to control *Bactrocera oleae*. In Italy, the parasitoid achieves considerable suppression levels by inundative or inoculative releases. Reviewing the literature on successful biological control programs on Tephritidae reveals that documented successes are scarce.

Box 1-2 | The history of *Fopius arisanus* in the control of fruit fly pests*

Fopius arisanus most probably was among the parasitoid species introduced into Hawaii between 1947 and 1952 to control the oriental fruit fly *Bactrocera dorsalis* (Haramoto & Bess 1970). *Fopius arisanus* not only established but also became the most abundant parasitoid species. The Hawaiian control program is still the most successful in the history of *F. arisanus* as a biological control agent.

Fopius arisanus has established in French Polynesia and recent surveys show that the parasitoid reaches more than 50 % parasitism of *B. dorsalis* in fruits collected from the field (Vargas *et al.* 2012a). It also reaches up to 30 % parasitism on *B. passiflorae* and *B. xanthodes* in the Fiji Islands (Bautista, Harris & Vargas 2001).

However, the success of *F. arisanus* is restricted to some cases while in others it has failed to establish or only caused low parasitism in the targeted species. For example parasitism reported from Samoa (to control *B. kirki* and *B. xanthodes*) does not exceed 10 %. Also in Australia the success of *F. arisanus* in parasitizing *B. tryoni* seems to be regional (Purcell 1998).

The attempt to use *F. arisanus* for the control of *Anastrepha suspensa* in Florida, USA failed (Rousse, Harris & Quilici 2005). *Fopius arisanus* has been introduced to several countries for laboratory studies to evaluate its potential as control agent. However, reports on failed releases are rather scarce.

Furthermore, *F. arisanus* is considered for biological control ventures to suppress fruit fly populations in Mexico, Guatemala, Israel (Rousse, Harris & Quilici 2005).

*This is not a complete list of all attempts but rather a compilation of selected examples to show the diversity in the success obtained by introducing *F. arisanus*.

The case of the Hawaiian fruit fly management program remains by far the best documented example. Further examples of biological control efforts with *F. arisanus* are given in Box 1-2.

Some of the above mentioned programs, including the control of *B. dorsalis* in Hawaii, were based on mass releases of several natural enemies. From 1947 to 1952 a total of 24 parasitoid species were introduced into Hawaii to control *B. dorsalis*. In the case of *B. tryoni* nine parasitoids were introduced into Australia in the 1950's of which only *F. arisanus* and *D. longicaudata* became established. Although, no harm has been reported from the introduced control agents, I hold the opinion that future programs should focus on the most promising candidates.

Some predators have been reported to cause mortality upon fruit flies as well. Birds and ants are causing substantial mortality on pupae of *Bactrocera oleae* (Bigler *et al.* 1986;

Orsini *et al.* 2007). Recently ant species within the genus *Oecophylla* seem to have gained a growing attention in the context of fruit fly management. Ants seem to provide two major services. They prey upon fruit fly larvae and pupae, and secondly, ant cues seem to have a repellent effect on flies searching for oviposition sites (van Mele *et al.* 2009). *Oecophylla smaragdina* leads to lower abundances of pupae and adults of *Bactrocera jarvisi* in Northern Australia (Peng & Christian 2006). Mango growers in Benin yield more uninfested mangoes when the weaver ant *Oecophylla longinoda* inhabits the orchards (van Mele *et al.* 2007). In Kenya, current investigations study the effect of weaver ants on the oviposition behaviour of tephritid flies and on *F. arisanus* (Migani pers. comm.). In Kenya the introduction of the weaver ants into mango orchards is considered as part of an integrated pest management program. The use of more than one agent for biological control requires a great knowledge on the biology of the agents involved and their interactions. Therefore, this study on biological traits of *F. arisanus* laid a foundation for current researches addressing the biological control of *B. invadens* with more than one natural enemy.

1.4. Objectives

The main objective was to study the density dependence and its causes in the host-parasitoid interaction of *Fopius arisanus* and *Bactrocera invadens*. The attained knowledge should provide new insights in the mechanisms that generate density-dependent responses in host-parasitoid systems and how these mechanisms relate to biological control efforts. Therefore this study aimed at answering the following questions.

1) What type of the functional response does *F. arisanus* show when foraging on mangos infested with different densities of host eggs? The functional response describes the dependency of a parasitoid's attack rate on the host density, therefore, I investigated if a host density dependent refuge to the hosts can cause the host density dependence in the response of *F. arisanus*.

2) Does mutual interference play an important role in this species and thus causes parasitoid density dependence in the attack rate? The distribution of host eggs can lead to the aggregation of parasitoids, which would then increase the effect of mutual interference. Thus I also tested if the strength of interference varies according to the host distribution.

3) How does *F. arisanus* adapt to varying habitats and what are the mechanisms that drive foraging decisions in this parasitoid. Therefore, I tested the effect of experience (e.g. by host encounters, host distribution) on the tendency of *F. arisanus* to leave a patch.

2

General Methods

2.1. The insects

Bactrocera invadens Drew, Tsuruta and White (Diptera, Tephritidae) has first been recorded in 2003 from the coastal area in Kenya (Lux *et al.* 2003; Drew, Tsuruta & White 2005) and subsequent reports confirm its current distribution throughout sub-Saharan Africa (Drew, Tsuruta & White 2005; De Meyer *et al.* 2010). The knowledge about the biology of *B. invadens* is still fragmentary and mainly concentrates on the trades that verify its pest status (Ekési, Nderitu & Rwomushana 2006; Rwomushana *et al.* 2008a; Rwomushana *et al.* 2008b; De Meyer *et al.* 2009).



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Figure 2-1: Female *Bactrocera invadens* from Kenya (laboratory population, icipe)

Female flies (Figure 2-1) oviposit their eggs in ripening fruits, in doing so they drill holes with their ovipositor or use previous oviposition sites as well as otherwise damaged fruit areas. *Bactrocera invadens* is highly polyphagous but seems to have a preference for mango fruits (Rwomushana *et al.* 2008a; Goergen *et al.* 2011).

The larvae feed on the fruit pulp and pass three larval stages before pupating in the soil. After approximately two weeks the flies emerge from the puparium and undergo a pre-reproductive phase.

Fruits collected from the field can contain more than hundred larvae of *B. invadens* (personal observation) but the actual distribution of egg clusters in the field is unknown. However, it is likely that eggs are distributed in large clusters as reported from *Bactrocera dorsalis* (Christenson & Foote 1960; Bateman 1972). Under laboratory rearing conditions *B. invadens* lays huge clusters of eggs when provided with punctured mango domes or punctured plastic beakers.

The remarkable ability of *B. invadens* to expand to and establish in new habitats, paired with a high fecundity and with a polyphagous lifestyle contributes to its status as one of the most devastating horticultural pests in Africa. The actual and potential danger posed by this species for the horticultural sector and the availability of a vast amount of

information on the biology of the genus *Bactrocera* make it a favourable species for conceptual studies towards knowledge based biological control.

Fopius arisanus (Sonan) (Hymenoptera, Braconidae) is one among few opiine egg-pupal parasitoids associated with tephritid flies. *Fopius arisanus* is indigenous to the Indo-Pacific region with reports from Taiwan, Borneo, India, Malaysia and Thailand (Chinajariyawong *et al.* 2000). The first intentional transfer of this species was from Malaysia to Hawaii to control *B. dorsalis* (van den Bosch & Haramoto 1951).



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Figure 2-2: Female *Fopius arisanus* probing a mango dome infested with host eggs.

Fopius arisanus is synovigenic and ovipositions enhance the maturation of eggs (Wang & Messing 2003c). The fact that the hosts of *F. arisanus* are concealed in fruits requires that females are able to locate places, which contain host eggs. To find potential host patches *F. arisanus* uses colour and odour cues (Vargas *et al.* 1991). Upon encounter with a suitable host the female deposits a single egg inside the host egg (Figure 2-2). In experienced females an encounter with a previously parasitized egg will most likely lead to the rejection of that host (Wang & Messing 2008). *Fopius arisanus* is koinobiont, and hence allows further development of its host. The parasitoid primarily develops within the fruit fly pupa.

Individuals used for the experiments were obtained from the colony at *icipe*, Nairobi, Kenya. This colony of *F. arisanus* originates from an initial cohort that was obtained from the University of Hawaii at Manoa, Honolulu, Hawaii, and has been maintained at the insect rearing facilities at *icipe* (Mohamed, Ekesi & Hanna 2010). The colony was kept on *B. invadens* for more than 40 generations as described by Mohamed and colleagues (2010). Female wasps used for experiments were kept in cohorts of 30 freshly emerged females (emergence within 15 hours) and 30 four- to six-day old males for mating. The insects were kept at room temperature (25-26 °C) and a photoperiod of 12 L:12 D. The cages were equipped with fine drops of pure honey and moistened cotton wool.

2.2. Experimental set-up

Collection of host eggs

Eggs of *Bactrocera invadens* were collected one day prior to exposure to *Fopius arisanus*. This ensured that all host eggs used for the pre-experience treatment and the observations were between 17 and 28 h. In order to collect eggs, mango fruits were cut in half and the pulp was carefully removed. The remaining dome was pierced with a needle to facilitate oviposition by *B. invadens*. After the exposure of mango domes to *B. invadens* for two hours, the domes were covered and kept in an incubator at 20 ± 1 °C (to decelerate the egg development).

Preparation of host patches

A host patch consisted of a mango (*Mangifera indica* var. Apple) dome with a single clutch of eggs at the centre. Patches were prepared one day before exposure to the wasps. The mango domes were cut into circular pieces of different diameters (specific sizes are given in the respective chapters). In order to simulate an oviposition puncture, the fruit domes were pierced with a fine entomological pin at the centre.



Figure 2-3: Eggs of *Bactrocera invadens* placed at an artificial oviposition puncture.

The eggs of *B. invadens* were placed at the artificial puncture on the inside of the dome (Figure 2-3) using a single hair brush. The prepared domes were fixed with beeswax to a Plexiglas plate to prevent dehydration of the eggs and to avoid parasitoid searching at the cut surface of the fruit. The domes were covered with a glass bowl and stored in an incubator at 20 ± 1 °C until use.

Observation arena

A plain Plexiglas cage (20 x 7 x 20 cm) served as an observation arena (Figure 2-4). The host patch consisted either of a single mango dome or of four mango domes, for the study of host density dependence and mutual interference, respectively. An aspirator was appropriate to introduce a single wasp into the arena, but for the introduction of up to

four parasitoids the release cap worked better. After the parasitoids were released onto the host patch the arena was closed and the behaviour of wasps was recorded using the software *The Observer* (Noldus 1991).

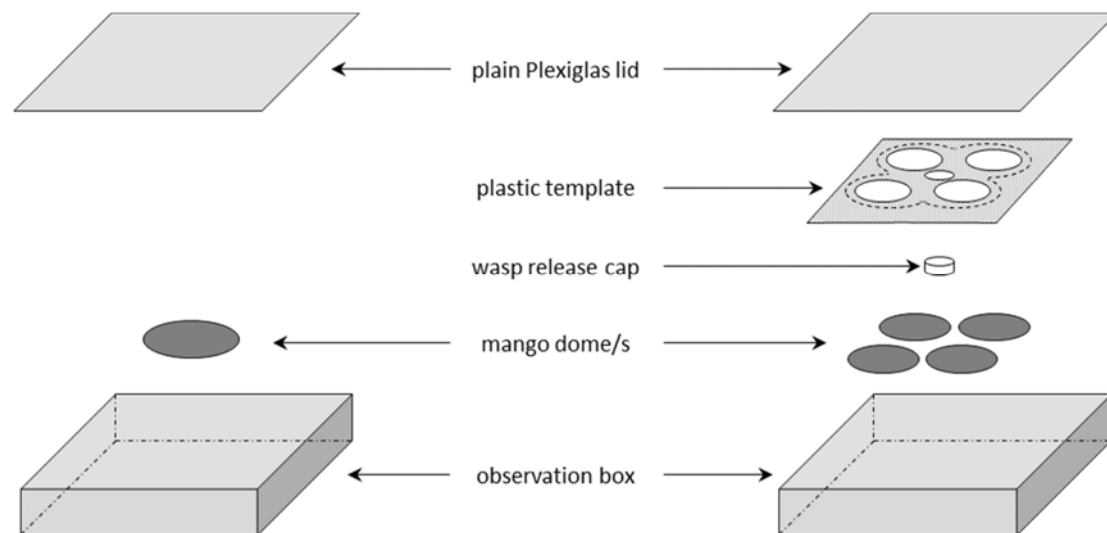


Figure 2-4: Set-up of arena for behavioural observations. The left side shows the set-up for the functional response study (chapter three). A single mango dome infested with varying densities of 3-81 *Bactrocera invadens* eggs was placed in the centre of the arena (observation box). After a single wasp was released from the tip of an aspirator on top of the dome the arena was covered with the plain Plexiglas lid. The right side pictures the set-up for the mutual interference study (chapter four) where a set of four mango domes was placed in the centre. The mango domes contained in total 80 host eggs which were either aggregated (one of the domes contained a cluster of 80 eggs) or regular (each of the four domes contained a cluster of 20 eggs). The total fruit surface area and the distance between fruit patches were kept constant for all observations. Therefore, a rectangular plastic template with four holes (diameter = 3.5 cm) and one hole for the wasp release cap (diameter = 1.8) was placed above the mango domes. The area within the dotted line on the plastic template defines the host patch. To release the parasitoids into the arena the parafilm was removed from the wasp release cap.

Estimation of parasitism

The eggs of *B. invadens* were examined, to count the number of eggs parasitized by *F. arisanus* and to confirm that females had laid eggs prior to the observation. To count the parasitized eggs, the fruit fly eggs were gently transferred into a drop of 2.5 % Sodium-Hypochlorite on a microscopic slide using a single hair brush. After approx. 2 minutes the Sodium-Hypochlorite was removed and the eggs were thoroughly rinsed with water using a pipette. This procedure removed the chorion of the host eggs so that the sickle-shaped eggs of *F. arisanus* were clearly visible inside. Parasitized eggs were counted using a binocular microscope with illuminator and fortyfold magnification.

3

Compensation for spatial host refuges by reward-dependent mechanisms

3.1. Introduction

The functional response of parasitoids describes changes of the per capita attack rate according to the host density. Ecological models, like the random parasite equation (Rogers 1972), are based on a specific set of assumptions about the underlying mechanisms. It is established that other than the assumed mechanisms may lead to the observed relationship (Holling 1959b; Hassell 1978). Therefore, it may be misleading to draw conclusions about behavioural responses from the basic mathematical model alone. For example, Holling's (1959b) famous simulation of predator-prey interactions with sandpaper discs showed how the effect of handling time leads to a functional response type II. Although, Holling (1959b) himself highlighted the possibility of alternative components to shape the functional response, the type II has often been attributed to the fact that parasitoids spend time handling a host, ever after (Patel & Habib 1993; Lee & Kang 2004). While this may be true for many systems studied, other mechanisms, e.g. interference (Hassell 1978) and adaptation of patch residence time (Abrams 1990) have been reported to result in similar responses. An observed functional response pattern can be caused by different mechanisms. Consequently, alternative factors have to be included in functional response studies in order to interpret the results biologically meaningful and use the results as predictive tool.

Female parasitoids maximize their fitness by optimizing the time they spent searching host patches. Parasitoids exhibit mechanisms to process information from immediate perception of the habitat, past experiences and/or their innate physiological state to adjust their behaviour within the boundaries of the individual range of behavioural plasticity (Mery & Burns 2010; Froissart *et al.* 2012). Female parasitoids face the risk of either time or egg limitation whereby the limiting factor may vary during the course of their life (Heimpel, Mangel & Rosenheim 1998). Synovigenic parasitoids that possess high egg maturation rates throughout their lifetime are very likely to be primarily time limited. Under these circumstances the parasitoids should optimize the time they spent on exploiting a patch so that the rate of encounters with healthy hosts is maximized within the span of their life (Hubbard & Cook 1978). This means that a parasitoid should theoretically leave a patch when the rate of oviposition opportunities it encounters drops

below the average rate that can be attained in the surrounding habitat (Charnov 1976; Wajnberg *et al.* 2006). As a consequence female parasitoids would leave a patch area despite the presence of unparasitized hosts leading to a refuge for the hosts.

Parasitoids are not omniscient and hence have to rely on past and current information to drive their decisions. Iwasa and colleagues (1981) demonstrated how the distribution of prey in the habitat alters the qualitative value of prey encounters and hence promotes the adaptation of different strategies. They considered the use of the following three foraging strategies in order to maximize a forager's fitness gain under different host distributions (Iwasa, Higashi & Yamamura 1981): A) the fixed time strategy, where the forager spends the same amount searching at all encountered patches; B) the fixed number strategy, where the forager leaves the patch after having encountered a fixed number of prey and; C) the fixed giving-up time, where the forager leaves the patch when the time interval between the last prey encounter until the next encounter exceeds a fixed time. According to their stochastic model they concluded that (Iwasa, Higashi & Yamamura 1981): A) the fixed time strategy is superior over the other two when hosts are distributed randomly across the habitat; B) a forager should attack a fixed number of prey in each patch when the hosts are distributed regular and; C) the fixed giving-up time outcompetes the other two strategies when the prey is aggregated.

While Iwasa and colleagues (Iwasa, Higashi & Yamamura 1981) studied the optimal strategy by a stochastic model, Waage (1979) developed a deterministic model to study the way parasitoids use information on patch quality to adjust their searching time. Waage (1979) first constructed a model to describe the behavioural mechanisms determining patch time in the thelytokous parasitoid, *Venturia canescens*, by the interaction of incremental and decremental processes. Waage (1979) assumes that when encountering a patch parasitoids have an initial "responsiveness to the patch edge" that declines over time but is sensitive to events (e.g. ovipositions) having incremental or decremental effects. As mentioned above it depends on the host distribution, which is the optimal effect of host encounters on the patch residence time (Wajnberg 2006): Parasitoids should employ A) no effect of host encounters if hosts are randomly distributed; B) a decremental patch time mechanism, which responds similar to a fixed number strategy, when host distribution is regular and; C) an incremental mechanism in

patchy environments that, like the giving-up time strategy, leads to an increased time investment on higher quality patches than compared to patches of poor quality.

The mechanism used by a parasitoid can vary within and among species depending on habitat characteristics and their predictability. Female parasitoids are known to use diverse mechanisms based on e.g. physiological state, previous experience, abiotic conditions and within patch experience in order to optimize their search efforts on patches (see Wajnberg 2006, for a review).

Despite the host's refuge that exists through the tendency of parasitoids to leave as the patch gets less profitable their might be an additional spatial refuge. Hosts that aggregate in patches may have different probabilities of being encountered by a parasitoid to the point of zero risk for hosts that are inaccessible due to their spatial distribution. The presence of spatial refuges and their importance for stability of population models has been widely acknowledged (Berryman & Hawkins 2006), while it is rarely included in empirical studies of functional responses. The concept of aggregation to lower the risk of predation is widely spread in the animal kingdom. The idea that insect eggs in the centre of a cluster may be inaccessible for predators or parasitoids seems intuitive. Yet, there is only little evidence from empirical studies supporting the idea of spatial host refuges in insect egg masses (Damman & Cappuccino 1991; Faraji, Janssen & Sabelis 2002). Only few studies investigated the impact of size and location of egg masses on the effectiveness of natural enemies. For example the tropical armyworm, *Spodoptera litura* (F.), has been shown to successfully escape attacks by the egg parasitoid *Telenomus remus* through larger egg batches (Braune 1982). The butterfly *Asterocampa clyton* deposits pyramidal egg clutches that produce exponentially growing refuges (Friedlander 1985).

Approaches to model the effects of host induced refuges on parasitoids functional response mostly consider the extreme case of a constant number or a constant proportion refuge (Hassell & May 1973; McNair 1986; González-Olivares & Ramos-Jiliberto 2003) leading to theoretically infinite increasing numbers of available hosts within one cluster. However, most naturally occurring refuges are not that simple (Sih 1987). In insects eggs that are clustered together are likely to be disposed to parasitoid attack at different levels of risk depending on their position to the exposed surface and

parasitoid morphological traits (e.g. ovipositor length). It is rather difficult to estimate the exact proportion of eggs that is safe from attacks thus refuge estimates are at best approximations derived from host exploitations by their enemies (Hochberg & Holt 1999).

In the light of classical biological control parasitism studies of *Fopius arisanus* (Sonan) on several fruit flies have been conducted (Quimio & Walter 2001; Bautista *et al.* 2004; Harris *et al.* 2010; Mohamed, Ekesi & Hanna 2010) but apart from two studies (Wang & Messing 2003a; Wang & Messing 2003b) they do not address the response towards different host densities and the underlying mechanism. Considering the characteristics of the interaction between *F. arisanus* and *B. invadens* the functional response of female parasitoids may be affected by several factors. A density dependent response towards host density may be caused by the parasitoids handling time, the adaptation of foraging times and host refuges. The presented experiment tested how the parasitism rate changes when varying numbers of *B. invadens* eggs are presented in a cluster to individually searching *F. arisanus* females and the effect of the above mentioned factors on the parasitoid's response.

To understand the role of behavioural events in patch time allocation by *F. arisanus* the observation data were analysed with a Cox proportional hazards model. Depending on the host density *F. arisanus* should adjust the time it spends searching so that more time is spent on host rich patches. If so, changes of females motivation should be effected by information gathered while searching a patch. Since host eggs are assumed to be aggregated in the field *F. arisanus* should display an incremental mechanism towards oviposition and hence prolong its searching time on the patch. The informative value of oviposition and rejection differs and *F. arisanus* exhibits a sophisticated discrimination behaviour towards parasitized hosts (Ramadan, Wong & Beardsley 1992; Wang & Messing 2008) thus compared to ovipositions, rejections should decrease the patch residence time (Rosenheim & Mangel 1994; Wajnberg *et al.* 2003). Similar to successive visits to the same patch encounters with a previously attacked host cluster within one patch visit should decrease the patch time as they are a measure of patch depletion. Furthermore, the parasitoids response could be based on the sequence of the above events to each other. These hypotheses were tested by analysing the observation data with a Cox proportional hazards model.

3.2. Materials and methods

Patch preparation

Mango domes with a single egg mass at the centre of the inner part served as host patches. Therefore, circular pieces (pre-experience patch: diameter = 3.5 ± 0.5 cm; experimental patch: diameter = 6.7 ± 1 cm) were cut from ripe mango fruits, *Mangifera indica* var. Apple. The flesh of the fruit was removed to obtain a dome with a very thin layer of flesh under the fruit skin. In order to simulate an oviposition puncture, the fruit domes were pierced at the centre. A predetermined number of *B. invadens* eggs (pre-experience patch: 10 eggs; experimental patch: 3, 6, 9, 18, 27, 54 or 81 eggs) were placed at the inside of the dome, in the immediate vicinity of the artificial puncture. To prevent dehydration of the eggs and to avoid parasitoid searching at the cut surface of the fruit the domes were fixed onto a Plexiglas slide. The domes were covered with a glass bowl and stored in the incubator at $20 \pm 1^\circ\text{C}$ until usage at the following day.

Pre-experience

Six day old female wasps were exposed to a pre-experience patch infested with ten host eggs to allow for experience in locating and handling hosts. Therefore, a single wasp was transferred into a glass vial (21 x 70 mm), which was placed on top of a mango dome so that the artificial oviposition puncture was in the centre of the bottom. The vial with the female was removed from the dome after the wasp stopped probing or spent over an hour without showing oviposition behaviour. The females that displayed oviposition behaviour were separately kept in the vials provided with moist cotton wool and a streak of honey until the experiment at the following day. To ensure that female wasps used for observations were experienced the fruit fly eggs had been checked for parasitized eggs and only females that laid eggs were considered in further investigations.

Experiment

The observation arena consisted of a plain Plexiglas cage (20 x 7 x 20 cm) with a host patch in the centre. A single wasp was kept in the tip of an aspirator for five minutes to allow the wasp to settle. The time for an observation started when the wasp left the aspirator tip and walked onto the outer margin of the host patch. Variable-time

approaches best comprise the fact that the functional response of parasitoids is sensitive to the time a parasitoid is willing to search a patch area. Therefore, an observation was terminated when *F. arisanus* left and spent more than 30 s off the mango dome. Wasps that did not encounter the oviposition puncture within 10 min were disregarded from further analysis.

The patch residence time (PRT), host encounters and returns to the oviposition puncture were recorded using the event-recording software *The Observer* (Noldus 1991). The recorded parameters are given in **Table 3-1**.

Table 3-1: Observed behaviour. The duration and frequency of the parameters listed in the table were recorded.

Behaviour	Description
I) patch residence time (PRT)	the total time that the wasp spent on the dome counting from the moment the wasp leaves the aspirator tip to walking or flying off the patch.
II) encounters with puncture	all visits of the mango domes in the fruit patch area
III) probing	the time wasps spent probing the oviposition puncture by inserting the ovipositor into the oviposition puncture and moving the abdomen up and down (Wang & Messing 2003a).
IV) host encounters	events where the parasitoid remained motionless in a distinct pose for some time and the female's antennae are raised in an acute angle as described by Wang & Messing (2003a). Since the eggs are covered oviposition and rejection can only be separated by the time of the motionless behaviour. Thus for each observation all events of host encounters were ranked by time and events with the highest times were taken as ovipositions so that the number of events corresponds to the counted number of parasitized eggs.

To estimate the parasitism, eggs were gently transferred into a drop of 2.5 % Sodium-Hypochlorite on a microscopic slide and then rinsed with water. With the chorion removed the sickle-shaped eggs of *F. arisanus* were clearly visible inside the fruit fly egg.

The functional response model

Holling's (1959b) type II functional response describes rising parasitism to a maximum at a constantly decelerating rate with increasing host density due to the effect of time spent on handling an object, which in return reduces the proportion of time a parasitoid spent

searching. In his work, Rogers (1972) inserts Holling's disc equation into Nicholson and Bailey's (1935) equation for random search of non-depletable patches giving the random parasite equation:

$$N_a = N_t \left(1 - e^{\frac{-a' T P_t}{1 + a' T_h N_t}} \right) \quad \text{eqn 1}$$

Here the number of attacked hosts N_a is a function of the initial host density N_t and the number of simultaneously searching parasitoids P_t . T is the total time that hosts are exposed to parasitoid attack, T_h is the time parasitoids spent handling encountered hosts and a' is the parasitoids search rate that is assumed to be constant. The random parasite equation (eqn 1) accounts for the host refuge generated by the parasitoids behaviour.

For spatial refuges the initial number of hosts is reduced to the number of hosts accessible for parasitoid attack. Assuming, that fruit fly eggs are aggregated rather spherical inside a fruit the resulting refuge should grow exponentially with the host density of the eggs up to a critical maximum. Above the critical value every additional host is inaccessible for parasitoids and thus, positioned in an explicit refuge. An approximation of the accessible hosts regarding an exponentially growing refuge with a maximum can thus be derived from:

$$N'_t = r_{max} (1 - r_s^{N_t}) \quad \text{eqn 2}$$

where the number of initially accessible hosts N'_t is a function of N_t and rises at a decelerating rate r_s towards a maximum. The asymptote r_{max} denotes a critical value above, which all additional hosts can be considered to be in an explicit refuge from parasitoid attack. Substituting N_t of eqn 1 by N'_t gives:

$$N_a = r_{max} (1 - r_s^{N_t}) \left(1 - e^{\frac{-a' T P_t}{1 + a' T_h r_{max} (1 - r_s^{N_t})}} \right) \quad \text{eqn 3}$$

The functional response described by eqn 3 is bound to an upper limit determined by r_{max} .

Data analysis

With the exception of Figure 3-1, which was created in SigmaPlot version 11.0 (Systat_Software 2008) all statistical tests and figures were generated with the software R (R_Core_Team 2014).

The shape of the functional response curve was analysed by logit regression of the parasitism rate on log-transformed host densities (Trexler, McCulloch & Travis 1988). A stepwise backward elimination was performed starting from the model with the log of host density cubed as highest order term (Model 1) until significance in the lack-of-fit statistic was obtained. The linear coefficient of the regression depicts the slope near the origin and thus allows to separate between functional responses of type II or III by negative or positive estimates of the coefficient, respectively (Trexler, McCulloch & Travis 1988; Juliano 2001). If a negative density dependence of the parasitoid response on host density and hence a functional response type II was found eqns 1 and 3 were fitted to the data. Instead of a fixed time parameter T the equations were fitted using the actual patch residence time as a variable. To estimate the equation parameters a non-linear least-square regression was performed (Schenk & Bacher 2002) using the nls procedure from the 'nlrwr' package (Ritz & Streibig 2008). The models were simplified by deleting non-significant parameters.

The existence of a spatial host refuge should lead to a lower parasitism rate with increasing patch depletion as females encounter more previously parasitized eggs than expected from random search. To illustrate this effect of a spatial refuge in the studied system the observed and the expected (without a spatial refuge) parasitism are displayed as a function of the cumulative time wasps spent probing (eqn 4), which can be easily derived from the Nicholson and Bailey model (Nicholson & Bailey 1935).

$$N_a = (N_t - 1) * \left(1 - e^{-\frac{\epsilon T_{probing}}{N_t}} \right) \quad \text{eqn 4}$$

where the constant ϵ is the parasitoids encounter rate with hosts and, $T_{probing}$ is the time a female wasp spends probing the puncture in the fruit.

The time until the first oviposition varied greatly among observations. Therefore, only the time for probing and, the encounters or successful ovipositions after the first until the last oviposition event were considered. The number of ovipositions within the lowest host density of three was too low and thus excluded. The encounter rate ϵ_i for each female was calculated by dividing the number of all encounters by the total time wasps spent probing and then the mean encounter rate ϵ was estimated for each group of initial host density. The expected parasitism at all host densities if no spatial refuge exists is presented by inserting the mean encounter rate into eqn 4. The curve for the observed parasitism was fitted by a non-linear least-square regression.

To identify the events that influence patch time decisions the data were analysed with a Cox proportional hazards model using the `coxph` function of the 'survival' package (Therneau 2014). The observed events, i.e. oviposition, rejection and returns to the oviposition puncture, were included as time dependent covariates using the counting process of the Cox model (Haccou et al. 1991; Therneau & Grambsch 2000). The model assumptions were checked following the procedure described by Fox (2011).

3.3. Results

Out of 80 observations 50 *Fopius arisanus* achieved a total of 576 successful oviposition events with only two occurrences of superparasitism. The criteria of leaving the patch for more than 30 s seemed sufficient since 45 wasps just flew away and settled elsewhere, while short excursions off the patch occurred in seven observations only.

Table 3-2: Logit regression to estimate functional response type. Maximum likelihood estimates and F-statistics of logit regression on the proportion of parasitized host eggs on log-transformed host densities.

	Model 1			Model 2			Model 3		
	df	F	P	df	F	P	df	F	P
F statistic	46	0.05	0.813	47	3.66	0.062	48	9.07	0.004*
Parameter	Estimates	SE	P	Estimates	SE	P	Estimates	SE	P
N_0	5.384	5.30		4.231	1.81		1.175	0.54	
$\log(N_t)$	- 3.728	5.60	0.487	- 2.448	1.13	0.021*	- 0.421	0.14	0.003*
$\log(N_t^2)$	0.750	1.88	0.683	0.311	0.17	0.056			
$\log(N_t^3)$	- 0.047	0.20	0.812						

The functional response

After eliminating the non-significant parameters from the logistic regression model the intercept N_0 and the linear parameter $\log(N_t)$ (GLM with binomial error distribution corrected for overdispersion: $d = 1, \chi^2 = 9.1, P = 0.003$) remained in the model (Table 3-2). The negative estimate for the linear predictor indicates a type II functional response, with the proportion of hosts parasitized decreasing with increasing host density (Figure 3-1).

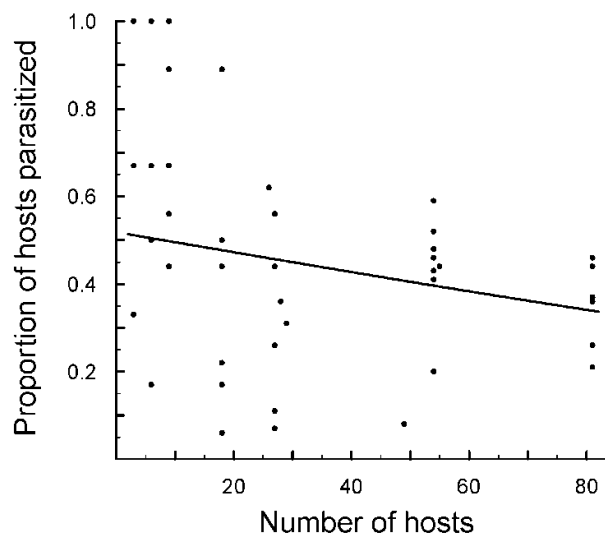


Figure 3-1: The observed proportion of hosts parasitized by *F. arisanus* at different egg densities of *B. invadens* with the fitted regression line.

Table 3-3: Parameter estimates of non-linear least square regression for eqns 1 and 3. The handling time was set to zero ($b=0$).

Parameter	reduced random parasite equation			reduced modified equation		
	Estimate	\pm SE	P	Estimate	\pm SE	P
a'	$7.0E^{-5}$	$\pm 5.4E^{-6}$	$< 2E^{-16}$	0.0003	± 0.00012	0.017
r_{max}	–			42.73	± 9.308	$3.3E^{-5}$
r_s	–			0.9824	± 0.008	$< 2E^{-16}$
summary	RSS		AIC	RSS		AIC
	1757.8		328.9	1125.0		305.6

For both, eqns 1 and 3, the parameter estimate for b ($b = a'T_h$) was not significant (Non-linear regression; eqn 1: $P = 0.17$; eqn 3: $P = 0.9$). Consequently b was removed from the models. The parameter estimates for the reduced models are shown in Table 3-3. The residual sum of squares (RSS) for the reduced models show that the model modified to account for a refuge explains 35% more variance than the random parasite equation (eqn 1: RSS = 1757.8; eqn 3: RSS = 1125.0) and thus best fitted the observed data. The fit of the reduced modified model is shown in Figure 3-2.

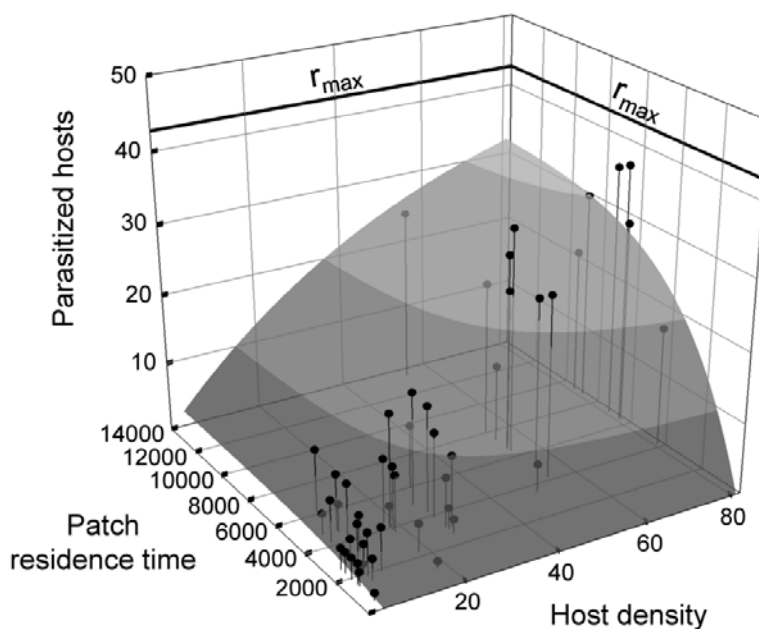


Figure 3-2: Functional response of *F. arisanus*. Circles depicts observed data and the grey area shows the fitted regression surface of eqn 3 (Type II functional response caused by a host refuge) with: $r_{max} = 42.73$, $r_s = 0.9824$, $a' = 0.0003$ and $b = 0$.

The observed parasitism deviated noticeable from the parasitism that would be expected from a random search considering the observed encounter rates with hosts. The curves of the observed versus the expected parasitism in Figure 3-3 showed that the observed increase in parasitism tended to be at a lower rate than for the expected parasitism except for the host density of six fruit fly eggs.

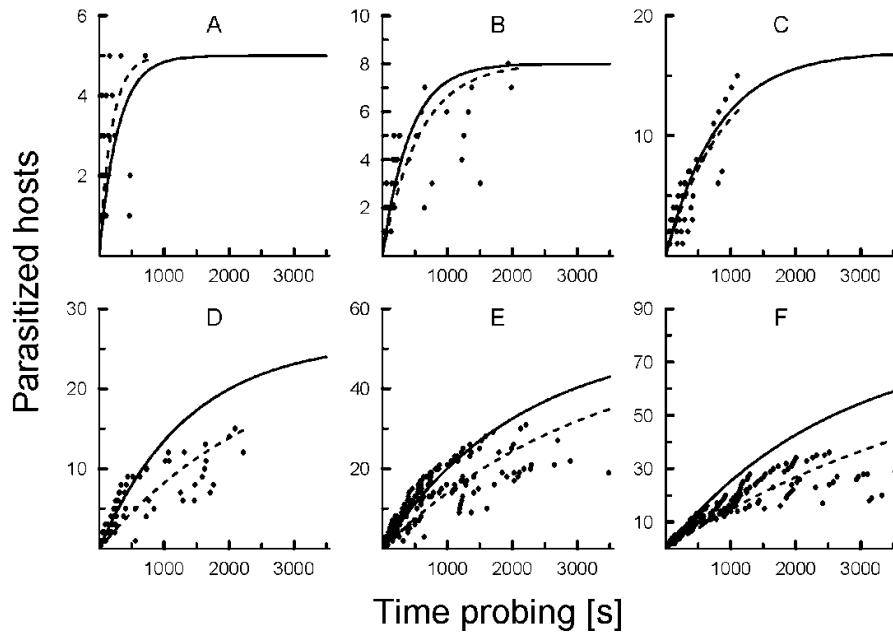


Figure 3-3: Relationship between the cumulative number of host eggs parasitized and the cumulative time parasitoids spent searching between ovipositions at six host densities: A = 6, B = 9, C = 18, D = 27, E = 54, F = 81. Circles are the raw data, standardized by setting the first oviposition of each female to zero. The solid line represents the expected cumulative parasitism if no spatial refuge is present and the dashed line depicts the fitted non-linear regression of the observed oviposition events.

The patch residence time

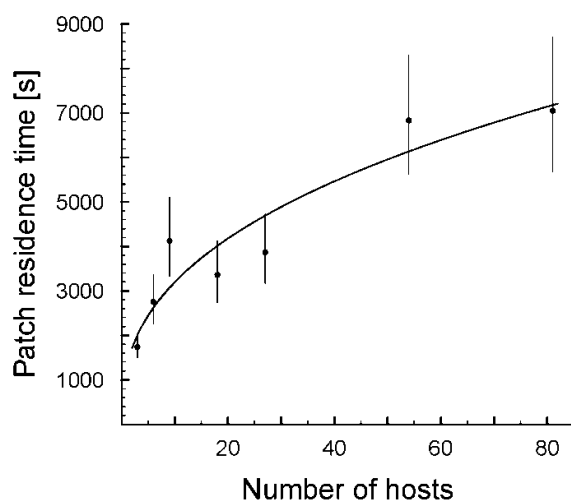


Figure 3-4: Patch residence time of *F. arisanus* at different densities of *B. invadens* eggs. Circles are the mean estimates (\pm SE) for the seven tested densities and the line represents the regression fit.

The effect of host density on the patch residence time was analysed with a GLM using a Gamma distribution and a log link function. Females of *F. arisanus* spent more time on patches with higher host densities (Likelihood ratio test: $\chi^2 = 53.3$, $df = 1$, $P < 0.001$; Figure 3-4). To detect the mechanisms involved in patch time allocation by female wasps the observation data were analysed using a Cox proportional hazards model with three time-dependent covariates and their interactions: oviposition, host rejection and returns to oviposition puncture.

Thus, the full model consisted of 7 parameters and was reduced stepwise until all parameters within the model were significant. The minimum adequate model included all three covariates (Likelihood ratio test: $\chi^2 = 78.1$, $df = 3$, $P < 0.001$) while no significant effect was found for any of their interactions. The model diagnostics regarding proportional hazards, influential data points and nonlinearity in the relationship between the log hazard and the covariates showed no major discrepancy in the model assumptions. The estimated effects of the significant parameters are shown in Table 3-4 whereby a hazard ratio ($\exp(\beta)$) above one denotes an increase and a value below one indicates a decrease in the female's tendency to leave a patch. Ovipositions as well as rejections had a decremental effect on the leaving tendency of *F. arisanus*. A single oviposition decreased the tendency to leave by a factor of 0.93 and thus had a slightly stronger effect than host rejection with a hazard ratio of 0.96. In contrast, anytime females returned to an oviposition puncture the risk of leaving increased by a factor of 1.52.

Table 3-4: Cox proportional hazards regression. Estimated regression coefficients (β), hazard ratios ($\exp(\beta)$), standard error ($SE(\beta)$) and likelihood ratio test of covariates.

covariates	β	$\exp(\beta)$	$SE(\beta)$	χ^2 (d.f.)	P
(1) oviposition	-0.074	0.92	0.027	8.36 (1)	0.004
(2) host rejection	-0.037	0.96	0.012	9.90 (1)	0.002
(3) returns to oviposition puncture	0.424	1.52	0.060	43.46 (1)	4.3E ⁻¹¹
(1) * (2)	0.0016	1.00	0.001	2.12 (1)	0.15
(1) * (3)	-0.0035	1.00	0.013	0.07 (1)	0.79
(2) * (3)	0.0036	1.00	0.003	1.45 (1)	0.23
(1) * (2) * (3)	-0.0005	1.00	0.0004	1.25 (1)	0.26

3.4. Discussion

The functional response of *Fopius arisanus* on mango domes infested by *Bactrocera invadens* was inverse density dependent and thus displays a type II functional response. Wang and Messing (2003b) found a similar relationship when exposing egg clusters of *Ceratitis capitata* on papaya fruits to *F. arisanus*, while they found a constant rate of parasitism on *C. capitata* in coffee berries (Wang & Messing 2003a). Despite the different fruits used in their experiment the different results may be mainly caused by the relative fruit size and thus the smaller range of tested egg densities in coffee berries. Results presented by a field study on interactions between four parasitoids also suggested an increased efficiency of *F. arisanus* at low host densities (Purcell *et al.* 1998).

Although, type II functional responses have often been attributed to the fact that parasitoids parasitism success is constrained by the handling time (Holling 1959b; Getz & Mills 1996) this assumption is restricted. In most cases the proportion of time spent handling hosts to the lifespan of a parasitoid is negligible in the field and thus, renders handling time rather unimportant (Hassell & May 1973; Hassell 1978). Fitting parasitism data with negative dependency towards host density for a fixed time to the random parasite equation will inevitably produce a type II response through the parameter handling time. However, it remains questionable to, which extend the equation parameter handling time responds to the biological process of handling hosts by parasitoids (Hassell 1978; Abrams 1990). It has been discussed by several authors that other mechanisms, e.g. interference (Hassell 2000b) or adaptation of foraging time (Abrams 1990) may lead to similar response patterns as the handling time and thus offer alternative explanations to type II responses. The presented study tested individual females so that interference with conspecifics can be excluded.

In contrast to most functional response studies we used a variable time approach, hence parasitoids could compensate for cumulative longer handling times at higher host densities by prolonged patch residence times. *Fopius arisanus* adapted the time spent on patches to the local host density by extending their patch residence time with increasing host numbers as expected by optimal foraging theory for time limited parasitoids (McNair

1982; Wajnberg *et al.* 2006). This pattern had been reported for *F. arisanus* attacking *C. capitata* (Wang & Messing 2003a) and *Anastrepha* spec. (Montoya *et al.* 2009). Nevertheless, the increase in the patch residence time could not fully compensate for the increase in host density thus the proportion of parasitized hosts decreased. Therefore, the results support the assumption that foraging decisions i.e. patch time allocation, can induce a functional response type II. This was further supported by the improved fit of the model with the handling time removed and hence, the patch residence time presented a better explanation for the observed parasitism by *F. arisanus*.

Hosts inaccessible for parasitoid attack potentially alter the functional response depending on the absolute abundance of hosts outside the spatial refuge. The inclusion of a spatial host refuge substantially improved the explanatory power of the functional response model. The data suggest that the decrease in the relative parasitism of *F. arisanus* with increasing host densities is considerably affected by a host refuge. A similar explanation was given by Wang & Messing (2003b) who found that the number of egg clusters that were not entirely parasitized increased with size of the clusters. From the parasitoids perspective the actual patch quality is reduced. This may further lead to a decreased efficiency of a parasitoids foraging strategy depending on the used information to assess the initial motivation to stay on a patch. Parasitoids searching in habitats with partial refuges should possess strategies in order to optimize their lifetime fecundity especially if hosts can be detected but not reached (White & Andow 2007).

Females facing time limitation should avoid to waste time searching for hosts in an explicit refuge. Thus, effects of the within patch experience may provide an appropriate tool to ensure that female wasps leave a patch if the rate of ovipositions to rejections decreases. Female *F. arisanus* seem to use an incremental mechanism for host encounters (oviposition and rejection) whereby each encounter results in a decreased tendency to leave the current patch. This mechanism seems straight forward for oviposition events in healthy hosts and has been shown earlier for other parasitoids, e.g. *Anaphes victus* (van Baaren, Boivin & Outreman 2005) and *Leptopilina heterotoma* (Haccou *et al.* 1991; Varaldi *et al.* 2005). To enhance the searching effort within a patch upon successful host encounters is adaptive for parasitoids in a heterogeneous environment (Iwasa, Higashi & Yamamura 1981; van Alphen, Bernstein & Driessen 2003).

The incremental mechanism for host rejections is rather surprising, especially since *F. arisanus* possesses a highly sensitive discrimination behaviour against previously parasitized hosts (Wang & Messing 2008). The opposite mechanism has been repeatedly assumed to be adaptive as rejections indicate a decreasing patch quality hence patches should be abandoned earlier (Hemerik, Driessen & Haccou 1993; van Alphen, Bernstein & Driessen 2003; Wajnberg *et al.* 2003; Kolss, Hoffmeister & Hemerik 2006). The fact that the effect of rejections on the tendency to leave is smaller than compared to the effect of ovipositions supports that *F. arisanus* discriminates between these two behavioural events. Even though rejections cause an increment in patch time this increment decreases when the proportion of rejections to oviposition increases. A similar mechanism for the effect of oviposition and rejection has been shown in the leafminer parasitoid, *Opius dimidiatus* (Nelson & Roitberg 1995). While the adaptive value of acquiring information on patch quality through oviposition experience is quite well understood theoretical and empirical, investigations on the value of encounters with parasitized hosts are still scarce. In a simulation experiment Kolss, Hoffmeister & Hemerik (2006) demonstrated that in most situations a patch leaving strategy with consideration of parasitized hosts is marginally superior to a strategy where no information is gained from encounters with parasitized hosts. However, an incremental mechanism of host rejections on patch residence time has not been shown to optimal, yet. With exception of an elaborate study on the genus *Trichogramma* by Wajnberg *et al.* (2003) investigations of the effect of rejections are few (see Wajnberg 2006, for review). Thus, more studies on the effect of rejections on the patch residence time in different parasitoid species are needed to make assumptions about the potential value of an incremental mechanism. In this study no differentiation between rejecting parasitized or unparasitized hosts was possible and host eggs may be rejected for several reasons besides parasitism, e.g. sterile eggs or eggs of a less favourable age. Encountering such unparasitized but otherwise unsuitable hosts may still indicate healthy hosts nearby.

The leaving tendency of female wasps increased with the number of returns to the oviposition puncture hence *F. arisanus* uses a decremental mechanism on the patch residence time. This is in agreement with previous findings of successive visits to the same patch in *F. arisanus* (Wang & Messing 2003a) and other parasitoid wasps (Haccou *et*

al. 1991; Outreman *et al.* 2005). This mechanism may prevent the parasitoids from wasting time in previously exploited patches. The observations suggest that *F. arisanus* exhibits different mechanisms based on within patch experiences to adjust its patch residence time to varying host densities, which in turn affects its functional response.

In contrast to the handling time the alternatively considered mechanisms, i.e. foraging time and spatial host refuge, as explanations for density dependent parasitism may play an important role as limiting factors to the efficiency of *F. arisanus* in the field. The existence of spatial refuges should depend on the structure of the fruit fly community in e.g. fly species and abundance and, the available host plants in the environment. In most regions the structure of the fruit fly community underlies seasonal and annual changes (Aluja *et al.* 2012; Vargas *et al.* 2012b) hence being a generalist comes with enormous challenges. The size of egg clusters of fruit flies within smaller fruits is limited by the available resources for larval development, which in turn reduces the number of host eggs that are in an explicit refuge from parasitoid attack. This may explain the differences in parasitism when comparing hosts in coffee berries to hosts in papaya or mango. The first obstacle for *F. arisanus* in the field is to detect hosts inside different plant species through olfactory location (Rousse *et al.* 2007). The second is to optimize its foraging success under the special conditions of each fruit habitat, which requires a very flexible adjustment of behaviour. This study suggests that *F. arisanus* apparently uses several mechanisms to handle the problem of a complex environment.

4

Mutual interference in *Fopius arisanus*

4.1. Introduction

Parasitoids that search an area at the same time often interact directly or indirectly, which frequently results in a reduction of the parasitisation success. With increasing parasitoid density, and thus a higher frequency of interactions, the negative effect on the parasitisation rate gets stronger. This reduction of the attack rate due to interference with other individuals has been reported for several parasitoid species from cage and semi-field studies (Hassell 1978; Fernández-Arhex & Corley 2010; de Jong *et al.* 2011). Yet, to estimate how interference among parasitoids constrains host suppression in the field is rather complex, hence reliable estimates are rare (Hassell 1978). Broadhead & Cheke (1975) report that the efficiency of the parasitoid *Alaptus fuscus* attacking eggs of *Mesopsocus unipunctatus* in the field is considerably reduced due to mutual interference. Several field studies also support that mutual interference essentially affects the interaction of predator-prey/ host-parasitoid systems in nature (Reeve 1997; Jost *et al.* 2005; Spataro *et al.* 2012; Liljesthröm, Cingolani & Rabinovich 2013). Thus, interference in host-parasitoid systems may considerably alter the dynamics of host and parasitoid populations.

Many theoretical studies focus on the processes that enhance the stability of host-parasitoid systems. Among others (e.g. parasitoid and host distribution, refuges), interference has been demonstrated to enhance system stability in otherwise unstable host-parasitoid populations (Hassell & May 1973; Beddington, Free & Lawton 1978). Stability in host-parasitoid interactions allows that species co-occur in naturally existing systems for prolonged periods. However, in the scope of biological control Murdoch *et al.* (1985) argue that system stability is not a necessity for successful control of a pest species. The searching efficiency of a parasitoid is often considered to be closely linked to the success of biological control agents (Waage & Hassell 1982; Ives 1995; Mills & Wajnberg 2008). Therefore, studies concerned with applied problems should have a higher focus on processes leading to changes in the searching efficiency and the degree of their impact.

The change in the searching efficiency of a parasitoid due to the simultaneous exploitation of a specific area by conspecifics is commonly referred to as interference.

This can be induced by any direct behavioural response that alters the proportion of time spent searching within a patch. For example, a parasitoid that encounters a conspecific may invest time in aggressive behaviour, e.g. fighting or chasing (Lawrence 1981), which leads to a reduced search time. Such an effect is termed direct mutual interference (Free, Beddington & Lawton 1977; Visser, Jones & Driessen 1999). The time spent with activities others than searching due to another wasp exploiting the same area is lost for searching and hence, decreases the searching efficiency of a parasitoid (Hassell 1978). Thus, direct mutual interference is marked by changes of the searching efficiency in patches of varying parasitoid densities (Visser, Jones & Driessen 1999).

The searching efficiency can be estimated as a function of the number of hosts parasitized per unit time. Thus, changes of the searching efficiency may result from: A) time intensive behaviours that lead to a reduction of the relative search time on the patch or, B) alterations in the time wasps allocate to a patch due to conspecifics.

Many of the direct behavioural activities related to conspecifics, e.g. antennating, fighting or chasing, usually last only few seconds. Hence, the temporal costs of such activities may be considered of minor importance for the searching efficiency because the proportion of lifetime “wasted” upon them may be negligible (Free, Beddington & Lawton 1977; Hassell 1978). Changes of a female’s intrinsic motivation to stay on a patch upon encounter with conspecifics/conspecific cues, however, may be more pronounced. A parasitoid encountering conspecifics on a host patch may prolong the time allocated to the current patch, e.g. as a consequence of guarding behaviour. In contrast, a female wasp encountering a conspecific on a patch may leave in order to search for unexploited patches. The decision to leave results in an increase of the proportion of time spent traveling and/or in a change of the parasitoids distribution pattern (Visser & Driessen 1991; Visser, Jones & Driessen 1999). This change of the searching efficiency due to a change of inter-patch activities is termed indirect mutual interference (Visser, Jones & Driessen 1999). Although, indirect mutual interference leads to an unfavourable reduction of the searching efficiency on the one hand, it may enhance the parasitoid dispersal rate on the other.

Compared to mutual interference, where the change of the searching efficiency stems from behavioural interactions among parasitoids, pseudo-interference arises from parasitoid aggregation (Free, Beddington & Lawton 1977). In patchily environments parasitoids tend to allocate more time for searching in areas of higher host densities and thus underexploit patches of lower host densities. However, the distribution of parasitoids remains the same at all parasitoid densities and hence is not a behavioural response towards conspecifics.

The distinction of the causes that lead interference may be of minor interest from a population's point of view, but essentially contributes to the understanding of the direct mechanisms that shape population processes (Visser, Jones & Driessen 1999). Many of the existing models have been shown to adequately describe population dynamics in the field, but attempts to use them as predictive tools for applied problems is prone to failure. A detailed knowledge is needed of the proximate mechanisms that shape individual behaviour and how this in turn is reflected at the population level. The diversity of mechanisms that can trigger population dynamics suggests that there is not a single model that serves as an ultimate solution. Thus, the choice between several models should be based on the mechanisms that drive the dynamics of the population in question.

This chapter addresses the role of and the proximate mechanisms underlying mutual interference among females of *Fopius arisanus* searching for eggs of its host, *Bactrocera invadens*.

A multi-patch experiment was conducted to investigate the effect of interference on the searching efficiency of *F. arisanus*, searching in groups or alone. To investigate the potential influence of the spatial distribution of resources on the searching efficiency, the hosts were distributed either aggregated within one egg batch or equally among four egg batches (hereafter, referred to as aggregated and uniform, respectively). The searching efficiency of *F. arisanus* was expected to decrease with increased numbers of parasitoids searching the area. Furthermore, this effect was anticipated to be stronger in trials with females searching an aggregated host patch, and thus a higher probability of encounters among parasitoids, than compared to trials with the uniform distribution.

To maximize the fitness gain parasitoids, which search in groups and hence face interference, should adjust the time they spent searching a patch. Under competition a female wasp may respond in two ways: A) she prolongs her stay on the patch to defend her offspring or to defend the remaining resources and B) she leaves in order to find unexploited patches. The decision for one of the two strategies should depend on the previous investment and the anticipated future fitness gain on this patch. To optimize their behaviour, in a heterogeneous environment, female wasps have been shown to use past and current experiences to reach their decisions under several circumstances (van Alphen, Bernstein & Driessen 2003; Thiel & Hoffmeister 2004; Outreman *et al.* 2005; Koppik, Thiel & Hoffmeister 2014). A model presented by Waage (1979) and advances of statistical tools, like the Cox proportional hazards regression, led to a recent interest in the study of the proximate mechanisms that are involved in decision making by animals and humans (Wajnberg 2006; Hutchinson, Wilke & Todd 2008; Louâpre, van Alphen & Pierre 2010).

The perception of the patch quality has been shown to be of major importance for patch time decisions (van Baaren, Boivin & Outreman 2005; Liu, Thiel & Hoffmeister 2009). The experiences wasps gather while searching a patch strongly affects the perception of the patch quality, which in turn influences the decision on time investment for this patch (see Wajnberg 2006 for a review). The results presented in the third chapter suggest that the patch residence time (PRT), the time wasps spent on a host patch, in *F. arisanus* depended on host encounters. Females were sensible to cues that served as indicators of the patch quality. This is in line with previous findings by Wang and Messing (Wang & Messing 2003a), who studied the effect of ovipositions on PRT in *F. arisanus* attacking *Ceratitis capitata*. Furthermore, the presence of conspecifics has been shown to effect the time female parasitoids spent on a patch in other braconid wasps (Hassell 1971; Visser, Alphen & Nell 1990; Goubault *et al.* 2005). I hypothesized that *F. arisanus* uses contacts with conspecifics to gather information about the current patch and adjust their PRT thereafter.

According to game theory females facing competition should enter into a “war of attrition”, especially in species where superparasitism commonly occurs (Haccou, Glaiot & Cannings 2003; Wajnberg, Curty & Colazza 2004). In solitary parasitoids a host can only

yield one offspring, hence leaving a patch while superparasitizing conspecifics are still present may incur high fitness losses. Consequently, remaining on the patch until all other wasps have left may lead to a considerable payoff for the remaining wasp. *F. arisanus* rarely superparasitizes hosts, but host eggs that are probed more than once seem to suffer a higher mortality rate (Calvitti *et al.* 2002; Wang & Messing 2003b). Thus, females of *F. arisanus* that have already laid eggs on the patch may suffer severe fitness costs by conspecifics exploiting the same patch. I expected that, females, which have previously invested in the current patch “defend” it from conspecifics and therefore, extend their PRT upon encounters with other wasps. On the other hand, I expected that wasps respond to encounters with conspecifics by abandoning the patch, if they have not encountered hosts before.

4.2. Material and methods

An experiment with a multipatch area (Figure 2-4) was conducted to study the mutual interference among parasitoids simultaneously searching for hosts in areas with aggregated or uniform host distribution.

Patch preparation

Mango domes were used to simulate fruit patches that are either infested by *Bactrocera invadens* or free of host eggs. Circular pieces (diameter = 4.0 ± 0.5 cm) were cut from ripe mango fruits, *Mangifera indica* var. Apple. The fruit flesh was removed to obtain a dome with a very thin layer of flesh under the fruit skin. The fruit domes were pierced at the centre and depending on the treatment twenty, eighty or no host eggs were placed in the immediate vicinity of the artificial puncture. The prepared patches were placed inside Petri dishes to keep the mango peel fresh and stored in an incubator until use. To decelerate host development the incubator was set at 20 ± 1 °C.

Wasp preparation

On day four post emergence female *Fopius arisanus* were chilled on ice and marked using nail polish. After the marking procedure females were kept individually in glass vials (2.1 x 7.0 cm) provided with a streak of honey and moistened cotton wool. One day prior to the experiment females were placed in cohorts of four, two or one on top of a fruit patch infested by twenty host eggs to facilitate foraging experience. The females were given experience depending on the designated wasp density within the observation. The parasitoids were allowed to attack a host puncture once and the experience period was stopped after the female stopped probing the puncture or after one hour. Females that were given experience in groups were observed to identify the female laying the eggs. To ensure that all wasps, which were used in the observations were experienced the host eggs were dissected and checked for parasitoid eggs. Only wasps that laid more than one egg were used for the experiments.

Bioassay

Plain Plexiglas cages with four fruit patches served as observation arena. The setting of the observation arena is shown in (Figure 2-4). A rectangular template, which was placed

above the mango domes ensured that the total fruit surface area and the distance between domes was kept constant among all observations. A total of 80 host eggs was distributed among four fruit patches, either A) aggregated: one dome infested with one cluster of 80 eggs and three host free domes, or B) uniform: four infested patches with one cluster of twenty eggs per dome.

Seven day-old females of *F. arisanus* were transferred into a plastic cap, such that females (one, two or four females) could be simultaneously released into the arena. The cap was closed with Parafilm and placed in the centre of the arena. Before opening the release cap, wasps were allowed to settle for about five minutes. The observation period started with the first wasp entering one of the mango domes. The females were removed from the observation arena with an aspirator, after leaving the fruit patch area for more than five consecutive minutes. Therefore, the observation was terminated after the last wasp spent more than five minutes off the patches. The fruit fly eggs were dissected subsequent to the experiments to determine the number of parasitoid eggs laid.

Table 4-1: Observed behaviour. The duration and frequency of the parameters listed in the table were recorded for each wasp within a trial.

Behaviour	Description
I) patch residence time	the time wasps spent from the first entry on a fruit patch until leaving the fruit patch area for more than five consecutive minutes (duration only)
II) patch encounters	all visits of the mango domes in the fruit patch area
III) probing	the time wasps spent probing the oviposition puncture by inserting the ovipositor into the oviposition puncture and moving the abdomen up and down (Wang & Messing 2003a)
IV) host encounters	events where the ovipositor was inserted into an oviposition puncture and the parasitoid remained motionless in a distinct pose for more than three seconds and the female's antennae are raised in an acute angle as described by Wang & Messing (2003a)
V) encounters with conspecifics	The direct contact of any body parts of two females (frequency only) a) contacts without previous oviposition: the focal wasp had not laid an egg since the start of the observation until the time of the contact b) contacts with previous oviposition: at the time of the contact event the focal wasp had already oviposited into a host egg

The parameters recorded using the event-recording software *The Observer* (Noldus 1991) are listed in **Fehler! Verweisquelle konnte nicht gefunden werden.** The wasps displayed similar behaviour for ovipositions and rejections, which mainly differ in the duration of the event. Therefore, these two behavioural categories have been recorded as host encounters and have been classified subsequent to the observation. To do so all events of host encounters on one fruit patch were ranked by time and the events with the highest times taken as ovipositions so that the number of events corresponds to the counted number of parasitized eggs. The effect of contacts with other females was expected to vary with different oviposition experiences in the current patch area. To account for this difference all contact events were subsequently categorized as: Va) contacts without previous oviposition and Vb) contacts with previous oviposition.

Determination of Mutual interference

The search rate a' is a measure of the fraction of the total area that is searched by a parasitoid in one unit of time. A reduction of a' as a result of increased parasitoid density indicates interference among parasitoids. The search rate was estimated, using the equation first proposed by Hassell (1978), as follows:

$$a' = \frac{A}{T_{P_i}} * \ln \frac{N}{N - N_a} \quad \text{eqn 5}$$

where A is the total area available for search, which was kept constant for all observations and thus was taken to be 1. T_{P_i} denotes the total time the i th parasitoid spend searching the fruit patch area until leaving. N and N_a are the total number of hosts within the fruit patch area and the number of hosts parasitized at the end of the observation, respectively.

Statistical analysis

To estimate the interference coefficient m the relationship between the search rate ($\text{Log}_{10} a'$) and parasitoid density ($\text{Log}_{10} P$) was analysed by a linear model. To test for differences in this relationship due to the host distribution, the interaction term between the log of parasitoid density and the host distribution was included in the analysis.

The effect of the parasitoid density and the host distribution on the total number of hosts parasitized in a trial was analysed with a zero-altered negative binomial (ZANB) for zero inflated data with overdispersion in the count part (Zuur *et al.* 2009). It is not surprising that trials with single wasps more often resulted in zero parasitism than trials with two or four searching wasps. To exclude observations without parasitism from the analysis would lead to an overestimation of the parasitism, especially in trials with only one

Box 4-1 | ZANB models (zero-altered negative binomial models)

In their book “Mixed effects models and extensions in ecology with R” Zuur and colleagues (2009) provide a detailed review on models for zero-inflated data. Here, I will only give a brief summary on ZANB models.

Count data in ecological research often contain many zeroes. The Poisson or negative binomial data distribution of samples with a small sample mean contain zero counts within the data. However, if the frequency of zeroes exceeds the expected zero frequency for a Poisson or negative binomial distribution one speaks from zero inflation. Several models exist to deal with zero-inflated data that differ in their assumption on the data distribution in the count part and the assumption about the sources for the observed zeroes. The ZANB model tests for the covariates that best explain the probability to observe a zero but it does not assume different sources for zeroes.

The ZANB models are also commonly referred to as hurdle models or two-part models. ZANB models simply consist of two parts:

Part I: In a first step, the ZANB analyses the probability that a zero is observed with a binomial model that considers the data as zeroes and non-zeroes. In the simplest case the binomial model is an intercept only model but it can also contain covariates.

Part II: Secondly, a truncated negative binomial model is used to analyse the non-zero observations. The negative binomial model for the count part should be used when the count data excluding the zeroes are overdispersed.

A comparison of the ZANB model to the output of a binomial GLM and of a negative binomial GLM should reveal the same parameters. The advantage of the ZANB model over separately fitting a binomial GLM and a negative binomial GLM is that you obtain one AIC value for both parts.

female wasp. To prevent this bias a ZANB model was used to deal with the zeros in the data (see Box 4-1).

The Cox proportional hazards model was used to test for the effects of the within-patch experiences on the patch residence time. Oviposition, rejection, probing and contact with or without previous oviposition experience were included as time-dependent covariates. The host distribution was entered as time-independent covariate into the model.

4.3. Results

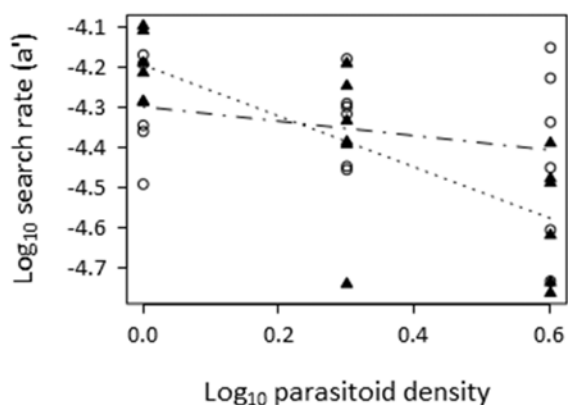


Figure 4-1: The relationship between the searching efficiency ($\text{Log}_{10} a'$) and parasitoid density for all trials where parasitism occurred. The filled triangles and hollow circles present the estimated searching efficiency for aggregated and uniform host distributions, respectively. The dotted and the dotted-dashed line show the predicted relationship for aggregated and uniform host distributions, respectively.

When comparing the search rate of *Fopius arisanus* females that search on mango patches alone or in the presence of other females, it becomes evident that in trials with successful oviposition events the search rate ($\text{Log}_{10} a'$) decreased with increasing parasitoid density ($\text{Log}_{10} P$) Figure 4-1. For the tested parasitoid densities a linear relationship best explained the observed results. The negative estimates for the interference coefficient ($m_{\text{aggregated}} = -0.64$;

$m_{\text{uniform}} = -0.18$) suggest interference between females searching simultaneously. The interference was stronger for wasps searching in patch areas with hosts aggregated on a single mango dome than in patch areas with uniform host distribution across the four mango domes (LM, parasitoid density \times host distribution: $F_{1,31} = 4.66$, $P = 0.034$).

The ZANB model was used to analyze the influence of parasitoid density and host distribution on the total number of hosts parasitized within trials. Both parameters, i.e. host distribution and parasitoid density, influenced the total number of parasitized hosts for trials with parasitism (count part of the model), whereas the proportion of trials with no parasitism (logistic part) was only influenced by parasitoid density. The proportion of zero-observations decreased with increasing parasitoid density (logistic part of the ZANB model, parasitoid density: $\chi^2 = 12.433$, $df = 1$, $P < 0.001$), regardless of the host distribution in the fruit patch area (logistic part of the ZANB model, host distribution: $\chi^2 = 2.2992$, $df = 1$, $P = 0.13$) (Figure 4-2A). However, the number of hosts parasitized by *F. arisanus* at different parasitoid densities was affected by the host distribution (count model, parasitoid density \times host distribution: $\chi^2 = 10.246$, $df = 1$, $P < 0.002$). At patches with uniformly distributed hosts, parasitism increased with increasing parasitoid density,

whereas parasitism decreased under the aggregated host distribution Figure 4-2B. Combining the count and logistic part of the model the parasitism by *F. arisanus* showed an almost linear increase of hosts parasitized when hosts were uniformly distributed across the four mango domes and a curvilinear relationship that levels off at densities above two parasitoids when host eggs were aggregated in a single mango dome Figure 4-2B. Despite the interference of wasps, the total number of host eggs parasitized increased with increasing parasitoid density, at least on uniformly distributed hosts (Figure 4-2B).

Table 4-2: Cox proportional hazards regression. Estimated regression coefficients (β), hazard ratios ($\exp(\beta)$), standard error ($SE(\beta)$) and likelihood ratio test of time-independent and time-dependent covariates in the Cox proportional hazards model.

Covariates	β	$\exp(\beta)$	$SE(\beta)$	χ^2 (d.f.)	P
Effect of time-independent covariates					
(1) Distribution uniform	- 0.8107	0.4446	0.2403	12.01 (1)	< 0.001
Effect of time-dependent covariates					
(2) Oviposition	- 0.0981	0.9065	0.0210	23.16 (1)	< 0.001
(3) Rejection	- 0.0242	0.9761	0.0084	8.73 (1)	0.003
(4) Probing	0.1029	1.1084	0.0444	5.16 (1)	0.023
(5) Contacts with previous ovipositions	- 0.1175	0.8892	0.0588	4.48 (1)	0.034
(6) Contacts without previous oviposition	0.4419	1.5556	0.0960	0.08 (1)	0.773
(7) Contacts without previous oviposition:time	- 0.0036	0.9999	0.00003	24.85 (1)	< 0.001

Among all the observations were 57 females that did not lay any eggs during the observation period. Since, so many females left the patch without laying eggs it was tested, if females in patches of higher parasitoid densities have an increased tendency to leave the patch without oviposition and if this effect is stronger on aggregated patches. The comparison of the probability of females to leave the fruit patch area without laying eggs showed no significant effect of parasitoid density (LME: $df = 2$, $P = 0.997$). However, females foraging the aggregated patches exhibited a significantly lower probability to oviposit than females foraging the patches with uniformly distributed hosts ($Estimate_{aggregated} = 0.33$, $Estimate_{uniform} = 0.67$; LME: $df = 1$, $P < 0.001$) (Figure 4-3).

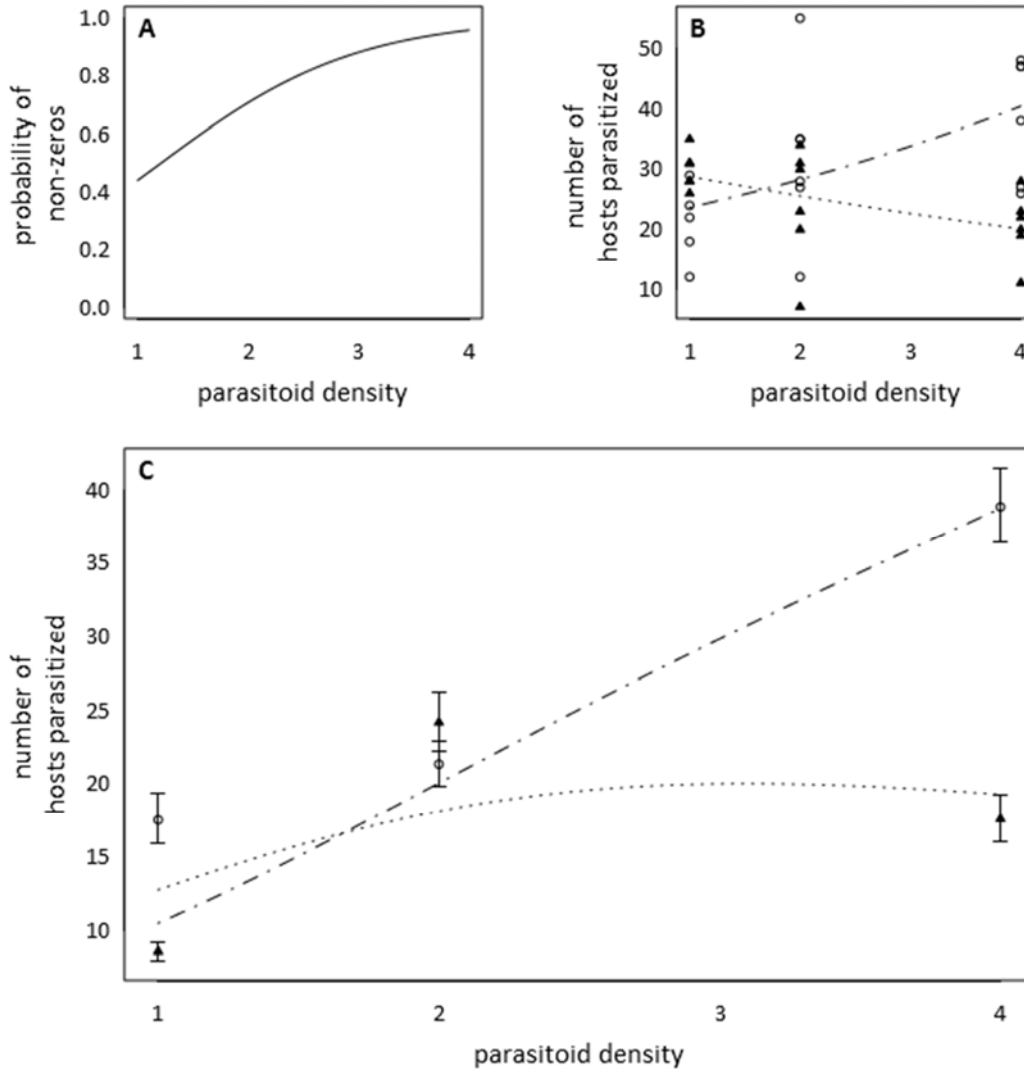


Figure 4-2: Parasitisation response per trial at different parasitoid densities. A) The probability of parasitism, B) the number of parasitized hosts in trials with parasitism, thus excluding zero counts (raw data and regression lines) C) the number of parasitized hosts for all trials (means \pm SE and regression lines). The dotted and dotted-dashed lines in B and C depict the predicted parasitism for the “aggregated” and “uniformly” distributed hosts, respectively; filled triangles correspond to the “aggregated” and hollow circles to the “uniform” host distribution.

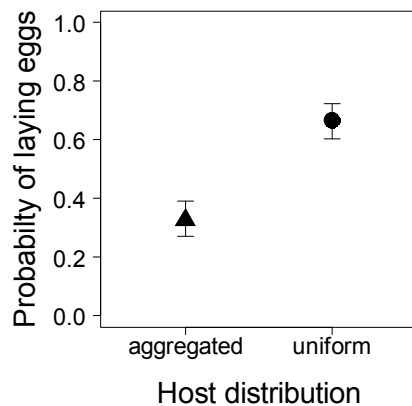


Figure 4-3: Estimated probability that females oviposit before leaving the fruit patch area at different host distributions.

When analysing the patch time allocation of foraging wasps with Cox proportional hazard models, it became obvious in the model diagnostics of hazards that the hazard ratio for contacts without previous oviposition events is non-proportional ($X^2 = 7.745$, $P = 0.005$) and thus violates the proportionality criterion of these models. Thus, an interaction between the covariate contacts without previous oviposition events and time was included. The statistics for the extended Cox proportional hazards model are given in Table 4-2.

The patch leaving tendency of wasps searching patches with uniformly distributed hosts was reduced by 55 % in comparison with wasps searching for aggregated hosts. The time dependent covariates are characterized by cumulative changes of their status within the foraging time of a wasp. Among these, ovipositions into hosts, rejections of hosts and contacts with conspecifics influenced the leaving tendency of wasps. Interestingly, the direction in, which contacts with other wasps affected the leaving tendency switched if wasps had oviposited earlier on this patch. The leaving tendency of searching wasps was reduced when the focal wasp oviposited into hosts, rejected hosts or encountered conspecifics after having at least one oviposition on the patch. For single events the effect was strongest for a contact with a conspecific forager and weakest for host rejections. In contrast, the first probing event increased the risk of leaving by 10.8 %. For contacts of wasps with conspecifics when the focal wasp had no previous ovipositions on the mango patch, a significant interaction between the regression coefficient for contacts and time searching on the patch was found. This shows that the coefficient itself varied over time. Therefore, the interpretation of the coefficient is more complex. The hazard ratio of 1.56 indicates that at the start of an observation the first contact without previous ovipositions increased a female's leaving tendency by 56 %. However, this initial effect decreased over time at a very low rate, which is indicated by the negative coefficient of the interaction term close to one.

4.4. Discussion

4.4.1. Mutual interference

Female *Fopius arisanus* displayed an incremental mechanism, an increase of the PRT, upon host encounters (see results of Cox regression in 3.3 and 4.3), which resulted in longer PRTs at higher host densities (see chapter 3). This mechanism helps to maximize the lifetime fitness of female *F. arisanus* as it ensures that the parasitoids search patches with high host abundance longer than patches with few hosts available. However, under high parasitoid densities, this mechanism may promote an aggregation of attacks and hence, higher rates of interference.

The observed parasitism under different competition levels showed that female *F. arisanus* interfered with each other when simultaneously searching for hosts (Figure 4-1). This is in line with previous studies on interference within many parasitoid species (Hassell & Varley 1969; Broadhead & Cheke 1975; Luo *et al.* 2014). The estimated interference coefficients were -0.18 and -0.64 at patches with uniformly distributed hosts and patches with aggregated hosts, respectively. In comparable studies on other parasitoid species, the estimates of the interference coefficients range from -0.2 to -1.2 (see Hassell 1978; Chong & Oetting 2006). Parasitoids searching the arena with the uniform host distribution faced minor interference, only. This is implied by the low interference coefficient and the almost linear increase of parasitized hosts (Figure 4-2C). In contrast, females that searched patches with highly aggregated hosts displayed an intermediate degree of interference, which led to a decelerating increase of parasitized hosts (Figure 4-2C). Thus, the spatial host distribution in this study considerably affected the degree of interference among *F. arisanus* females. The finding of interference among searching *F. arisanus* also supports that the behavioural mechanism displayed by individually searching wasps may be disadvantageous under competition. To minimize their fitness losses through competition *F. arisanus* should use additional information to compensate for the incremental mechanism upon host encounter. Females should update their assessment of a patch when encountering conspecifics and adjust their behaviour accordingly.

4.4.2. Encounters with conspecifics

Parasitoids that search environments with high parasitoid densities have to decide whether to stay on the patch or to leave (Hassell & Rogers 1972; Cheke 1974). Assuming that parasitoids adjust their behaviour according to the experienced competition, they may extend the time on the patch to either defend the resource or to defend their previous investment in offspring (Visser, Alphen & Nell 1990; Sjerps & Haccou 1994). This behaviour can lead to a war of attrition whereby two or more contestants compete for a resource by remaining on the patch and thus accumulating costs over the duration of the contest (Maynard Smith 1974). On the other hand, a higher frequency of encounters with conspecifics may lead to reduced PRTs (Hassell 1971) and therefore, to a higher proportion of time spend for traveling among patches. Both effects of conspecifics on the PRT have been shown to exist in parasitoids. The presence of conspecifics in *Leptopilina heterotoma* results in an increased PRT (Visser, Alphen & Nell 1990), while the PRT decreases in *Venturia canescens* (Hassell 1971), *Trissolcus basalis* (Wajnberg, Curty & Colazza 2004) and *Pachycrepoideus vindemmiae* (Goubault *et al.* 2005).

Female *F. arisanus* that encountered conspecifics on a patch without having laid eggs within the patch before showed an increased tendency to leave (Table 4-2). In contrast, females that encountered conspecifics after laying eggs within the patch increased the time they spent on the patch (Table 4-2). The contradictory effects of encounters with conspecifics as a function of oviposition experience in the patch suggest that *F. arisanus* not only used a variety of cues but also combined the information about the environmental cues. This indicates that there was interdependency among the events that influenced the PRT, which caused a switch from a decremental to an incremental patch time mechanism upon contacts with conspecifics. Similar observations have been made on the dependency of mechanisms for host encounters on other environmental cues or the physiological state of parasitoids. The Valence population of *V. canescens* shows a switch from a decremental to an incremental patch leaving mechanism for host encounters depending on the availability of food (Lucchetta *et al.* 2007). In females of *Aphidius rhopalosiphi* the egg load triggers a switch from an incremental to a decremental mechanism for host encounters (Outreman *et al.* 2005).

These findings indicate that the patch-leaving mechanisms for specific cues can be adjusted according to the presence of other cues. Under competition, the observed mechanism in *F. arisanus* allows for the switch of behavioural strategies depending on their previous investment in offspring. Parasitoids that encounter conspecifics have to decide between two strategies: A) to stay and exploit the current patch or B) to leave and search for unexploited patches. Under high parasitoid densities any female, always following strategy II would suffer from severe fitness costs. It would “waste” a high amount of time travelling among patches and thus reducing the foraging time on patches by simultaneously increasing the risk of predation. On the other hand, females that always apply strategy I would “waste” time on already overexploited patches. To include the successful investment on the current patch into the decision to stay or leave after the occurrence of conspecifics seems to provide a mechanism to counterbalance between fitness losses due to overexploitation and travel costs.

4.4.3. Host spatial distribution

Interactions in host-parasitoid systems highly depend on the spatial distribution of hosts. The effect of host distribution in the habitat on the degree of interference is known from several studies (Hassell 1971; Free, Beddington & Lawton 1977). The parasitoid *Alaptus fuscus* shows a higher rate of interference when searching for randomly distributed hosts compared to searching for clumped hosts (Cheke 1974).

In *F. arisanus* the effect of interference on the search rate was stronger in trials with the aggregated host distribution (Figure 4-1). In trials with an aggregated host distribution more than 60 % of the females left the patch without oviposition, compared to less than 40 % in trials with uniform host distribution (Figure 4-3). The fact that this effect was equally shown over all parasitoid densities including females searching the patch alone suggests that the effect is a result of the host distribution itself. This behaviour may lead to a higher dispersal rate in areas where interference is stronger due to host aggregation, than in areas with low interference.

Results from the exploited patches further indicate that female *F. arisanus* parasitized hosts in patches with highly aggregated hosts more efficiently than in patches with

uniformly distributed hosts. However, this relation changes when more than one female forages on a patch. In the presented study the patches with the uniformly distributed hosts were exploited more efficiently.

4.4.4. Conclusion

Considering releases of *F. arisanus* to control fruit fly populations, interference may play an important role as wasps are commonly released at high densities. Especially in habitats with highly aggregated host distributions, the aggregation of *F. arisanus* at release sites is likely to induce a locally reduced efficiency in host parasitisation rates through interference. Despite the negative interference effect displayed in the presented results, the total number of hosts parasitized in trials with two or four searching parasitoids was higher than compared to individually searching wasps. Thus, the presence of conspecifics at the tested parasitoid densities did not lead to a reduction of the total number of hosts parasitized.

Further, the results indicate that *F. arisanus* employs behavioural mechanisms to prevent high interference levels. *Fopius arisanus* showed an increased tendency to leave the patch when they encountered conspecifics and had not previously laid an egg on the patch. This behaviour may facilitate dispersal of *F. arisanus* in the field. Theoretical and empirical studies have shown that high levels of interference can result in farther dispersal of agents (Otronen & Hanski 1983; Albrechtsen & Nachman 2001; Bowler & Benton 2005). The finding that *F. arisanus* encountering another wasp on the patch without a previous oviposition, showed a decremental patch time mechanism supports that high densities may lead to higher dispersal rates. It seems likely that females searching for hosts in areas of high parasitoid density may distribute in order to reduce future fitness losses through intraspecific competition.

This study highlights the importance of a detailed knowledge of the habitat characteristics in order to develop successful biological control strategies. In the presented system, the distribution of fruit fly eggs in the habitat may be a key factor for the success or failure of introduced parasitoid, *F. arisanus*. In a homogenous environment, the variability of the host distribution can be expected to be low. In contrast, in a heterogeneous environment,

where fruit flies can inhabit areas of different structures and with different host plants the distribution pattern may vary greatly. This has to be taken into account when developing pest management strategies. The disregard of habitat structure and hence, fruit fly distribution in the field, may in part explain the failed biological control programs using *F. arisanus*.

5

General Discussion

5.1. Density dependent processes

Density dependence in host-parasitoid relationships can result from many biological processes, nevertheless the occurrence of density dependence is commonly accepted among ecologists (Beddington 1975; Lessells 1985; Abrams & Ginzburg 2000). This study was aimed at identifying the causes that led to density dependence in the response of *Fopius arisanus* on its host *Bactrocera invadens* and, hence, may influence the level of suppression achieved by *F. arisanus*. Therefore, I studied the functional response of and the effect of parasitoid density dependence caused by mutual interference on *F. arisanus*.

In line with previous studies, using different tephritid hosts (Purcell *et al.* 1998; Wang & Messing 2003b), the functional response of *F. arisanus* on *Bactrocera invadens* displayed a Type II response. Thus, an increased host density led to an increased number of eggs attacked by *F. arisanus* at a decelerating rate Figure 3-2. In Holling's (1959b) disc experiment the decelerating rate was caused by the time spent on handling a disc. It has been argued that a high proportion of the reported Type II responses in parasitoids are caused by the fixed time the insects were presented to their hosts. However, the functional response of *F. arisanus* displayed a type II response despite the variable time approach that was terminated after a wasp had left the patch. Furthermore, the results presented in chapter three suggests that in my study the handling time was of minor importance and did not considerably contribute to the decelerating parasitism rate of *F. arisanus*. This is in line with the statement that the proportion of time spend handling hosts may be negligible compared to the proportion of time available for search, especially when the functional response is estimated over a long period, e.g. the whole lifespan of a parasitoid (Holling 1961; Hassell 1978). In this study, this holds true even though the total time taken for the functional response was just the patch residence time of the wasp. A host density dependent refuge to the hosts seemed to better explain the observed parasitism (Table 3-3), which therefore, suggests that the density dependent response of *F. arisanus* was at least partly caused by a spatial host refuge. This is an important finding considering the use of *F. arisanus* for biological control (see section 5.3 below).

The experiment to study the mutual interference revealed that the search rate decreased with increased parasitoid densities Figure 4-1. The effect was rather weak in patches with the regular host distribution but in patches with an aggregated host distribution mutual interference led to an approx. tenfold reduction of the search rate when comparing a single wasp to four searching wasps. The results suggest that the drastic decrease in the search rate is at least partly caused by a higher tendency of females to migrate from such patches. This behaviour may have influences on the dispersal behaviour of *F. arisanus* and therefore, is an important characteristic for release strategies (see section 5.3 below).

Host-dependence and mutual interference seemed important components of the interactions in the studied system and both processes may potentially affect the efficiency of *F. arisanus* in the field. Furthermore, the results indicate that spatial host refuges considerably effect the parasitoid-host interaction. Future studies should address the occurrence and importance of spatial host refuges in the field. The fact that *F. arisanus* is a generalized endoparasitoid (Rousse, Harris & Quilici 2005) that attacks tephritid flies within various fruits suggests that refuges may vary greatly between different habitats, i.e. crop systems.

5.2. Behavioural adaptations

The foraging success of parasitoids, searching for resources which are subject to constant change, highly depends on their ability to use information and adapt their behaviour accordingly. Habitats with patches of different quality require that female parasitoids estimate the quality of the current patch. Females should adapt the time they spend on a patch to varying patch qualities in order to maximize their fitness. To study the foraging decisions of female wasps the experiments on the functional response and the mutual interference were complemented by behavioural observations. *Fopius arisanus* used information on host encounters, probings, returns to patches, occurrence of conspecifics and on host distribution to adapt its behaviour. An experience with the named cues resulted in a change of the tendency to leave the current patch and hence adaptive behaviour.

Fopius arisanus displayed an incremental patch time mechanism towards ovipositions. This result confirms the theory that parasitoids co-evolved with aggregated hosts should increase their search effort upon host encounters, in order to maximize their fitness (Iwasa, Higashi & Yamamura 1981). Surprisingly, the rejection of a host egg also resulted in a decreased tendency to leave the patch and hence increased the patch time. In the light of adaptive strategies such a mechanism seems counterintuitive (Kolss, Hoffmeister & Hemerik 2006). The opposite, that is to say an higher tendency to leave patches after host rejections has been proposed as an adaptive strategy (Kolss, Hoffmeister & Hemerik 2006) and repeatedly reported for many parasitoid species (van Lenteren 1991; Wajnberg, Fauvergue & Pons 2000; Wang & Keller 2004). The observed mechanism may ensure that female wasps encountering unsuitable hosts search that patch longer than an empty patch, which may increase the probability of finding better quality hosts nearby. Once females encounter a patch containing suitable and unsuitable hosts the rate of oviposition to rejection may lead to a damping effect on the tendency to stay, with higher patch depletion (Nelson & Roitberg 1995). A careful interpretation of the effect is needed since the mechanism may also be an artefact of the experiment. Since host eggs were covered there was no differentiation between the rejection of already parasitized and otherwise unsuitable (unhealthy) hosts. Thus, the mean response to host rejection could

be a combined effect that resulted from different mechanisms towards parasitized and unsuitable hosts. It is further possible that the incremental mechanism is closely linked with another trait (e.g. the incremental mechanism towards oviposition) which has a stronger effect on the fitness. In that case selection would favour individuals which are better adapted to the more fitness relevant trait and thus maintain the not optimal mechanism towards host rejections.

In trials with simultaneously searching parasitoids, females adjusted their search effort according to the presence of conspecifics. Females that encountered conspecifics without a previous oviposition on that patch showed an increased tendency to leave. The mechanism resulted in a number of females that left the patch shortly after entering. This is in line with the prediction of optimal patch time allocation under competition which states that there is a maximum number above which all remaining individuals should leave at once (Sjerps & Haccou 1994). Therefore, this mechanism may allow for roughly optimal foraging decisions under competition. In contrast, females stayed longer after encounters with conspecifics if they had parasitized eggs before the encounter. In conclusion, *F. arisanus* seemed to be able to adapt its foraging decisions according to the previous investment within the patch.

Despite some ambiguities, regarding the mechanism towards rejection, the results show that *F. arisanus* employs a diverse repertoire of mechanisms which allows it to respond to complex and changing habitats. Some of the presented mechanisms that drive foraging decisions of *F. arisanus* provide new insights in traits of this species that may have further implications on biological control projects (see section 5.3 below).

5.3. Classical biological control

The development of classical biological control strategies builds on the knowledge of population dynamics, and its causes. The presented study provides a deeper understanding of the mechanisms and processes that are important for the introduction of *Fopius arisanus* into new regions. First of all, the rate of parasitism by *F. arisanus* was inversely density dependent, but still reached more than 30 % even at the highest host density. A maximum parasitism of at least 30 % in the field has been related to considerable suppression of host populations (Hawkins, Thomas & Hochberg 1993; Hawkins & Cornell 1994). Although, this study was conducted in the laboratory, and thus the observed parasitism was most likely overestimated, the estimates together with the reported success of *F. arisanus* in Hawaii, suggest that *F. arisanus* is a suitable candidate for the biological control of tephritid flies in general. I further consider *F. arisanus* a promising candidate for the biological control of *B. invadens* in Kenya, however based on the results of this studies a significant suppression of fly populations in mango due to parasitism by *F. arisanus* may not be achieved directly. The existence of a spatial density dependent refuge to hosts indicates that the parasitism achieved by *F. arisanus* may be reduced in crop systems of plants with large fruits, e.g. mango. If that is the case a high proportion of fly eggs may escape from parasitism by *F. arisanus* in mango orchards. Therefore, strategies aiming at suppressing *B. invadens* should include increasing the parasitoid density by augmentative releases during the off-season of mangos, when the flies have to switch to alternative hosts that produce smaller fruits. Furthermore, information on the distribution of *B. invadens* larvae in mangos are readily available, but it remains unclear how aggregated the eggs are within the fruits. Information about the egg distribution would be important in order to predict the proportion of flies that will escape from parasitism.

Efforts to establish natural enemies in new regions usually apply mass releases of the agents, and thus locally generate extremely high parasitoid densities. The result presented in chapter four shows that such an approach may lead to severe mutual interference which in turn hampers the efficiency of *F. arisanus*. The results suggest that the high interference coefficient was at least partly caused by an increased tendency of

females to leave the patch. This implies that mass releases, especially in environments with a highly aggregated host distribution lead to a high dispersal rate, which may in turn reduce the agent's density in the target region below the minimum density necessary for establishment. The dispersal behaviour of control agents has been related to their successful establishment and to their ability to follow the pest populations. Heimpel and Asplen (2011) propose that an intermediate level of dispersal is optimal for successful biological control. This and the presented results, which showed that females of *F. arisanus* responded to the experienced density of conspecifics, highlight the need for future studies on the effect of experiences on the dispersal behaviour in this species.

On the basis of this study I recommend that: 1) the efforts in utilizing *F. arisanus* as a biological control agent of tephritid flies continue and 2) that those efforts are accompanied by extensive studies on the dispersal behaviour of this species and in particular the effect of experiences on dispersal. Due to the potential host refuge in mangos I further suggest that release strategies for *F. arisanus* include releases during the off-season of mango when the flies have to switch to smaller fruits. The problem that *F. arisanus* alone may not achieve a sufficient control of *B. invadens* should be solved by the use of additional management approaches. Despite the use of bait sprays or pesticides, the suppression of the fruit fly population within mango orchards may be enhanced by the introduction of weaver ants. An enterprise that currently is under investigation in some African countries and shows some promising results (van Mele *et al.* 2009).

Conclusion

The presented study on the density dependence in the host-parasitoid interaction of *Fopius arisanus* and *Bactrocera invadens* revealed new insights into the mechanisms causing the density dependence in the response of *F. arisanus*. The host density dependence seemed to be affected by a spatial density dependent refuge to the host. This suggests that the level of host suppression achieved by *F. arisanus* is related to the distribution of host eggs in the field. Therefore, my study provides essential information about the number of hosts that may escape a control by the parasitoid when combined with necessary future studies on the host distribution in the field. Intuitively, *F. arisanus* should be more efficient in crop systems of plants with small fruits, which prevent the formation of highly aggregated host distributions, but further research is needed to confirm this.

To achieve a significant level of pest suppression the maximum level of parasitism should, at least, be 30 % (Hawkins & Cornell 1994). More than 30 % parasitism caused by *F. arisanus* on specific plants has been reported from field surveys in Hawaii, Fiji Islands, French Polynesia (see Vargas *et al.* 2012a for a review). The fact that *Fopius arisanus* is one of the most successful natural enemies of tephritid flies within the genus *Bactrocera* was supported by the results of this study. Individually searching females parasitized more than 30 % of the provided eggs even at the highest host density tested. However, not all biological control efforts that included the introduction of *F. arisanus* have been successful. Some attempts failed at the step of establishment, whereas others led to establishment but have not yet reported a considerable reduction of the pest population (see Rousse, Harris & Quilici 2005 for a review). The study at hand provides potential processes, i.e. mutual interference, dispersal behaviour and host refuges, that should be considered in order to explain the different successes of *F. arisanus*. Under competition, parasitism by *F. arisanus* was reduced to less than 20 % of the host eggs. On the other hand, the presence of conspecifics also led to the migration of wasps from the host patch, a behaviour response that may be important for the dispersal behaviour of *F. arisanus*. The study suggests that mutual interference and dispersal behaviour are closely connected. The biological traits investigated do not only provide information for releases of *F. arisanus* in Eastern Africa, but also for biological control efforts elsewhere.

Valuable insights into the proximate mechanisms that result in adaptations of the foraging behaviour were obtained from the behavioural observations of *F. arisanus*. First of all, the results support the prediction of foraging theory that parasitoids searching in environments with aggregated hosts should react to a successful host encounter with an increased time spent searching on that patch. In addition to ovipositions, *F. arisanus* used several sources of information, e.g. host rejections, encounters with conspecifics and host distribution, and adapted its behaviour accordingly. The response to conspecifics depended on the earlier investment by the female on the patch. Interestingly, a change of the investment status, from not having laid an egg on the patch to having laid eggs, led to a switch from an increasing to a decreasing effect on the patch time, respectively.

Finally, what can be concluded from this study? Some of the mechanisms used by *F. arisanus* to forage optimally across diverse host densities on fruits have been clarified that: 1) suggest that releases of wasps should not be performed at very high densities to avoid the immediate dispersal of wasps due to interference and 2) point to the fact that especially in large fly egg aggregations, a high proportion of hosts will always escape parasitism through the spatial refuge that acts in an increasing density dependent fashion. The latter suggests that *F. arisanus* alone will not be sufficient to control *B. invadens* thus further techniques should be implemented to fight the fly pest. On the other hand the finding of the density dependent refuge suggests that outside the mango season, when the fly has to use much smaller hosts, *F. arisanus* may be a highly efficient control agent. This could make it much harder for the fly to re-establish on mango in following seasons. Yet, this study has also led to new important questions. Patch leaving in *F. arisanus* is not only a function of foraging success on a given fruit, but also strongly impacted by the presence of conspecifics. Those females that do not find host eggs before encountering conspecifics seem to have a high tendency to disperse from the patch. The open question is whether dispersal distance from patches is a function of the experience made on a patch. This may influence the spatial distribution of the parasitoid in the field and would make an interesting and important research question coming up from the study presented here.

Appendix

References

- Abrams, P.A. (1982) Functional responses of optimal foragers. *The American Naturalist*, **120**, 382-390.
- Abrams, P.A. (1990) The effects of adaptive behavior on the type-2 functional response. *Ecology*, **71**, 877-885.
- Abrams, P.A. & Ginzburg, L.R. (2000) The nature of predation: prey dependent, ratio dependent or neither? *Trends in Ecology & Evolution*, **15**, 337-341.
- Abrams, P.A. & Kawecki, T.J. (1999) Adaptive host preference and the dynamics of host-parasitoid interactions. *Theoretical Population Biology*, **56**, 307-324.
- Albrechtsen, B. & Nachman, G.S. (2001) Female-biased density-dependent dispersal of a tephritid fly in a fragmented habitat and its implications for population regulation. *Oikos*, **94**, 263-272.
- Aluja, M. & Norrbom, A.A.L. (2000) *Fruit flies (Tephritidae): phylogeny and evolution of behavior*. Crc Press.
- Aluja, M., Ordano, M., Guillén, L. & Rull, J. (2012) Understanding long-term fruit fly (Diptera: Tephritidae) population dynamics: implications for areawide management. *Journal of Economic Entomology*, **105**, 823-836.
- Anderson, R.M. & May, R.M. (1981) The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **291**, 451-524.
- Arditi, R. & Akçakaya, H.R. (1990) Underestimation of mutual interference of predators. *Oecologia*, **83**, 358-361.
- Bach, C.E. (1991) Direct and indirect interactions between ants (*Pheidole megacephala*), scales (*Coccus viridis*) and plants (*Pluchea indica*). *Oecologia*, **87**, 233-239.
- Barratt, B.I.P., Howarth, F.G., Withers, T.M., Kean, J.M. & Ridley, G.S. (2010) Progress in risk assessment for classical biological control. *Biological Control*, **52**, 245-254.
- Bartlett, B.R., Clausen, C.P., DeBach, P., Goeden, R.D., Legner, E.F., Mc Murtry, J.A., Oatman, E.R., Bay, E.C. & Rosen, D. (1978) *Introduced parasites and predators of arthropod pests and weeds : a world review*. Washington DC.
- Bateman, M.A. (1972) The ecology of fruit flies. *Annual Review of Entomology*, **17**, 493-518.
- Bautista, R.C., Harris, E.J. & Vargas, R.I. (2001) The fruit fly parasitoid *Fopius arisanus*: reproductive attributes of pre-released females and the use of added sugar as a potential food supplement in the field. *Entomologia Experimentalis et Applicata*, **101**, 247-255.
- Bautista, R.C., Harris, E.J., Vargas, R.I. & Jang, E.B. (2004) Parasitization of melon fly (Diptera: Tephritidae) by *Fopius arisanus* and *Psyttalia fletcheri* (Hymenoptera: Braconidae) and the effect of fruit substrates on host preference by parasitoids. *Biological Control*, **30**, 156-164.

- Beddington, J.R. (1975) Mutual interference between parasites or predators and its effect on searching efficiency. *Journal of Animal Ecology*, **44**, 331-340.
- Beddington, J.R., Free, C.A. & Lawton, J.H. (1978) Characteristics of successful natural enemies in models of biological control of insect pests. *Nature*, **273**, 513-519.
- Berryman, A.A. & Hawkins, B.A. (2006) The refuge as an integrating concept in ecology and evolution. *Oikos*, **115**, 192-196.
- Bigler, F., Bale, J.S., Cock, M.J.W., Dreyer, H., Greatrex, R., Kuhlmann, U., Loomans, A.J.M. & van Lenteren, J.C. (2005) Guidelines on information requirements for import and release of invertebrate biological control agents in European countries. *Biocontrol News and Information*, **26**, 115N-123N.
- Bigler, F., Neuenschwander, P., Delucchi, V. & Michelakis, S. (1986) Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. II. Impact on olive fly populations. *Bollettino del Laboratorio di Entomologia Agraria" Filippo Silvestri", Italy*, **43**, 79-96.
- Bo, W., Ahmad, S., Dammalage, T., Tomas, U.S., Wornoyayporn, V., Ul Haq, I., Caceres, C., Vreysen, M.J.B., Hendrichs, J. & Schutze, M.K. (2014) Mating compatibility between *Bactrocera invadens* and *Bactrocera dorsalis* (Diptera: Tephritidae). *Journal of Economic Entomology*, **107**, 623-629.
- Bowler, D.E. & Benton, T.G. (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, **80**, 205-225.
- Braune, H.J. (1982) Einfluß der Gelegestruktur des Wirtes auf die Wirksamkeit eines Eiparasiten von *Spodoptera litura* (F.) (Lepidoptera, Noctuidae). *Drosera*, **10**.
- Broadhead, E. & Cheke, R.A. (1975) Host spatial pattern, parasitoid interference and the modelling of the dynamics of *Alaptus fuscus* (Hym.: Mymaridae), a parasitoid of two *Mesopsocus* species (Psocoptera). *Journal of Animal Ecology*, **44**, 767-793.
- Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovář, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A. & Roy, D.B. (2008) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. *BioControl*, **53**, 5-21.
- Caltagirone, L.E. (1981) Landmark examples in classical biological control. *Annual Review of Entomology*, **26**, 213-232.
- Calvitti, M., Antonelli, M., Moretti, R. & Bautista, R.C. (2002) Oviposition response and development of the egg-pupal parasitoid *Fopius arisanus* on *Bactrocera oleae*, a tephritid fruit fly pest of olive in the Mediterranean basin. *Entomologia Experimentalis et Applicata*, **102**, 65-73.
- Charnov, E.L. (1976) Optimal foraging, the Marginal Value Theorem. *Theoretical Population Biology*, **9**, 129-136.
- Cheke, R.A. (1974) Experiments on the effect of host spatial distribution on the numerical response of parasitoids. *Journal of Animal Ecology*, **43**, 107-113.

- Chinajariyawong, A., Clarke, A.R., Jirasurat, M., Kritsaneepiboon, S., Lahey, H.A., Vijaysegaran, S. & Walter, G.H. (2000) Survey of opiine parasitoids of fruit flies (Diptera: Tephritidae) in Thailand and Malaysia. *Raffles Bulletin of Zoology*, **48**, 71-101.
- Chong, J.-H. & Oetting, R.D. (2006) Functional response and progeny production of the Madeira mealybug parasitoid, *Anagyrus* sp. nov. nr. *sinope*: The effects of host and parasitoid densities. *Biological Control*, **39**, 320-328.
- Chown, S.L., Slabber, S., McGeoch, M.A., Janion, C. & Leinaas, H.P. (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society Series B-Biological Sciences*, **274**, 2531-2537.
- Christenson, L.D. & Foote, R.H. (1960) Biology of fruit flies. *Annual Review of Entomology*, **5**, 171-192.
- Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K. & Yeates, D.K. (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology*, **50**, 293-319.
- Clarke, B., Murray, J. & Johnson, M.S. (1984) The extinction of endemic species by a program of biological control. *Pacific Science*, **38**, 97-104.
- Clausen, C.P., Clancy, D.W. & Chock, Q. (1965) *Biological control of the oriental fruit fly (Dacus dorsalis Hendel) and other fruit flies in Hawaii*. Agricultural Research Service. US Dept. of Agriculture.
- Cock, M.J.W., van Lenteren, J.C., Brodeur, J., Barratt, B.I.P., Bigler, F., Bolckmans, K., Consoli, F.L., Haas, F., Mason, P.G. & Parra, J.R.P. (2010) Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? *BioControl*, **55**, 199-218.
- Cooper, W.R. & Rieske, L.K. (2007) Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America. *Annals of the Entomological Society of America*, **100**, 236-244.
- Coote, T. & Loève, É. (2003) From 61 species to five: endemic tree snails of the Society Islands fall prey to an ill-judged biological control programme. *Oryx*, **37**, 91-96.
- Damman, H. & Cappuccino, N. (1991) 2 forms of egg defense in a chrysomelid beetle: egg clumping and excrement cover. *Ecological Entomology*, **16**, 163-167.
- Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**, 419-431.
- Davis, C. & Butler, G. (1964) Introduced enemies of the giant African snail, *Achatina fulica* Bowdich, in Hawaii (Pulmonata: Achatinidae). *Proceedings of the Hawaiian Entomological Society*, **18**, 14.
- De Clercq, P., Mason, P. & Babendreier, D. (2011) Benefits and risks of exotic biological control agents. *BioControl*, **56**, 681-698.

- de Jong, P.W., Hemerik, L., Gort, G. & van Alphen, J.J. (2011) Rapid establishment of a regular distribution of adult tropical *Drosophila* parasitoids in a multi-patch environment by patch defence behaviour. *Plos One*, **6**, e20870.
- De Meyer, M., Robertson, M.P., Mansell, M.W., Ekesi, S., Tsuruta, K., Mwaiko, W., Vayssieres, J.F. & Peterson, A.T. (2009) Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). Cambridge University Press.
- De Meyer, M., Robertson, M.P., Mansell, M.W., Ekesi, S., Tsuruta, K., Mwaiko, W., Vayssieres, J.F. & Peterson, A.T. (2010) Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). *Bulletin of Entomological Research*, **100**, 35-48.
- Drew, R.A.I., Tsuruta, K. & White, I.M. (2005) A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology*, **13**, 149-154.
- Ekesi, S., Nderitu, P.W. & Chang, C.L. (2007) Adaptation to and small-scale rearing of invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on artificial diet. *Annals of the Entomological Society of America*, **100**, 562-567.
- Ekesi, S., Nderitu, P.W. & Rwomushana, I. (2006) Field infestation, life history and demographic parameters of the fruit fly *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research*, **96**, 379-386.
- El-Arnaouty, S.A., Beyssat-Arnaouty, V., Ferran, A. & Galal, H. (2000) Introduction and release of the coccinellid *Harmonia axyridis* Pallas for controlling *Aphis craccivora* Koch on faba beans in Egypt. *Egyptian Journal of biological pest control*, **10**, 129-136.
- Estoup, A. & Guillemaud, T. (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, **19**, 4113-4130.
- Fagan, W.F., Lewis, M.A., Neubert, M.G. & Van Den Driessche, P. (2002) Invasion theory and biological control. *Ecology Letters*, **5**, 148-157.
- Faraji, F., Janssen, A. & Sabelis, M.W. (2002) The benefits of clustering eggs: the role of egg predation and larval cannibalism in a predatory mite. *Oecologia*, **131**, 20-26.
- Farrokhi, S., Ashouri, A., Shirazi, J., Allahvari, H. & Huigens, M.E. (2010) A comparative study on the functional response of *Wolbachia*-infected and uninfected forms of the parasitoid wasp *Trichogramma brassicae*. *Journal of Insect Science*, **10**, 167.
- Fellowes, M.D.E., van Alphen, J.J.M. & Jervis, M.A. (2005) Foraging behaviour. *Insects as natural enemies: A practical perspective* (ed. M.A. Jervis). Springer.
- Fenner, F. (1983) The Florey lecture, 1983: biological control, as exemplified by smallpox eradication and myxomatosis. *Proceedings of the Royal Society Series B-Biological Sciences*, **218**, 259-285.
- Fernandez-Arhex, V. & Corley, J.C. (2003) The functional response of parasitoids and its implications for biological control. *Biocontrol Science and Technology*, **13**, 403-413.

- Fernández-Arhex, V. & Corley, J.C. (2010) The effects of patch richness on con-specific interference in the parasitoid *Ibalia leucospoides* (Hymenoptera: Ibalidae). *Insect Science*, **17**, 379-385.
- Field, S.A. & Calbert, G. (1998) Patch defence in the parasitoid wasp *Trissolcus basalis*: When to begin fighting? *Behaviour*, **135**, 629-642.
- Fletcher, B.S. (1987) The biology of dacine fruit flies. *Annual Review of Entomology*, **32**, 115-144.
- Fox, J. & Weisberg, S. (2011) Cox proportional-hazards regression for survival data in R. *An Appendix to An R Companion to Applied Regression, Second Edition*.
- Free, C.A., Beddington, J.R. & Lawton, J.H. (1977) On the inadequacy of simple models of mutual interference for parasitism and predation. *Journal of Animal Ecology*, **46**, 543-554.
- Friedlander, T.P. (1985) Egg mass design relative to surface-parasitizing parasitoids, with notes on *Asterocampa clyton* (Lepidoptera: Nymphalidae). *Journal of Research on the Lepidoptera*, **24**, 250-257.
- Froissart, L., Bernstein, C., Humblot, L., Amat, I. & Desouhant, E. (2012) Facing multiple information sources while foraging on successive patches: how does a parasitoid deal with experience? *Animal Behaviour*, **83**, 189-199.
- Gaigher, R., Samways, M., Henwood, J. & Jolliffe, K. (2011) Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. *Biological Invasions*, **13**, 1717-1721.
- Gaigher, R. & Samways, M.J. (2013) Strategic management of an invasive ant-scale mutualism enables recovery of a threatened tropical tree species. *Biotropica*, **45**, 128-134.
- Getz, W.M. (1996) A hypothesis regarding the abruptness of density dependence and the growth rate of populations. *Ecology*, **77**, 2014-2026.
- Getz, W.M. & Mills, N.J. (1996) Host-parasitoid coexistence and egg-limited encounter rates. *The American Naturalist*, **148**, 333-347.
- Goergen, G., Vayssières, J.-F., Gnanvossou, D. & Tindo, M. (2011) *Bactrocera invadens* (Diptera: Tephritidae), a new invasive fruit fly pest for the afro-tropical region: host plant range and distribution in West and Central Africa. *Environmental Entomology*, **40**, 844-854.
- Goetz, D.W. (2009) Seasonal inhalant insect allergy: *Harmonia axyridis* ladybug. *Current Opinion in Allergy and Clinical Immunology*, **9**, 329-333.
- Gompertz, B. (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical Transactions of the Royal Society of London*, **115**, 513-583.
- González-Olivares, E. & Ramos-Jiliberto, R. (2003) Dynamic consequences of prey refuges in a simple model system: more prey, fewer predators and enhanced stability. *Ecological Modelling*, **166**, 135-146.

- Gordon, R.D. (1985) The Coccinellidae (Coleoptera) of America north of Mexico. *Journal of the New York Entomological Society*, **93**, 1-912.
- Goubault, M., Outreman, Y., Poinso, D. & Cortesero, A.M. (2005) Patch exploitation strategies of parasitic wasps under intraspecific competition. *Behavioral Ecology*, **16**, 693-701.
- Grasman, J., van Herwaarden, O.A., Hemerik, L. & van Lenteren, J.C. (2001) A two-component model of host-parasitoid interactions: determination of the size of inundative releases of parasitoids in biological pest control. *Mathematical Biosciences*, **169**, 207-216.
- Haccou, P., Glaizot, O. & Cannings, C. (2003) Patch leaving strategies and superparasitism: an asymmetric generalized war of attrition. *Journal of Theoretical Biology*, **225**, 77-89.
- Haccou, P. & Hemerik, L. (1985) The influence of larval dispersal in the cinnabar moth (*Tyria jacobaeae*) on predation by the red wood ant (*Formica polyctena*) - an analysis based on the proportional hazards model. *Journal of Animal Ecology*, **54**, 755-769.
- Haccou, P., Vlas, S.J.D., Alphen, J.J.M.V. & Visser, M.E. (1991) Information processing by foragers: effects of intra-patch experience on the leaving tendency of *Leptopilina heterotoma*. *Journal of Animal Ecology*, **60**, 93-106.
- Haramoto, F.H. & Bess, H.A. (1970) Recent studies on the abundance of the oriental and mediterranean fruit flies and the status of their parasites. *Proceedings of the Hawaiian Entomological Society*, **20**, 16.
- Hardy, I.C.W. & Blackburn, T.M. (1991) Brood guarding in a bethylid wasp. *Ecological Entomology*, **16**, 55-62.
- Harris, E.J., Bautista, R.C., Vargas, R.I., Jang, E.B., Eitam, A. & Leblanc, L. (2010) Suppression of melon fly (Diptera: Tephritidae) populations with releases of *Fopius arisanus* and *Psytalia fletcheri* (Hymenoptera: Braconidae) in North Shore Oahu, HI, USA. *BioControl*, **55**, 593-599.
- Hassell, M.P. (1971) Mutual interference between searching insect parasites. *Journal of Animal Ecology*, **40**, 473-486.
- Hassell, M.P. (1978) *The dynamics of arthropod predator-prey systems*. Princeton University Press.
- Hassell, M.P. (2000a) Host-parasitoid population dynamics. *Journal of Animal Ecology*, **69**, 543-566.
- Hassell, M.P. (2000b) *The spatial and temporal dynamics of host-parasitoid interactions*. Oxford Univ. Press, Oxford [u.a.].
- Hassell, M.P., Lawton, J.H. & Beddington, J.R. (1977) Sigmoid functional responses by invertebrate predators and parasitoids. *Journal of Animal Ecology*, **46**, 249-262.
- Hassell, M.P. & May, R.M. (1973) Stability in insect host-parasite models. *Journal of Animal Ecology*, **42**, 693-726.

- Hassell, M.P. & Rogers, D.J. (1972) Insect parasite responses in the development of population models. *Journal of Animal Ecology*, **41**, 661-676.
- Hassell, M.P. & Varley, G.C. (1969) New inductive population model for insect parasites and its bearing on biological control. *Nature*, **223**, 1133-&.
- Hastings, A. (2000) Parasitoid spread: lessons for and from invasion biology. *Parasitoid Population Biology* (eds M.E. Hochberg & A.R. Ives), pp. 70-82. Princeton University Press.
- Hawkins, B.A. & Cornell, H.V. (1994) Maximum parasitism rates and successful biological control. *Science*, **266**, 1886-1886.
- Hawkins, B.A., Thomas, M.B. & Hochberg, M.E. (1993) Refuge theory and biological control. *Science*, **262**, 1429-1432.
- Heimpel, G.E. & Asplen, M.K. (2011) A 'Goldilocks' hypothesis for dispersal of biological control agents. *BioControl*, **56**, 441-450.
- Heimpel, G.E., Mangel, M. & Rosenheim, J.A. (1998) Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. *The American Naturalist*, **152**, 273-289.
- Hemerik, L., Driessen, G. & Haccou, P. (1993) Effects of intra-patch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes*. *Journal of Animal Ecology*, **62**, 33-44.
- Henne, D.C., Johnson, S.J. & Cronin, J.T. (2007) Population spread of the introduced red imported fire ant parasitoid, *Pseudacteon tricuspis* Borgmeier (Diptera : Phoridae), in Louisiana. *Biological Control*, **42**, 97-104.
- Hochberg, M.E. & Holt, R.D. (1999) The uniformity and density of pest exploitation as guides to success in biological control. *Theoretical Approaches to Biological Control* (eds H.V. Cornell & B.A. Hawkins), pp. 71-88. Cambridge University Press, Cambridge.
- Holland, B.S., Chock, T., Lee, A. & Sugiura, S. (2012) Tracking behavior in the snail *Euglandina rosea*: First evidence of preference for endemic vs. biocontrol target pest species in Hawaii. *American Malacological Bulletin*, **30**, 153-157.
- Holling, C. (1961) Principles of insect predation. *Annual Review of Entomology*, **6**, 163-182.
- Holling, C.S. (1959a) The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *The Canadian Entomologist*, **91**, 293-320.
- Holling, C.S. (1959b) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, **91**, 385-398.
- Holling, C.S. & Buckingham, S. (1976) Behavioral model of predator-prey functional responses. *Behavioral Science*, **21**, 183-195.

- Holt, R.D. & Kimbrell, T. (2007) Foraging and population dynamics. . *Foraging: Behavior and Ecology* (eds D.W. Stephens, J.S. Brown & R.C. Ydenberg), pp. 365-395. University of Chicago Press, Chicago & London.
- Howarth, F.G. (1983) Classical biocontrol: panacea or Pandora's box. *Proceedings of the Hawaiian Entomological Society*, **24**, 239-244.
- Howarth, F.G. (1991) Environmental impacts of classical biological control. *Annual Review of Entomology*, **36**, 485-509.
- Huang, H.T. & Yang, P. (1987) The ancient cultured citrus ant. *Bioscience*, **37**, 665-671.
- Hubbard, S.F. & Cook, R.M. (1978) Optimal foraging by parasitoid wasps. *Journal of Animal Ecology*, **47**, 593-604.
- Humphries, E.L., Hebblethwaite, A.J., Batchelor, T.P. & Hardy, I.C.W. (2006) The importance of valuing resources: host weight and contender age as determinants of parasitoid wasp contest outcomes. *Animal Behaviour*, **72**, 891-898.
- Hutchinson, J.M.C., Wilke, A. & Todd, P.M. (2008) Patch leaving in humans: can a generalist adapt its rules to dispersal of items across patches? *Animal Behaviour*, **75**, 1331-1349.
- Irvin, N., Suarez-Espinoza, J. & Hoddle, M. (2009) Functional response of *Gonatocerus ashmeadi* and the "New Association" parasitoid *G. tuberculifemur* attacking eggs of *Homalodisca vitripennis*. *Environmental Entomology*, **38**, 1634-1641.
- Ives, A.R. (1995) Spatial heterogeneity and host-parasitoid population dynamics: Do we need to study behavior? *Oikos*, **74**, 366-376.
- Iwasa, Y., Higashi, M. & Yamamura, N. (1981) Prey distribution as a factor determining the choice of optimal foraging strategy. *The American Naturalist*, **117**, 710-723.
- Jost, C., Devulder, G., Vucetich, J.A., Peterson, R.O. & Arditi, R. (2005) The wolves of Isle Royale display scale-invariant satiation and ratio-dependent predation on moose. *Journal of Animal Ecology*, **74**, 809-816.
- Juliano, S.A. (2001) Non-linear curve-fitting: predation and functional response curves. *Design and Analysis of Ecological Experiments* (eds S.M. Scheiner & J. Gurevitch), pp. 178-196. Chapman & Hall, New York.
- Kareiva, P. (1996) Special feature: contributions of ecology to biological control. *Ecology*, **77**, 1963-1964.
- Kenis, M., Auger-Rozenberg, M.-A., Roques, A., Timms, L., Péré, C., Cock, M.J.W., Settele, J., Augustin, S. & Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. *Ecological impacts of non-native invertebrates and fungi on terrestrial ecosystems* (eds D. Langor & J. Sweeney), pp. 21-45. Springer Netherlands.
- Kidd, D. & Amarasekare, P. (2012) The role of transient dynamics in biological pest control: insights from a host-parasitoid community. *Journal of Animal Ecology*, **81**, 47-57.
- Kidd, N.A. & Jervis, M.A. (2005) Population dynamics. *Insects as Natural Enemies: A Practical Perspective*, pp. 435-523. Springer.

- Koch, R.L. (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, **3**, 32.
- Koch, R.L. & Galvan, T.L. (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. *BioControl*, **53**, 23-35.
- Kolss, M., Hoffmeister, T.S. & Hemerik, L. (2006) The theoretical value of encounters with parasitized hosts for parasitoids. *Behavioral Ecology and Sociobiology*, **61**, 291-304.
- Konradsen, F., van der Hoek, W., Cole, D.C., Hutchinson, G., Daisley, H., Singh, S. & Eddleston, M. (2003) Reducing acute poisoning in developing countries - options for restricting the availability of pesticides. *Toxicology*, **192**, 249-261.
- Koppik, M., Thiel, A. & Hoffmeister, T.S. (2014) Adaptive decision making or differential mortality: what causes offspring emergence in a gregarious parasitoid? *Entomologia Experimentalis et Applicata*, **150**, 208-216.
- Lawrence, P.O. (1981) Interference competition and optimal host selection in the parasitic wasp, *Biosteres longicaudatus*. *Annals of the Entomological Society of America*, **74**, 540-544.
- Lee, J.H. & Kang, T.J. (2004) Functional response of *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae) to *Aphis gossypii* Glover (Homoptera : aphididae) in the laboratory. *Biological Control*, **31**, 306-310.
- Lessells, C.M. (1985) Parasitoid foraging: Should parasitism be density dependent? *Journal of Animal Ecology*, **54**, 27-41.
- Liljeström, G., Cingolani, M. & Rabinovich, J. (2013) The functional and numerical responses of *Trissolcus basalis* (Hymenoptera: Platygasteridae) parasitizing *Nezara viridula* (Hemiptera: Pentatomidae) eggs in the field. *Bulletin of Entomological Research*, **103**, 441-450.
- Liu, Y.-Q., Thiel, A. & Hoffmeister, T.S. (2009) Odor-mediated patch choice in the parasitoid *Venturia canescens*: temporal decision dynamics. *Entomologia Experimentalis et Applicata*, **132**, 110-117.
- Lombaert, E., Guillemaud, T., Cornuet, J.M., Malausa, T., Facon, B. & Estoup, A. (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. *Plos One*, **5**.
- Louâpre, P., van Alphen, J.J.M. & Pierre, J.S. (2010) Humans and insects decide in similar ways. *Plos One*, **5**.
- Louda, S.M., Arnett, A.E., Rand, T.A. & Russell, F.L. (2003a) Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. *Conservation Biology*, **17**, 73-82.
- Louda, S.M., Pemberton, R.W., Johnson, M.T. & Follett, P.A. (2003b) Nontarget effects - The Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, **48**, 365-396.

- Lucchetta, P., Desouhant, E., Wajnberg, E. & Bernstein, C. (2007) Small but smart: the interaction between environmental cues and internal state modulates host-patch exploitation in a parasitic wasp. *Behavioral Ecology and Sociobiology*, **61**, 1409-1418.
- Luo, S., Li, H., Lu, Y., Zhang, F., Haye, T., Kuhlmann, U. & Wu, K. (2014) Functional response and mutual interference of *Peristenus spretus* (Hymenoptera: Braconidae), a parasitoid of *Apolygus lucorum* (Heteroptera: Miridae). *Biocontrol Science and Technology*, **24**, 247-256.
- Lux, S.A., Copeland, R.S., White, I.M., Manrakhan, A. & Billah, M.K. (2003) A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *International Journal of Tropical Insect Science*, **23**, 355-361.
- Malthus, T. (1798) *An essay on the principle of population*.
- Massad, E. (1987) Transmission rates and the evolution of pathogenicity. *Evolution*, **41**, 1127-1130.
- May, R.M. (1978) Host-parasitoid systems in patchy environments: a phenomenological model. *Journal of Animal Ecology*, **47**, 833-844.
- May, R.M., Hassell, M.P., Anderson, R.M. & Tonkyn, D.W. (1981) Density dependence in host-parasitoid models. *Journal of Animal Ecology*, **50**, 855-865.
- Maynard Smith, J. (1974) The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology*, **47**, 209-221.
- McNair, J.N. (1982) Optimal giving-up times and the Marginal Value Theorem. *The American Naturalist*, **119**, 511-529.
- McNair, J.N. (1986) The effects of refuges on predator-prey interactions - a reconsideration. *Theoretical Population Biology*, **29**, 38-63.
- Mery, F. & Burns, J. (2010) Behavioural plasticity: an interaction between evolution and experience. *Evolutionary Ecology*, **24**, 571-583.
- Messing, R.H. & Wright, M.G. (2006) Biological control of invasive species: solution or pollution? *Frontiers in Ecology and the Environment*, **4**, 132-140.
- Michaux, B. (1996) The origin of southwest Sulawesi and other Indonesian terranes: A biological view. *Palaeogeography Palaeoclimatology Palaeoecology*, **122**, 167-183.
- Mills, N. (2005) Selecting effective parasitoids for biological control introductions: codling moth as a case study. *Biological Control*, **34**, 274-282.
- Mills, N.J. & Getz, W.M. (1996) Modelling the biological control of insect pests: a review of host-parasitoid models. *Ecological Modelling*, **92**, 121-143.
- Mills, N.J. & Kean, J.M. (2010) Behavioral studies, molecular approaches, and modeling: methodological contributions to biological control success. *Biological Control*, **52**, 255-262.
- Mills, N.J. & Wajnberg, E. (2008) Optimal foraging behaviour and efficient biological control methods. *Behavioural ecology of insect parasitoids: from theoretical*

- approaches to field applications* (eds E. Wajnberg, C. Bernstein & J.J.M. van Alphen), pp. 3 - 30. Blackwell.
- Mohamed, S.A., Ekesi, S. & Hanna, R. (2010) Old and new host-parasitoid associations: parasitism of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) and five African fruit fly species by *Fopius arisanus*, an Asian opiine parasitoid. *Biocontrol Science and Technology*, **20**, 183-196.
- Montoya, P., Suárez, A., López, F. & Cancino, J. (2009) *Fopius arisanus* oviposition in four *Anastrepha* fruit fly species of economic importance in Mexico. *BioControl*, **54**, 437-444.
- Moriya, S., Shiga, M. & Adachi, I. (2002) Classical biological control of the chestnut gall wasp in Japan. *1st International Symposium on Biological Control of Arthropods* (ed. R.G. Van Driesche), pp. 407-415. Honolulu, Hawaii.
- Murdoch, W., Briggs, C.J. & Swarbrick, S. (2005) Host suppression and stability in a parasitoid-host system: experimental demonstration. *Science*, **309**, 610-613.
- Murdoch, W.W. (1973) The functional response of predators. *Journal of Applied Ecology*, **10**, 335-342.
- Murdoch, W.W. & Briggs, C.J. (1996) Theory for biological control: Recent developments. *Ecology*, **77**, 2001-2013.
- Murdoch, W.W., Chesson, J. & Chesson, P.L. (1985) Biological control in theory and practice. *American Naturalist*, **125**, 344-366.
- Murdoch, W.W., Nisbet, R.M., Luck, R.F., Godfray, H.C.J. & Gurney, W.S.C. (1992) Size-selective sex-allocation and host feeding in a parasitoid-host model. *Journal of Animal Ecology*, **61**, 533-541.
- Murdoch, W.W., Swarbrick, S.L., Luck, R.F., Walde, S. & Yu, D.S. (1996) Refuge dynamics and metapopulation dynamics: an experimental test. *American Naturalist*, **147**, 424-444.
- Nelson, J.M. & Roitberg, B.D. (1995) Flexible patch time allocation by the leafminer parasitoid, *Opius dimidiatus*. *Ecological Entomology*, **20**, 245-252.
- Neuenschwander, P. (2001) Biological control of the cassava mealybug in Africa: a review. *Biological Control*, **21**, 214-229.
- Nicholson, A.J. & Bailey, V.A. (1935) The balance of animal populations - Part I. *Proceedings of the Zoological Society of London*, **105**, 551-598.
- Noldus, L.P.J.J. (1991) *The Observer*. Noldus Information Technology b.v., Wageningen.
- Oliveira, C.M., Auad, A.M., Mendes, S.M. & Frizzas, M.R. (2013) Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, **137**, 1-15.
- Orsini, M.M., Daane, K.M., Sime, K.R. & Nelson, E.H. (2007) Mortality of olive fruit fly pupae in California. *Biocontrol Science and Technology*, **17**, 797-807.
- Otronen, M. & Hanski, I. (1983) Movement patterns in *Sphaeridium*: Differences between species, sexes, and feeding and breeding individuals. *Journal of Animal Ecology*, **52**, 663-680.

- Outreman, Y., Le Ralec, A., Wajnberg, E. & Pierre, J.-S. (2005) Effects of within- and among-patch experiences on the patch-leaving decision rules in an insect parasitoid. *Behavioral Ecology and Sociobiology*, **58**, 208-217.
- Patel, P.N. & Habib, M.E.M. (1993) *Microcharops anticarsiae* (Hym.: Ichneumonidae) parasitoid of *Anticarsia gemmatilis* (Lep.: Noctuidae): Host age preference, sex ratio effects and functional response. *Entomophaga*, **38**, 511-517.
- Peng, R.K. & Christian, K. (2006) Effective control of Jarvis's fruit fly, *Bactrocera jarvisi* (Diptera : Tephritidae), by the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), in mango orchards in the Northern Territory of Australia. *International Journal of Pest Management*, **52**, 275-282.
- Pimentel, D. (2005) Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*, **7**, 229-252.
- Purcell, M.F. (1998) Contribution of biological control to integrated pest management of tephritid fruit flies in the Tropics and Subtropics. *Integrated Pest Management Reviews*, **3**, 63-83.
- Purcell, M.F., Herr, J.C., Messing, R.H. & Wong, T.T.Y. (1998) Interactions between augmentatively released *Diachasmimorpha longicaudata* (Hymenoptera : Braconidae) and a complex of opiine parasitoids in a commercial guava orchard. *Biocontrol Science and Technology*, **8**, 139-151.
- Quimio & Walter (2001) Host preference and host suitability in an egg-pupal fruit fly parasitoid, *Fopius arisanus* (Sonan) (Hym., Braconidae). *Journal of Applied Entomology*, **125**, 135-140.
- R_Core_Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramadan, M.M., Wong, T.T.Y. & Beardsley, J.W. (1992) Reproductive behavior of *Biosteres arisanus* (Sonan) (Hymenoptera: Braconidae), an egg-larval parasitoid of the oriental fruit fly. *Biological Control*, **2**, 28-34.
- Reeve, J.D. (1997) Predation and bark beetle dynamics. *Oecologia*, **112**, 48-54.
- Reeve, J.D. & Murdoch, W.W. (1985) Aggregation by parasitoids in the successful control of the California red scale: a test of theory. *Journal of Animal Ecology*, **54**, 797-816.
- Reeve, J.D. & Murdoch, W.W. (1986) Biological control by the parasitoid *Aphytis melinus*, and population stability of the California red scale. *Journal of Animal Ecology*, **55**, 1069-1082.
- Ritz, C. & Streibig, J.C. (2008) *Nonlinear Regression with R*. Springer.
- Robinson, A. & Hooper, G. (1989) *Fruit flies: their biology, natural enemies and control*. Elsevier Science Publishers BV.
- Rogers, D. (1972) Random search and insect population models. *Journal of Animal Ecology*, **41**, 369-383.

- Roitberg, B.D., Sircom, J., Roitberg, C.A., van Alphen, J.J.M. & Mangel, M. (1993) Life expectancy and reproduction. *Nature*, **364**, 108-108.
- Rosenheim, J.A. & Mangel, M. (1994) Patch-leaving rules for parasitoids with imperfect host discrimination. *Ecological Entomology*, **19**, 374-380.
- Rousse, P., Chiroleu, F., Veslot, J. & Quilici, S. (2007) The host- and microhabitat olfactory location by *Fopius arisanus* suggests a broad potential host range. *Physiological Entomology*, **32**, 313-321.
- Rousse, P., Harris, E.J. & Quilici, S. (2005) *Fopius arisanus*, an egg-pupal parasitoid of Tephritidae. Overview. *Biocontrol News and Information*, **26**, 59N - 69N.
- Roy, H. & Wajnberg, E. (2008) From biological control to invasion: the ladybird *Harmonia axyridis* as a model species. *BioControl*, **53**, 1-4.
- Rwomushana, I., Ekesi, S., Gordon, I. & Ogot, C.K.P.O. (2008a) Host plants and host plant preference studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, a new invasive fruit fly species in Africa. *Annals of the Entomological Society of America*, **101**, 331-340.
- Rwomushana, I., Ekesi, S., Ogot, C.K.P.O. & Gordon, I. (2008b) Effect of temperature on development and survival of immature stages of *Bactrocera invadens* (Diptera: Tephritidae). *Journal of Applied Entomology*, **132**, 832-839.
- Schenk, D. & Bacher, S. (2002) Functional response of a generalist insect predator to one of its prey species in the field. *Journal of Animal Ecology*, **71**, 524-531.
- Sih, A. (1987) Prey refuges and predator-prey stability. *Theoretical Population Biology*, **31**, 1-12.
- Simberloff, D. (2012) Risks of biological control for conservation purposes. *BioControl*, **57**, 263-276.
- Simberloff, D. & Stiling, P. (1996) Risks of species introduced for biological control. *Biological Conservation*, **78**, 185-192.
- Sjerps, M. & Haccou, P. (1994) Effects of competition on optimal patch leaving: a war of attrition. *Theoretical Population Biology*, **46**, 300-318.
- Sol, D., Maspons, J., Vall-Ilosera, M., Bartomeus, I., García-Peña, G.E., Piñol, J. & Freckleton, R.P. (2012) Unraveling the life history of successful invaders. *Science*, **337**, 580-583.
- Solomon, M.E. (1949) The natural control of animal populations. *Journal of Animal Ecology*, **18**, 1-35.
- Spataro, T., Bacher, S., Bersier, L.-F. & Arditi, R. (2012) Ratio-dependent predation in a field experiment with wasps. *Ecosphere*, **3**, art124.
- Stals, R. (2010) The establishment and rapid spread of an alien invasive lady beetle: *Harmonia axyridis* (Coleoptera: Coccinellidae) in southern Africa. *IOBC/wprs Bulletin*, **58**, 125-132.

- Stals, R. & Prinsloo, G. (2007) Discovery of an alien invasive, predatory insect in South Africa: the multicoloured Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *South African Journal of Science*, **103**, 4.
- Systat_Software, I. (2008). San Jose, USA.
- Tan, K.H., Tokushima, I., Ono, H. & Nishida, R. (2011) Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species: *Bactrocera invadens*, *B. dorsalis*, *B. correcta* and *B. zonata*. *Chemoecology*, **21**, 25-33.
- Therneau, T.M. (2014) A Package for Survival Analysis in S. R package version 2.37-7.
- Therneau, T.M. & Grambsch, P.M. (2000) *Modeling survival data: extending the Cox model*.
- Thiel, A. & Hoffmeister, T.S. (2004) Knowing your habitat: linking patch-encounter rate and patch exploitation in parasitoids. *Behavioral Ecology*, **15**, 419-425.
- Thiel, A. & Hoffmeister, T.S. (2006) Selective information use in parasitoid wasps. *Animal Biology*, **56**, 233-245.
- Thiel, A. & Hoffmeister, T.S. (2009) Decision-making dynamics in parasitoids of *Drosophila*. *Advances in Parasitology* (ed. G. Prevost), pp. 45-66. Elsevier; Academic Press, Burlington.
- Trexler, J.C., McCulloch, C.E. & Travis, J. (1988) How can the functional response best be determined? *Oecologia*, **76**, 206-214.
- van Alphen, J.J.M., Bernstein, C. & Driessen, G. (2003) Information acquisition and time allocation in insect parasitoids. *Trends in Ecology and Evolution*, **18**, 81-87.
- van Baaren, J., Boivin, G. & Outreman, Y. (2005) Patch exploitation strategy by an egg parasitoid in constant or variable environment. *Ecological Entomology*, **30**, 502-509.
- van den Bosch, R. & Haramoto, F.H. (1951) *Opius oophilus* Fullaway, an egg-larval parasite of the oriental fruit fly discovered in Hawaii. *Proceedings of the Hawaiian Entomological Society*, **14**, 251-255.
- van Driesche, R.G. (2012) The role of biological control in wildlands. *BioControl*, **57**, 131-137.
- van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D.L., Blossey, B., Brancatini, V., Casagrande, R., Causton, C.E., Coetzee, J.A., Cuda, J., Ding, J., Fowler, S.V., Frank, J.H., Fuester, R., Goolsby, J., Grodowitz, M., Heard, T.A., Hill, M.P., Hoffmann, J.H., Huber, J., Julien, M., Kairo, M.T.K., Kenis, M., Mason, P., Medal, J., Messing, R., Miller, R., Moore, A., Neuenschwander, P., Newman, R., Norambuena, H., Palmer, W.A., Pemberton, R., Perez Panduro, A., Pratt, P.D., Rayamajhi, M., Salom, S., Sands, D., Schooler, S., Schwarzländer, M., Sheppard, A., Shaw, R., Tipping, P.W. & van Klinken, R.D. (2010) Classical biological control for the protection of natural ecosystems. *Biological Control*, **54**, Supplement 1, S2-S33.

- van Lenteren, J. (1991) Encounters with parasitized hosts: to leave or not to leave a patch. *Netherlands Journal of Zoology*, **41**, 144-157.
- van Lenteren, J.C. (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, **57**, 1-20.
- van Lenteren, J.C. & Bakker, K. (1976) Functional responses in invertebrates. *Netherlands Journal of Zoology*, **26**, 567-572.
- van Lenteren, J.C. & Bakker, K. (1978) Behavioural aspects of the functional responses of a parasite (*Pseudeucoila bochei* Weld) to its host (*Drosophila melanogaster*). *Netherlands Journal of Zoology*, **28**, 213-233.
- van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T. & Loomans, A.J.M. (2006) Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology*, **51**, 609-634.
- van Mele, P., Vayssieres, J.F., Adandonon, A. & Sinzogan, A. (2009) Ant cues affect the oviposition behaviour of fruit flies (Diptera: Tephritidae) in Africa. *Physiological Entomology*, **34**, 256-261.
- van Mele, P., Vayssieres, J.F., Van Tellingen, E. & Vrolijk, J. (2007) Effects of an African weaver ant, *Oecophylla longinoda*, in controlling mango fruit flies (Diptera: Tephritidae) in Benin. *Journal of Economic Entomology*, **100**, 695-701.
- Varaldi, J., Fouillet, P., Boulétreau, M. & Fleury, F. (2005) Superparasitism acceptance and patch-leaving mechanisms in parasitoids: a comparison between two sympatric wasps. *Animal Behaviour*, **69**, 1227-1234.
- Vargas, R.I., Leblanc, L., Harris, E.J. & Manoukis, N.C. (2012a) Regional suppression of *Bactrocera* fruit flies (Diptera: Tephritidae) in the Pacific through biological control and prospects for future introductions into other areas of the world. *Insects*, **3**, 727-742.
- Vargas, R.I., Leblanc, L., Putoa, R. & Piñero, J.C. (2012b) Population dynamics of three *Bactrocera* spp. fruit flies (Diptera: Tephritidae) and two introduced natural enemies, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), after an invasion by *Bactrocera dorsalis* (Hendel) in Tahiti. *Biological Control*, **60**, 199-206.
- Vargas, R.I., Mau, R.F.L., Jang, E.B. & Wong, L. (2010) Area-wide fruit fly integrated pest management program: a model system. *8th International Symposium on Fruit Flies of Economic Importance* (eds B. Sabater-Munoz, V.N. Llopis & A.U. Garcia), pp. 171-178. Editorial Universitat Politècnica de Valencia, Valencia, Spain.
- Vargas, R.I., Stark, J.D., Prokopy, R.J. & Green, T.A. (1991) Response of oriental fruit fly (Diptera, Tephritidae) and associated parasitoids (Hymenoptera, Braconidae) to different color spheres. *Journal of Economic Entomology*, **84**, 1503-1507.
- Visser, M.E., Alphen, J.J.M.v. & Nell, H.W. (1990) Adaptive superparasitism and patch time allocation in solitary parasitoids: The influence of the number of parasitoids depleting a patch. *Behaviour*, **114**, 21-36.

- Visser, M.E. & Driessen, G. (1991) Indirect mutual interference in parasitoids. *Netherlands Journal of Zoology*, **41**, 214-227.
- Visser, M.E., Jones, T.H. & Driessen, G. (1999) Interference among insect parasitoids: A multi-patch experiment. *Journal of Animal Ecology*, **68**, 108-120.
- Waage, J.K. (1979) Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. *Journal of Animal Ecology*, **48**, 353-371.
- Waage, J.K. & Hassell, M.P. (1982) Parasitoids as biological-control agents - a fundamental approach. *Parasitology*, **84**, 241-268.
- Wajnberg, E. (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. *Behavioral Ecology and Sociobiology*, **60**, 589-611.
- Wajnberg, E., Bernhard, P., Hamelin, F. & Boivin, G. (2006) Optimal patch time allocation for time-limited foragers. *Behavioral Ecology and Sociobiology*, **60**, 1-10.
- Wajnberg, E., Curty, C. & Colazza, S. (2004) Genetic variation in the mechanisms of direct mutual interference in a parasitic wasp: consequences in terms of patch-time allocation. *Journal of Animal Ecology*, **73**, 1179-1189.
- Wajnberg, E., Fauvergue, X. & Pons, O. (2000) Patch leaving decision rules and the Marginal Value Theorem: an experimental analysis and a simulation model. *Behavioral Ecology*, **11**, 577-586.
- Wajnberg, E., Gonsard, P.-A., Tabone, E., Curty, C., Lezcano, N. & Colazza, S. (2003) A comparative analysis of patch-leaving decision rules in a parasitoid family. *Journal of Animal Ecology*, **72**, 618-626.
- Wang, X. & Messing, R.H. (2003a) Foraging behavior and patch time allocation by *Fopius arisanus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies. *Journal of Insect Behavior*, **16**, 593-612.
- Wang, X. & Messing, R.H. (2003b) Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. *Biological Control*, **27**, 251-259.
- Wang, X.G. & Keller, M.A. (2004) Patch time allocation by the parasitoid *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). III. Effects of kairomone sources and previous parasitism. *Journal of Insect Behavior*, **17**, 761-776.
- Wang, X.G. & Messing, R.H. (2003c) Egg maturation in the parasitoid *Fopius arisanus* (Hymenoptera: Braconidae): Do host-associated stimuli promote ovarian development? *Annals of the Entomological Society of America*, **96**, 571-578.
- Wang, X.G. & Messing, R.H. (2008) Role of egg-laying experience in avoidance of superparasitism by fruit fly parasitoid *Fopius arisanus* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America*, **101**, 656-663.
- White, I.M. & Elson-Harris, M.M. (1992) *Fruit flies of economic significance: their identification and bionomics*. CAB International.

- White, J.A. & Andow, D.A. (2007) Foraging for intermittently refuged prey: theory and field observations of a parasitoid. *Journal of Animal Ecology*, **76**, 1244-1254.
- Xu, C.L. & Boyce, M.S. (2005) Dynamic complexities in a mutual interference host-parasitoid model. *Chaos Solitons & Fractals*, **24**, 175-182.
- Zeddies, J., Schaab, R.P., Neuenschwander, P. & Herren, H.R. (2001) Economics of biological control of cassava mealybug in Africa. *Agricultural Economics*, **24**, 209-219.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed effects models and extensions in ecology with R*. Springer.

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ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

“Patch exploitation behaviour of the tephritid parasitoid *Fopius arisanus*, a candidate for the biological control of mango flies”

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

(Unterschrift)