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Modelling the effects of host searching
behaviour on the efficiency of the parasitic wasps
Cotesia glomerata and *Cotesia rubecula* in both
simple and more complex environments

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Study programme: MBI

Period: September – January

Thesis ENT

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Abstract

Upon herbivory, plants release chemicals as an attractant for the natural enemies of the herbivore. Multiple herbivory leads to changed headspace compositions, and thereby affects the attraction of natural enemies. In this thesis, the effect of multiple herbivory by both a host caterpillar, *Pieris rapae*, and a non-host caterpillar, *Mamestra brassicae*, on the parasitoids *Cotesia glomerata* and *Cotesia rubecula* is studied. Upon intrinsic competition, occurring in *P. rapae*, *C. rubecula* is known to be superior. It is expected that *C. glomerata* is superior on another facet, which would facilitate their coexistence. Here, by studying the behaviour of both species, it is shown that the host finding efficiency of *C. glomerata* is less affected by the presence of pure non-host patches than *C. rubecula*, which might facilitate coexistence. However, on patches containing both *P. rapae* and *M. brassicae*, no differential effects on the efficiencies of both species are observed.

In addition, a model is created using parameters from the behavioural experiments of both species. This model can be used to study the relative effects of different factors. Learning behaviour seems to have no large effect, whereas the host searching behaviour of the different species seems to have a significant effect on the number of parasitized hosts. For that reason, the more efficient host searching behaviour of *C. glomerata* might have facilitated its coexistence with *C. rubecula*.

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Introduction

In nature, individuals that are better adapted to their environment are more likely to survive and reproduce than less adapted congeners. This process of natural selection is also known as survival of the fittest. The fitness of an individual is strongly dependent on the amount of reproduction, and for that reason reproduction is important in this process of natural selection.

In case of parasitoids, the larvae develop exclusively on or in the body of another arthropod, its host. Immature endoparasitoids feed from the inside of parasitized hosts, which are almost exclusively insects. Since successful parasitoid development results in the emergence of adult parasitoids (Godfray, 1994), the finding of suitable hosts is a crucial aspect for reproduction. For that reason, host searching behaviour in parasitoids is thought to be subjected to strong natural selection (Gols *et al.*, 2005; Vet & Dicke, 1992). Furthermore, the ability of parasitoids to discriminate between poor hosts and more suitable hosts is important, as immature survival and overall adult fitness is strongly dependent on the physiological suitability and nutritional value of the host (Slansky, 1986; Brodeur *et al.*, 1998).

In addition, since each parasitized host is killed and cannot contribute to the growth of the host population, parasitoid searching efficiency is central to parasitoid-host population dynamics, to the evolution of parasitoid and host behaviour, and to the application of parasitoids as natural enemies of insect pests in biological control (Vet, 2001).

In this thesis, the host searching behaviours of *Cotesia glomerata* and *Cotesia rubecula* will be studied in simple environments containing only hosts, and in more complex environments containing both hosts and non-hosts.

In the Netherlands, *Cotesia rubecula* and *Cotesia glomerata* coexist and share the same host, which is *Pieris rapae* (Geervliet & Brodeur, 1992). For that reason, competition for available hosts is likely to occur. Environmental complexity, which is caused by presence of hosts and non-hosts on these two species, is known to influence the host searching behaviour (Vos *et al.*, 2001; Gols *et al.*, 2005). Differential effects of complexity on the host finding efficiencies of these species might provide more insights in their coexistence.

Because of strong interactions, parasitoids strongly influence the density of their hosts. For that reason, parasitoids play a major role in natural and agricultural ecosystems (Pearce *et al.*, 2006). Gaining more knowledge of the interactions between different species might be helpful in biological control programmes.

Model organisms

The parasitoids *Cotesia rubecula* (Marshall) and *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) will be studied. In general, parasitoids can be divided into two classes based on host specificity: specialists and generalists. As the names imply, specialists are specialized on just one host species, whereas generalists are able to use several host species for oviposition (Pearce *et al.*, 2006). *Cotesia rubecula* is a specialist of the small cabbage white, *Pieris rapae* (L.) (Lepidoptera: Pieridae). In contrast, *Cotesia glomerata* is a generalist, ovipositing in a wider range of hosts, consisting of several Pieridae species (Laing & Levin, 1982).

Another difference between *Cotesia glomerata* and *C. rubecula* is the clutch size: *C. rubecula* lays only one egg per host, whereas *C. glomerata* lays twenty to thirty eggs per host (le Masurier, 1991). For this reason, lower host densities might influence both species differentially.

Cotesia glomerata and *C. rubecula* are both reported to use *Pieris rapae* as host (Geervliet & Brodeur, 1992), which leads to competition when they occur in the same habitat (Fisher, 1961). An important form of competition between these species is known to be intrinsic competition as result from multiparasitism, in which one caterpillar is parasitized by both *C. glomerata* and *C. rubecula* (Laing & Levin, 1982). In this larval competition, *C. rubecula* is known to be superior (Laing & Corrigan, 1987).

Cotesia glomerata showed a higher degree of behavioural plasticity towards acceptance of *Pieris* host species than did *C. rubecula*, indicating a higher ability to adapt to changing environments. The host species which are most frequently used by *C. glomerata* are *Pieris rapae* and *Pieris brassicae* (L.) (Lepidoptera: Pieridae) (Brodeur *et al.*, 1996).

Pieris brassicae and *P. rapae* use the same food plants, mainly consisting of cultivated crucifers (Ohsaki & Sato, 1990). Both *P. rapae* and *P. brassicae* are common crop pests of brassica species, and *C. rubecula* as well as *C. glomerata* have been used successfully in biological control programmes (Cameron & Walker, 2002; Pearce *et al.*, 2006).

Host searching

The process of host searching consists of several phases: habitat location, host location, host acceptance, host suitability and host regulation. During the first three steps, host selection takes place. In every step, the area and habitats searched will be restricted, eventually leading to the suitable host (Vinson, 1976).

Different trophic levels in the parasitoid's environment, such as the herbivorous host or host's food plant, emit chemical cues that are often used in the host-searching process (Price, 1981; Vinson, 1976). Parasitoids are able to use these cues to assess important characteristics of the spatial distribution of their hosts, for example which food plants hosts are feeding from, the distribution of the hosts on these plants, what their density is and what quality the patch is (Vet, 2001).

The process of host searching can be divided in two phases: first the long-distance location of the host, and short-distance location after landing on an infested plant. Several studies have shown that parasitoids use volatiles from herbivore-infested plants for the long-distance location of their victims. These herbivore-induced plant volatiles (HIPVs) are known to be highly detectable (Price, 1981; Vet *et al.*, 1991; Vet & Dicke, 1992). In short-distance location, host-derived stimuli are thought to be important (Nealis, 1986).

Because of their role in the long-distance location, plants play an important role in the spatial guidance of foraging parasitoids (Vet *et al.*, 2001). De Moreas *et al.* (1998) have shown that some plants can emit specific volatile blends for different herbivore species. In this way, parasitoids might be selectively attracted. However, other studies have shown that several parasitoid species are also attracted to volatiles of plants under attack by non-host herbivores (Agelopoulos & Keller, 1994; Geervliet *et al.* 1996). This might be explained by non-specific volatile blends from these plants, or similar volatiles for different herbivore species (Vos *et al.*, 2001).

In a study of Geervliet *et al.* (1996), it has been shown that neither *C. rubecula* nor *C. glomerata* discriminated between plants infested by different caterpillar species. They did not discriminate between host and non-host species. In addition, artificial damage also led to the attraction of parasitoid females (Geervliet *et al.*, 1994). This inability to distinguish between host and non-host herbivores is thought to decrease the host searching efficiency in

environments containing both host and non-host herbivores, as parasitoids waste time on non-hosts (Vet, 2001).

The degree of specialization on both herbivore host range and host plant range is expected to influence the foraging strategies of parasitoids. Specialist parasitoids are thought to have more restricted preferences for plant-host complexes, compared to generalist parasitoids (Vet & Dicke, 1992).

Apart from playing a major role in host selection, these same chemicals are expected to play an important role in the biology of the host. As the emission of host-derived chemicals provides information about the host's location, natural selection on hosts will act against the emission of those volatiles (Vinson, 1976).

In addition, as plant species, cultivars, genotypes or even plant parts differ in attractivity to parasitoids, it is beneficial for herbivores to choose less attractive plants, because these are less visited by the parasitoid (Vet & Dicke, 1992, Vet 2001).

As herbivore-induced plant volatiles play a major role, this subject will be discussed in more detail.

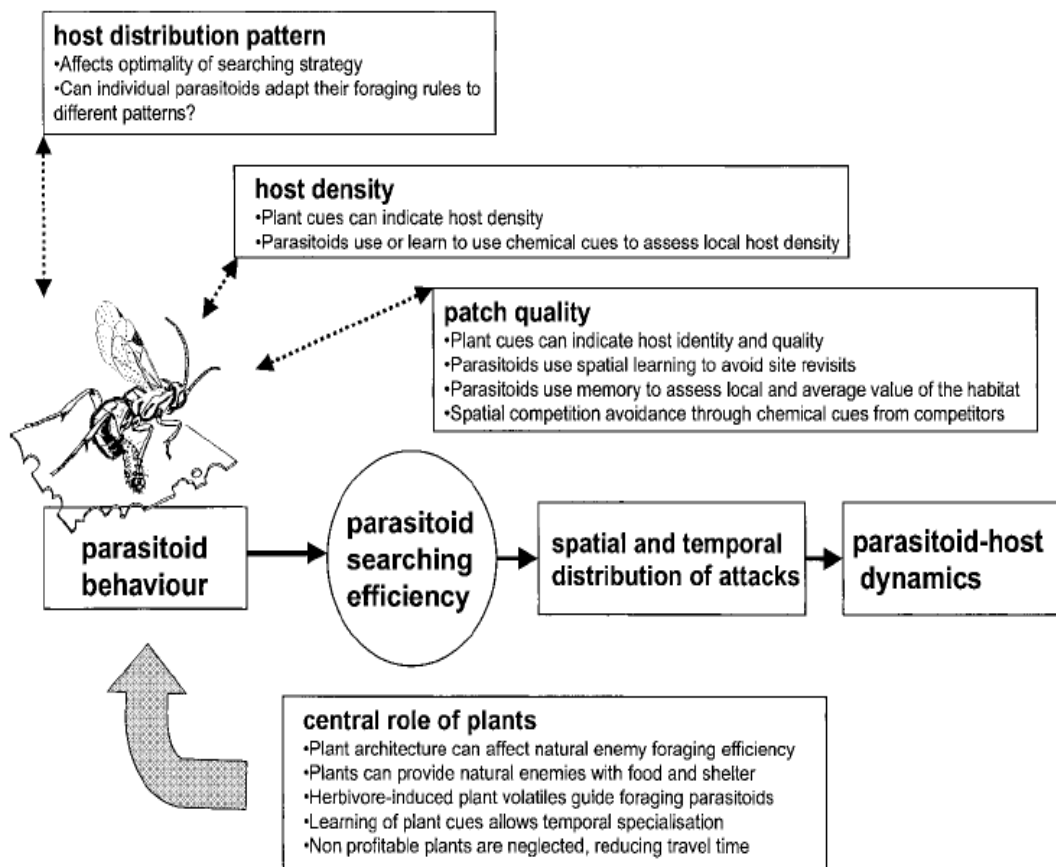


Fig. 1. Parasitoid searching efficiency links parasitoid behaviour to population processes. Black arrows specify how this process proceeds from parasitoid behaviour to parasitoid-host dynamics. Plants play a central role in this linking process through its strong influence on parasitoid behaviour. The dotted arrows specify the dynamic relationship between spatial characteristics

Source: Vet (2001)

Herbivore-induced plant volatiles

Volatiles from herbivore-damaged plants are an important source of information for parasitoids searching for these herbivores. Variation in headspace composition that is affected by herbivore species is relevant to the parasitoid female, because it provides information on the herbivore's identity (Vet & Dicke, 1992). This may also be true for variation due to plant species or cultivar, because the plant may affect the herbivore's suitability for parasitoid development (Godfray, 1994).

Plants are known to produce a diversity of secondary metabolites after attack or egg deposition by herbivores (Dicke *et al.*, 2009), which are used by parasitoids to detect their herbivore hosts (Price, 1981; Vet *et al.*, 1991; Vet & Dicke, 1992). These metabolites are called HIPVs, herbivore induced plant volatiles, and mainly comprise terpenoids, fatty acid derivatives, phenyl propanoids and benzenoids (Dudareva *et al.*, 2004). HIPVs can either be compounds that a plant does not biosynthesize unless it is damaged, or compounds that are also synthesised by undamaged plants but in different amount by damaged plants (Dicke & van Loon, 2000).

Herbivores belonging to different feeding guilds activate different signal-transduction pathways. Three pathways are known, respectively leading to emission of jasmonic acid (JA), salicylic acid (SA) or ethylene (ET) (Zhang *et al.*, 2009). Interestingly, it has been found that SA suppresses JA-dependent defense gene expression, and also negative effects of JA on SA-dependent defense gene expression are found (BRON). In this study, both herbivores *Pieris rapae* and *Mamestra brassicae* mainly induce the JA-dependent pathway (Marcel Dicke, personal communication). In a choice study between patches containing both *P. rapae* and *M. brassicae*, both *Cotesia glomerata* and *C. rubecula* are found to be more attracted to patches containing both herbivores (Bukovinszky *et al.* unpublished). Both herbivore species inducing the same signal-transduction pathways might explain this preference for mixed patches. Exposure of a plant to the attack of two herbivore species that induce the same signalling pathway is found to either enhance the attraction of carnivores, or it will not impair it, compared to the attraction to plants exposed to infestation by one species. For example, in case of double infestation by a spider mite and a leaf-chewing caterpillar, attraction of carnivores has found to be enhanced, compared to single infestation of one species (Dicke *et al.*, 2009). However, from a study on interactions between the two lepidopterans *Plutella xylostella* and *Pieris rapae*, it is clear that more studies should be done on this subject. Both species induce the jasmonic acid pathway, thus it would be expected that presence of both species would increase the attraction of natural enemies. The study looked at the effect of the presence of both species on the attraction of parasitic wasps *Cotesia vestalis*, attacking *P. xylostella*, and *C. glomerata*, attacking *P. rapae*. *Cotesia vestalis* showed a preference for plants exclusively infested by only its host compared to plants infested with both species. This leads to a lower mortality of *P. xylostella* caterpillars in presence of *P. rapae*, and it is therefore useful for *P. xylostella* to preferentially oviposit on plants infested by *P. rapae* (Dicke *et al.*, 2009; Shiojiri *et al.*, 2001).

In addition, HIPVs emitted by neighbouring plants (Baldwin *et al.*, 2006), root feeding insects, and abiotic conditions affect the emission of HIPV, and consequently the attraction of natural enemies of herbivores (Dicke *et al.*, 2009).

In addition to being used by parasitoids to detect herbivores, these HIPVs affect the behaviour of several community members. Apart from parasitoids, herbivorous arthropods (Halitschke *et al.*, 2008) and pollinators have been shown to use these volatiles (Kessler & Halitschke, 2007). Through these effects, HIPVs can strongly influence community dynamics (Dicke *et al.*, 2009; Bruinsma & Dicke, 2008). For that reason, it is important to know the exact effects of these HIPVs.

Spatial distribution of hosts

HIPVs play a major role in the long-distance location of hosts. After landing on a plant, host-related cues are used to find their hosts. In order to be efficient, the parasitoid should adapt its host-searching behaviour to the spatial distribution of the hosts (Vos *et al.*, 1998). Herbivore hosts have two types of spatial distribution: clustered vs. evenly distributed. Grouping is often used by animals to protect themselves against predation or disturbance (Mooring & Hart, 1992). In contrast, herbivorous hosts may be relatively inconspicuous when they occur solitary on plants (Mauricio & Bowers, 1990). However, in large aggregates, hosts may experience a reduced per capita risk of parasitism (Hunter, 2001). This might be due to group defence behaviour (Stamp, 1981), or to dilution effects and the limited accessibility of central individuals within a cluster (Turchin & Kareiva, 1989).

In the tritrophic system studied, remarkable differences can be observed between the spatial distributions of both *Pieris* species. The small white butterfly, *P. rapae* (L.), lays its eggs regularly distributed throughout the leaf, often only one or a few per leaf. This leads to a regular distribution of the larvae. In contrast, the large white butterfly, *P. brassicae* (L.), lays its eggs in clusters of 7-150 eggs, leading to a more heterogeneous distribution of the larvae. Clusters of *P. brassicae* are embedded in a silk mat, protecting them from falling off the leaf after mechanical disturbance (Brodeur *et al.*, 1996).

Feeding of a cluster of *P. brassicae* results in one large feeding damage site on a leaf, while leaves infested with low densities of the regularly distributed *P. rapae* contain several small feeding damage sites (Vos *et al.*, 1998). Parasitoids might use these visual cues in their search for hosts (Wäckers, 1994).

Host-searching behaviour should be adapted to the spatial distribution of the hosts in order to achieve a high efficiency (Vos *et al.*, 1998). The better the host searching behaviour is adapted to the spatial distribution of a particular host, the more individuals of this host species will be encountered.

Coexistence

Differential host searching efficiency might have facilitated coexistence between *C. glomerata* and *C. rubecula*. In this case, a trade-off exists between exploitative and interference competition exists, which means that one species is more efficient at finding unparasitized hosts, while the other species is more efficient in direct interactions such as larval competition (Holt & Polis, 1997; Briggs, 1993; Amarasekare, 2000). In contrast, another hypothesis on parasitoid coexistence states that two parasitoid species may be able to coexist via a trade-off between dispersal and competition, leading to metapopulations. In this case, one species is superior in competition, whereas the other species is able to escape from this competition by establishing patches where the first species is not present (Hastings, 1980; Amarasekare, 2000).

In case of the system studied, *Cotesia rubecula* is found to be superior over *C. glomerata* in case of multiparasitism, nevertheless these species both occur in the same habitat in the Netherlands (Geervliet & Brodeur, 1992). In order to see how this coexistence is possible, Vos and Hemerik (2003) compared the Dutch population of *C. glomerata* to a population that does not contain *C. rubecula*. *Cotesia glomerata* has been introduced into North America from Europe in 1884, about 350 generations of this parasitoid ago. By looking at the North American variant, the optimal strategy in absence of *C. rubecula* can be observed, as this latter parasitoid is absent there (Vos & Hemerik, 2003).

In America, in absence of *P. brassicae*, *P. rapae* is the only host of *C. glomerata*. *P. rapae* has a negative binomial distribution and, for this distribution, the foraging strategy of the North American population resulted in a higher lifetime reproductive success than the European strategy. This indicates that *C. glomerata* has adapted its host-searching strategy to *P. rapae* (Vos & Hemerik, 2003).

In Europe, *C. rubecula* is specialized on *P. rapae*. In contrast, *C. glomerata* is a generalist, being able to use several hosts, and parasitizes both *P. brassicae* and *P. rapae* (Vos & Hemerik, 2003). However, the foraging behaviour in Europe seems to be adapted to the gregarious larvae of *P. brassicae*, and is much less efficient on solitary feeding *P. rapae* larvae (Wiskerke & Vet, 1994). By adapting its host searching behaviour, *C. glomerata* has been able to escape from competition with *C. rubecula*, which might have contributed to the possibility to coexist.

Competition with *C. rubecula* might have played a role in the evolution of the host searching behaviour of *C. glomerata* in Europe. The superiority of *C. rubecula* after multiparasitism in *P. rapae* might have forced *C. glomerata* to restrict its host range to only *P. brassica* (Laing & Corrigan, 1987). In addition, the specialist *C. rubecula* hardly ever parasitized *P. brassicae* larvae, leading to the availability of this host species for *C. glomerata*, and therefore the possibility to avoid competition (Geervliet *et al.*, 2000).

In addition, *C. glomerata* is known to be able to recognize plants on which *C. rubecula* is parasitizing by using odours, and avoids flying to these plants (Geervliet *et al.*, 2002). This is also a way of avoiding competition with *C. rubecula*.

Coming back on the earlier mentioned hypotheses, no metapopulations have been observed. By specializing on another species, *C. glomerata* was able to escape from competition. However, the second hypothesis, in which a trade-off between exploitative and interference competition is considered, might also have contributed to the possibility of coexistence.

Competition

In coexistence, competition plays a major role. Intrinsic competition is by far the most important form of competition between *C. glomerata* and *C. rubecula* (Fisher, 1961). This occurs when different parasitoid species attack one host, also called multiparasitism, which results in intrinsic competition for host's resources. Only one of these parasitoids survives (Fisher, 1961).

Different mechanisms for intrinsic competition of parasitoids have been suggested, including direct physical attack, physiological suppression or starvation through lack of nutrients or oxygen, and the introduction of a toxic substance by one of the parasitoids (Fisher, 1961).

Both *C. glomerata* and *C. rubecula* are known to use *P. rapae* as a host (Geervliet & Brodeur, 1992), and for that reason intrinsic competition between these parasitoids plays a role. In case of multiple parasitism by *C. glomerata* and *C. rubecula* in a *P. rapae*-host, *C. rubecula* is found to be superior (Laing & Corrigan, 1987).

Patch use

In the search for hosts, choices have to be made about for example where to forage and when to leave the current patch (Pierre, 2010). Decision variables such as patch residence time seem to play a major role in increasing the host finding efficiency (Charnov, 1976). Several authors have considered what mechanisms might be employed to achieve optimal patch residence times (Vos *et al.*, 1998).

In nature, two types of mechanisms for decision making can be distinguished: incremental and decremental mechanisms (van Alphen *et al.*, 2003). In both cases, the initial willingness of staying on a patch is proportional to the host density, and decreases with time spent on the patch. However, the effect of ovipositions is different in the two mechanisms. When a parasitoid uses the incremental mechanism for decision making, each oviposition increases the probability of staying in the patch (van Alphen *et al.*, 2003; Driessen & Bernstein, 1999). This incremental mechanism is expected to be beneficial when foraging for a clustered host (Iwasa *et al.*, 1981), because when hosts occur in clusters, after encountering a host, the chance on encountering another host in the vicinity is relatively large.

In contrast, in the decremental mechanism for patch leaving decision, the probability of staying in the patch decreases with increased number of ovipositions (van Alphen *et al.*, 2003; Driessen & Bernstein, 1999). This mechanism is thought to be efficient for parasitoids searching for regularly distributed hosts (Iwasa *et al.*, 1981). Encountering a host decreases the quality of the patch, and therefore leads to a higher probability to leave the patch.

In the comparison of *C. glomerata* and *C. rubecula*, *Cotesia rubecula* is expected to use a decremental mechanism as it is specialized on the evenly distributed *Pieris rapae*. For *Cotesia glomerata*, the used mechanism is expected to differ depending on the host species used in that particular environment. In America, *P. brassicae* is absent, and *P. rapae* is the only host of *C. glomerata* (Vos & Hemerik, 2003). In this environment, *C. glomerata* is expected to use a decremental mechanism. However, when *C. glomerata* coexists with *C. rubecula*, *Cotesia glomerata* adapts its host searching behaviour to finding more *P. brassicae* (Vos & Hemerik, 2003). In the latter environment, it would be more efficient to use an incremental mechanism (Iwasa *et al.*, 1981).

Vos *et al.* (1998) performed a proportional hazards analysis in order to describe the patch exploitation mechanisms of *C. rubecula* and *C. glomerata*. Both *Cotesia glomerata* and *C. rubecula* use olfactory information during the flight to set a 'basic leaving tendency'. After *C. rubecula* has landed on a plant, the olfactory and/or visual information of feeding damage is perceived, leading to a decreased leaving tendency on a damaged leaf and an increased leaving tendency on a clean leaf. In addition, the leaving tendency was strongly decreased on a leaf containing the type of damage which is caused by the preferred host species. Thus, for *C. rubecula*, the presence of host- or non-host species is considered in this last step (Vos *et al.*, 1998).

In coexistence with *C. rubecula*, it would be beneficial for *C. glomerata* to learn to discriminate between the olfactory information released from *P. brassicae*- and *P. rapae*-infested plants in habitats containing both host species, and land on leaves with *P. brassicae* in an attempt to escape from intrinsic competition with *C. rubecula*. For that reason, an important difference with *C. rubecula* is that *C. glomerata* should discriminate between host and non-host species before landing on a leaf (Vos *et al.*, 1998). The specialization on *P. brassicae* in coexistence with *C. rubecula* leads to a further adaption of the exploitation mechanism of *C. glomerata*. After landing on a leaf, the leaving tendency decreases with ovipositions. In addition, the cumulative number of ovipositions experienced, the number of infested patches visited, and time seem to have an effect on the leaving tendency. The exact balance of these factors depends on

the amount of heterogeneity in the environment and may change as the parasitoid moves into richer or poorer parts of its environment (Vos *et al.*, 1998).

In order to find *P. brassicae*, it is efficient to invest more time and energy in exploration flights in order to increase the chance of encountering its rare clusters in the European environment (Vos, 2001). This exploration behaviour leads to less encounters with *P. rapae* than would be possible under more exploitative foraging behaviour. When focussing on the exploitation of *P. rapae*, the parasitoids may risk to miss a high-quality cluster of *P. brassicae*. However, when focussing on rare *P. brassicae* clusters, they may risk having no offspring at all. In conclusion, in the European strategy, more time is spent on exploration in order to locate the clustered *P. brassicae*. This caused reduced parasitism rates of *P. rapae*. The American strategy seems to have adapted to the environment by spending more time and energy on exploitation instead of exploration. Exploitation is more beneficial for regularly distributed hosts, and thus for finding *P. rapae* (Vos & Hemerik, 2003).

Wiskerke and Vet (1994) also compared host searching behaviour of *C. glomerata* and *C. rubecula*. *Cotesia glomerata* displayed area-restricted search, a repeated cycle of attack and oviposition, and a significantly shorter time between ovipositions. All of these are characteristics of an incremental mechanism for making decisions, and are beneficial for feeding on gregariously distributed hosts. In addition, the encounter rate of *C. glomerata* with *P. brassicae* was much higher than with *P. rapae*, which confirms the adaptation on this host species. However, the propensity to search for and parasitize larvae did not differ between both host species for *C. glomerata*. This indicates that this parasitoid species does not show a preference for one of both host species, but it has solely adapted its host searching behaviour in such a way that it encounters more *P. brassicae* (Wiskerke & Vet, 1994).

In contrast, *C. rubecula* shows a higher propensity to search for *P. rapae* than for *P. brassicae*, thereby showing a preference for the former species. In a choice situation where both parasitoids could parasitize both *P. rapae* and *P. brassicae*, *C. glomerata* did not show a preference, whereas *C. rubecula* showed a clear preference for *P. rapae* (Wiskerke & Vet, 1994).

Influence of complexity on host-finding efficiency

The complexity of a habitat is increased by the presence of several plant species (plants polyculture) or several insect species. The composition and the structure of the vegetation have an influence on the host-finding efficiency of parasitoids. A more complex vegetation reduces parasitoid host-finding efficiency due to physical and chemical characteristics of the plants located close to the host-infested plant (Gols *et al.*, 2005).

A study of Geervliet *et al.* (1996) revealed that both *C. glomerata* and *C. rubecula* do not discriminate between the host species *Pieris rapae* and the non-host species *Mamestra brassicae*. This inability to discriminate is expected to result in wasting time, spent on locating non-host species instead of host species. In the field, most plant species will be attacked by a complex of several herbivore species. For that reason, this decreased efficiency is expected to have profound effects on the community (Futuyma & Gould, 1979). In addition, a decreased host-finding efficiency of the parasitoids will be advantageous for the herbivores. By making plant information less informative, each herbivore in such a complex might be relatively protected from predation. These refuges are dynamically important, since they lead to variability in the intensity of parasitoid attacks on hosts within a given domain and allow hosts to benefit from enemy-free space (Holt & Lawton, 1994).

Behavioural studies of host location and patch time allocation have shown that the presence of both host and non-host species indeed results in a decreased host-finding efficiency of *C. glomerata*. In a situation with only host-species present on *Brassica oleracea* plants, the parasitoid has no problem with finding hosts. However, when both host and non-host species were present on these plants, the host-finding efficiency is significantly decreased due to considerable time waste on these leaves. The reason for this difference is that plant information is non-specific, and therefore no information about herbivore species can be obtained from it (Vos *et al.*, 2001).

For *C. glomerata*, it has been shown that the residence time on a non-host patch is significantly shorter than on a pure host patch or a mixed patch. However, for this same species, the residence time on mixed patches has shown to be larger than on pure host patches, but interestingly, the relative number of ovipositions per time unit was lower in the mixed patch compared to the host only patch. In addition, *C. glomerata* has shown to parasitize non-host caterpillars on mixed patches, leading to loss of eggs and time (Bukovinszky *et al.*, unpublished). This indicates that *C. glomerata* not only wastes time on pure non-host patches, but also loses time as a result of a decreased efficiency on mixed patches.

From a model, designed by Vos *et al.* (2001), it can be concluded that a decreased host searching efficiency weakens the interaction strength between parasitoid and host, which is suggested to have large effects on the persistence of the complete parasitoid-host community. This model predicts that increasing herbivore diversity initially has a positive effect, by promoting the persistence of parasitoid communities. However, a certain threshold has been predicted, above which the parasitoid would become extinct. At that point, the efficiency of finding hosts has decreased to a point where the parasitoid is unable to parasitize sufficient hosts to maintain the population (Vos *et al.*, 2001).

When the number of herbivore species has reached the threshold, extinction of parasitoids will occur. According to the model, the first species to go extinct are those that relatively waste most time on non-hosts. Interestingly, 'perfect' parasitoids, with no wasting time on non-hosts at all, will go extinct as well because of strong fluctuations in both host and parasitoid populations (Vos *et al.*, 2001). These 'perfect' parasitoids are strongly specialized on one host, and therefore cannot change to another host when the abundance of the preferred host decreases. In addition, as a result of the large pressure, the abundance of the preferred host is expected to decrease.

From this model of Vos *et al.* (2001), it can be concluded that on one hand natural selection takes place, eliminating parasitoids that waste too much time on non-host species, and on the other hand chance operates on small number of parasitoids that are perfectly efficient. Extinctions would stop when the number of species would get back below the threshold. This would result in communities with parasitoid species that waste relatively little time on non-hosts (Vos *et al.*, 2001).

This decreased host-finding efficiency is expected to have a larger effect on specialist parasitoids than on generalists (Vos *et al.*, 2001). For that reason, it might also influence the relative abundance of different parasitoid species.

In the system studied, the specialist *Cotesia rubecula* is expected to be more affected by increased complexity than the generalist *C. glomerata* (Laing & Levin, 1982). This might change the relative abundance in favour of *C. glomerata*, and the interactions between both species.

From a field study (MSc thesis Ana Efremova, Wageningen University, 2009) it can be concluded that *C. glomerata* is indeed less affected by increased complexity than *Cotesia rubecula*. In the control situation, in which plants with *Pieris rapae* were alternated with plants lacking caterpillars, *C. rubecula* has found to be superior. In the other treatments, the complexity is increased by the presence of the non-host *Mamestra brassicae*. In the second treatment, the presented plants contained alternatingly *Pieris rapae* and

Mamestra brassicae, and in the last treatment the plants contained alternately *Mamestra brassicae* and both *M. brassicae* and *P. rapae*. For both *Cotesia* species, the fraction of caterpillars parasitized decreased for the last two treatments. Interestingly, the effect was larger for *C. rubecula*, which has led to superiority of *C. glomerata* over *C. rubecula* in the treatments with increased complexity. This confirms the hypothesis that increased complexity has a larger effect on *C. rubecula* than on *C. glomerata*.

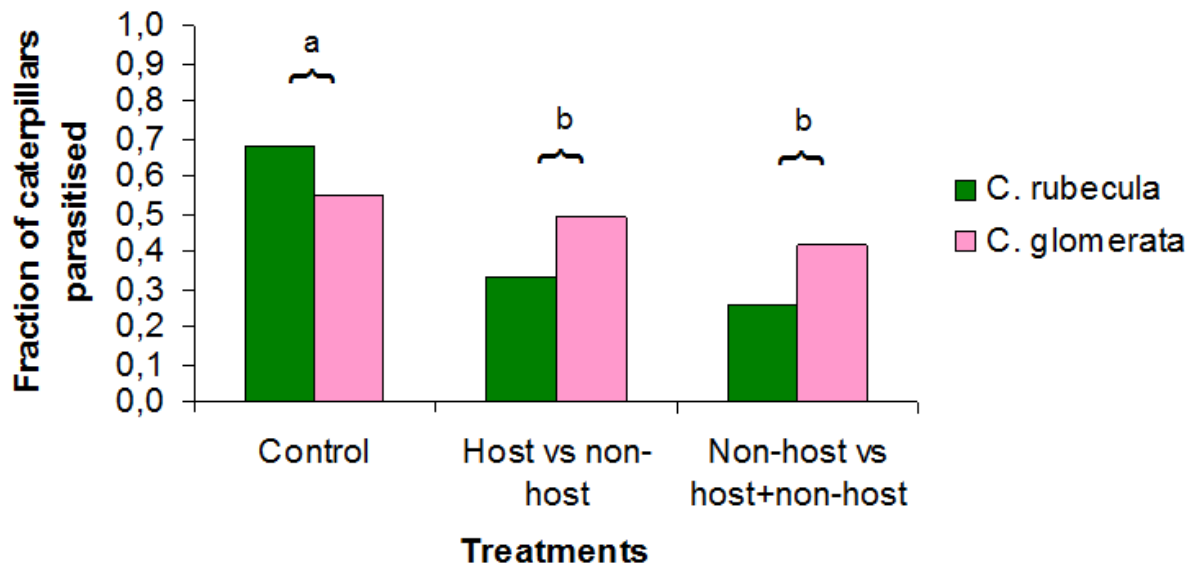


Figure 2. Effect of different environment on the fraction parasitized caterpillars on *Cotesia rubecula* and *Cotesia glomerata*. Source: MSc thesis Anna Efremova.

From the thesis of Ana Efremova, it was concluded that this difference could not be explained by differences in learning ability. However, different host searching strategies might be the reason.

In this thesis, I will investigate the differences in host-searching behaviour between *C. glomerata* and *C. rubecula*. From these results, a conclusion can be drawn on the role of host-searching behaviour on explaining the different effects of complexity on the fraction of caterpillars parasitized of these two species.

Scientific Importance

It is important to study the interactions between parasitoid populations and their host populations, as these populations directly affect each other (Pearce *et al.*, 2006).

During the last decades, much research has been done about host-searching behaviour in parasitoids. However, in most cases, experiments are carried out with solely host species, while in nature, plants will often contain non-host species next to host-species (Futuyma & Gould, 1979). This presence of both host- and non-host species has been thought to induce the production of less informative volatile blends, compared to herbivory by a single herbivore (Vos *et al.*, 2001).

In this thesis, the influence of the presence of both host and non-host herbivores on the searching behaviour of *C. rubecula* will be investigated. In addition, the relative numbers of non-hosts will be varied to test if this has an effect. When such an effect of non-host species on its parasitoid seems to exist, this might be of importance for the use of parasitoids in the control of pests. In

order to understand the spatial variation in parasitoid attack rates, we certainly need to have behavioural data that deal with assessment of host distributions and patch profitability, and movement between patches, as these are both important components of searching efficiency (Vet, 2001).

In addition, conclusions will be drawn on the comparison of the effect of increased complexity on *C. rubecula* and *C. glomerata*. When these species are differentially affected by increased complexity, this might change the relative abundance and interactions between *C. rubecula* and *C. glomerata*. For that reason, it might (partly) explain the possibility for coexistence.

Research Questions and Hypotheses

In this thesis, behavioural experiments will be carried out with *Cotesia rubecula*. The behaviour of this parasitoid will be compared to the behavior of *Cotesia glomerata*, on which data are already available from Bukovinszky *et al* (unpublished).

The relative number of resp. non-host and host herbivores will be varied in four different treatments:

- 1) 10 hosts
- 2) 10 non-hosts
- 3) 5 hosts, 5 non-hosts
- 4) 2 hosts, 8 non-hosts

The behavioural elements which will be distinguished are the following: fly, walk, attack, oviposit, stand still and preen. The same behavioural elements are used in the study of Bukovinszky *et al.* (unpublished).

From these behavioural data, several parameters will be calculated and used in a model. In this way, conclusions can be drawn about the relative importance of host searching behaviour.

Research questions:

1. Does the presence of both host and non-host species have an effect on the host searching behaviour of *C. rubecula*?
2. Is the presence of both host and non-host species expected to have a different effect on *C. rubecula* compared to the effect on *C. glomerata*?
3. Concluding from a simulation model, might differences in host searching behaviour have facilitated the coexistence of both species?

Hypotheses:

1. The host searching behaviour of *C. rubecula* is expected to be affected by the presence of both host and non-host species.
 - This is based on the fact that the presence of both host and non-host species has been thought to induce the production of less informative volatile blends, compared to herbivory by a single herbivore (Vos *et al.*, 2001).
2. The presence of both host and non-host species is expected to have a larger effect on *C. rubecula*, compared to *C. glomerata*.
 - Increased complexity is expected to have a stronger effect on specialists than on generalists (Gols *et al.*, 2005; Vos *et al.*, 2001). As *C. rubecula* is a specialist species, and *C. glomerata* is a generalist, the host searching behaviour of *C. rubecula* is expected to be stronger affected by the presence of both non-host and host species.

- However, it can also be expected that *C. rubecula* has evolved better strategies in the discrimination of different hosts, which would lead to less wasted time on non-host. If this is true, *C. rubecula* will be less affected by increased complexity compared to *C. glomerata*.
3. Increased complexity is expected to have relatively smaller effects on *C. glomerata* than on *C. rubecula*, which might have facilitated the coexistence of both species.
- See hypothesis 2.

Materials and Methods

Plants

The plants that were used in the experiment were *Brassica oleracea cv capitata* Cyrus. These were grown in a greenhouse at a temperature of 22 (± 2) °C, a relative humidity of 60%, and a light-dark pattern of 16:8 L:D. During the experiment, all plants were 7-8 weeks old, and contained 6-7 leaves. Prior to the observations, plants were infested with caterpillars according to the treatment. The time lapse between infestation and the observations was 16-24 hours.

Insects

For the experiment, only *Cotesia rubecula* was used, as data on *Cotesia glomerata* were already available from an experiment by Bukovinszky *et al* (unpublished). The parasitoids were reared on larvae of *Pieris brassicae*, the large white butterfly. Laboratory cultures of both *C. rubecula* and *P. rapae* were used, which were maintained at 20 (± 2) °C, a relative humidity of 60 (± 10) % and a L16-D8 photoperiod.

In order to compensate for different amounts of foliar damage, the larvae of *P. rapae* used in the experiment were in their early second instar whereas larvae of *M. brassicae* were in their late second instar.

Caterpillars were reared on Brussels sprouts plants (*Brassica oleracea gemmifera cv. Cyrus*).

The total number of caterpillars per plant was set on 10, using four different treatments varying the relative numbers of host and non-host individuals. 16 - 24 hours after infestation with caterpillars, the plants were used in the experiment.

Cocoons were collected from separate cages without hosts, host-related cues or plant material. After emergence, the parasitoids were provided *ad libitum* with water and honey, and were allowed to mate. In the experiment, naïve wasps of 5-7 days old were used.



Figure 3: The experimental setup

Experiment

The experiment took place in a greenhouse compartment. In this compartment, a construction was made of white sheets (330 x 175 x 280 cm), in order to obtain a contrasting background for flying parasitoids (see figure 3). An air flow was created by placing two fans covered with a piece of netting at the end of the

table. Within the greenhouse compartment, temperature was maintained at 22 (± 2) °C, the relative humidity was 60%, and a L16- D8 photoperiod was used. Additional illumination was provided by metal halide bulbs of 400W. Four plants were placed in a square with 50 cm distance between plants. The release site of the parasitoids was at 15 cm height from the surface of the table, and at 50cm distance from the two nearest experimental plants. It consisted of an excised leaf with feeding damage caused by *P. rapae*, but without hosts.

Every day, new plants were used for every treatment. Parasitized larvae were replaced by unparasitized ones, as presence of parasitized larvae influences the host searching behaviour of parasitoids (Kusano & Kitano, 1974). After each observation the plants that were visited by the parasitoid, were replaced by a new one.

Three different treatments were used:

- 10 hosts
- 10 non-hosts
- 2 hosts, 8 non-hosts

C. rubecula is known to have specialized on *P. rapae*, and for that reason, *P. rapae* is used as host. As non-host, *Mamestra brassicae* was used.

The different behavioural elements were recorded on a laptop with The Pocket Observer 5.0 (Noldus Information Technology, 2003a). The behavioural elements which will be distinguished are the following: fly, walk, attack, oviposit, stand still and preen. The same behavioural elements are used in the study of Bukovinszky *et al.* (unpublished). Walking also included searching. Preening is defined as brushing of the antennae, thorax, abdomen, ovipositor or wings with the legs, while stationary. The behavioural element 'attack' includes charging at the herbivore with raised antennae and wings, and coiled abdomen. Oviposition is recorded when the parasitoid inserted its ovipositor into the herbivore.

The simulation model

In the model, the environments used in the field experiment of Ana Efremova (MSc thesis Wageningen University, 2009) are simulated. These environments consisting of sixteen patches can be seen in figure 4, in which the circles represent plants, and the different colors represent different treatments.

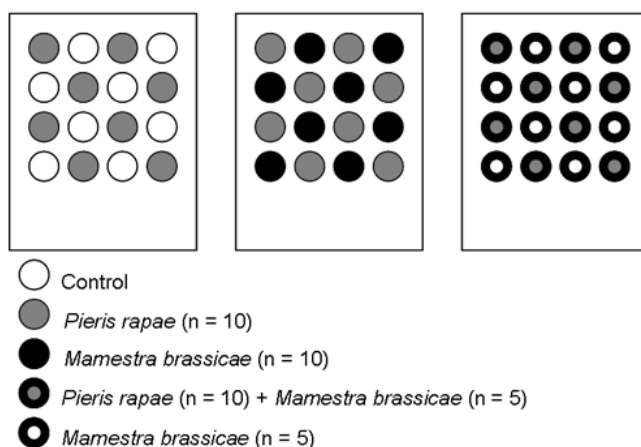


Figure 4. Source: MSc thesis Ana Efremova, Wageningen University.

In the construction of the model, data from my own experiment with *C. rubecula* and a similar experiment with *C. glomerata* (Bukovinszky *et al.*, unpublished) are used. The data from these experiments provide information about the intervals between encounters and the giving up times for both *Cotesia rubecula* and *Cotesia glomerata*. In this way, a comparison can be made between the

efficiency of these two species in environments with different complexity.

After arrival on a plant, both a 'giving up time' (GUT) and an 'interval between encounters' (IBE) are drawn from an exponential distribution, in which mean $GUT = 1/(\text{leaving rate})$ and mean $IBE = 1/(\text{encounter rate})$. Every encounter of a host is assumed to result in oviposition. For encounters with non-hosts, this is not the case, as it has been observed from both the experiment with *C. rubecula* and *C. glomerata*, *Mamestra brassicae* is not parasitized in this patch-type. In contrast, in mixed patches *M. brassicae* is known to be parasitized. *Cotesia glomerata* and *Cotesia rubecula* are both not able to discriminate between the non-host *Mamestra brassicae* and the host species *Pieris rapae* (Geervliet *et al.* 1996). On a non-host patch no host-related cues are present, which are present in patches containing both hosts and non-hosts. In the latter situation, the parasitoid seems to have difficulty in discriminating hosts and non-hosts, resulting in the oviposition of non-hosts as well as hosts.

The encounter rates in the different patch types are assumed to be equal. In addition, on mixed patches, the encounter rate of hosts and non-hosts are assumed to be the same.

If the drawn IBE is larger than the drawn GUT, the parasitoid stops searching (i.e. gives up) before a host or non-host is encountered, and leaves the patch. In contrast, when IBE is smaller than GUT, oviposition takes place, after which a new GUT and IBE are being drawn until the GUT is shorter than the IBE. These new IBE and GUT are again compared, again resulting in leaving the patch or encounter of a caterpillar.

On an empty patch, the parasitoid always leaves after the drawn GUT, as there are no caterpillars to be parasitized. This is also the case for patches containing only non-hosts, as these are not parasitized in a pure non-host patch.

On a mixed patch, two drawn IBE's are taken into account: the interval between oviposition of a host (IBE_h) and the interval between oviposition of a non-host (IBE_{nh}). When IBE_h is smaller than both IBE_{nh} and GUT, a host is parasitized. In contrast, when IBE_{nh} is smaller than IBE_h and GUT, oviposition of a non-host takes place.

The residence time, i.e. the total time on one patch, is calculated for every patch. For every oviposition taking place, a handling time is drawn. After one oviposition, the residence time will be the sum of all IBE's and the handling time. When the parasitoid parasitizes another time, the new IBE and handling time will be added. However, when the parasitoid leaves the patch, the GUT is added to the residence time. In addition, after leaving the patch, the travel time between patches is calculated.

When the parasitoid leaves the patch, the new patch is chosen by taking into account the attractivity of the different neighboring patches. From a windtunnel experiment by Poelman & Bukovinszky, in which parasitoids choose between empty, host only, non-host only and mixed patches, the relative preferences of both *Cotesia glomerata* and *Cotesia rubecula* were calculated. These preference values are taken into account in the model when the parasitoid chooses a new patch.

The baseline leaving tendency is defined as the leaving tendency on a pure non-host patch. This is calculated by using the formula of Vos and Hemerik (2003):

$$\text{leaving rate} = \frac{\#gut}{t.t.o.t.}$$

in which t.t.o.t. represents the total time on test and #gut is the number of patch leavings (and therefore also the number of realised gut's).

The effects of oviposition and host presence on the giving up time are calculated by using a Cox survival analysis in R, and significant effects are taken into

account in the model. A fixed time analysis is used, as this gave more realistic results compared to the time-dependent analysis.

The encounter rate is assumed to be constant for the different patch types. The calculation is similar to the calculation of the leaving rate, with #ibe as the number of realized encounters:

$$\text{encounter rate} = \frac{\#ibe}{ttot}$$

An important difference between *C. rubecula* and *C. glomerata* is that only one larva of *C. rubecula* can develop per host, whereas a host can contain multiple larvae of *C. glomerata*. When the last mentioned species parasitizes one host multiple times, this results in more offspring. In contrast, when *C. rubecula* parasitizes one host multiple times, no extra offspring is produced, leading to egg loss of this parasitoid.

In the model, the effect of using the parasitoid or predator approach on the number of parasitized hosts is calculated for *Cotesia rubecula*. In the predator approach, the number of hosts counts down after parasitism. In this way, parasitized hosts are unavailable for superparasitism. In contrast, in the parasitoid approach, the hosts are still available for superparasitism. *Cotesia glomerata* is always treated as a parasitoid, as no difference is expected of oviposition in a host which is already parasitized or an unparasitized host. Superparasitism leads to extra offspring for the latter species.

This model runs for two hours, after which the effects of environment, preference and species on the number of parasitized hosts are calculated. The effect of preference is taken into account, as learning behavior might change the ability to discriminate between patches differing in profitability, and might therefore change the patch choices based on a changed preference.

Results

Increased complexity is thought to influence the foraging efficiency of parasitoid wasps. In this thesis, two aspects are studied.

As *Cotesia glomerata* and *Cotesia rubecula* both are not able to distinguish between the host species *Pieris rapae* and non-host *Mamestra brassicae* (Geervliet *et al.*, 1996), they are expected to land on patches containing only non-hosts, which leads to wasting time.

In addition, this inability to distinguish between these species is expected to lead to time loss on mixed patches.

Time loss on non-host patches

As a result of choosing to land on a non-host patch, the efficiency will be decreased. By comparing the preferences of both *Cotesia glomerata* and *Cotesia rubecula*, the probability of choosing the non-host patch can be predicted.

The preferences of both *C. glomerata* and *C. rubecula* are calculated from a choice experiment carried out by Bukovinszky *et al.* (unpublished). From these preferences, it can be concluded that *C. glomerata* will choose the non-host patch 16 times when you let her choose 84 times, which is 19%. In case of *C. rubecula*, 5 of the 19 times the non-host patch will be chosen, which is 26,3%. The latter species lands more often on a non-host patch.

Table 1: Relative preferences of *Cotesia rubecula* and *Cotesia glomerata* in a choice situation between the host species *Pieris rapae*, the non-host *Mamestra brassicae*, mixed patches containing both *P. rapae* and *M. brassicae*, and empty patches containing no caterpillars. Calculated from unpublished data of T. Bukovinszky

	<i>C. rubecula</i>	<i>C. glomerata</i>
<i>P. rapae</i>	7	25
<i>M. brassicae</i>	5	16
Mixed	6	42
Empty	1	1

In addition, the residence time of *Cotesia glomerata* on a pure non-host patch is significantly shorter (Appendix: Output 1, Kruskal-Wallis: $P=0,000$) than on a pure host patch. In contrast, for *Cotesia rubecula*, no significant difference was found between the residence time on a non-host patch and on a pure host patch (Appendix: Output 2). This suggests that *Cotesia glomerata* is able to adjust its residence time to the patch type, whereas *C. rubecula* is not able to do this.

However, the difference in residence times of both species on non-host patches is not significant (Appendix: Output 3). For that reason, despite of being able to adjust its residence time to the patch type, *C. glomerata* does not stay significantly shorter on these unfavourable patches .

The comparison of the preferences for host only and non-host only patches suggest that the presence of non-host patches has a stronger negative effect on the host finding efficiency of *C. rubecula* than on the efficiency of *Cotesia glomerata*. Residence time on non-host patches does not differ significantly.

Time loss on mixed patches

The presence of mixed patches is also thought to have a negative effect on the host finding efficiency, as the parasitoid is expected to have more difficulty in finding hosts when non-hosts are also present (Vos *et al.*, 2001). Both *C. glomerata* and *C. rubecula* were shown to parasitize non-hosts in mixed patches, which does not occur in non-host only patches. The parasitoids are thought to be distracted by the presence of host-related cues (e.g. feces, frass).

For both species, the residence time on mixed patches was not significantly different from the residence time on host only patches. However, for both *C. glomerata* and *C. rubecula*, the number of hosts parasitized per time unit was significantly lower in the mixed patch containing two hosts and eight non-hosts, compared to the host only patch (Appendix: output 4 and 5, Kruskal Wallis: $P=0,003$ and $P=0,020$ respectively for *C. glomerata* and *C. rubecula*). This latter indicates that the presence of mixed patches results in a lower host finding efficiency for both species.

The residence time of *C. glomerata* and *C. rubecula* on mixed patches did not differ significantly (Appendix: Output 6), indicating no differential effect of landing on mixed patches on the time loss of both species.

No clear differences in time loss on mixed patches between *C. glomerata* and *C. rubecula* can be concluded from these data. The host finding efficiency of both species is thought to be negatively affected by the presence of mixed patches, but no clear differences between these species can be observed.

Model parameters

Effect of oviposition on giving up time

Cotesia glomerata:

For *C. glomerata*, a significant difference (Appendix, output 7: cox regression R, $P < 0.05$) was found between the giving up time after no parasitizations and the giving up time after one or more parasitizations of hosts. After one or more ovipositions, the leaving rate was decreased with 52% (Appendix, output 7: $e^{\beta} = \exp(\text{coeff}) = 0.5204$) compared to the leaving rate after no ovipositions, leading to a larger giving up time, and therefore a larger residence time. Oviposition in non-hosts did not have an effect on the leaving tendency (Appendix, output 8).

Cotesia glomerata mainly uses *Pieris brassicae* as host, which occurs in clusters. For that reason, it is beneficial to stay on the patch after oviposition as the chance of another encounter is thought to be increased. This lower leaving tendency after oviposition is indeed observed from the analysis.

Cotesia rubecula:

For *C. rubecula*, a similar effect has been observed. The giving up time after one or more ovipositions of hosts was significantly different (Appendix, output 9: cox regression R, $P < 0.005$) from the giving up time after zero ovipositions. The hazard ratio was 0.4087, indicating that the leaving rate after one or more ovipositions was 0.4087 times the hazard rate after no ovipositions, resulting in an increased residence time after one or more ovipositions in hosts. Oviposition in non-hosts does not have a significant effect on the giving up time (Appendix, output 10).

As *Cotesia rubecula* is known to be specialized on the evenly distributed *Pieris rapae*, a decremental mechanism would be expected. However, this hypothesis is not confirmed by these results. A positive effect of the number of parasitizations on the giving up time is observed, which is characteristic for the incremental mechanism.

Effect of presence of hosts on giving up time

For both species, the residence time on non-host patches differs significantly from the residence time on the host only patches and mixed patches (Appendix: output 1 and 2; Figure 5 and 6 for resp. *Cotesia rubecula* and *Cotesia glomerata*). The residence time on mixed patches and patches containing only hosts did not differ significantly (Appendix: output 4 and 5). For that reason, the effect of the presence of hosts is included in the model. On patches containing hosts (host only patches and mixed patches), the leaving rate was significantly lower than on pure non-host patches. From a Cox survival analysis including only the effect of host presence (Appendix: output 11 and 13), the hazard ratio (e^{β}) of the leaving rates turned out to be 0.4431 and 0.1163 respectively for *C. rubecula* and *C. glomerata*, indicating a leaving rate on patches containing hosts of 0.4431 times the leaving rate on pure non-host patches for *C. rubecula* and 0.1163 times the leaving rate on pure non-host patches for *C. glomerata*. For both species, this lower leaving rate in presence of hosts resulted in a longer residence time, which increases the host finding efficiency.

Including both the effect of host presence and the effect of oviposition on the giving up time resulted in a significant effect of both factors for *C. glomerata* (Appendix: output 14), and only a significant effect of host presence for *C. rubecula* (Appendix: output 12). For that reason, in case of *C. glomerata* both factors are included in the model, whereas for *C. rubecula* only the effect of host presence is taken into account. For *C. glomerata*, coefficients from output 14 are used ($e^{\beta} = 8.60321$), and for *C. rubecula* the coefficients from output 11

($e^{\beta}=0.4431$). The insignificance of the effect of oviposition on the giving up time of *C. rubecula* when including both factors can be explained by overlap of the effect of both factors. Host presence partly explains the effect of oviposition, leading to insignificance of the latter factor for *Cotesia rubecula*.

Figure 5: Survival graph of residence time of *Cotesia rubecula* on patches with different host densities. In this graph, the event is patch leaving, and is plotted as a function of the time. The vertical axis shows the proportion individuals that has not left the patch after the time displayed at the horizontal axis.

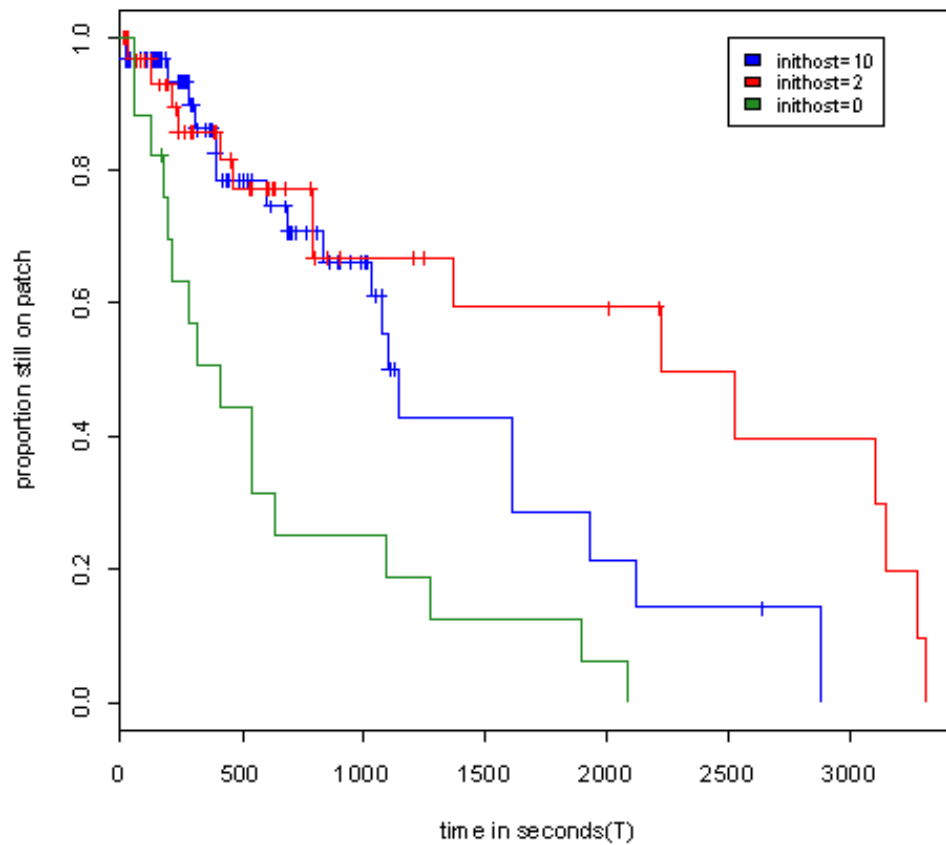
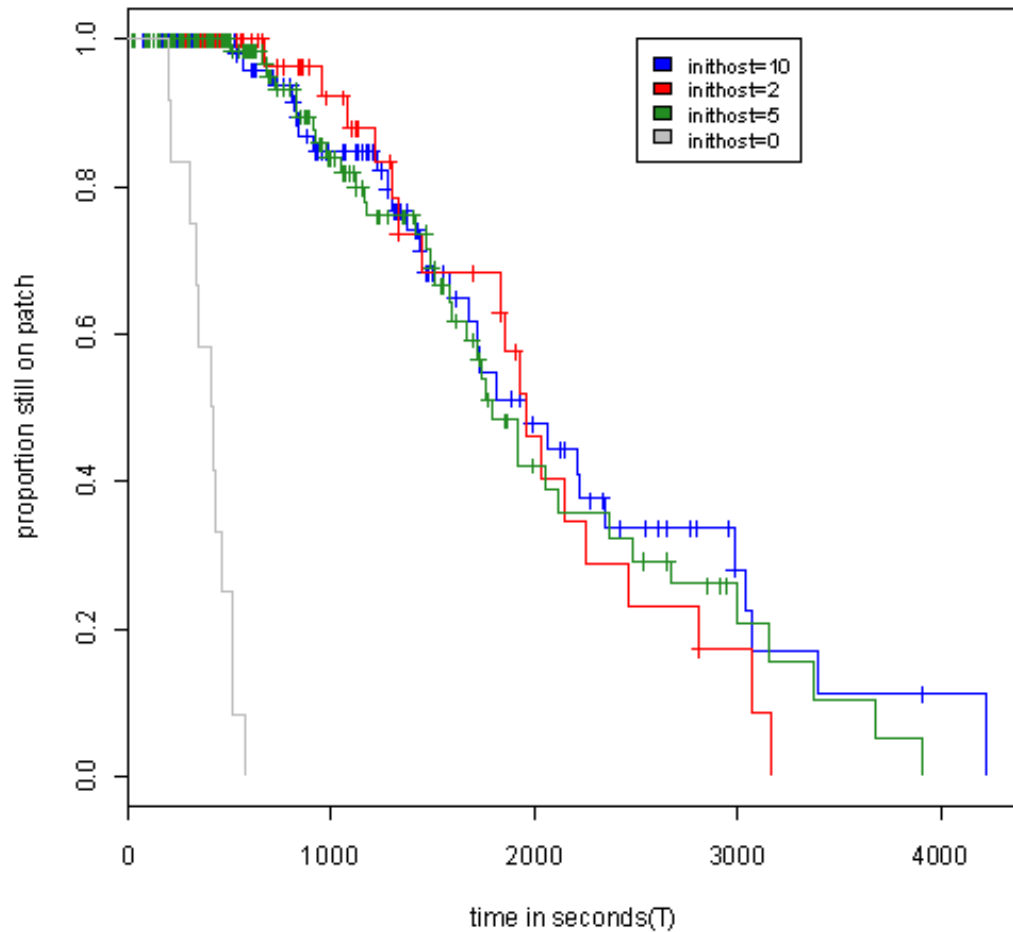


Figure 6: Survival graph of residence time of *Cotesia glomerata* on patches with different host densities. In this graph, the event is patch leaving, and is plotted as a function of the time. The vertical axis shows the proportion individuals that has not left the patch after the time displayed at the horizontal axis.



Model calculations

Effect predator and parasitoid approach

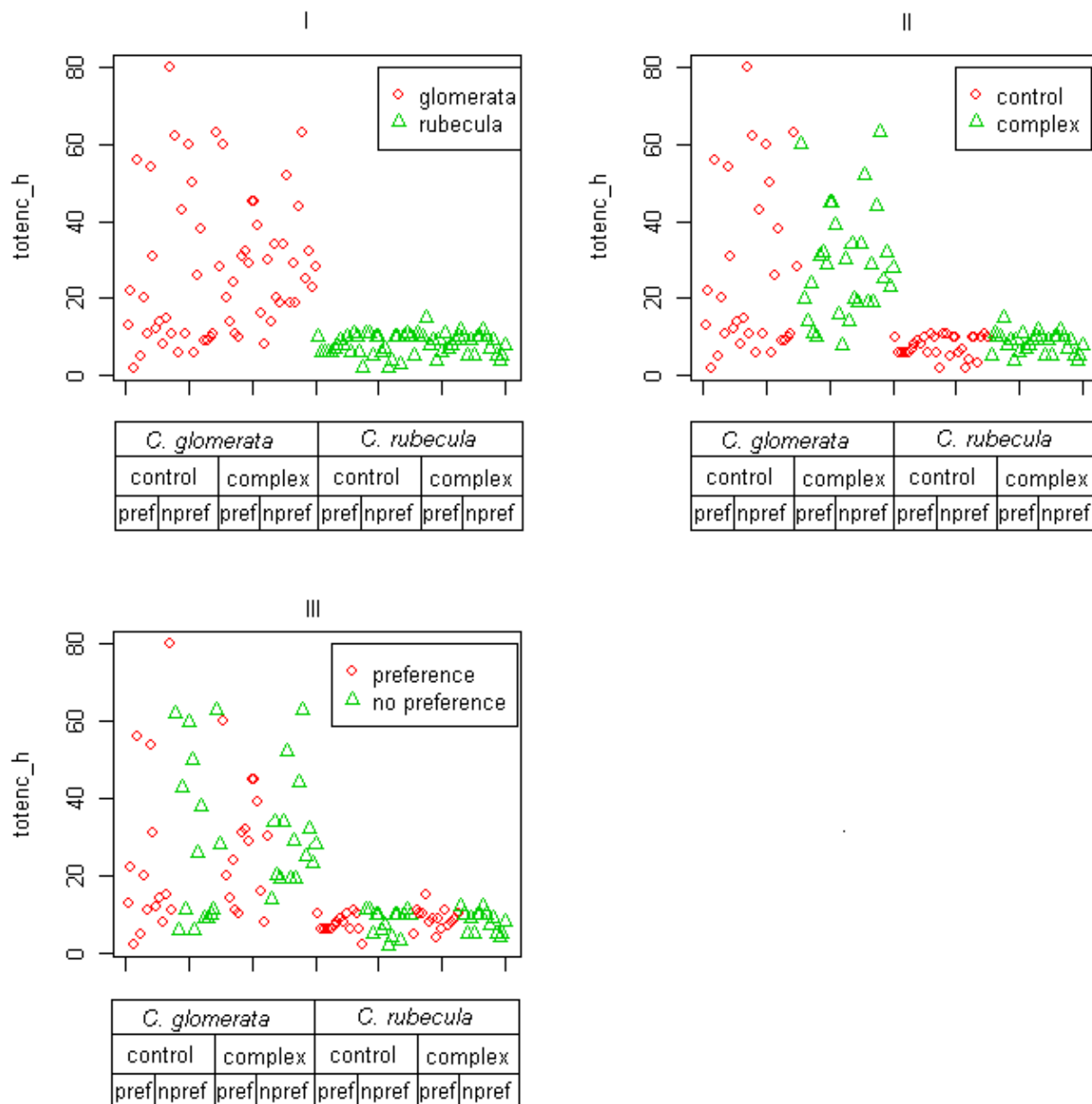
The effect of using the parasitoid or predator approach on the number of parasitized hosts is calculated for *Cotesia rubecula*. In the predator approach, the number of hosts counts down after parasitization. In this way, hosts are unavailable after parasitization. In contrast, in the parasitoid approach, the hosts are still available after parasitization. *Cotesia glomerata* is always treated as a parasitoid, as no difference is expected of oviposition in a host which is already parasitized or an unparasitized host. Superparasitism leads to extra offspring for the latter species.

Using the predator approach for *Cotesia rubecula* resulted in a significant difference (Appendix, output 15, $P=0.0197$) in total parasitized hosts compared to the parasitoid approach, with a higher number of ovipositions in the parasitoid approach. As in this species no extra offspring is gained after superparasitism of one host, the parasitoid approach is used for the model calculations, in which for every oviposition one host is counted down and is therefore no longer available.

Influence species, environment and preference

The number of host parasitizations turned out to be significantly affected by the factor species (Appendix: output 16, $P<0.000$; figure 7), indicating a significant effect of the host-searching behaviour of the two species over all treatments, with a larger number of parasitizations for *Cotesia glomerata*. No significant effect of environment and preference has been observed.

Figure 7: Modelled results of the effect of species (I), environment (II) and the ability to distinguish between different patch types (III) on the total number of encountered hosts. The vertical axis represents the number of encounter hosts, and the horizontal axis represents the treatments used in the model as shown below the graphs, with pref for the situation with differential preference for the different environments and npref for the situation with no preference.



Discussion and Conclusions

The results presented here show that the host finding efficiencies of *C. rubecula* and *C. glomerata* are differentially affected by the presence of non-host patches. *C. rubecula* lands relatively more often on non-host patches compared to *C. glomerata* (19% vs. 26,3%; Table 1), suggesting a stronger impact of presence of non-host patches on *C. rubecula* compared to *C. glomerata*. No significant difference between both species is found for the residence time on non-host patches (Appendix: Output 3).

On mixed patches, no clear differences in time loss between *C. glomerata* and *C. rubecula* can be concluded from the results of the analysis. The host finding efficiency of both species is negatively affected by the presence of mixed patches, but no differences between these species can be concluded.

As *Cotesia rubecula* is known to be specialized on the evenly distributed *Pieris rapae*, a decremental mechanism would be expected. However, when modelling the behaviour of *C. rubecula*, it was found that oviposition seems to have a negative effect on the leaving tendency (output 9), which is characteristic for an incremental mechanism.

For *Cotesia glomerata*, oviposition seems to have a negative effect on the leaving tendency, resulting in a longer residence time (output 7). As this species mainly uses *Pieris brassicae* as host, which occurs in clusters, this is expected to be efficient.

However, additional inclusion of the effect of host presence led to changed results. The giving up times of both *Cotesia glomerata* and *C. rubecula* decreased in presence of hosts. The effect on *C. glomerata* (hazard ratio = 0.1163, Output 13) has found to be larger than the effect on *C. rubecula* (hazard ratio = 0.4431, Output 11).

Inclusion of the effects of both oviposition and host presence leads to a significant effect of oviposition on the giving up times for *Cotesia glomerata*, whereas the effect of oviposition on *C. rubecula* is not significant. Comparison of leaving rates of *C. glomerata* after no ovipositions with leaving rates after one or more ovipositions results in a hazard ratio of 8.60321. This indicates that oviposition leads to an increased leaving rate and thereby a shorter residence time, in contrast to earlier results. This contrast is caused by overlapping effects of oviposition and host presence. Apparently, the presence of hosts partly explains the effect of oviposition.

From the calculation with the results of the model, it can be concluded that the number of host parasitizations is significantly affected by the factor species (Appendix: output 16, $P < 0.000$), indicating a significant effect of host searching behaviour of the two species over all treatments, with a larger number of parasitizations for *Cotesia glomerata*. This result suggests that the host searching behaviour of *Cotesia glomerata* is more efficient than that of *C. rubecula*. This might have facilitated the coexistence. In a thesis by A. Efremova (MSc thesis, Wageningen University, 2009), *C. glomerata* has found to be superior in more complex environments containing both hosts and non-hosts, while *C. rubecula* was superior in simple environments. In simple environments, the effect of intrinsic competition might have been large, and thereby have resulted in superiority of *C. rubecula*. In contrast, because of the less efficient host searching behaviour of *C. rubecula* in more complex environments, the effect of intrinsic competition is expected to decrease in more complex environments, which might have facilitated the superiority of *C. glomerata*. Poelman *et al.* (unpublished data) analysed the fraction multiparasitism in different environments, and indeed found a decrease upon adding a non-host, which supports this hypothesis.

Both the environment and the ability to discriminate between patch types (preference) did not have a significant effect on the number of parasitized hosts.

The insignificance of the effect of the environment is in contrast with the results of the thesis of A. Efremova (MSc thesis, Wageningen University, 2009), in which *C. rubecula* has found to be superior in simple environments and *C. glomerata* in more complex environments.

The insignificance of the ability to discriminate between patch types suggests that development of a stronger preference will not significantly increase the number of parasitized hosts. For that reason, learning behaviour of *C. glomerata* is expected to have no significant effect. However, more experiments with both naïve and experienced wasps are recommended.

It should be mentioned that the behavioural data for *C. glomerata* used in this thesis were obtained from another study. The environmental factors are attempted to be the same. However, for example the use of a different greenhouse compartment and a different observer might have influenced the results. In the greenhouse compartment used for *C. glomerata*, an air-circulation was present, whereas this was not present in the greenhouse compartment used for *C. rubecula*. For that reason, in the latter situation, a fan is used to circulate the air.

This, and possibly other differences, might have affected the parasitoid behaviour as the responsiveness of parasitoids towards plant volatiles is influenced by a range of abiotic and biotic conditions (Poelman *et al.*, 2009). In addition, problems in the rearing of *C. rubecula* led to a low number of wasps. For that reason, some individuals of *C. rubecula* might have been used on two subsequent days. However, since the effect of learning in *C. rubecula* after one experience is expected to be small, this is not expected to have influenced the results strongly.

Future research should be done on the effect of *Pieris brassicae*. In nature, plants may contain both *P. brassicae* and *P. rapae*. As both caterpillar species are known to be used as host by *C. glomerata*, presence of both species might have a strong influence on the interactions between *C. glomerata* and *C. rubecula*. In addition, the use of another non-host species might have a different effect. In these experiments, *Mamestra brassicae* is used. Both *C. rubecula* and *C. glomerata* are not able to distinguish between pure patches containing only *M. brassicae* and pure patches containing only *P. rapae* (Geervliet *et al.*, 1996). This might be caused by similarity of the volatiles emitted by plants after damage by these caterpillar species, as both species induce the jasmonic acid pathway (Dicke *et al.*, 2009). However, other non-host species might have a different effect by inducing a different pathway. For that reason, results from this study should not be generalized for all non-host species. Additional studies should be done with different non-host species, forming an environment comparable to the field situation. From this, conclusions can be drawn on the effects of non-hosts on both species in nature.

Another area for future research is performing experiments with both parasitoid species. In this thesis, experiments were performed with one parasitoid at the time. However, presence of the other parasitoid might influence the behaviour of both *C. rubecula* and *C. glomerata*. As a result of being inferior in intrinsic competition with *C. glomerata*, presence of both species might lead to a lower number of offspring for *C. rubecula*. However, *C. rubecula* has been observed to avoid flying to plants on which *C. glomerata* is already present (Geervliet *et al.*, 2000). In order to draw more realistic conclusions on the interaction between both species, similar experiments should be done with both *C. rubecula* and *C. glomerata* simultaneously.

Interestingly, in the experiments with *C. rubecula*, the parasitoid seemed to be more strongly attacked by *Mamestra brassicae* than by *Pieris rapae*. Attacks of *Mamestra brassicae* frequently were carried out by two or three individuals, all approaching *C. rubecula*. After these attacks by *M. brassicae*, the parasitoid seemed to have difficulty with walking and kept standing still for a considerable time lapse. This might lead to an extra negative effect of the presence of non-hosts. In addition to losing eggs and time, the parasitoid might

also lose energy and extra time needed for recovery. In this thesis, this effect is not taken into account. Additional experiments should be performed to reveal the effect of these attacks on both parasitoid species.

In order to draw conclusions from the model on the effect of increased complexity on the relative abundance of *Cotesia rubecula* and *C. glomerata*, the model should be further optimized. No difference between the two environments has been found, which does not agree with field observations (MSc thesis Anna Efremova, Wageningen University, 2009). It seems that assumptions have been made which do not fit to reality. The assumption of linearity should be criticized.

Several assumptions have been made in the model. No experimental data were available for both *C. glomerata* and *C. rubecula* on empty patches. For that reason, in the model, it is assumed that the leaving tendency of the parasitoids on an empty patch was equal to the leaving tendency on a patch containing only non-hosts. However, presence of non-hosts might alter the leaving tendency, and thereby the residence time. Additional experiments should be done to study the effect of non-host encounters on the leaving tendency.

Another important assumption made in the model is the encounter rate of hosts and non-hosts being independent of the number of hosts and non-hosts present on the patch. This might have strongly affected the outcome of the model calculations. For that reason, the model should be further optimized for the different patch-types.

In addition, the encounter rate of hosts was assumed to be equal to the encounter rate of non-hosts. Additional calculations should be done to verify the if this assumption is applicable to the situation in nature.

Still, this model might be very useful in predicting the relative contribution of different factors to the outcome of the competition between *C. glomerata* and *C. rubecula*. For example, when more data on the effect of *Pieris brassicae* are available, the model can easily be adapted to include this other host species of *C. glomerata*.

In conclusion, in this thesis it has been shown that *C. glomerata* displays a more efficient host searching behaviour than *C. rubecula*. In complex situations, the negative effect of intrinsic competition on *C. glomerata* might have decreased as a result of the less efficient host searching behaviour of *C. rubecula*. For that reason, differences in host searching behaviour might have facilitated the coexistence between both species.

Acknowledgements

I would like to thank Erik Poelman and Lia Hemerik for supervising me during this thesis. Their help has made this thesis very educative. In addition, I would like to thank everyone at entomology for giving me a pleasant time there.

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Appendix

Outputs statistical tests

Output 1: Residence time of *Cotesia glomerata* on patches containing only non-hosts (10NH) or only hosts (10H)

Mann-Whitney Test

		Ranks		
	treatment	N	Mean Rank	Sum of Ranks
residence_time	10NH	12	8,83	106,00
	10H	28	25,50	714,00
	Total	40		

Test Statistics^b

	residence_time
Mann-Whitney U	28,000
Wilcoxon W	106,000
Z	-4,132
Asymp. Sig. (2-tailed)	,000
Exact Sig. [2*(1-tailed Sig.)]	,000 ^a

a. Not corrected for ties.

b. Grouping Variable: treatment

Output 2: Residence time of *Cotesia rubecula* on patches containing only non-hosts (10NH) or only hosts (10H)

Mann-Whitney Test

		Ranks		
	treatment	N	Mean Rank	Sum of Ranks
residence_time	10H	18	19,72	355,00
	10NH	16	15,00	240,00
	Total	34		

Test Statistics^b

	residence_time
Mann-Whitney U	104,000
Wilcoxon W	240,000
Z	-1,380
Asymp. Sig. (2-tailed)	,168
Exact Sig. [2*(1-tailed Sig.)]	,175 ^a

a. Not corrected for ties.

b. Grouping Variable: treatment

Output 3: Comparison of residence times of *Cotesia glomerata* and *Cotesia rubecula* on patches containing only non-hosts

Group Statistics

	species	N	Mean	Std. Deviation	Std. Error Mean
residence_time	glomerata	12	284,7350	123,46888	35,64240
	rubecula	17	580,9765	635,20207	154,05913

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95%
								Confidence Interval
residence_time	10,493	,003	-1,586	27	,124	-296,24147	186,74218	-6
Equal variances assumed								
Equal variances not assumed			-1,873	17,685	,078	-296,24147	158,12842	-6

Output 4: Residence times and number of ovipositions per time unit for *Cotesia glomerata* on mixed patches containing two hosts and eight non-hosts (2H8NH), and on patches containing only hosts (10H)

Kruskal-Wallis Test

Ranks			
	treatment	N	Mean Rank
time_interval	2H8NH	18	23,72
	10H	28	23,36
	Total	46	
hostvertime	2H8NH	18	16,28
	10H	28	28,14
	Total	46	

Test Statistics^{a,b}

	time_interval	hostvertime
Chi-Square	,008	8,563
df	1	1
Asymp. Sig.	,928	,003

a. Kruskal Wallis Test

b. Grouping Variable: treatment

Output 5: Residence times and number of ovipositions per time unit for *Cotesia rubecula* on mixed patches containing two hosts and eight non-hosts (2H8NH), and on patches containing only hosts (10H)

Kruskal-Wallis Test

Ranks			
	treatment	N	Mean Rank
time_interval	2H8NH	16	18,88
	10H	18	16,28
	Total	34	
hostvertime	2H8NH	16	13,31
	10H	18	21,22
	Total	34	

Test Statistics^{a,b}

	time_interval	hostvertime
Chi-Square	,576	5,414
df	1	1
Asymp. Sig.	,448	,020

a. Kruskal Wallis Test

b. Grouping Variable: treatment

Output 6: Comparison of residence times of *Cotesia glomerata* and *Cotesia rubecula* on patches containing two hosts and eight non-hosts

Group Statistics

	species	N	Mean	Std. Deviation	Std. Error Mean
residence_time	glomerata	59	524,8742	518,38122	67,48749
	rubecula	55	415,0302	702,44679	94,71791

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
residence_time	,590	,444	,954	112	,342	109,84406	115,09376	-	337,88759
								Equal variances assumed	118,19948
residence_time	,590	,444	,944	98,992	,347	109,84406	116,30152	-	340,61172
								Equal variances not assumed	120,92361

Output 7: Cox Survival in R on the effect of oviposition in hosts on giving up time of *C. glomerata*, no parasitizations vs. one or more parasitizations

```
coxph(formula = Surv(tstart, tstop, fc) ~ factor(oviposition))
```

```
n= 371
```

	coef	exp(coef)	se(coef)	z	Pr(> z)
factor(oviposition)1	-0.6532	0.5204	0.3064	-2.132	0.0330 *

Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

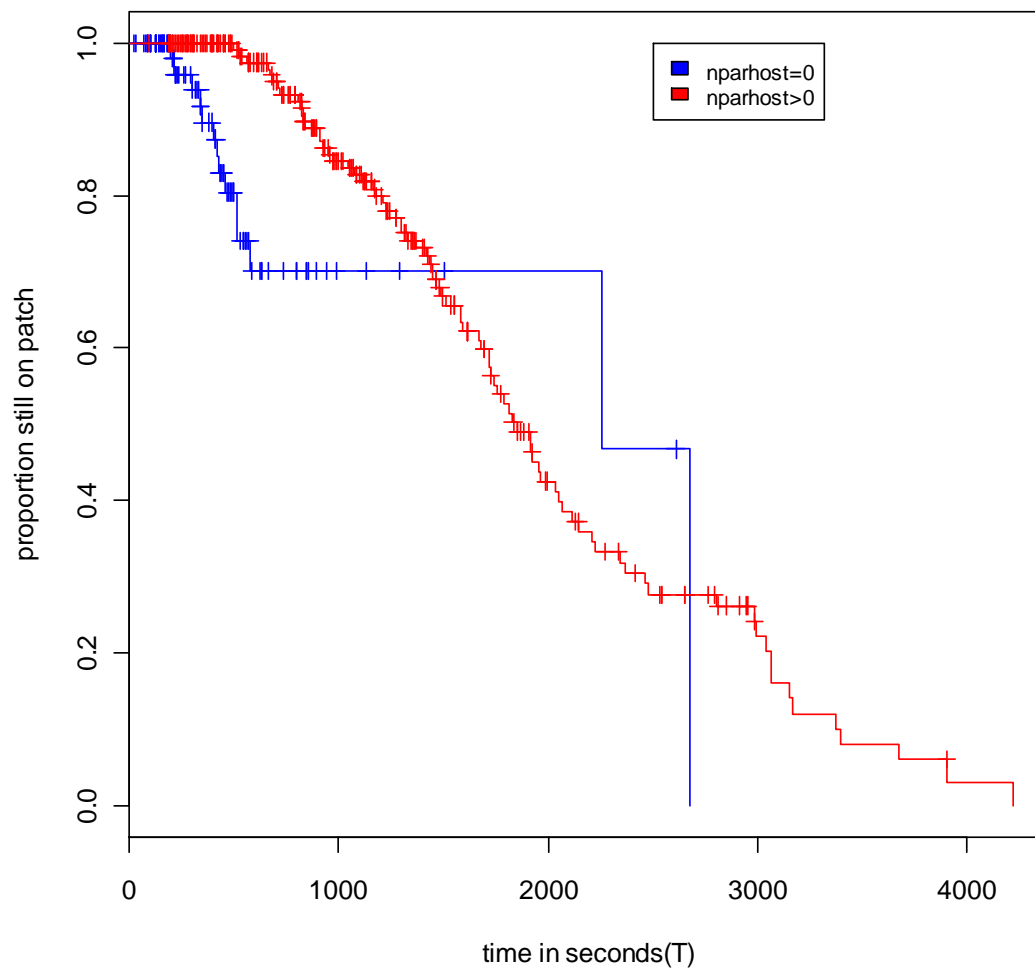
	exp(coef)	exp(-coef)	lower .95	upper .95
factor(oviposition)1	0.5204	1.922	0.2854	0.9488

Rsquare= 0.011 (max possible= 0.864)

Likelihood ratio test= 4 on 1 df, p=0.04558

Wald test = 4.54 on 1 df, p=0.03304

Score (logrank) test = 4.68 on 1 df, p=0.03048



Output 8: Cox survival on the effect of oviposition in the non-host *Mamestra brassicae* on the giving up time of *C. glomerata*

```
coxph(formula = Surv(tstart, tstop, fc) ~ factor(nparnhost))

n= 371

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(nparnhost)1 -0.1880   0.8286  0.3990 -0.471   0.637
factor(nparnhost)2 -0.4109   0.6630  0.3990 -1.030   0.303
factor(nparnhost)3 -0.3558   0.7006  1.0104 -0.352   0.725
factor(nparnhost)4 -0.9661   0.3806  1.0156 -0.951   0.341

              exp(coef) exp(-coef) lower .95 upper .95
factor(nparnhost)1   0.8286     1.207   0.37909   1.811
factor(nparnhost)2   0.6630     1.508   0.30332   1.449
factor(nparnhost)3   0.7006     1.427   0.09670   5.076
factor(nparnhost)4   0.3806     2.628   0.05199   2.786

Rsquare= 0.007 (max possible= 0.864 )
Likelihood ratio test= 2.52 on 4 df, p=0.641
Wald test              = 2.13 on 4 df, p=0.7127
Score (logrank) test = 2.2 on 4 df, p=0.6983
```

Output 9: Cox Survival on the effect of oviposition on giving up time of *C. rubecula*, no parasitizations vs. one or more parasitizations

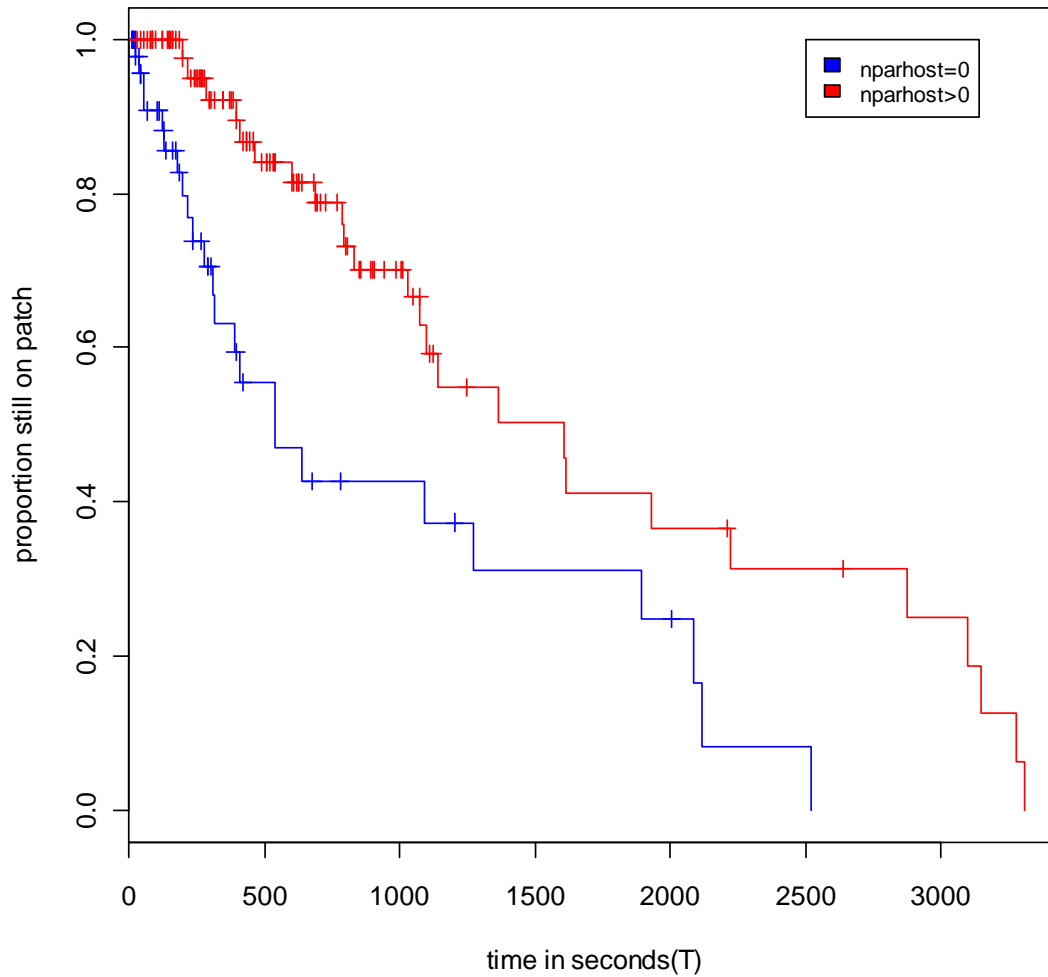
```
> t_efron<-coxph(Surv(tstart,tstop,fc)~factor(oviposition))
> summary(t_efron)
Call:
coxph(formula = Surv(tstart, tstop, fc) ~ factor(oviposition))

n= 162

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(oviposition)1 -0.8949   0.4087  0.3105 -2.882  0.00395 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
factor(oviposition)1   0.4087     2.447   0.2224   0.751

Rsquare= 0.05 (max possible= 0.864 )
Likelihood ratio test= 8.27 on 1 df, p=0.004023
Wald test              = 8.31 on 1 df, p=0.003952
Score (logrank) test = 8.8 on 1 df, p=0.003013
```



Output 10: Cox survival on the effect of oviposition in the non-host *Mamestra brassicae* on the giving up time of *C. rubecula*

```
coxph(formula = Surv(tstart, tstop, fc) ~ factor(nparnhost))
```

```
n= 162
```

	coef	exp(coef)	se(coef)	z	Pr(> z)
factor(nparnhost)1	0.05756	1.05925	0.53104	0.108	0.914
factor(nparnhost)2	-0.10931	0.89645	0.72993	-0.150	0.881
factor(nparnhost)3	-0.18118	0.83429	1.02401	-0.177	0.860

	exp(coef)	exp(-coef)	lower .95	upper .95
factor(nparnhost)1	1.0592	0.944	0.3741	2.999
factor(nparnhost)2	0.8965	1.116	0.2144	3.748
factor(nparnhost)3	0.8343	1.199	0.1121	6.208

```
Rsquare= 0 (max possible= 0.864 )
```

```
Likelihood ratio test= 0.07 on 3 df, p=0.9951
```

```
Wald test = 0.07 on 3 df, p=0.9953
```

```
Score (logrank) test = 0.07 on 3 df, p=0.9953
```

Output 11: Cox survival on the effect of presence of host on the giving up time of *C. rubecula*

```
coxph(formula = Surv(tstop - tstart, fc) ~ factor(init_nhost < 10))

n= 162

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(init_nhost < 10)TRUE -0.8139   0.4431  0.3166 -2.571  0.0101 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
factor(init_nhost < 10)TRUE   0.4431     2.257   0.2383   0.8241

Rsquare= 0.037 (max possible= 0.883 )
Likelihood ratio test= 6.03 on 1 df,  p=0.01409
Wald test              = 6.61 on 1 df,  p=0.01014
Score (logrank) test = 6.96 on 1 df,  p=0.008335
```

Output 12: Cox survival on the effect of both presence of host and oviposition on the giving up time of *C. rubecula*

```
coxph(formula = Surv(tstop - tstart, fc) ~ factor(init_nhost < 10) +
oviposition)

n= 162

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(init_nhost < 10)TRUE -0.88011   0.41474  0.43611 -2.018  0.0436 *
oviposition                 0.09266   1.09709  0.41436  0.224  0.8230
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
factor(init_nhost < 10)TRUE   0.4147     2.4112   0.1764   0.975
oviposition                 1.0971     0.9115   0.4870   2.471

Rsquare= 0.037 (max possible= 0.883 )
Likelihood ratio test= 6.08 on 2 df,  p=0.0479
Wald test              = 6.64 on 2 df,  p=0.03609
Score (logrank) test = 7 on 2 df,  p=0.03022
```

Output 13: Cox survival on the effect of presence of host on the giving up time of *C. glomerata*

```
coxph(formula = Surv(tstop - tstart, fc) ~ factor(init_nhost < 10))

n= 371

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(init_nhost < 10)TRUE -2.1516   0.1163   0.3292 -6.536 6.31e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
factor(init_nhost < 10)TRUE   0.1163   8.599   0.061   0.2217

Rsquare= 0.072 (max possible= 0.889 )
Likelihood ratio test= 27.89 on 1 df,  p=1.281e-07
Wald test              = 42.72 on 1 df,  p=6.307e-11
Score (logrank) test = 61.79 on 1 df,  p=3.775e-15
```

Output 14: Cox survival on the effect of both presence of host and oviposition on the giving up time of *C. glomerata*

```
coxph(formula = Surv(tstop - tstart, fc) ~ factor(init_nhost<10) +
oviposition)

n= 371

              coef exp(coef) se(coef)      z Pr(>|z|)
factor(init_nhost < 10)TRUE -4.11797   0.01628   0.77107 -5.341 9.26e-08 ***
oviposition                 2.15214   8.60321   0.71653  3.004 0.00267 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
factor(init_nhost < 10)TRUE   0.01628   61.4341   0.003591   0.07378
oviposition                 8.60321   0.1162   2.112269   35.04066

Rsquare= 0.119 (max possible= 0.889 )
Likelihood ratio test= 47.04 on 2 df,  p=6.105e-11
Wald test              = 46.8 on 2 df,  p=6.864e-11
Score (logrank) test = 73.3 on 2 df,  p=1.110e-16
```

Output 15: Difference between predator and parasitoid approach on total parasitized hosts for *Cotesia rubecula*

```
lm(formula = totenc_h ~ parpred)

Residuals:
    Min       1Q   Median       3Q      Max
-10.1667  -3.0917  -0.1667   1.9333  15.8333

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   8.0667     0.6280  12.845  <2e-16 ***
parpred       2.1000     0.8881   2.365   0.0197 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 4.864 on 118 degrees of freedom
Multiple R-squared:  0.04524,    Adjusted R-squared:  0.03715
F-statistic: 5.591 on 1 and 118 DF,  p-value: 0.01968
```

Output 16: Effect of species, environment and preference (volatiles) on total parasitized hosts for both *Cotesia rubecula* and *C. glomerata*

```
Call:
lm(formula = totenc_h ~ factor(species) + factor(environment) +
factor(volatiles))

Residuals:
    Min       1Q   Median       3Q      Max
-23.733  -5.779  -0.217   2.833  54.267

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   27.68      2.41  11.489  < 2e-16 ***
factor(species)2  -19.52      2.41  -8.100  6.2e-13 ***
factor(environment)2  1.75      2.41   0.726   0.469
factor(volatiles)1  -1.95      2.41  -0.809   0.420
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 13.2 on 116 degrees of freedom
Multiple R-squared:  0.3654,    Adjusted R-squared:  0.349
F-statistic: 22.26 on 3 and 116 DF,  p-value: 1.863e-11
```

Model:

```
n=1          #defining the filename for the output of the totout matrix
filename=c("control_rub_pref_pred","control_rub_pref_par","control_rub_npref_p
red","control_rub_npref_par","control_glom_pref_pred","control_glom_pref_par",
control_glom_npref_pred","control_glom_npref_par","env2_rub_pref_pred","env2
_rub_pref_par","env2_rub_npref_pred","env2_rub_npref_par","env2_glom_pref_p
red","env2_glom_pref_par","env2_glom_npref_pred","env2_glom_npref_par")
filename[n]

environment=2    #env1 is 10H vs. empty (=control environment); env2 is mix55
vs 10NH
species=2    #1 for glomerata, 2 for rubecula
volatiles=0   #0 for no difference in preference for the different patches (=npref,
no effect of volatiles), 1 for different preferences (=pref)
parpred=1     #0 for predator (pred), 1 for parasitoid approach (par); counting
down hosts only in predator approach

tottime=2*3600      #simulation time is 2 hours
out=matrix(data=0,ncol=6,nrow=0) # after every patch visit the necessary
output will be attached
colnames(out)=c("Npatch","sumres", "restime", "t_travel", "n_ench", "inithostd")

outd=matrix(data=0,ncol=10,nrow=0) # after every part of a patch visit the
necessary output will be attached

colnames(outd)=c("Npatch","patchnr","inithostd","hostd","nhostd","decis","ibe","g
ut","(ibe<gut)","th")

totendout=matrix(data=0,ncol=17,nrow=0)
colnames(totendout)=c("environment","species","volatiles","parpred","tot_enc","t
otenc_h","totenc_nh","restim_empty","restim_host","restim_nohost","restim_mix1
","restim_mix2","tot_offspring","meanoffspr","longterm_rate_offspr_prod","fractio
n_realized_ovi","nreplicates")

library(MASS)
# set-up the environment

# nr of rows in set-up is nro
nro= 4
# nr of columns in set-up is nco
nco= 4

nreplicates=0    #in total 15 replicates

while((nreplicates<15)){

totpatch=nro*nco
nrs=1:(nco*nro)
struct=matrix(data=nrs,ncol=nco,nrow=nro)
nrs=matrix(data=nrs,ncol=1,nrow=totpatch)
```

```

neighb= matrix(data=0, ncol=13, nrow=totpatch)
neighb[,1]=nrs
dum=cbind(0,rbind (0,struct,0),0)
dum1=dum[,c(2:(2+nco),1)]
dum2=dum[c(2:(2+nro),1),]
dum3=dum1[c(2:(2+nro),1),]
dum4=dum[,c((2+nco), 1:(1+nco))]
dum5=dum[c((2+nro), 1:(1+nro)),]
dum6=dum2[,c((2+nco), 1:(1+nco))]
dum7=dum1[c((2+nro), 1:(1+nro)),]
dum8=dum5[,c((2+nco), 1:(1+nco))]

# create matrix with for every plant number its 8 nrs of neighbours in column
2:9
neighb[,2]=dum1[2:(1+nro),2:(1+nco)]
neighb[,3]=dum2[2:(1+nro),2:(1+nco)]
neighb[,4]=dum3[2:(1+nro),2:(1+nco)]
neighb[,5]=dum4[2:(1+nro),2:(1+nco)]
neighb[,6]=dum5[2:(1+nro),2:(1+nco)]
neighb[,7]=dum6[2:(1+nro),2:(1+nco)]
neighb[,8]=dum7[2:(1+nro),2:(1+nco)]
neighb[,9]=dum8[2:(1+nro),2:(1+nco)]

# add the number of host and non-host in columns 10 and 11 of neighb
if(environment==1) {
alternatingly host only and empty
neighb[,10]=c(0,10,0,10,10,0,10,0)
neighb[,11]=0 }

if(environment==2) {
neighb[,10]=c(0,5,0,5,5,0,5,0)
neighb[,11]=c(10,5,10,5,5,10,5,10) }

# add the coding for 1 empty, 2 host only, 3 non-host only, 4 mixed patch 2:8,
and 5 mixed patch 5:5 in column 12 of neighb
t=neighb[,10]+neighb[,11]
a=t==0
a=a+2*((neighb[,11]==0)&(t>0))
a=a+3*((neighb[,10]==0)&(t>0))
a=a+4*(a==0)
a=a+(neighb[,10]==5)
neighb[,12]=a

# add the coding for predator and parasitoid (with resp. decline number of host
or possibility to superparasitize)
if(parpred==0) {neighb[,13]=0}
if(parpred==1) {neighb[,13]=1}

totout=matrix(data=0,ncol=11,nrow=0)

colnames(totout)=c("Npatch","patchnr","tot_enc","totenc_host","totenc_nohost","r
estim_empty","restim_host","restim_nohost","restim_mix1","restim_mix2","tot_re
stime")

```



```

# add leaving rates
if(species==1) { #glomerata
h0=0.000696 #baseline hazard, leaving rate empty patch and
nonhost patch
hh=0.01628 * h0 #leaving rate host only, mix1 and mix2 patches; no
ovipositions
hpar=hh * 8.60321 #leaving rate host only, mix1 and mix2 patches;
after 1 or more ovipositions
}
if(species==2) { #rubecula
h0=0.000982553 #baseline hazard, leaving rate empty patch and
nonhost patch
hh=0.4431 * h0 #leaving rate host only, mix1 and mix2 patches
#for rubecula no significant effect of number of parasitizations on the leaving
rate
hpar=hh #leaving rate host only, mix1 and mix2 patches; after 1 or
more ovipositions
}

# add encounter rates
if(species==1) { #glomerata
h_ench=0.002052247 #baseline encounter rate hosts, on host only and
mixed patches
h_encnh=h_ench #encounter rate hosts is assumed to be equal to
encounter rate nonhosts
}
if(species==2) { #rubecula
h_ench=0.002217839 #baseline encounter rate hosts, on host only and
mixed patches
h_encnh=h_ench #encounter rate hosts is assumed to be equal to
encounter rate nonhosts
}

h_handh=1/20 #handling rate host
h_handnh=1/20 #handling rate non-host, assumed to be equal to handling rate
hosts
h_travel=1/5 #travel rate between patches

# when leaving a patch the choice between neighbouring plants will be weighed
by preference:
# empty patches(code 1),
# non-host only patches (code 3)
# host only patches (code 2) and
# mixed patches with 2 hosts and 8 nonhosts (code 4)
# mixed patches 5 hosts and 5 nonhost (code 5)

if(volatiles==1) { #including the effect of preference
if(species==1) { pref=c(1,25,16,42,42) } #preference of glomerata
(empty,host_only,nonhost_only,mix55,mix28)
if(species==2) { pref=c(1,7,5,6,6) } #preference of rubecula
(empty,host_only,nonhost_only,mix55,mix28)
}
if(volatiles==0) { #assuming that the parasitoids have no preference for
different patch-types
pref=c(1,1,1,1,1) }

```

```

# choose first patch randomly
curr_p=sample(neighb[,1],1)
hostd=neighb[curr_p,10] #evaluating the number of hosts and non-hosts on the
first patch
nhostd=neighb[curr_p,11]

Npatch=0 # number of visited patches
sumres=0 #sumres in seconds
n_ench=0 #nr of encountered hosts on current patch
totenc_host=0 #total nr of encountered hosts over all patches = nr of
parasitized hosts
totenc_nohost=0 #total nr of encountered nonhosts over all patches
tot_enc=0 #tot_enc=totenc_host+totenc_nohost
restim_empty=0 #residence time on empty patch
restim_host=0 #residence time on host only patch
restim_nohost=0 #residence time on nonhost only patch
restim_mix1=0 #residence time on mix1 (=5 hosts, 5 nonhosts)
restim_mix2=0 #residence time on mix2 (=2 hosts, 8 nonhosts)
totdecis=0 #total number of decisions (nr of parasitizations + nr of patch
leavings)
t_traveltot=0 #total travel time
totgut=0 #sum of all giving up times
totibe=0 #sum of all intervals between encounters (host and nonhost)
t_travel=0 #travel time to first patch
inithostd=hostd #nr of hosts present at the beginning

while((sumres<totime)) {
# determine host and non-host density on the current patch
hostd=neighb[curr_p,10]
nhostd=neighb[curr_p,11]
Npatch=Npatch+1

#1 on empty patch
if (neighb[curr_p,12]==1) {
inithostd=0
gut= rgamma(1, shape=1, rate=h0)
decis=1 #number of decisions on this patch, always leaves the patch so always
1
totdecis=totdecis+1 #total number of decisions on all patches
th=0 #no handling time, as no parasitization occurs
restime=gut
ibe=1+gut # this is a trick to ensure leaving
n_ench=0
n_encnh=0 # no host or non-host encountered

restim_empty=restim_empty+restime
tot_enc=totenc_host+totenc_nohost #no encounters on mixed patch, so no
change with previous number

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)

newd=c(Npatch,curr_p, inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)
sumres= sumres+restime

```

```

new=c(Npatch, sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)
totgut=totgut+gut

neighb[curr_p,10]=hostd
# go to neighbouring plant
}

#2 on patch with positive density of host
if (neighb[curr_p,12]==2) {

inithostd=10
n_ench=0
n_encnh=0 # no host or non-host encountered
ibe=-1
gut=0
decis=0
restime=0

while ((ibe<gut)&(hostd>0)) {
decis=decis+1
totdecis=totdecis+1
# decision proces
# draw an encounter time
ibe=rgamma(1, shape=1, rate=h_ench)
# draw a giving up time
if(n_ench==0){
gut=rgamma(1, shape=1, rate=hh)}
if(n_ench>0) { gut=rgamma(1, shape=1, rate=hpar)}

if ((ibe<gut)&(hostd>0)) {
th= rgamma(1, shape=1, rate=h_handh)
n_ench=n_ench+1
totenc_host=totenc_host+1
totibe=totibe+ibe
    if(neighb[curr_p,13]==0){
        hostd=hostd-1}
restime=restime+ibe+th
}

sumres=sumres+restime
restim_host=restim_host+restime

tot_enc=totenc_host+totenc_nohost          #total encountered caterpillars (host
and nonhost)

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
newd=c(Npatch,curr_p,inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)
new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)
} #whileloop (ibe<gut)

```

```

if ((ibe<gut)&(hostd==0)) {th= 0#if all hosts are parasitized (in predator-
approach), parasitoid leaves the patch after gut
  restime=restime+gut
  ibe=gut+1

  sumres=sumres+restime
  restim_host=restim_host+gut

tot_enc=totenc_host+totenc_nohost

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
newd=c(Npatch,curr_p, inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)

new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)}

totgut=totgut+gut

neighb[curr_p,10]=hostd      #in predator approach, the number of
unparasitized hosts is saved for the next visit
} # end host only patch

# go to neighbouring plant

#3 on non-host patch
if (neighb[curr_p,12]==3) {
  inithostd=0
  gut= rgamma(1, shape=1, rate=h0)
  ibe=gut+1
  decis= 0
  th=0
  restime=gut
  n_ench=0
  n_encnh=0
  decis=decis+1      #always decides to leave the patch, as no parasitizations
occur on nonhost patches
  totdecis=totdecis+1
  # decision proces
  # draw an encounter time

restim_nohost=restim_nohost+gut      #always leaves the patch, so gut is added
every time
totgut=totgut+gut
sumres=sumres+restime
tot_enc=totenc_host+totenc_nohost      #no host or non-host is parasitized on
nonhost patch

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
  newd=c(Npatch,curr_p, inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)
new=c(Npatch, sumres, restime, t_travel, n_ench, inithostd)

```

```

out=rbind(out,new)

neighb[curr_p,10]=hostd
#end nonhost patch
}
# go to neighbouring plant

#4 on patch with 5 hosts and 5 non-host
if (neighb[curr_p,12]==5) {      #mixed patch 55

  inithostd=5
  n_ench=0
  n_encnh=0 # no host or non-host encountered at start
  ibe=-1      #ibe<gut to ensure that the parasitoid makes a choice
  gut=0
  restime=0
  decis=0

  while ((ibe<gut)&(hostd>0)) {
    decis=decis+1
    totdecis=totdecis+1
    # decision proces
    # draw an encounter time with host
    ibeh=rgamma(1, shape=1, rate=h_ench)
    # draw an encounter time with non-host
    iben=rgamma(1, shape=1, rate=h_encnh)
    if(n_ench==0){ gut=rgamma(1, shape=1, rate=hh)}      #if no parasitization
    has taken place, leaving rate = hh
    if(n_ench>0) { gut=rgamma(1, shape=1, rate=hpar)}    #if parasitization has
    already taken place, leaving rate=hpar

    Min=min(ibeh,iben,gut)      #Min is the minimum of ibeh, iben and gut

    if(Min==ibeh) {      #parasitization host
      ibe=ibeh
      if(neighb[curr_p,13]==0){hostd=hostd-1}      #in parasitoid approach: counting
      down of parasitized hosts
      th= rgamma(1, shape=1, rate=h_handh)
      n_ench=n_ench+1      #number of encountered hosts
      totenc_host=totenc_host+1      #total number of encountered non-hosts
      restime=restime+ibe+th
      totibe=totibe+ibe
    }

    if(Min==iben) {      #parasitization nonhost
      ibe=iben
      th= rgamma(1, shape=1, rate=h_handnh)
      n_encnh=n_encnh+1      #number of encountered non-hosts
      totenc_nohost=totenc_nohost+1      #total number of encountered non-hosts
      restime=restime+ibe+th
      totibe=totibe+ibe
    }

    if(Min==gut) {      #leaving patch
      th=0
      ibe=ibeh
      restime=restime+gut
      totgut=totgut+gut
    }
  }
}

```

```

}      #end while(ibe<gut)&(hostd>0)

sumres= sumres+restime
restim_mix1=restim_mix1 + restime

tot_enc=totenc_host+totenc_nohost

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)
newd=c(Npatch,curr_p,inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)

if ((hostd==0) & (nhostd>0)) { #no unparasitized hosts left, leaving patch (only
when treating like predator, in case of parasitoid no counting down of hosts)
if(n_ench==0) {gut=rgamma(1, shape=1, rate=hh)}
if(n_ench>0) { gut=rgamma(1, shape=1, rate=hpar)}
restime = gut
sumres = sumres+restime
restim_mix1=restim_mix1+restime
totgut=totgut+gut
th=0

decis=decis+1
totdecis=totdecis+1

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)
newd=c(Npatch,curr_p,inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd) } #end if(hostd==0 en nhostd>0)

neighb[curr_p,10]=hostd
# go to neighbouring plant

} # end mixed patch 5:5

#5 on patch with positive density of host and non-host
if (neighb[curr_p,12]==4) { #mixed patch 2:8

inithostd=2
n_ench=0
n_encnh=0 # no host or non-host encountered
ibe=-1
gut=0
restime=0
decis=0

```

```

while ((ibe<gut)&(hostd>0)) {
  decis=decis+1
  totdecis=totdecis+1
  # decision proces
  # draw an encounter time with host
  ibeh=rgamma(1, shape=1, rate=h_ench)
  # draw an encounter time with non-host
  iben=rgamma(1, shape=1, rate=h_encnh)
  if(n_ench==0) {gut=rgamma(1, shape=1, rate=hh)}
  if(n_ench>0) { gut=rgamma(1, shape=1, rate=hpar)}
  Min=min(ibeh,iben,gut)

  if((Min==ibeh)&(hostd>0)) {          #parasitization hosts
    ibe=ibeh
    if(neighb[curr_p,13]==0) {
      hostd=hostd-1}
    th= rgamma(1, shape=1, rate=h_handh)
    n_ench=n_ench+1
    totenc_host=totenc_host+1
    restime=restime+ibe+th
    totibe=totibe+ibe
  }

  if((Min==iben)&(hostd>0)) {          #parasitization non-host
    ibe=iben
    th= rgamma(1, shape=1, rate=h_handnh)
    n_encnh=n_encnh+1
    totenc_nohost=totenc_nohost+1
    restime=restime+ibe+th
    totibe=totibe+ibe
  }

  if((Min==gut)&(hostd>0)) {          #leaving patch
    ibe=ibeh
    th=0          #no handling time
    restime=restime+gut    #leaves the patch after giving up time
    totgut=totgut+gut
  }

  sumres= sumres+restime
  restim_mix2=restim_mix2+restime

  tot_enc=totenc_host+totenc_nohost

  totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
  host,restim_nohost,restim_mix1,restim_mix2,sumres)
  totout=rbind(totout,totnew)
  new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
  out=rbind(out,new)
  newd=c(Npatch,curr_p,inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
  outd=rbind(outd,newd)
} #whileloop (ibe<gut)

if ((hostd==0) & (nhostd>0)) { #no unparasitized hosts left, only in predator-
  approach
  if(n_ench==0) {gut=rgamma(1, shape=1, rate=hh)}
  if(n_ench>0) { gut=rgamma(1, shape=1, rate=hpar)}

  restime = gut    #always leaves the patch

```

```

sumres=sumres+restime
restim_mix2=restim_mix2+restime
ibe=1+gut
decis=decis+1
totdecis=totdecis+1
totgut=totgut+gut
tot_enc=totenc_host+totenc_nohost

totnew=c(Npatch,curr_p,tot_enc,totenc_host,totenc_nohost,restim_empty,restim_
host,restim_nohost,restim_mix1,restim_mix2,sumres)
totout=rbind(totout,totnew)
new=c(Npatch,sumres, restime, t_travel, n_ench, inithostd)
out=rbind(out,new)
newd=c(Npatch,curr_p,inithostd,hostd,nhostd,decis,ibe,gut,(ibe<gut),th)
outd=rbind(outd,newd)      #end if((hostd==0)&(nhostd>0))

neighb[curr_p,10]=hostd
} # end mixed patch 2:8
# go to neighbouring plant

#here the parasitoid chooses from neighbouring plants
nb=sort(neighb[curr_p,2:9])
nb=nb[which(nb>0)]          #defining neighbours
prnb=neighb[nb,12]         #preference of different neighbours
is taken into account
s=matrix(0,nrow=1,ncol=1+length(nb))
for (i in 1:length(nb)){
s[1,1+i]=s[1,i]+pref[prnb[i]]}
rand=runif(1)
curr_p=nb[sum(rand > s*(1/sum(pref[prnb])))] #new patch is choosen

# adjust time with traveltime
t_travel= rgamma(1, shape=1, rate=h_travel)
sumres=sumres+t_travel
t_traveltot=t_traveltot+t_travel

if(species==1) {tot_offspring=20*totenc_host}          #glomerata (sp1) 20 ei
gem per host
if(species==2) {tot_offspring = totenc_host }          #rubecula (sp2) 1 ei per
host
averageoffspring_patch=tot_offspring/Npatch
E=tot_offspring/sumres
frac_realovi=tot_enc/totdecis

#totout laten wegschrijven

naam=paste(filename[n],as.character(nreplicates),".txt")
naam=paste("M:/My Documents/minor thesis/analyse R/totout r/",naam)
write.matrix(totout,naam,sep=" ")
}      #end while loop sumres<tottime

nreplicates=nreplicates+1

```



```
totendnew=c(environment,species,volatiles,parpred,tot_enc,totenc_host,totenc_n
ohost,restim_empty,restim_host,restim_nohost,restim_mix1,restim_mix2,tot_offs
pring,averageoffspring_patch,E,frac_realovi,nreplicates)
  totendout=rbind(totendout,totendnew)

}      #end 15 replicates

write.matrix(totendout,file ="M:/My Documents/minor thesis/analyse R/output
model.txt ", sep = " ")
```