RENATA VIEIRA MARQUES

DIET CHOICE AND HABITAT STRUCTURE MEDIATE COEXISTENCE OF PREDATORY MITES ON *Jatropha curcas*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

VIÇOSA
MINAS GERAIS – BRASIL
2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T

Marques, Renata Vieira, 1989-

M357d Diet choice and habitat structure mediate coexistence of
2019 predatory mites on *Jatropha curcas* / Renata Vieira Marques. – Viçosa, MG, 2019.

100 f. : il. ; 29 cm.

Texto em inglês.

Orientador: Angelo Pallini Filho.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Ácaros - Controle biológico. 2. *Euseius concordis*. 3. *Iphiseiodes zulluagai*. 4. Animais predadores. 5. Dieta. I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22. ed. 595.42

RENATA VIEIRA MARQUES

DIET CHOICE AND HABITAT STRUCTURE MEDIATE COEXISTENCE OF PREDATORY MITES ON *Jatropha curcas*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

APROVADA: 28 de fevereiro de 2019.

Arnoldus Rudolf Maria Janssen
(Co-advisor)

Morgana M.F. Brito
Morgana Maria Fonseca

Madelaine Venzon

Renato de Almeida Sarmento

Angelo Pallini
(Advisor)

AGRADECIMENTOS

Primeiramente gostaria de agradecer a Deus pelo dom da vida e por me permitir seguir forte durante toda a jornada do doutoramento.

Aos meus Pais que sempre me apoiaram e me mostraram que eu era capaz de realizar meus sonhos e muito mais. Sempre foram minha base e me apoiaram nos momentos mais difíceis. Mas também se fizeram presentes nos momentos de conquistas.

Ao meu marido Victor Vidal faço um agradecimento muito especial, por sempre me incentivar a crescer e conseguir alcançar meus objetivos. Com toda paciência permaneceu ao meu lado nos momentos difíceis. A minha pequena Manuella, gostaria de dizer que junto com seu nascimento nasceu uma mulher mais dedicada, esforçada, pontual, organizada, capaz de conseguir alcançar seus sonhos. Obrigada por me completar e me fazer feliz!

Aos mestres incríveis que tive a oportunidade de trabalhar junto esses anos. Angelo Pallini, meu muito obrigada por me acolher em Viçosa, pelos ensinamentos e por me apoiar em todos os momentos do meu doutorado. Arne Jansen, pessoa excepcional que mesmo a distância sempre me ajudou com muita paciência em todos os momentos do doutorado, com ensinamentos e me incentivando a crescer. Ao professor Renato Sarmento por me apresentar ao mundo científico e me acompanhar durante essa jornada, sem citar anos pois assim me sentirei velha. Muito obrigada por todos os ensinamentos e paciência durante todos estes anos!

Aos meus queridos companheiros de laboratório em Viçosa, por todas as ajudas que recebi e por todo o tempo de vida compartilhado em alegres cafezinhos: Aline Francês, Felipe Lemos, Célia Ferraz, Laila Rezende, Henry, Ana Bernardo, Cleide Dias, Cleber, Felipe Colares, Morgana, João, Marcus Duarte, André.

Durante meu doutoramento tive a oportunidade de também fazer parte do laboratório de Ecologia Funcional e Aplicada, coordenado pelo professor Renato Sarmento em Gurupi, Tocantins. Gostaria de deixar aqui meus agradecimentos para aqueles que fizeram e fazem parte deste grupo e me ajudaram durante este período. Em especial ao subgrupo do pinhão manso: Marçal Pedro Neto, Adriana Gonçalves, Thaysa, Dara, Laila.

Formalmente agradeço as instituições de fomento que financiaram minha pesquisa CNPq e CAPES. Aos membros da banca de avaliação da tese e seus suplentes, o Programa de Pós-Graduação em Entomologia, Universidade Federal de Viçosa e a Universidade Federal do Tocantins.

BIOGRAFIA

RENATA VIEIRA MARQUES, filha de Renato Carneiro Marques e Katia Rosanne Vieira Marques é natural de Araguari, Minas Gerais e nasceu em 14 de novembro de 1989. Em agosto de 2008 ingressou no curso de Engenharia Florestal na Universidade Federal do Tocantins. Em 2009 iniciou suas atividades no Laboratório de Ecologia coordenado pelo Prof. Renato Sarmento. Em janeiro de 2013 graduou-se em Engenharia Florestal pela UFT e em março do mesmo ano ingressou no mestrado em Ciências Florestais e Ambientais na UFT sob orientação do Prof. Renato Sarmento. Em fevereiro de 2015 terminou o mestrado e em março do mesmo ano ingressou o doutorado no Programa de Pós-Graduação em Entomologia na Universidade Federal de Viçosa, em Viçosa – MG sob orientação do Prof. Angelo Pallini.

ABSTRACT

MARQUES, Renata Vieira, D.Sc., Universidade Federal de Viçosa, February, 2019. **Diet choice and habitat structure mediate coexistence of predatory mites on *Jatropha curcas*.** Advisor: Angelo Pallini Filho. Co-advisor: Arnoldus Rudolf Maria Janssen.

Many animals select their diet based on the nutrient composition of their food, aiming to increase their performance. There are several studies showing that animals have a higher fitness when they feed on a mixed diet instead of feeding on single diets. Here, I studied two species of predatory mites, *Iphiseiodes zuluagai* and *Euseius concordis,* that showed a higher fitness when they fed on a diet composed of mixtures of the two *natural* prey species. Most importantly, these predators actively forage to obtain such a mixed diet. These two predators are being evaluated for their capacity to control the pest mites *Polyphagotarsonemus latus* and *Tetranychus bastosi* in plantations of the biodiesel plant *Jatropha curcas*. In the first chapter I assessed the performance of the predator *E. concordis* with a decreasing number of either of the two prey species to assess the optimal consumption of each prey. I found that the predator *E. concordis* selected a diet that maximized its reproduction. However, in nature, food items are usually patchily distributed, and with an increased distance between nutritionally complementary food types, the foraging animal needs to spend more energy and time to commute between different food sources. Thus, in the second chapter, I investigated whether the predator *E. concordis* does actively mix their diet when this requires foraging over longer distances than in the previous study, and I evaluated the effect on their fitness using their natural prey. Furthermore, the structure of the natural habitat of the prey is more complex than the structure offered in the experiments of Chapter 2. Thus, the foraging costs will also increase with increasing habitat complexity. In chapter 3, I therefore evaluated if the predator *E. concordis* mixed its diet when the two prey species occured spatially separated on a small *J. curcas* plant and if it benefitted from this. Although the predator had to spend time and energy to cover long distances to obtain a mixed diet, on small, intact plants, the predator actively foraged for a mixed diet, resulting in an increased oviposition. The structure of communities in nature as well as in biological control systems is not only affected by the interactions between plants and their pests, but also by interactions among their natural enemies. In theory, some interactions between the predators (such as intraguild predation) are predicted to disrupt pest control. Therefore, I investigated the possible interactions between the predatory mites *E. concordis* and *I. zuluagai* in Chapter 4. First, I show that the two predator species co-occur on the same *J. curcas* plants in the field. Second, I show that the two species are involved in reciprocal intraguild predation. Subsequently, I show that increasing the spatial structure and supplying alternative food resulted in increased coexistence of the two intraguild predators. In conclusion, these results suggest that to improve the persistence of predators in the field, it is important to increase the spatial structure of the habitat and offer a diverse predator diet.

RESUMO

MARQUES, Renata Vieira Marques, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **A escolha de dieta e a estrutura de habitat mediam a coexistência de ácaros predadores em pinhão manso**. Orientador: Angelo Pallini Filho. Coorientador: Arnoldus Rudolf Maria Janssen.

Muitos animais selecionam sua dieta baseado na composição dos nutrientes visando aumentar sua performance. Existem diversos estudos que mostram que os animais têm seu *fitness* aumentado quando eles se alimentam de uma dieta mista ao invés de uma dieta simples. Nesta tese, foram estudados dois ácaros predadores, *Iphiseiodes zuluagai* e *Euseius concordis,* que apresentaram maior fitness quando se alimentaram de uma dieta composta por uma mistura de duas espécies de presa naturais. Mais impressionante, estes predadores forragearam em busca de obter a dieta mista. Estas espécies de predadores estão sendo avaliadas pela sua capacidade de controlar os ácaros pragas *Polyphagotarsonemus latus* e *Tetranychus bastosi* em plantações de pinhão manso (*Jatropha curcas*), de onde se extrai biodiesel. No primeiro capítulo foi avaliado a performance do predador *E. concordis* em função da diminuição do número de cada uma de suas espécies de presas para se verificar o consumo ótimo de cada presa. Foi verificado que o predator *E. concordis* selecionou a dieta que maximizou sua reprodução. Entretanto, na natureza os itens alimentares estão espacialmente distribuídos. A medida que se aumenta a distância entre os alimentos que nutricionamente completam sua dieta, o animal precisa gastar maior energia e tempo em busca dos seus recursos alimentares. Sendo assim, no segundo capítulo foi investigado se o predador *E. concordis* percorre longas distâncias para misturar sua dieta e avaliou-se o efeito disto em seu fitness. Na natureza a complexidade do habitat das presas é maior do que foi avaliado em laboratório e as presas podem estar mais afastadas umas das outras. Sabe-se que o custo aumentará com a maior complexidade do habitat. Por isso, no terceiro capítulo foi avaliado se o predador *E. concordis* mistura sua dieta quando as suas presas estão separadas espacialmente em plantas de *Jatropha curcas* e se os predadores se beneficiam disto. Apesar do tempo e gasto energético durante a procura por uma dieta mista. Em plantas pequenas, o predador demonstrou procurar ativamente por uma dieta mista e se beneficiou dela. A estrutura das comunidades na natureza, assim como, em sistemas de

controle biológico, não é somente influenciada pelas interações entre as plantas e suas pragas, mas também em função das interações entre seus inimigos naturais. Na teoria, algumas interações entre os predadores (como a predação intraguilda) são descritas como perturbadoras do efetivo controle das pragas. Por essa razão, no capítulo 4 foi avaliada a possibilidade de ocorrer a predação intraguilda entre as duas espécies de ácaros predadores. Para isto, primeiramente foi verificado coocorrência dos ácaros *E. concordis* e *I. zuluagai* nas mesmas plantas em campo. Em seguida, mostrou-se que os predadores estavam envolvidos em predação intraguilda recíproca. Subsequentemente, foi visto que o aumento das estruturas espaciais e a presença de fontes alternativas de alimento parece ser uma estratégia viável para manter a coexistência entre estes predadores. Em conclusão, estes resultados sugerem que para aumentar a persistência dos predadores no campo é importante aumentar a estrutura espacial do habitat e os alimentos alternativos da dieta do predador.

SUMÁRIO

GENERAL INTRODUCTION

There are many direct and indirect interactions among plants, pests and their natural enemies and these interactions are important in structuring communities. Basically, the structure of the communities is determined by consumption, such as herbivory, predation, parasitism and competition (Chase et al., 2002) and mutualistic interactions such as those between plants and pollinators (Kearns et al. 1998) and between plants and natural enemies of the herbivores (Price et al. 1980). However, other kinds of interactions can also occur, such as apparent competition, intraguild predation, (Messelink et al., 2012) and interactions among herbivores through induced plant defences. While the pests can interact through exploitative competition for the plant, the plant can mediate interactions between herbivores through plant defense responses. This may occur because the presence of one species could promote a plant defense that can affect the other species (Karban and Carey, 1984).

Another possible interaction is apparent competition, defined as an indirect interaction among prey that is mediated by a shared predator population (Holt and Lawton, 1994; Janssen et al., 1999; Harmon and Andow, 2004; Veen et al., 2006). The density of one prey increases the density of a predator, which subsequently decreases the density of a second species of prey. Thus, the dynamics of both prey resemble that of two species that are competing for resources, however, the interaction is mediated by a shared predator (Holt, 1977). In the long term, this can reduce the pest population, positively affecting biological control of this pest (Messelink et al., 2008).

Apparent competition is intrinsically related to diet choice, and theory has included the switching of predators between diet items and optimal diet choice into population dynamical models of two prey sharing a predator population (Abrams, 1993; Holt, 1977; Abrams and Matsuda, 2004, 2003). However, positive effects of mixed diets on predator performance have not been included, to the best of my knowledge. Nevertheless, there are several studies showing that the animals have a higher fitness when they feed on mixed diets instead of feeding on single diet (Messelink et al., 2008; Lefcheck et al., 2013; Marques et al., 2015; Ko et al., 2017).

Here I studied a system of predators and prey, where the predators have a higher fitness when they feed on a mixture of the two prey species. The system is composed of two species of predatory mites belonging to the family Phytoseiidae: *Iphiseiodes zuluagai* Denmark & Muma and *Euseius concordis* Chant. These two phytoseiids are being evaluated for their capacity to control the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) in plantations of the biodiesel plant *Jatropha curcas* L. in Brazil (Pedro Neto et al., 2013; Sarmento et al., 2011). These predators feed on the two herbivores as well as on *J. curcas* plant material (pollen), and are therefore typical examples of omnivores (Figure 1).

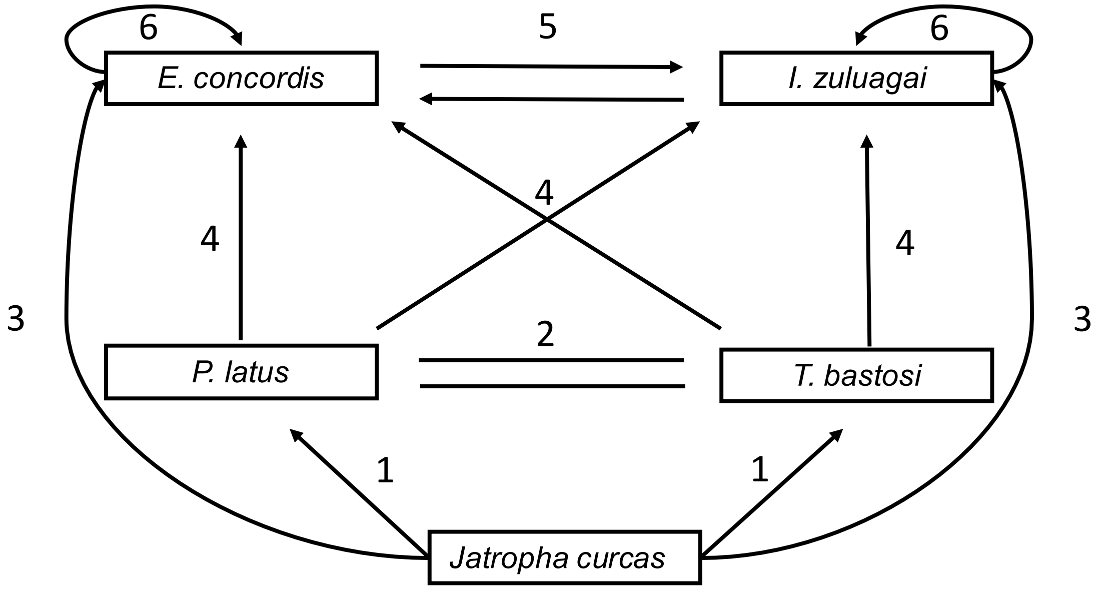


Figure 1. Simplified food web of arthropods inhabiting *Jatropha curcas* plants. Arrows indicate flow of energy. Interactions are herbivory (1), apparent competition and competition (2), omnivory (3), predation (4), intraguild predation and cannibalism (6).

The two predatory mites showed a higher fitness when they fed on a diet composed of mixtures of the two prey species mentioned above (Marques et al., 2015). Several studies use the geometrical framework to construct a fitness landscape as function of the nutrient contents of specific, often artificial, diets (Simpson and Raubenheimer, 1995Simpson et al., 2004, 2006; Behmer and Joern, 2008; Lee et al., 2008; Behmer, 2009; Jensen et al., 2012;). Constructing such fitness landscapes using natural prey has not been done yet. In Chapter 1, I constructed a fitness landscape as function of the proportion of the two prey species consumed. We assessed the ratio of prey that resulted in the best performance of the predator *E. concordis.*

In nature, food items are commonly spatially distributed (Godin and Keenleyside, 1984). Therefore, as the distance between nutritional complementary food sources increases, the energy cost and time spent on traveling between patches with different prey types to mix a predator's diet also increases (Abrams, 1993; Lima and Dill, 1990). Hence, I expect that there should be a distance between patches of different prey where the energy cost and time associated to search for a mixed diet is higher than the benefits of it. There are some studies that considered the spatial separation of the diet sources (Behmer et al., 2003; Mody et al., 2007; Ko et al., 2017). So, in Chapter 2, I investigated if the predator *E. concordis* covers longer distances (compared to Marques et al., 2015) to obtain a mixed diet.

The structure of the natural habitat of the prey is more complex than the structure offered in the experiments of Chapter 2. Hence, the foraging costs will also increase with increasing habitat complexity (Brown and Kotler, 2004), but the predators may be more adapted to deal with this habitat complexity than that offered in Chapter 2. Therefore, I evaluated in Chapter 3 if the predator *E. concordis* mixes its diet when the two prey species occur spatially separated on a small plant of *J. curcas* and if it benefits from this.

The natural enemies cited above can also interact with each other. Predators that kill and eat individuals of another species of natural enemy are referred to as intraguild predators; the victim is called the intraguild prey (Polis et al., 1989; Holt and Polis, 1997). Intraguild predation (IGP) is described as a mix of competition and predation between species that use similar resources and occurs when individuals of one species kill and eat the individuals of the other species (Polis and Holt, 1992). Theoretical models of intraguild predation usually consider the shared resource, the intraguild prey, and the intraguild predator. These models predict that the three species can only coexist if the intraguild prey is the better competitor for the shared resource (Holt and Polis, 1997). Even then, however, coexistence is only possible at intermediate levels of productivity (Holt and Polis, 1997; Diehl and Feißel, 2000; Mylius et al., 2001), but it is common in nature. This discrepancy between theory and reality may be explained by factors not included in the original theory, such as temporal variation of the refuge, alternative prey for the intraguild predator, and spatial structure (Mylius et al., 2001;Amarasekare, 2007; Holt and Huxel, 2007; Janssen et al., 2007; Daugherty et al., 2007; Rosenheim, 2007; Rudolf, 2007; Vance-Chalcraft et al., 2007).

Aiming to study the intraguild interactions between the two species of predatory mites, I first assessed the possible co-occurrence of *I. zuluagai* and *E. concordis* on the same *J. curcas* plants in the field (Chapter 4). Second, I investigated whether the two predators were involved in IGP or reciprocal IGP. Subsequently, I investigated whether spatial structure promoted the coexistence of these two predators.

REFERENCES

Abrams, P., Matsuda, H., 2004. Consequences of behavioral dynamics for the population dynamics of predator-prey systems with switching. Population Ecology 46. https://doi.org/10.1007/s10144-003-0168-2

Abrams, P.A., 1993. Optimal traits when there are several costs: the interaction of mortality and energy costs in determining foraging behavior. Behavioral Ecology 4, 246–259. https://doi.org/10.1093/beheco/4.3.246

Abrams, P.A., Matsuda, H., 2003. Population dynamical consequences of reduced predator switching at low total prey densities. Population Ecology 45, 175–185. https://doi.org/10.1007/s10144-003-0159-3

Amarasekare, P., 2007. Spatial dynamics of communities with intraguild predation: The role of dispersal strategies. The American Naturalist 170, 819–831. https://doi.org/10.1086/522837

Behmer, S.T., 2009. Insect herbivore nutrient regulation. Annual Review of Entomology 54, 165–187. https://doi.org/10.1146/annurev.ento.54.110807.090537

Behmer, S.T., Cox, E., Raubenheimer, D., Simpson, S.J., 2003. Food distance and its effect on nutrient balancing in a mobile insect herbivore. Animal Behaviour 66, 665–675. https://doi.org/10.1006/anbe.2003.2243

Behmer, S.T., Joern, A., 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. Proceedings of the National Academy of Sciences 105, 1977–1982. https://doi.org/10.1073/pnas.0711870105

Brown, J.S., Kotler, B.P., 2004. Hazardous duty pay and the foraging cost of predation: Foraging cost of predation. Ecology Letters 7, 999–1014. https://doi.org/10.1111/j.1461-0248.2004.00661.x

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M., Case, T.J., 2002. The interaction between predation and competition: a review and synthesis. Ecology Letters 5, 302–315. https://doi.org/10.1046/j.1461-0248.2002.00315.x

Diehl, S., Feißel, M., 2000. Effects of enrichment on three-level food chains with omnivory. The American Naturalist 155, 200–218. https://doi.org/10.1086/303319

Godin, J.-G.J., Keenleyside, M.H.A., 1984. Foraging on patchily distributed prey by a cichlid fish (Teleostei, Cichlidae): A test of the ideal free distribution theory. Animal Behaviour 32, 120–131. https://doi.org/10.1016/S0003-3472(84)80330-9

Harmon, J., Andow, D.A., 2004. Indirect effects between shared prey: Predictions for. BioControl 49, 605–626. https://doi.org/10.1007/s10526-004-0420-5

Holt, R.D., 1977. Predation, apparent competition, and the structure of prey communities. Theoretical Population Biology 12, 197–229. https://doi.org/10.1016/0040-5809(77)90042-9

Holt, R.D., Huxel, G.R., 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88, 2706–2712. https://doi.org/10.1890/06-1525.1

Holt, R.D., Lawton, J.H., 1994. The ecological consequences of shared natural enemies. Annual Review of Ecology and Systematics 25, 495–520. https://doi.org/10.1146/annurev.es.25.110194.002431

Holt, R.D., Polis, G.A., 1997. A theoretical framework for intraguild predation. The American Naturalist 149, 745–764. https://doi.org/10.1086/286018

Janssen, A., Pallini, A., Venzon, M., Sabelis, M.W., 1999. Behaviour and indirect interactions in food webs of plant-inhabiting arthropods, in: Bruin, J., van der Geest, L.P.S., Sabelis, M. W. (Eds.), Ecology and Evolution of the Acari. Springer Netherlands, Dordrecht, pp. 231–249. https://doi.org/10.1007/978-94-017-1343-6_16

Janssen, A., Sabelis, M.W., Magalhães, S., Montserrat, M., van der Hammen, T., 2007. Habitat structure affects intraguild predation. Ecology 88, 2713–2719. https://doi.org/10.1890/06-1408.1

Jensen, K., Mayntz, D., Toft, S., Clissold, F., Hunt, J., Raubenheimer, D., J Simpson, S., 2012. Optimal foraging for specific nutrients in predatory beetles. https://doi.org/10.1098/rspb.2011.2410

Kearns, C.A., Inouye, D.W., Waser, N.M., 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29, 83–112. https://doi.org/10.1146/annurev.ecolsys.29.1.83

Karban, R., Carey, J.R., 1984. Induced resistance of cotton seedlings to mites. Science 225, 53–54. https://doi.org/10.1126/science.225.4657.53

Ko, A.E., Jensen, K., Schal, C., Silverman, J., 2017. Effects of foraging distance on macronutrient balancing and performance in the German cockroach *Blattella* *germanica*. The Journal of Experimental Biology 220, 304–311. https://doi.org/10.1242/jeb.146829

Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in Drosophila: New insights from nutritional geometry. Proceedings of the National Academy of Sciences 105, 2498–2503. https://doi.org/10.1073/pnas.0710787105

Lefcheck, J.S., Whalen, M.A., Davenport, T.M., Stone, J.P., Duffy, J.E., 2013. Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology 94, 565–572. https://doi.org/10.1890/12-0192.1

Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68, 619–640. https://doi.org/10.1139/z90-092

Marques, R.V., Sarmento, R.A., Lemos, F., Pedro-Neto, M., Maurice W. Sabelis, Venzon, M., Pallini, A., Janssen, A., 2015. Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. Functional Ecology 29, 1317–1324. https://doi.org/10.1111/1365-2435.12439

Messelink, G.J., Maanen, R. van, van Steenpaal, S.E.F., Janssen, A., 2008. Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biological Control 44, 372–379. https://doi.org/10.1016/j.biocontrol.2007.10.017

Mody, K., Unsicker, S.B., Linsenmair, K.E., 2007. Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. Ecology 88, 1012–1020. https://doi.org/10.1890/06-1338

Mylius, S.D., Klumpers, K., de Roos, A.M., Persson, L., 2001. Impact of intraguild predation and stage structure on simple communities along a productivity gradient. The American Naturalist 158, 259–276. https://doi.org/10.1086/321321

Pedro Neto, M., Sarmento, R.A., Oliveira, W.P. de, Picanço, M.C., Erasmo, E.A.L., 2013. Biologia e tabela de vida do ácaro-vermelho *Tetranychus bastosi* em pinhão-manso. Pesquisa Agropecuária Brasileira 48, 353–357. https://doi.org/10.1590/S0100-204X2013000400001

Polis, G.A., Holt, R.D., 1992. Intraguild predation: The dynamics of complex trophic interactions. Trends in Ecology & Evolution 7, 151–154. https://doi.org/10.1016/0169-5347(92)90208-S

Polis, G.A., Myers, C.A., Holt, R.D., 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20, 297–330. https://doi.org/10.1146/annurev.es.20.110189.001501

Rosenheim, J.A., 2007. Intraguild predation: new theoretical and empirical perspectives 1. Ecology 88, 2679–2680. https://doi.org/10.1890/07-0790.1

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., Weis, A.E., 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Annu. Rev. Ecol. Syst. 11, 41–65.

Rudolf, V.H.W., 2007. The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology 88, 2697–2705. https://doi.org/10.1890/06-1266.1

Sarmento, R.A., Rodrigues, D.M., Faraji, F., Erasmo, E.A.L., Lemos, F., Teodoro, A.V., Kikuchi, W.T., dos Santos, G.R., Pallini, A., 2011. Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus* *latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. Experimental and Applied Acarology 53, 203–214. https://doi.org/10.1007/s10493-010-9396-2

Simpson, S.J., Raubenheimer, D., 1995. The geometric analysis of feeding and nutrition: a user’s guide. Journal of Insect Physiology 41, 545–553. https://doi.org/10.1016/0022-1910(95)00006-G

Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., Raubenheimer, D., 2004. Optimal foraging when regulating intake of multiple nutrients. Animal Behaviour 68, 1299–1311. https://doi.org/10.1016/j.anbehav.2004.03.003

Simpson, S.J., Sword, G.A., Lorch, P.D., Couzin, I.D., 2006. Cannibal crickets on a forced march for protein and salt. Proceedings of the National Academy of Sciences 103, 4152–4156. https://doi.org/10.1073/pnas.0508915103

Vance-Chalcraft, H.D., Rosenheim, J.A., Vonesh, J.R., Osenberg, C.W., Sih, A., 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88, 2689–2696. https://doi.org/10.1890/06-1869.1

Veen, F.J.F. van, Memmott, J., J. Godfray, H.C., 2006. Indirect effects, apparent competition and biological control, in: Brodeur, J., Boivin, G. (Eds.), Trophic and guild in biological interactions control. Springer Netherlands, Dordrecht, pp. 145–169. https://doi.org/10.1007/1-4020-4767-3_7

CHAPTER I

Optimal diet in a generalist predatory mite

Renata Vieira Marques[1], Angelo Pallini[1], Renato Almeida Sarmento[2], Adriana Golçalves Oliveira[2], Dara Emily Silva Ferreira[2], Thaysa Ferreira Alves[2] & Arne Janssen[3]

In preparation for submission to the Journal of Animal Ecology

[1]Department of Entomology, Federal University of Viçosa (UFV), CEP 36570-000, Viçosa, MG, Brazil. Email: renatamarques.ufv@gmail.com, pallini@ufv.br

[2]Federal University of Tocantins (UFT), PO Box 66, Gurupi, TO, Brazil. Emails: rsarmento@mail.uft.edu.br, drika_eafa@hotmail.com, daraferreira@mail.uft.edu.br, thaysaferreiraalves@gmail.com

[3]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands. Email: Arne.Janssen@uva.nl

Abstract

1. Many animals actively redress nutrient imbalances in their diet to maximize their performance.
2. We recently found that females of two species of phytoseiids showed a higher oviposition rate on a mixture of co-occurring phytophagous mites than when feeding on either of these prey species separately. We also found that the predators try to obtain such a mixed diet.
3. Here, we investigated the relation between diet composition and diet choice of individuals with their performance by quantifying the reproduction of the predatory mite *Euseius concordis* on diets with various proportions of the two prey.
4. Oviposition of the predator was lower on single diets than on mixtures. When female predators were offered both prey in different proportions, they selected a diet that maximized their oviposition rate.

Key words: balanced diet, foraging behaviour, nutritional ecology, prey quality, phytoseiids

Introduction

Many predator species consume more than one prey species (Sabelis, 1992), whereas many herbivorous arthropods are specialized on a single host plant species or genus (Jaenike, 1990). For several years, studies on diet choice of predators were based on the premise that predatory arthropods optimized the rate of prey capture or energy intake per time spent on a prey (Charnov, 1976; Stephens & Krebs, 1986) instead of selecting prey according to nutrient composition (but see Belovsky, 1978; Bilde & Toft, 1994; Greenstone, 1979; Rapport, 1980; Tinbergen, 1981). There are two explanations for the consumption of a mixed diet, and they are not mutually exclusive. One is that the animals need to mix their diet to dilute toxins present in one or more of the diet components (Toft & Wise, 1999). Another explanation is that different prey species contain different amounts of nutrients and the predatory arthropods mix their diet to redress specific nutritional imbalances (Mayntz, Raubenheimer, Salomon, Toft, & Simpson, 2005). Both theories predict that animals should have higher fitness when they feed on a mixed diet than when feeding on single diets.

Although there are several studies showing that animals actively redress nutrient imbalances in their diet, these studies often use semi-artificial diets or prey that were specifically reared to contain different amounts of nutrients (Mayntz et al., 2005; Raubenheimer, Mayntz, Simpson, & Tøft, 2007; Dussutour & Simpson, 2009; Jensen et al., 2012; Vaudo et al., 2016; Raubenheimer & Simpson, 2018). Evidence that predators do actively forage for a mixed diet consisting of several types of *natural* prey is scare. We previously showed that two species of predatory mites benefit from a mixed diet consisting of two coexisting natural prey and actively forage for a mixed diet (Marques et al., 2015). It is as yet unknown whether the two prey species are complementary in nutrient contents or whether toxins are present in the prey species. Thus, it is not possible yet to control the quality of the prey to construct a fitness landscape (that is, relating the diet choice of individuals with their fitness) as a function of specific nutrients, as has been demonstrated for other species, using the geometric framework (Simpson & Raubenheimer, 1995; Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004; Simpson, Sword, Lorch, & Couzin, 2006; Behmer & Joern, 2008; Lee et al., 2008; Behmer, 2009; Jensen et al., 2012). We therefore constructed a fitness landscape as function of the proportion of the two prey species offered. As stand-in measure for fitness, we used the oviposition rate of predators, which is closely related to the population growth rate and life-time reproduction of predatory mites (Janssen & Sabelis, 1992). We show that the predators often consume prey in different proportions than those offered, and that this maximizes their oviposition rate.

Material and methods

The study system

We studied the diet choice of the phytoseiid *Euseius concordis* Chant and its two prey, the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae). These herbivorous mites and predators co-occur in plantations of the biodiesel plant *Jatropha curcas* L. in Brazil (Sarmento et al., 2011; Pedro-Neto, Sarmento, Oliveira, Picanço, & Erasmo, 2013;). Whereas the herbivores are known to cause severe yield reductions, the predators are currently being evaluated for their capacity to control the plant pests (Marques et al., 2018; Sarmento et al., 2011).

Cultures

Jatropha curcas plants were grown in plastic pots (5 L) containing a mixture of soil and bovine manure (3:1) and were watered once per day. The herbivorous mites *T. bastosi* and *P. latus* and the predatory mite *E. concordis* were obtained from natural populations on *J. curcas* plants in Gurupi, State of Tocantins, Brazil (11°45′47″S, 49°02′57″W) in 2009. *Tetranychus bastosi* was cultured on *J. curcas* plants. The plants were kept in cages (47.5 x 47.5 x 93.0 cm) that contained a plastic tray (45 x 30 x 8 cm) that were placed inside a second tray (55 x 40 x 10 cm), filled with detergent and water, which served to prevent mite escapes and invasion of other arthropods. A clean plant was added to these cultures once a week and infested leaves of old plants were placed on it to allow the mites to move onto the new plant. The broad mite *P. latus* was cultured on isolated new leaves (diameter c. 5 cm), placed inside a plastic tray (45 x 30 x 8 cm) filled with distilled water with a sponge in the center. A thin layer of wet cotton wool was placed around the leaves, which served to prevent mite escapes and to avoid desiccation of the leaves. Every four days, we replaced the leaves; the old leaves were placed on top of the new leaves. The predatory mite *E. concordis* was reared inside plastic boxes (11 × 11 cm) on flexible plastic disks (∅ = 6 cm) floating on distilled water. Small tent-like structures consisting of a folded piece of plastic with some cotton threads under it were supplied on the arenas, serving as shelter and oviposition site. Once per day, a small quantity of castor bean (*Ricinus communis*) pollen was supplied on the arenas as food for the predators (McMurtry & Scriven, 1964). All cultures were maintained in a climate room at 25 ± 2 °C, 65 –70% R.H. and a 12 hr L/12 hr D photoperiod.

The optimal diet

We measured oviposition rates of adult female predators aged 9 days old since the egg stage. Using adult females of the same age is essential because the oviposition rate of predatory mites varies with age (Janssen & Sabelis, 1992). Experiments were carried out on leaves (diameter c. 5 cm) inside plastic boxes (11 x 11 cm) filled with distilled water with a sponge fixed in the center. A thin layer of wet cotton wool was placed around the leaves, which served to prevent mite escapes and to avoid desiccation of the leaves. We offered a mixture of eggs of both prey species in five different densities: 0/30, 5/25, 10/20, 15/15, 20/10, 25/5, 30/0, 60/0 (*T. bastosi*/*P. latus*). Thus, the performance of the predatory mite was assessed with an increasing number of one prey species and a decreasing number of the other. To exclude the possibility that lower oviposition on a diet of only *T. bastosi* was caused by insufficient food, we included the last treatment with double the number of eggs of *T. bastosi*. Each density combination was replicated 16 times. The experiment was carried out in several blocks in time, with all treatments in each block. We used eggs of the two prey species because mobile prey often display antipredator behaviour and this can affect the performance of the predator. To obtain these eggs, we transferred adult females of each prey species to a leaf with double the number of adults that was required for each density above. After 24 hours, we removed all adult females of both prey species, leaving only eggs of each species at their respective densities. The surplus of eggs was removed with the aid of a pin to obtain the exact densities. The number of prey eggs consumed during three consecutive days and the oviposition were assessed simultaneously. Because the oviposition of the first day is affected by the diet of previous days (Sabelis, 1990), we did not include oviposition and predation of this day in the analysis. The leaves with prey eggs were replaced daily with new ones with the same initial prey densities to prevent prey depletion.

Statistical analysis

The total numbers of eggs produced by the adult female predators during the last two days of the experiment and the total predation during the entire experiment were analysed with a linear mixed-effects model with treatment as fixed factor and block as random factor (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2017). Treatments were compared through model simplification by combining treatment levels (Crawley, 2013). The proportions of prey eaten vs. proportions of prey offered were analysed with a binomial test, and the oviposition of predators as function of the proportion of *T. bastosi* eaten was analysed with a linear and quadratic regression model. All statistical analyses were done with the statistical software R 3.3.3 (R Development Core Team, 2017), and models were compared with the anova function in R. All models were checked by plotting residuals against fitted values and checking normality of the error distribution.

Results

The total numbers of eggs consumed did not differ significantly among the densities of prey species (LME, $\text{Chi}^2 = 8.49$, d.f. = 1, $P > 0.05$). Thus, *E. concordis* consumed approximately equal numbers of eggs at all densities of prey species (Figure 1). We did not compare the consumption of eggs of the two species because the eggs of *P. latus* are smaller than those of *T. bastosi* and the eggs of the two species may differ in many other aspects. At high densities of *T. bastosi*, predators consumed eggs in the same proportion as they were offered (Figure 2). However, with an increasing proportion of *P. latus* eggs present, the predator consumed proportionally fewer *T. bastosi* eggs (Figure 2).

The oviposition rate of *E. concordis* feeding on prey eggs varied significantly with the diet offered (LME, $\text{Chi}^2 = 56.1$, d.f. = 1, $P > 0.001$). Oviposition was lower on a diet with only eggs of *T. bastosi* than on the other diets (Figure 3). For almost all prey densities, the oviposition rate of *E. concordis* was higher on the mixed diets than on single diets (Figure 3). A diet consisting of 83.3% of *T. bastosi* resulted in a significantly lower oviposition rate than the other mixed diets (Figure 3). Although the other mixed diets did not differ significantly from each other, the highest oviposition rate of *E. concordis* was found when it was offered 33% of *T. bastosi* and 67% of *P. latus* (Figure 3).

Because the proportion of *T. bastosi* offered was not the same as the proportion of *T. bastosi* eaten, we analysed the oviposition of *E. concordis* as a function of the proportion of *T. bastosi* eaten. The oviposition rate of *E. concordis* varied significantly with the composition of the diet consumed (LM, $\text{Chi}^2 = 22.4$, d.f. = 1, $P > 0.001$) and a quadratic model fitted the data significantly better than a linear model ($F_{1,125} = 22.4$, $P < 0.001$), showing that there was indeed an optimal diet. The oviposition was highest when the predator consumed a mixed diet consisting of 34% of *T. bastosi* and 66% of *P. latus* (Figure 4).

Discussion

We found an optimal diet consisting of a mixture of two natural co-occurring prey for the predatory mite *E. concordis*, with a diet composed of c. 34% of *T. bastosi* (66% *P. latus*) resulting in the highest oviposition rate of the predator (Figure 3, 4). At high densities of *T. bastosi*, the predators consumed eggs in the same proportion as they were offered (Figure 2). However, with an increasing proportion of *P. latus* eggs, the predators ate a lower proportion of *T. bastosi* eggs as was offered (Figure 2). This might be caused by the predators selecting for a mixed diet consisting of a higher proportion of *P. latus*. It is also possible that the eggs of *P. latus* were easier to find despite their smaller size. Elsewhere, we showed that *E. concordis* does actively search for a mixed diet (Marques et al., 2015). We therefore believe that the differences in the proportions of *T. bastosi* offered and consumed (Figure 2) are caused by the predators selecting a better diet.

For almost all prey combinations, a mixed diet of the two prey species resulted in a higher egg production rate (Figure 3). This is in agreement with an earlier study (Marques et al., 2015), which added these predatory mites to a growing list of predators that benefit from a mixed diet (Mayntz et al., 2005; Raubenheimer et al., 2007; Dussutour & Simpson, 2009; Jensen et al., 2012; Raubenheimer & Simpson, 2018).

When *E. concordis* fed on a diet mainly or exclusively consisting of *T. bastosi*, it had a lower oviposition rate than on other mixed diets and the oviposition rate of *E. concordis* was lower when the predators fed on single diets than on most of the mixed diets (Figure 3). The oviposition rate on the treatment with sixty eggs of *T. bastosi* was similar to that in the treatment with only thirty eggs of *T. bastosi*, showing that the lower oviposition when feeding on this prey was not caused by insufficient food. It is unclear whether this is because *T. bastosi* and *P. latus* are toxic to the predators or whether they are relatively deficient in some nutrients.

In nature, animals will not always find food of different quality in the same place. So, they may need to forage at different places to mix their diet. The two prey species studied here can be found at different parts of their host plant (*Jatropha curcas*). *Polyphagotarsonemus latus* attacks the apical leaves and *T. bastosi* is mainly found on the old leaves (Rosado et al., 2014). Therefore, the predators need to commute between plant parts to mix their diet. We previously showed that *E. concordis* covers short distances to mix their diet (Marques et al., 2015). Several studies have considered such spatial separation of the diet elements (Behmer, Cox, Raubenheimer, & Simpson, 2003; Mody, Unsicker, & Linsenmair, 2007), but these studies did not show if there will be a maximum distance that the animals are willing to cross to mix their diet. There must be a distance in which the costs of traveling to obtain a mixed diet are higher than the offset of mixing the diet to improve fitness. Future experiments should test the propensity of animals to cover larger distances to mix their diet.

Our results show a case of a polyphagous predator species that balances its diet by feeding on mixtures of their natural prey. However, figure 3 shows a flat curve, suggesting that any mixture is a good diet. Nevertheless, the predators did not consume prey in the ratios that they were offered (Figure 2), so they possibly obtained a diet closer to the optimal diet than when they would eat prey in the ratio offered, and this would obscure the existence of the optimum. Figure 4 indeed shows that there is an optimum mixture of the two prey, but the curve is still relatively flat, suggesting that selection for obtaining the exact optimal diet is probably not strong. Furthermore, there is considerable variation in oviposition rate independent of the diet (Figure 4). We suggest that there is selection for predators to mix some *T. bastosi* through their diet of *P. latus* and this resulted in predators that actively search for a mixed diet (Marques et al., 2015).

Acknowledgements

The National Council of Technological and Scientific Development, Brazil (CNPq) provided a master scholarship to the first author and RAS received a scholarship from CNPq (Produtividade em Pesquisa-Project: 306652/2018-8). CNPq and FAPEMIG sponsored AP. The Coordination of Improvement of Higher Education Personnel

(CAPES) provided financial support (PROCAD 2013). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annual Review of Entomology*, *54*, 165–187. doi:10.1146/annurev.ento.54.110807.090537

Behmer, S. T., Cox, E., Raubenheimer, D., & Simpson, S. J. (2003). Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Animal Behaviour*, *66*(4), 665–675. doi:http://dx.doi.org/10.1006/anbe.2003.2243

Behmer, S. T., & Joern, A. (2008). Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 1977–1982. doi:10.1073/pnas.0711870105

Belovsky, G. E. (1978). Diet optimization in a generalist herbivore: the moose. *Theoretical Population Biology*, *14*, 105–134.

Bilde, T., & Toft, S. (1994). Prey preference and egg production of the carabid beetle *Agonum dorsale*. *Entomologia Experimentalis Et Applicata*, *73*, 151–156.

Charnov, E. L. (1976). Optimal foraging: attack strategy of a mantid. *The American Naturalist*, *110*, 141–151.

Crawley, M. J. (2013). *The R book*. Chichester, UK: John Wiley & Sons.

Dussutour, A., & Simpson, S. J. (2009). Communal nutrition in ants. *Current Biology*, *19*, 740–744.

Greenstone, M. H. (1979). Spider feeding behaviour optimises dietary essential amino acid composition. *Nature*, *282*, 501–503.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, *21*, 243–273.

Janssen, A., & Sabelis, M. W. (1992). Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. *Experimental & Applied Acarology*, *14*, 233–250.

Jensen, K., Mayntz, D., Toft, S., Clissold, F. J., Hunt, J., Raubenheimer, D., & Simpson, S. J. (2012). Optimal foraging for specific nutrients in predatory beetles. *Proceedings of The Royal Society B-Biological Sciences*, *279*, 2212–2218. doi:10.1098/rspb.2011.2410

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., … Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 2498–2503.

Marques, R. V., Sarmento, R. A., Lemos, F., Pedro-Neto, M., Sabelis, M. W., Venzon, M., … Janssen, A. (2015). Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. *Functional Ecology*, *29*, 1317–1324. doi:10.1111/1365-2435.12439

Marques, R. V., Sarmento, R. A., Oliveira, A. G., Rodrigues, D. de M., Venzon, M., Pedro-Neto, M., … Janssen, A. (2018). Reciprocal intraguild predation and predator coexistence. *Ecology and Evolution*, *8*(14), 6952–6964. doi:10.1002/ece3.4211

Mayntz, D., Raubenheimer, D., Salomon, M., Toft, S., & Simpson, S. J. (2005). Nutrient-specific foraging in invertebrate predators. *Science*, *307*, 111–113.

McMurtry, J. A., & Scriven, G. T. (1964). Studies on the feeding, reproduction, and development of *Amblyseius hibisci* (Acarina: Phytoseiidae) on various food substances. *Annals of the Entomological Society of America*, *57*, 649–655.

Mody, K., Unsicker, S. B., & Linsenmair, K. E. (2007). Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. *Ecology*, *88*, 1012–1020.

Pedro-Neto, M., Sarmento, R. A., Oliveira, W. P. de, Picanço, M. C., & Erasmo, E. A. L. (2013). Biologia e tabela de vida do ácaro-vermelho *Tetranychus bastosi* em pinhão-manso. *Pesquisa Agropecuária Brasileira*, *48*, 353–357.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2017). *NLME: Linear and Nonlinear Mixed Effects Models. http://CRAN.R-project.org/package=nlme*. Retrieved from http://CRAN.R-project.org/package=nlme

R Development Core Team. (2017). R: A language and environment for statistical computing (Version 3.3.3). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Rapport, D. J. (1980). Optimal foraging for complementary resources. *American Naturalist*, *116*, 324–246.

Raubenheimer, D., Mayntz, D., Simpson, S. J., & Tøft, S. (2007). Nutrient-specific compensation following diapause in a predator: implications for intraguild predation. *Ecology*, *88*, 2598–2608.

Raubenheimer, D., & Simpson, S. J. (2018). Nutritional ecology and foraging theory. *Current Opinion in Insect Science*, *27*, 38–45. doi:https://doi.org/10.1016/j.cois.2018.02.002

Sabelis, M. W. (1990). How to analyze prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. *Oecologia*, *82*, 289–298.

Sabelis, M. W. (1992). Predatory arthropods. In M. J. Crawley (Ed.), *Natural enemies: the population biology of predators, parasites and diseases* (pp. 225–264). Oxford: Blackwell.

Sarmento, R. A., Rodrigues, D. M., Faraji, F., Erasmo, E. A. L., Lemos, F., Teodoro, A. V., … Pallini, A. (2011). Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. *Experimental and Applied Acarology*, *53*, 203–214. doi:10.1007/s10493-010-9396-2

Simpson, S. J., & Raubenheimer, D. (1995). The geometric analysis of feeding and nutrition: a user's guide. *Journal of Insect Physiology*, *41*, 545–553.

Simpson, Stephen J., Sibly, R. M., Lee, K. P., Behmer, S. T., & Raubenheimer, D. (2004). Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour*, *68*, 1299–1311.

Simpson, Stephen J., Sword, G. A., Lorch, P. D., & Couzin, I. D. (2006). Cannibal crickets on a forced march for protein and salt. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 4152–4156.

Stephens, D. W., & Krebs, J. R. (1986). *Foraging theory* (1st ed.). Princeton, New Jersey, USA: Princeton University Press.

Tinbergen, J. M. (1981). Foraging decisions in starlings. *Ardea*, *69*, 1–67.

Toft, S., & Wise, D. H. (1999). Growth, development, and survival of a generalist predator fed single- and mixed-species diets of different quality. *Oecologia*, *119*, 191–197.

Vaudo, A. D., Stabler, D., Patch, H. M., Tooker, J. F., Grozinger, C. M., & Wright, G. A. (2016). Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. *Journal of Experimental Biology*, *219*(24), 3962–3970. doi:10.1242/jeb.140772

Figure legends

Figure 1. Predation rate (± SE of the total) of eggs of two prey species (*Tetranychus bastosi*, grey bars, and *Polyphagotarsonemus latus*, white bars) by *E. concordis* during three days as function of the different proportion of prey eggs offered. Predation was assessed with an increasing number of *T. bastosi* and a decreasing number of *P. latus*.

Figure 2. Proportion of *T. bastosi* eggs consumed (±SE) by *E. concordis* on three consecutive days as a function of the proportion of *T. bastosi* offered (drawn line). Significant differences in the proportion of *T. bastosi* consumed vs offered are indicated by asterisks (Binomial test, $P < 0.05$).

Figure 3. Average oviposition rate (±SE) of *E. concordis* during three consecutive days feeding on a diet consisting of different proportions of the prey species *T. bastosi* and *P. latus*. 2x represent the treatment with double the number of eggs of *T. bastosi*. Significant differences in oviposition rate are indicated by letters below the averages (contrast after LME; $P < 0.05$).

Figure 4. The oviposition of *E. concordis* during three consecutive days as function of the proportion of the *T. bastosi* prey consumed. Dots represent the average oviposition rate of individual *E. concordis* during three consecutive days. The curve is a fitted quadratic model (Oviposition = $1.83 + 3.23x - 4.69x^2$, with x = the proportion of *T. bastosi* consumed, $F_{2,125} = 26.7$, $P < 0.0001$).

Figure 1

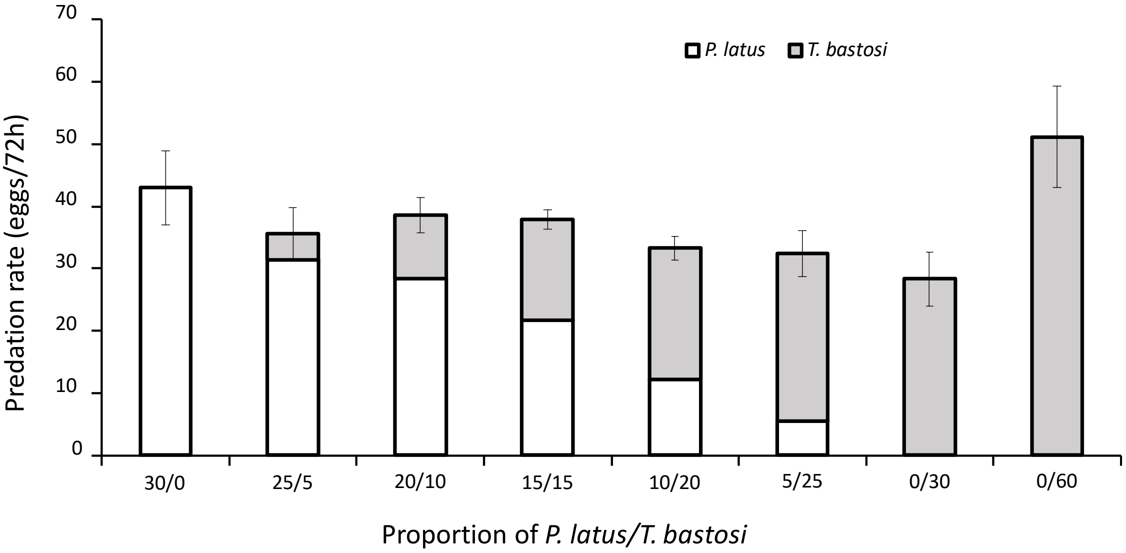

P. latus
T. bastosi
Predation rate (eggs/72h)
0
10
20
30
40
50
60
70
30/0
25/5
20/10
15/15
10/20
5/25
0/30
0/60
Proportion of P. latus/T. bastosi

Figure 2

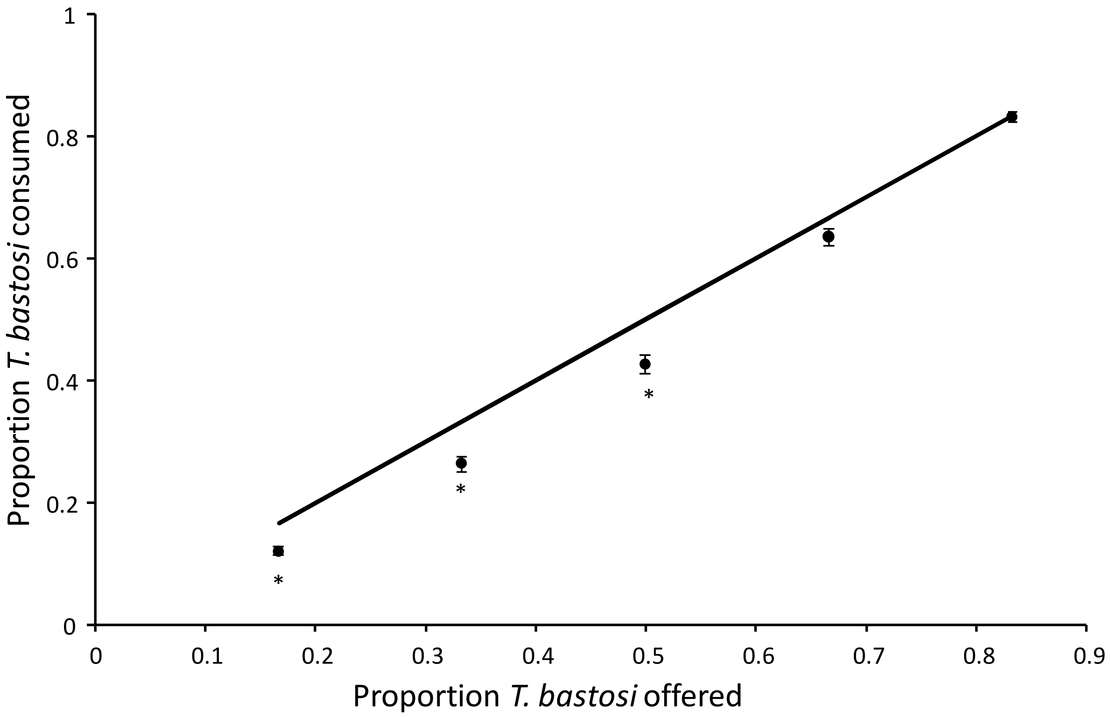

Proportion *T. bastosi* consumed
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Proportion *T. bastosi* offered
*
*
*

Figure 3

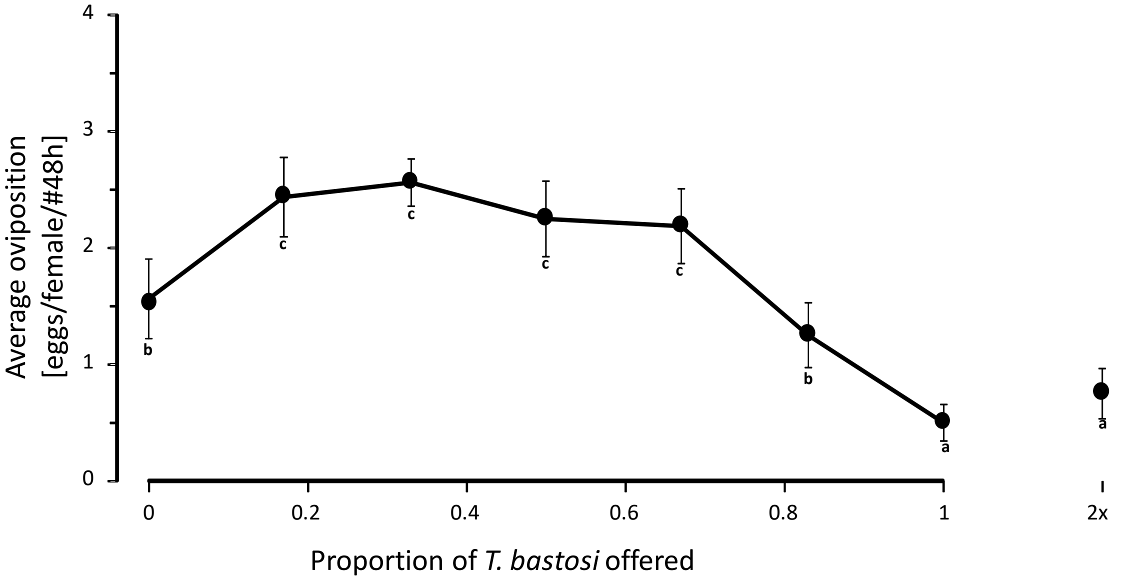
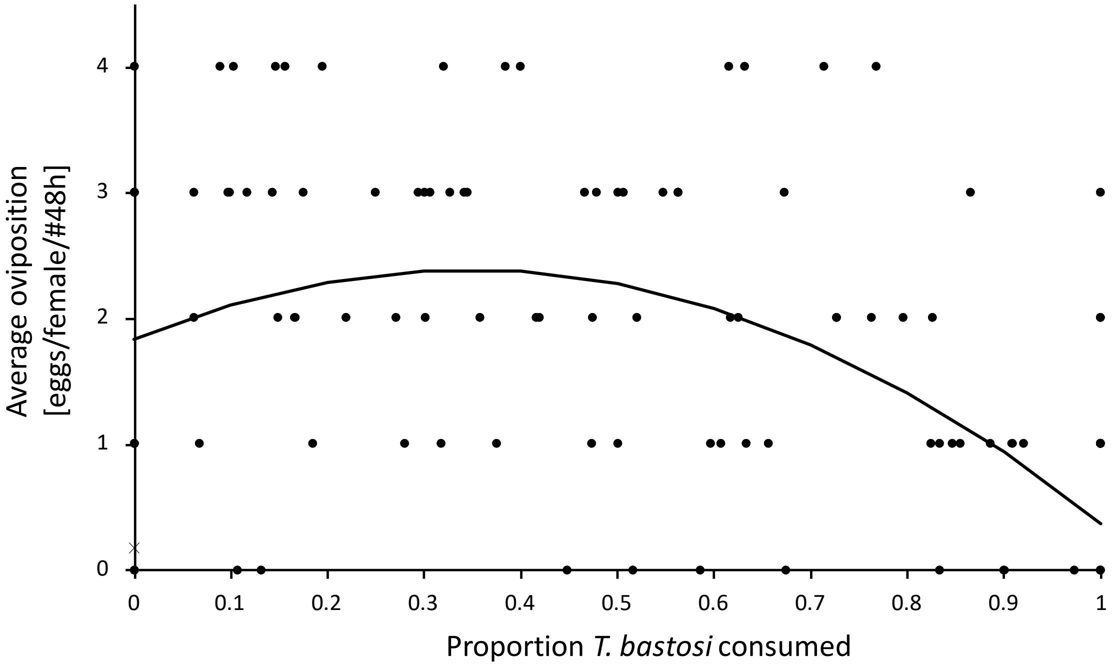

Average oviposition
[eggs/female/#48h]
0
1
2
3
4
0
0.2
0.4
0.6
0.8
1
2x
Proportion of *T. bastosi* offered
b
c
c
c
c
b
a
a

Figure 4


Average oviposition [eggs/female/#48h]
0
1
2
3
4
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Proportion *T. bastosi* consumed

CHAPTER II

Do predators cover distances to mix their diet?

Renata Vieira Marques[1], Angelo Pallini[1], Renato Almeida Sarmento[2], Adriana Golçalves Oliveira[2], Dara Emily Silva Ferreira[2], Thaysa Ferreira Alves[2] & Arne Janssen[3]

[1]Department of Entomology, Federal University of Viçosa (UFV), CEP 36570-000, Viçosa, MG, Brazil. Email: renatamarques.ufv@gmail.com, pallini@ufv.br

[2]Federal University of Tocantins (UFT), PO Box 66, Gurupi, TO, Brazil. Emails: rsarmento@mail.uft.edu.br, drika_eafa@hotmail.com, daraferreira@mail.uft.edu.br, thaysaferreiraalves@gmail.com

[3]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands. Email: Arne.Janssen@uva.nl

Abstract

Animals actively redress nutrient imbalances in their diet to increase their fitness. However, food items are usually patchily distributed in nature. As the distance between nutritionally complementary food increases, the foraging animal needs to spend more energy and time to commute between different food sources. The predatory mite *Euseius concordis* is known to cover short distances to mix its diet, resulting in an increased oviposition rate. Here, we investigated whether the predator *E. concordis* does actively mix its diet when this requires foraging over longer distances. We also assessed the effect on their fitness using their *natural* prey (*P. latus* and *T. bastosi*). Our results indicate that when the predators have to cover long distances, the positive effect of the mixed diet decreased. This may be because the predators have to spend more time and energy to commute between patches to obtain a mixed diet.

Key words: Foraging effort, balanced diet, foraging behavior, nutritional ecology, foraging distance.

Introduction

Many studies show that the nutrient composition of food plays an important role in diet choice of animals (see Rapport, 1980 for a review). Many animals actively redress nutrient imbalances in their diet to increase their fitness (Mayntz, 2005; Raubenheimer et al., 2007; Jensen et al., 2012; Vaudo et al., 2016; Raubenheimer and Simpson, 2018). However, in nature, food items are usually patchily distributed (Godin and Keenleyside, 1984). As the distance between nutritionally complementary food sources increases, the costs associated with obtaining a mixed diet also increase because the foraging animal needs to spend energy and time to commute from one food source to the other (Abrams, 1993; Lima and Dill, 1990). This suggests that there is a distance in which the costs of traveling to obtain a mixed diet are higher than the fitness benefits obtained by feeding on a mixed diet. Hence, we expect that animals will stop foraging for a mixed diet when the distance between the different diet elements becomes too long. Several studies have considered such spatial separation of diet elements (Behmer et al., 2003; Mody et al., 2007; Ko et al., 2017). For example, using artificial diet, Ko et al. (2017) found that when German cockroaches (*Blattella germanica*) had to cover longer distances to obtain a mixed diet, both males and females, as well as female oocytes became bigger. However, these studies did not show if there were costs associated with crossing long distances to obtain a mixed diet. Evidence that predators do actively mix their diet when this requires foraging over long distances and the effect on their fitness using *natural* prey is scare (Zee et al., 2002). This is why we investigated it here.

We previously found that the predatory mites *Euseius concordis* Chant and *Iphiseiodes zuluagai* actively cover short distances to obtain a mixed diet consisting of two types of natural prey, the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) (Marques et al., 2015). We also found that these predators increased their egg production when they fed on a mixed diet compared to a single diet (Marques et al., 2015). These predators and herbivorous mites co-occur in plantations of the biodiesel plant *Jatropha curcas* L. in Brazil (Sarmento et al., 2011; Pedro Neto et al., 2013). Whereas the herbivores are known to cause severe yield reductions, the predators have been evaluated for their capacity to control the plant pests (Sarmento et al., 2011; Marques et al., 2018;).

However, the two prey species are usually found on different parts of the host plant. *Polyphagotarsonemus latus* attacks the apical leaves and *T. bastosi* is mainly found on the old leaves (Rosado et al., 2014). Therefore, the predators need to commute between plant parts to mix their diet. Thus, is important to know if the benefits by feeding on a mixed diet are higher than the costs of traveling to obtain it. Here we investigated if the predator *E. concordis* covers long distances to obtain a mixed diet when it feeds its natural prey.

Material and methods

Organisms

Jatropha curcas plants were sown in plastic pots (5 L) filled with a mixture of soil and bovine manure (3:1) and irrigated once per day. We used the plants to feed the herbivore cultures and for experimental purposes. The cultures of herbivorous (*T. bastosi* and *P. latus*) and predatory mites *E. concordis* were started with naturally infested *J. curcas* plants in Gurupi, state of Tocantins, Brazil (11°45′47″S, 49°02′57″W), in 2009. The spider mite *T. bastosi* was cultured on *J. curcas* plants placed in plastic trays (45 x 30 x 8 cm). These trays were placed inside a larger tray (55 x 40 x 10 cm) filled with detergent and water that prevented contamination with other arthropods. Once per week, the infested leaves of the old plant were placed on a new plant. *Polyphagotarsonemus latus* was reared on detached leaves (diameter c. 5 cm) kept inside a plastic tray (45 x 30 x 8 cm). This tray contained distilled water with a sponge in the center. A thin layer of wet cotton wool was placed around the leaves, which served to maintain leaf turgor and prevent contamination between cultures. Every four days, new leaves were placed on top of the old leaves. Colonies of the predatory mites *E. concordis* were established inside plastic boxes (11 × 11 cm) on flexible PVC disks (Ø = 6 cm) floating on distilled water. Small tent-like structures consisting of a folded piece of plastic were supplied on the arenas, serving as shelter, and some cotton threads were supplied under them as oviposition sites. Every day, we fed the predatory mites with a small quantity of castor bean (*Ricinus communis*) pollen (McMurtry and Scriven, 1964). The cultures were maintained in a climate room (25 ± 2 °C, 65%–70% relative humidity) with controlled photoperiod (12:12 L:D).

Mixed-prey diets offered on distant patches

Here we investigated predation and oviposition of the predator when they had to cover the double of the distance used by Marques et al. (2015) to obtain a mixed diet. We used only eggs of *P. latus* and *T. bastosi* to be able to keep the two prey species spatially separated and to exclude antipredator behaviour in the prey. Each arena consisted of two plastic boxes (11 x 11 cm) filled with distilled water with a sponge fixed in the center. Each box contained one leaf (diameter 5 cm) on the sponge. A thin layer of wet cotton wool was placed around the leaves, which served to prevent mite escapes and leaf desiccation. The leaf in one box was connected to the leaf in the other box with a bridge made of transparent polyethylene terephthalate plastic (16 cm length, double the length that was used by Marques et al. 2015). Thirty adult females of *P. latus* were transferred to one of the leaves, and the other received thirty adult females of *T. bastosi*. After 24 hours, we removed all adult females of both species and left thirty eggs of each species on their respective leaf. An adult female of *E. concordis* (9 days old since egg stage) was placed on the bridge. The leaves with the eggs of the prey species were replaced daily with new ones to prevent prey depletion and the predators were returned to the bridge, where they had to choose between the two new prey patches.

Control treatments consisted of similar arenas with the same prey species (*P. latus* or *T. bastosi*) on both connected leaves. The experiment was carried out inside a climate room (28 ± 2 °C, 65-70% R.H. and 12h L/12h D photoperiod). Each treatment was replicated 10 times. During four days, the position of the predators, the number of eggs consumed and the oviposition of the predatory mites were recorded for each patch. Because oviposition of the first day has been affected by the diet of previous days (Sabelis, 1990), we excluded this oviposition and predation on the first day from further analysis.

The numbers of eggs produced by the adult female predators were analysed with a generalized linear model (GLM) with a Poisson (log link) error distribution with treatments (mixed diet vs single diet) as factor. The predation of the eggs of each species was analysed with a GLM with a quasi-Poisson error distribution (logit link) to correct for overdispersion. The proportion of predators that fed on each of the two patches was analysed with a GLM with a binomial error distribution. Treatments were compared through model simplification by combining treatment levels (Crawley, 2007).

All statistical analyses were done with the statistical software R 3.3.3 (R Development Core Team, 2017). All models were checked by plotting residuals against fitted values and checking for normality of the error distribution.

Results

There was a significant effect of time (Chi^2 = 20.3, d.f. = 2, $P < 0.001$) and diet offered (Chi^2 = 15.4, d.f. = 2, $P < 0.001$) on the proportion of predators that consumed prey on each of the two patches. There was no significant interaction between diet and time (Fig. 1, GLM: Chi^2 = 1.1, d.f. = 4, $P = 0.90$), showing that the effects of the diet offered were consistent throughout time. The proportion of predators that consumed prey on each of the two patches was significantly higher after 48h when both patches contained *T. bastosi* than when both patches contained *P. latus* (Fig. 1, contrasts after GLM). The same trend, but no longer significant, was observed after 72h, but not after 96h, and the proportion of predators that consumed prey on both patches was high at this last time (Fig. 1).

The numbers of *P. latus* eggs consumed during the entire experiment did not differ when only *P. latus* was offered compared when a mixed diet was offered (Fig. 2a, GLM: *48h* Chi^2 =3.8, d.f. = 1, $P = 0.51$; *72h* Chi^2 = 0.7, d.f. = 1, $P = 0.78$; *96h* Chi^2 = 8.3, d.f. = 1, $P = 0.12$). Similarly, the number of eggs of *T. bastosi* consumed when it was offered alone did not differ from when it was offered with *P. latus* on the other leaf (Fig. 2a, GLM: *48h* Chi^2 = 13.7, d.f. = 1, $P = 0.20$; *72h* Chi^2 = 1.2, d.f. = 1, $P = 0.66$; *96h* Chi^2 = 11.9, d.f. = 1, $P = 0.08$). The eggs of *P. latus* are smaller than those of *T. bastosi* and we therefore did not compare predation of the two prey species.

The rate of oviposition of *E. concordis* feeding on prey eggs varied significantly with diet (Fig. 3, GLM: Chi² = 9.7, d.f. = 2, *P* = 0.007). It was lower when eggs of *T. bastosi* were offered on both patches than when both patches contained eggs of *P. latus* or when the eggs of the two prey species were offered on separate patches (Figure 3). The oviposition of the predators was higher in the case where both patches contained eggs of *P. latus* than when eggs of the two prey species were offered on separate patches, but this difference was not significant (Figure 3).

Discussion

The predatory mite *E. concordis* is known to cover short distances to mix its diet, resulting in an increased oviposition rate (Marques et al., 2015). When *E. concordis* had to cover double the distance as in previous experiments in order to mix its diet (Marques et al. 2015), the highest oviposition by *E. concordis* was obtained when eggs of *P. latus* were offered on one or both leaves. Besides, the difference in oviposition on the mixed diet was not significantly higher than on a single diet of *P. latus*. In this experiment, the oviposition rate on the mixed diet was somewhat lower than in a previous experiment, where the predators needed to cover half the distance as here to obtain a mixed diet (1.5 eggs/female here vs 1.7 in Marques et al., 2015). Moreover, we previously showed in Chapter one that the oviposition rate on a mixed diet offered on the same patch was even higher (2.25 eggs/female), suggesting that the positive effect of the mixed diet decreased because now the predators had to spend more time and energy to commute between patches to obtain such diet.

The predatory mite had the lowest oviposition rate when feeding on a diet of only *T. bastosi* (Figure 3). This is in agreement with the results presented in the first chapter, where we showed a low oviposition rate of *E. concordis* when the predator fed on a diet consisting of only eggs of *T. bastosi* compared when the diet contained different proportions of *P. latus*. There are two possible explanations for the low oviposition on a diet of *T. bastosi*. First, *T. bastosi* may be toxic to the predator. Thus the predator would then suffer less adverse effects of this toxin when it is diluted by consuming *P. latus*. Another possibility is that the prey species have different nutritional compositions (Mayntz, 2005) and a diet composed by *T. bastosi* is in short supply of some nutrients. To increase its performance, *E. concordis* must then consume food in optimal ratios in order to optimally balance its intake of specific nutrients (Rapport, 1980; Lee et al., 2008; Jensen et al., 2012; Simpson et al., 2004, 2015). Notice that these two explanations are not mutually exclusive.

We expected that the predators would try to compensate deficiencies in the single diets by consuming higher amounts of the deficient diet (Simpson et al., 2004). However, we did not find differences in the numbers of eggs consumed by the predator of each species separately when feeding on a mixed diet compared to feeding on a single diet. This implicates that the predators ate more in total when feeding on a mixed diet than on a single diet.

We offered eggs of the two prey species on separate patches to prevent mobile prey stages from migrating to the other patch. Furthermore, mobile prey can display antipredator behaviour and this behaviour is affected by the diet of the predator . By using immobile prey (eggs), we prevented the effects of antipredator behaviour interfering with the diet choice of the predators.

Apparently, in a short period of time (first 48 hours), the predators that only found *T. bastosi* continued searching for better prey. Those predators that found only *P. latus* did not search for a mixed diet because it apparently was not present. After 72 hours, the same trend was found, however the difference was not significant. Finally, after 96 hours the predator has the necessity to search for a mixed diet even when they found only *P. latus* (Figure 1).

In nature, both prey occur on the same plant, but on different parts. The structure of the plants is more complex than the structure offered in our experiments, and distances between patches with the two prey can be longer than the distance tested here. Besides, the structure of the experimental arena is also not very natural. On a plant, the predator should go up to eat *P. latus* and down to find T. *bastosi* instead of just walking on a plastic bridge. Future experiments should investigate if the predator *E. concordis* forages to mix its diet on intact plants and if this predator can benefit from it.

Our results point in the direction that when the predators have to cover long distances, the positive effect of the mixed diet decreased because the predators have to spend more time and energy to commute between patches to obtain a mixed diet. However, despite this reduction of energetic gain, literature points in the direction that better control of pests is achieved thought apparent competition between two pests (Messelink et al., 2008). This occurs because predator densities increase due to the higher availability of food and also because of the effect of a mixed diet on the predator (Messelink et al., 2008).

Acknowledgements

The National Council of Technological and Scientific Development, Brazil (CNPq) provided a master scholarship to the first author and RAS received a scholarship from CNPq (Produtividade em Pesquisa-Project: 306652/2018-8). CNPq and FAPEMIG sponsored AP. The Coordination of Improvement of Higher Education Personnel

(CAPES) provided financial support (PROCAD 2013). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors declare no conflicts of interest.

References

Abrams, P.A., 1993. Optimal traits when there are several costs: the interaction of mortality and energy costs in determining foraging behavior. Behav. Ecol. 4, 246–259. https://doi.org/10.1093/beheco/4.3.246

Behmer, S.T., Cox, E., Raubenheimer, D., Simpson, S.J., 2003. Food distance and its effect on nutrient balancing in a mobile insect herbivore. Anim. Behav. 66, 665–675. https://doi.org/10.1006/anbe.2003.2243

Crawley, M.J., 2007. The R book. John Wiley & Sons, Ltd, Chichester, UK. https://doi.org/10.1002/9780470515075

Godin, J.-G.J., Keenleyside, M.H.A., 1984. Foraging on patchily distributed prey by a cichlid fish (Teleostei, Cichlidae): A test of the ideal free distribution theory. Anim. Behav. 32, 120–131. https://doi.org/10.1016/S0003-3472(84)80330-9

Jensen, K., Mayntz, D., Toft, S., Clissold, F., Hunt, J., Raubenheimer, D., J Simpson, S., 2012. Optimal foraging for specific nutrients in predatory beetles. Proc. R. Soc. B. https://doi.org/10.1098/rspb.2011.2410

Ko, A.E., Jensen, K., Schal, C., Silverman, J., 2017. Effects of foraging distance on macronutrient balancing and performance in the German cockroach *Blattella germanica*. J. Exp. Biol. 220, 304–311. https://doi.org/10.1242/jeb.146829

Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in Drosophila: New insights from nutritional geometry. Proc. Natl. Acad. Sci. 105, 2498–2503. https://doi.org/10.1073/pnas.0710787105

Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640. https://doi.org/10.1139/z90-092

Marques, R.V., Sarmento, R.A., Lemos, F., Pedro-Neto, M., Sabelis, M.W., Venzon, M., Pallini, A., Janssen, A., 2015. Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. Funct. Ecol. 29, 1317–1324. https://doi.org/10.1111/1365-2435.12439

Marques, R.V., Sarmento, R.A., Oliveira, A.G., Rodrigues, D. de M., Venzon, M., Pedro-Neto, M., Pallini, A., Janssen, A., 2018. Reciprocal intraguild predation and predator coexistence. Ecol. Evol. 8, 6952–6964. https://doi.org/10.1002/ece3.4211

Mathis, A., Smith, R.J.F., 1993a. Chemical labeling of northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows (*Pimephales promelas*). J. Chem. Ecol. 19, 1967–1979. https://doi.org/10.1007/BF00983800

Mathis, A., Smith, R.J.F., 1993b. Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. Anim. Behav. 46, 645–656. https://doi.org/10.1006/anbe.1993.1241

Mayntz, D., 2005. Nutrient-specific foraging in invertebrate predators. Science 307, 111–113. https://doi.org/10.1126/science.1105493

McMurtry, J.A., Scriven, G.T., 1964. Studies on the feeding, reproduction, and development of *Amblyseius hibisci* (Acarina: Phytoseiidae) on various food substances. Ann. Entomol. Soc. Am. 57, 649–655. https://doi.org/10.1093/aesa/57.5.649

Mody, K., Unsicker, S.B., Linsenmair, K.E., 2007. Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. Ecology 88, 1012–1020. https://doi.org/10.1890/06-1338

Pedro Neto, M., Sarmento, R.A., Oliveira, W.P. de, Picanço, M.C., Erasmo, E.A.L., 2013. Biologia e tabela de vida do ácaro-vermelho *Tetranychus bastosi* em pinhão-manso. Pesqui. Agropecuária Bras. 48, 353–357. https://doi.org/10.1590/S0100-204X2013000400001

Persons, M.H., Walker, S.E., Rypstra, A.L., Marshall, S.D., 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: Lycosidae). Anim. Behav. 61, 43–51. https://doi.org/10.1006/anbe.2000.1594

R Development Core Team, 2017. R: A language and environment for statistical computing (Version 3.3.3). R Foundation for Statistical Computing, Vienna, Austria.

Rapport, D.J., 1980. Optimal foraging for complementary resources. Am. Nat. 116, 324–346. https://doi.org/10.1086/283631

Raubenheimer, D., Mayntz, D., Simpson, S.J., Tøft, S., 2007. Nutrient-specific compensation following diapause in a predator: implications for intraguild predation. Ecology 88, 2598–2608. https://doi.org/10.1890/07-0012.1

Raubenheimer, D., Simpson, S.J., 2018. Nutritional ecology and foraging theory. Curr. Opin. Insect Sci. 27, 38–45. https://doi.org/10.1016/j.cois.2018.02.002

Rosado, J.F., Sarmento, R.A., Pedro-Neto, M., Galdino, T.V.S., Marques, R.V., Erasmo, E.A.L., Picanço, M.C., 2014. Sampling plans for pest mites on physic nut. Exp. Appl. Acarol. 63, 521–534. https://doi.org/10.1007/s10493-014-9804-0

Sabelis, M.W., 1990. How to analyse prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. Oecologia 82, 289–298. https://doi.org/10.1007/BF00317473

Sarmento, R.A., Rodrigues, D.M., Faraji, F., Erasmo, E.A.L., Lemos, F., Teodoro, A.V., Kikuchi, W.T., dos Santos, G.R., Pallini, A., 2011. Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. Exp. Appl. Acarol. 53, 203–214. https://doi.org/10.1007/s10493-010-9396-2

Simpson, S.J., Clissold, F.J., Lihoreau, M., Ponton, F., Wilder, S.M., Raubenheimer, D., 2015. Recent advances in the integrative nutrition of arthropods. Annu. Rev. Entomol. 60, 293–311. https://doi.org/10.1146/annurev-ento-010814-020917

Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., Raubenheimer, D., 2004. Optimal foraging when regulating intake of multiple nutrients. Anim. Behav. 68, 1299–1311. https://doi.org/10.1016/j.anbehav.2004.03.003

van Maanen, R., Broufas, G., De Jong, P., Aguilar-Fenollosa, E., Revynthi, A., Sabelis, M.W., Janssen, A., 2015. Predators marked with chemical cues from one prey have increased attack success on another prey species. Ecol. Entomol. 40, 62–68. https://doi.org/10.1111/een.12159

Vaudo, A.D., Stabler, D., Patch, H.M., Tooker, J.F., Grozinger, C.M., Wright, G.A., 2016. Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. J. Exp. Biol. 219, 3962–3970. https://doi.org/10.1242/jeb.140772

Venzon, M., Janssen, A., Pallini, A., Sabelis, M.W., 2000. Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Anim. Behav. 60, 369–375. https://doi.org/10.1006/anbe.2000.1483

Zee, B., Behmer, S.T., Simpson, S.J., 2002. Food mixing strategies in the desert locust: effects of phase, distance between foods, and food nutrient content. Entomol. Exp. Appl. 103, 227–237. https://doi.org/10.1046/j.1570-7458.2002.00978.x

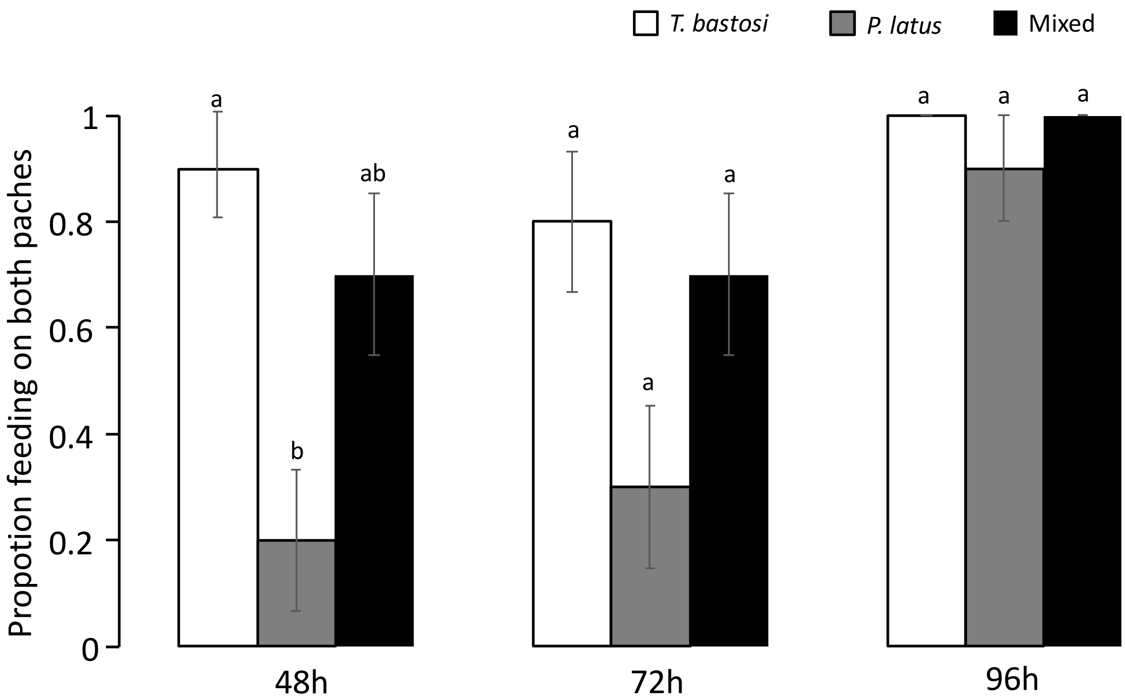
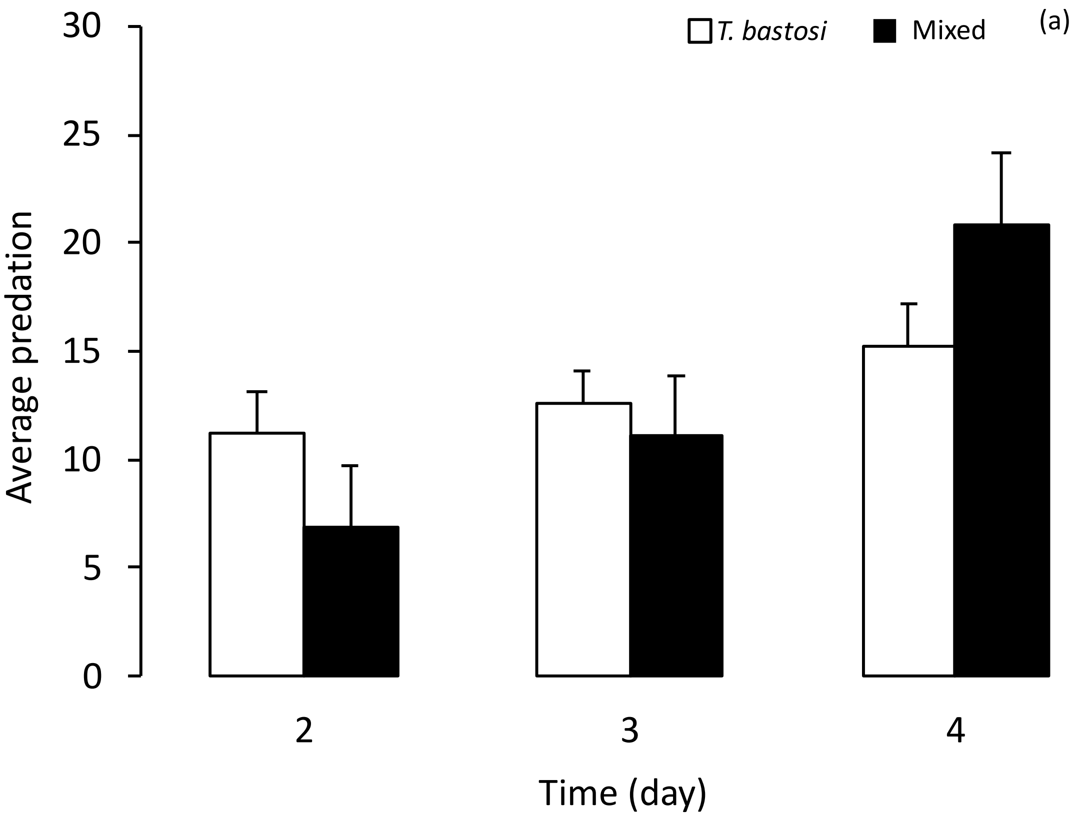


Figure 1. The average proportion (+SE) of predators that fed on prey eggs on both patches during three days (first day excluded). *Euseius concordis* was offered prey eggs on two patches connected with a bridge. Both patches contained either eggs of *T. bastosi*, eggs of *P. latus*, or one patch contained eggs of *T. bastosi* and the other eggs of *P. latus* (Mixed). Bars with different letters differ significantly within each day (contrasts after GLM, $P < 0.05$).


□ T. bastosi
■ Mixed
(a)
Average predation
30
25
20
15
10
5
0
2
3
4
Time (day)

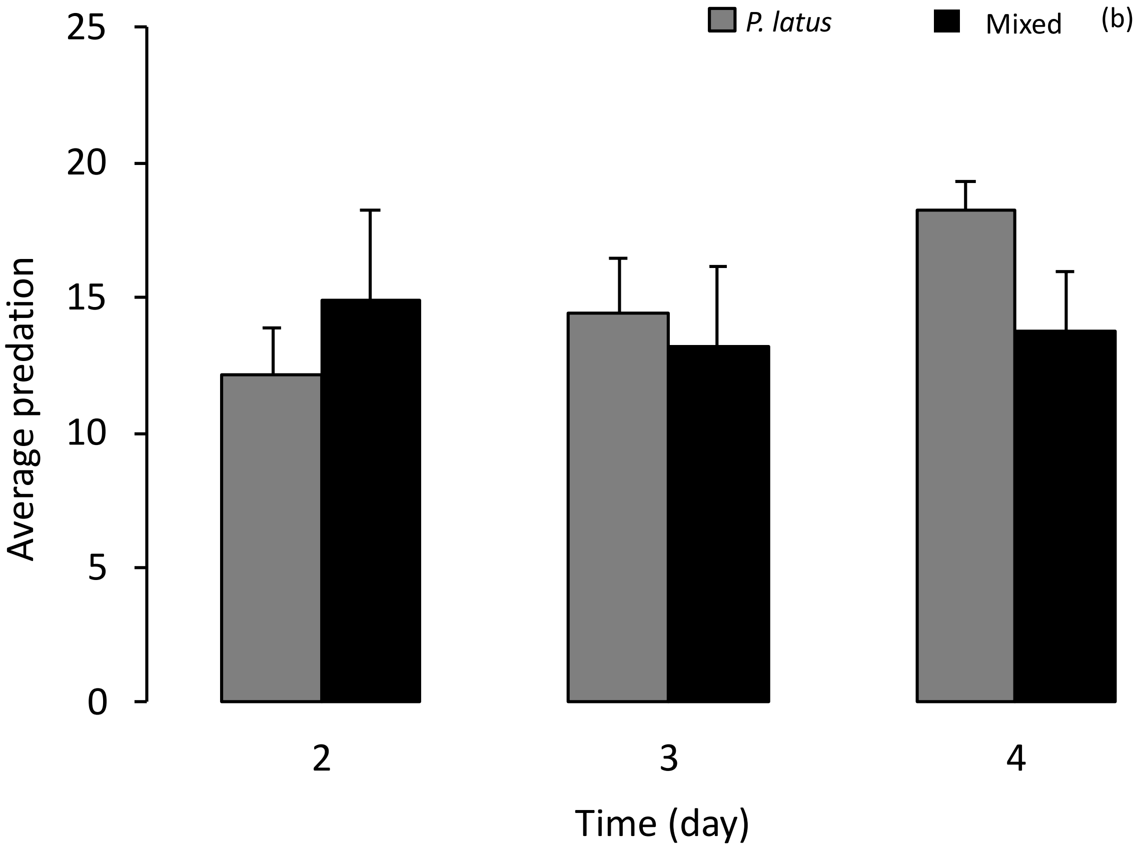

▨ P. latus
■ Mixed
(b)
Average predation
25
20
15
10
5
0
2
3
4
Time (day)

Figure 2. Predation on single- and mixed-prey diets in an arena with two connected patches. *Euseius concordis* was offered prey eggs on two patches connected with a bridge. Both patches contained either eggs of *T. bastosi*, eggs of *P. latus*, or one patch contained eggs of *T. bastosi* and the other eggs of *P. latus* (Mixed). Shown are the average numbers (+SE) of eggs of *T. bastosi* (a) and of *P. latus* (b) consumed on single and mixed diets respectively.

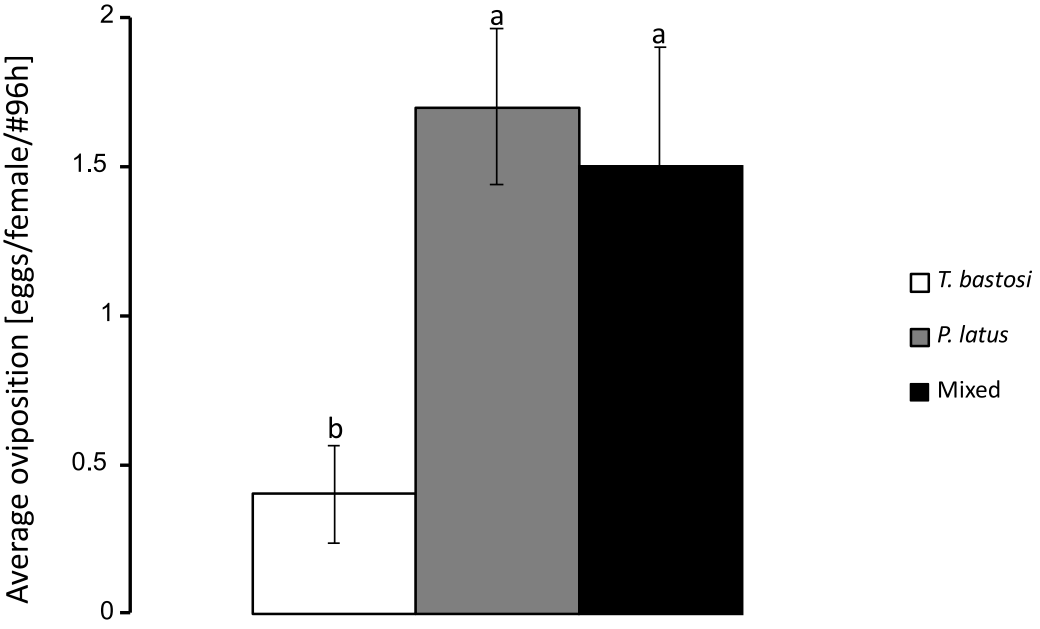


Figure 3. Oviposition on three different diets in an arena with two connected patches. *Euseius concordis* was offered prey eggs on two patches connected with a bridge. Both patches contained either eggs of *T. bastosi*, eggs of *P. latus*, or one patch contained eggs of *T. bastosi* and the other eggs of *P. latus* (Mixed). Shown are the average numbers (+SE) of eggs produced by *E. concordis* when feeding on eggs of *T. bastosi*, of *P. latus*, or a mixture of eggs (Mixed). Bars with different letters differ significantly (contrasts after GLM, $P < 0.05$).

CHAPTER III

Predatory mites forage on a plant to obtain a mixed diet and improve their performance

Renata Vieira Marques[1]*, Angelo Pallini[1], Renato Almeida Sarmento[2], Adriana Golçalves Oliveira[2], Dara Emily Silva Ferreira[2], Thaysa Ferreira Alves[2] & Arne Janssen[3]

[1]Department of Entomology, Federal University of Viçosa (UFV), CEP 36570-000, Viçosa, MG, Brazil. Email: renatamarques.ufv@gmail.com, pallini@ufv.br

[2]Federal University of Tocantins (UFT), PO Box 66, Gurupi, TO, Brazil. Emails: rsarmento@mail.uft.edu.br, drika_eafa@hotmail.com, daraferreira@mail.uft.edu.br, thaysaferreiraalves@gmail.com

[3]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands. Email: Arne.Janssen@uva.nl

Abstract

Animals choose their diet based on nutrient composition and presence of noxious compounds. However, different food items are commonly patchily distributed in nature. Thus, predators need to commute between plant parts to mix their diet. On artificial arenas, we found that the predatory mite *E. concordis* actively foraged for a mixed diet consisting of two types of natural prey, the *P. latus* and *T. bastosi*. However, in nature, the two prey can be found on different parts of their common host plant. *Polyphagotarsonemus latus* attacks the apical leaves and *T. bastosi* is mainly found on the old leaves of *Jatropha curcas*. Here, we investigate if the predator *E. concordis* forages for a mixed diet on a small plant with four leaves. Thus, an adult female of the predatory mite was placed on a plant with a single (*P. latus* or *T. bastosi*) or mixed-prey diets. We showed that the predator forages to mix its diet, and had high oviposition rates when feeding on both prey species. In conclusion, our results show that the predators actively search for a mixed diet on their host plants.

Key words: balanced diet, foraging behavior, nutritional ecology, habitat structure, diet choice.

Introduction

The nutrient composition of food plays an important role in diet choice of animals. There are several examples of animals that actively redress nutrient imbalances in their diet, but most of these studies concern (semi-)artificial diets or prey that were specifically prepared to contain different amounts of nutrients (Mayntz, 2005; Raubenheimer et al., 2007; Dussutour and Simpson, 2009 Jensen et al., 2012; Vaudo et al., 2016; Raubenheimer and Simpson, 2018).

We previous found that the predatory mites *E. concordis* Chant and *I. zuluagai* actively forage for a mixed diet consisting of two types of natural prey; i.e., the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae). On artificial arenas, the predatory mites showed a higher oviposition rate on a mixed diet consisting of the two prey species than on the two single diets (Marques et al., 2015). These herbivorous mites and predators co-occur on the biodiesel plant *Jatropha curcas* L. in Brazil (Pedro Neto et al., 2013; Sarmento et al., 2011). Because the herbivores cause severe yield reductions, the predators have been evaluated for their capacity to control the plant pests (Sarmento et al., 2011; Marques et al., 2018).

In nature, food items are commonly patchily distributed (Godin and Keenleyside, 1984). In the *Jatropha* system, for example, the two prey species are found on different parts of the host plant: *P. latus* attacks the apical leaves and *T. bastosi* is mainly found on the old leaves (Rosado et al., 2014). Therefore, the predators need to commute between plant parts to mix their diet. There are several factors that can influence the foraging behaviour and consequently animal performance. Foraging costs (i.e. time and energy spent on commuting among prey patches) will increase with increasing distance between patches (Abrams, 1993) and when the habitat is more complex (Brown and Kotler, 2004). This occurs because the structural complexity of the habitat could hamper the predators to find their prey. Therefore, predators will spend more time and energy to find their prey with increasing habitat structure.

Studies on diet mixing and its effects on fitness are normally conducted in small artificial arenas with the different types of food presented adjacent to each other, requiring limited activity and effort to obtain a balanced diet (Mody, Unsicker and Linsenmair, 2007). In nature, foraging efforts are required to obtain a mixed diet when the alternative food sources are patchily distributed and spatially separated. The predator then has to use some strategy to find its food and minimize its expenditure of time and energy.

Previous experiments showed that the predators are actively searching for prey on two different patches when the distance between these patches is relatively short (Marques et al. 2015). Subsequent experiments have shown that there is indeed an optimal diet, but that various mixtures of the two prey species resulted in similar performance (Chapter 1). When the distance between the patches was longer, the predators needed to spend more time and energy to obtain a mixed diet and this resulted in a decrease in the performance on the mixed diet, which was no longer profitable compared to the better of the two single diets (Chapter 2). The question we address here is whether the predators forage for a mixed diet on plants on which the two prey occur on different plant parts rather than on artificial arenas.

Material and methods

Organisms

The experiments were performed in Gurupi (Federal University of Tocantins, Gurupi, Tocantins, Brazil). For the plants, we used seeds provided by the *J. curcas* plantation localized at the Federal University of Tocantins. The populations of herbivores (*P. latus* and *T. bastosi*) and the predatory mites were collected in 2009 from naturally infested *J. curcas* plants in Gurupi (11°45′47″S, 49°02′57″W). Every week, we sowed *J. curcas* seeds directly in plastic pots (5L) containing a mixture of soil and bovine manure (3:1); plants were watered once per day. Colonies of the broad mite *P. latus* were kept inside a cage with PVC trays (45 x 30 x 8 cm) filled with water and a sponge in the center; the water served to prevent mite escapes and invasion of mites and other arthropods. We used detached leaves from *J. curcas* plants (diameter c. 5 cm) to feed the colonies of broad mites. To prevent desiccation, a thin layer of wet cotton wool surrounded fresh leaves used to feed the colonies. Colonies of the spider mite *T. bastosi* were reared directly on small plants (30-days-old) kept in trays (45 x 30 x 8 cm) filled with water and detergent, which served to prevent mite escapes. Colonies of the predatory mite *E. concordis* were reared inside a plastic box (11 × 11 cm) on flexible PVC disks (∅ = 6 cm) floating on distilled water. Cotton threads covered by a tent-shaped piece of plastic sheet were placed on the arena to serve as shelter and oviposition site. We fed the predatory mites daily with a small quantity of castor bean (*Ricinus communis*) pollen. All cultures were maintained in a climate room at (25 ± 2 °C, 65%–70% R.H. and a 12 hr L/12 hr D photoperiod).

Mixed-prey diets on plant

The experiments were carried out on 30-days-old *J. curcas* plants with four leaves. In general, the leaves are petiolate, alternate to subopposite with a spiral phyllotaxis.

Leaves were numbered from the youngest to the oldest leaf. The leaves attacked by *P. latus* and *T. bastosi* are the apical and middle third leaves, respectively (Rosado et al., 2014). Therefore, thirty adult females of *P. latus* were transferred to the apical leaf (leaf one), and the third leaf from above received thirty adult females of *T. bastosi*. Control treatments consisted of *J. curcas* plants with the same prey species (*P. latus* or *T. bastosi*). When the plant was infested with broad mites, we released them on the two apical leaves (leaves one and two) and when the plant was infested with *T. bastosi*, the mites were released on the two lower leaves (leaves three and four). Moistened cotton wool was placed on the leaf petioles that received the prey to avoid them moving to other leaves. After 24 hours, we removed the moistened cotton wool and all adult females of the two species, leaving the eggs produced by the females behind. Subsequently, one adult female of *E. concordis* (9 days old since egg stage) was placed between the two leaves on the stem of the plant. Each treatment was replicated 15 times. During four days, the oviposition of the predatory mite was recordedby carefully manipulating the plants under a stereomicroscope (Tecnival SQF-F, Brazil) and counting the eggs. We assessed the numbers of each prey species remaining on the leaves at the end of the experiment. Because the oviposition of the first day is affected by the diet of previous days (Sabelis, 1990), we did not include this in the analysis.

The numbers of eggs produced by the adult female predators were analysed with a generalized linear model (GLM) with a Poisson (log link) error distribution with treatments as fixed factor. The numbers of prey remaining per leaf at the end of the experiment were analysed with a GLM with a Gaussian error distribution. In the case of single diets, we used the average of the number of prey remaining on the two leaves of the same plant. Treatments were compared through model simplification by combining treatment levels and comparing models using the “anova” command in R (Crawley, 2007). All statistical analyses were done with the statistical software R 3.3.3 (2017). All models were checked by plotting residuals against fitted values and checking normality of the error distribution.

Results

Oviposition of *E. concordis* differed significantly among the treatments (Fig. 1, GLM: $Chi^2 = 29.0$, d.f. = 2, $P < 0.001$). Oviposition was significantly higher with a mixed diet than with the single diets, and oviposition on a diet of *T. bastosi* was the lowest (Figure 1).

The numbers of *P. latus* or *T. batosi* that remained per leaf at the end of the experiment did not differ among treatments (Fig. 2, GLM: *P. latus* F = 0.256, d.f. = 1, $P = 0.554$; *T. bastosi* F = 0.757, d.f. = 1, $P = 0.389$). For each prey species separately, there is no significant variation in the numbers of prey remaining at end of the experiment on plants harbouring single diets or mixed diets (Figure 2).

Discussion

Our results show that the predatory mite *E. concordis* benefits from mixing its diet on intact plants (Figure 1). Whereas it has been amply shown that animals perform better on a mixed diet when the two diets are offered close to each other (Mayntz, 2005; Raubenheimer et al., 2007; Dussutour and Simpson, 2009; Jensen et al., 2012; Raubenheimer and Simpson, 2018), there is not much knowledge on diet mixing in a more natural setting. In the experiment presented here, the predators had to search for the two prey on different plant parts, corresponding to the occurrence of these prey on small plants in the field. Although the predators presumably had to spend some time and energy to obtain a mixed diet, foraging for a mixed diet seems to be the better strategy for this predator. Still, the distance between the two prey species on the small plants used here is probably shorter than the distances that the predator must travel to obtain mixed diets on large plants in the field. Clearly, more studies are required to show what happens on a large, full-grown plant in the field, but our study shows that predators are likely to forage for a mixed diet on small plants in the field. By foraging on a mixed diet on such small plants, the predators increase their oviposition rate. This results in the build-up of larger populations, which will turn in better control of the two pest species on small and vulnerable plants. The difference in oviposition is especially pronounced between a mixed diet and a single diet of *T. bastosi*. Hence, we expect that the predators will control this pest better in the presence of the other pest. There are now several examples of natural enemies that perform better on a mixed diet (Dean and Schuster, 1995; Evans et al., 1999; Oelbermann and Scheu, 2002; Messelink et al., 2008; Muñoz-Cárdenas et al., 2014; Marques et al., 2015), and that such natural enemies may be more efficient at controlling pests when they can feed on a mixed diet (Messelink et al. 2008).

The predators had the lowest performance when the plant contained only *T. bastosi* (Figure 1), in agreement with data presented in the first and second chapter, where it was also shown that *E. concordis* had a low oviposition rate when it was feeding on a diet with only eggs of *T. bastosi*. This reinforces the idea that a diet of *T. bastosi* is an inferior diet for this predator.

When the predators change patch to mix their diet, they feed on both patches. If they cannot mix their diet, they could stay on the same patch, so they would eat more prey from one patch and less from the other patch with the same prey. We would then expect larger variation in the numbers of prey per leaf for the single diets than for the mixed diets at the end of the experiment. However, there is no indication that the variation in the numbers of prey was larger with single diets than with mixed diet (Figure 2).

This study represents a first step in describing a case of an omnivore that actively forages for a mixed diet on a natural arena, i.e. a plant, and benefits from it. Probably, better biocontrol of pests is achieved when these two prey co-occur in the field. However, future experiments are needed to enable implementation of results like these. Besides, conservation biological control techniques like the association of spontaneous plants within *J. curcas* plantations present a potential to maintain predatory mites on the cultivated plants (Cruz et al., 2012), especially in situations of prey shortage in the main crop (Saturnino et al., 2005).

Acknowledgements

The National Council of Technological and Scientific Development, Brazil (CNPq) provided a master scholarship to the first author and RAS received a scholarship from CNPq (Produtividade em Pesquisa-Project: 306652/2018-8). CNPq and FAPEMIG sponsored AP. The Coordination of Improvement of Higher Education Personnel (CAPES) provided financial support (PROCAD 2013). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors declare no conflicts of interest.

References

Abrams, P.A., 1993. Optimal traits when there are several costs: the interaction of mortality and energy costs in determining foraging behavior. Behavioral Ecology 4, 246–259. https://doi.org/10.1093/beheco/4.3.246

Brown, J.S., Kotler, B.P., 2004. Hazardous duty pay and the foraging cost of predation: Foraging cost of predation. Ecology Letters 7, 999–1014. https://doi.org/10.1111/j.1461-0248.2004.00661.x

Crawley, M.J., 2007. The R book. John Wiley & Sons, Ltd, Chichester, UK. https://doi.org/10.1002/9780470515075

Dean, D.E., Schuster, D.J., 1995. *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Macrosiphum euphorbiae* (Homoptera: Aphididae) as prey for two species of chrysopidae. Environmental Entomology 24, 1562–1568. https://doi.org/10.1093/ee/24.6.1562

Dussutour, A., Simpson, S.J., 2009. Communal nutrition in ants. Current Biology 19, 740–744. https://doi.org/10.1016/j.cub.2009.03.015

Evans, E.W., Stevenson, A.T., Richards, D.R., 1999. Essential versus alternative foods of insect predators: benefits of a mixed diet. Oecologia 121, 107–112. https://doi.org/10.1007/s004420050911

Godin, J.-G.J., Keenleyside, M.H.A., 1984. Foraging on patchily distributed prey by a cichlid fish (Teleostei, Cichlidae): A test of the ideal free distribution theory. Animal Behaviour 32, 120–131. https://doi.org/10.1016/S0003-3472(84)80330-9

Jensen, K., Mayntz, D., Toft, S., Clissold, F., Hunt, J., Raubenheimer, D., J Simpson, S., 2012. Optimal foraging for specific nutrients in predatory beetles. https://doi.org/10.1098/rspb.2011.2410

Marques, R.V., Sarmento, R.A., Lemos, F., Pedro-Neto, M., Maurice W. Sabelis, Venzon, M., Pallini, A., Janssen, A., 2015. Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. Functional Ecology 29, 1317–1324. https://doi.org/10.1111/1365-2435.12439

Marques, R.V., Sarmento, R.A., Oliveira, A.G., Rodrigues, D. de M., Venzon, M., Pedro-Neto, M., Pallini, A., Janssen, A., 2018. Reciprocal intraguild predation and predator coexistence. Ecology and Evolution 8, 6952–6964. https://doi.org/10.1002/ece3.4211

Mayntz, D., 2005. Nutrient-specific foraging in invertebrate predators. Science 307, 111–113. https://doi.org/10.1126/science.1105493

Messelink, G.J., Maanen, R. van, van Steenpaal, S.E.F., Janssen, A., 2008. Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biological Control 44, 372–379. https://doi.org/10.1016/j.biocontrol.2007.10.017

Muñoz-Cárdenas, K., Fuentes, L.S., Cantor, R.F., Rodríguez, C.D., Janssen, A., Sabelis, M.W., 2014. Generalist red velvet mite predator (*Balaustium* sp.) performs better on a mixed diet. Experimental and Applied Acarology 62, 19–32. https://doi.org/10.1007/s10493-013-9727-1

Oelbermann, K., Scheu, S., 2002. Effects of prey type and mixed diets on survival, growth and development of a generalist predator, *Pardosa lugubris* (Araneae: Lycosidae). Basic and Applied Ecology 3, 285–291. https://doi.org/10.1078/1439-1791-00094

Pedro Neto, M., Sarmento, R.A., Oliveira, W.P. de, Picanço, M.C., Erasmo, E.A.L., 2013. Biologia e tabela de vida do ácaro-vermelho *Tetranychus bastosi* em pinhão-manso. Pesquisa Agropecuária Brasileira 48, 353–357. https://doi.org/10.1590/S0100-204X2013000400001

R Development Core Team, 2017. R: A language and environment for statistical computing (Version 3.3.3). R Foundation for Statistical Computing, Vienna, Austria.

Raubenheimer, D., Mayntz, D., Simpson, S.J., Tøft, S., 2007. Nutrient-specific compensation following diapause in a predator: Implications for intraguild predation. Ecology 88, 2598–2608. https://doi.org/10.1890/07-0012.1

Raubenheimer, D., Simpson, S.J., 2018. Nutritional ecology and foraging theory. Current Opinion in Insect Science 27, 38–45. https://doi.org/10.1016/j.cois.2018.02.002

Rosado, J.F., Sarmento, R.A., Pedro-Neto, M., Galdino, T.V.S., Marques, R.V., Erasmo, E.A.L., Picanço, M.C., 2014. Sampling plans for pest mites on physic nut. Experimental and Applied Acarology 63, 521–534. https://doi.org/10.1007/s10493-014-9804-0

Sarmento, R.A., Rodrigues, D.M., Faraji, F., Erasmo, E.A.L., Lemos, F., Teodoro, A.V., Kikuchi, W.T., dos Santos, G.R., Pallini, A., 2011. Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. Experimental and Applied Acarology 53, 203–214. https://doi.org/10.1007/s10493-010-9396-2

Vaudo, A.D., Stabler, D., Patch, H.M., Tooker, J.F., Grozinger, C.M., Wright, G.A., 2016. Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. The Journal of Experimental Biology 219, 3962–3970. https://doi.org/10.1242/jeb.140772

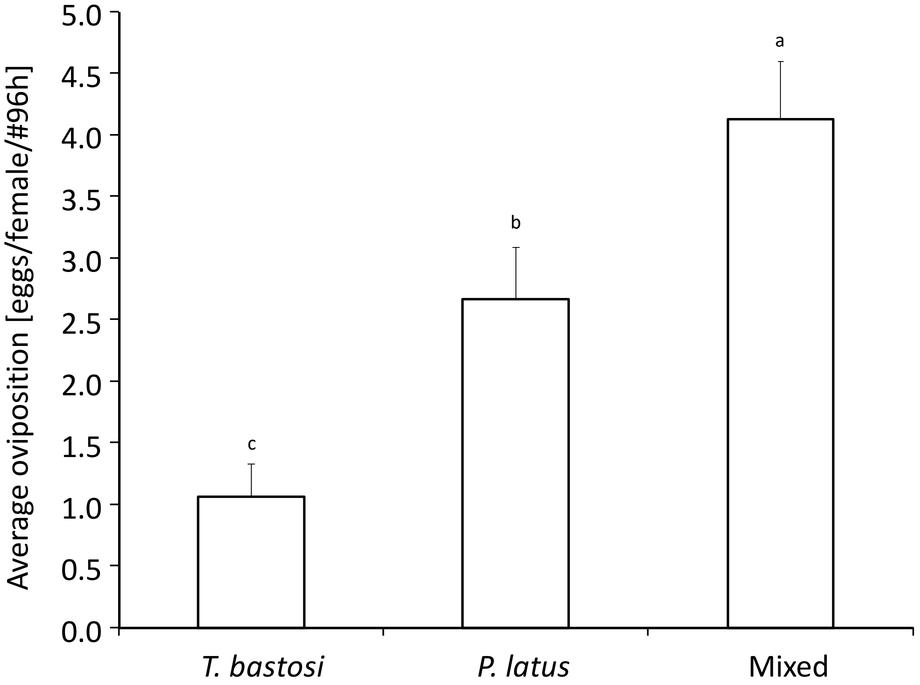


Figure 1. Oviposition of *E. concordis* on single- and mixed-prey diets on a plant. Plants contained *T. bastosi* plus *P. latus*, each on a different leaf (Mixed), or either of the two species on two leaves. Bars with different letters differ significantly (contrasts after GLM, $P < 0.05$).

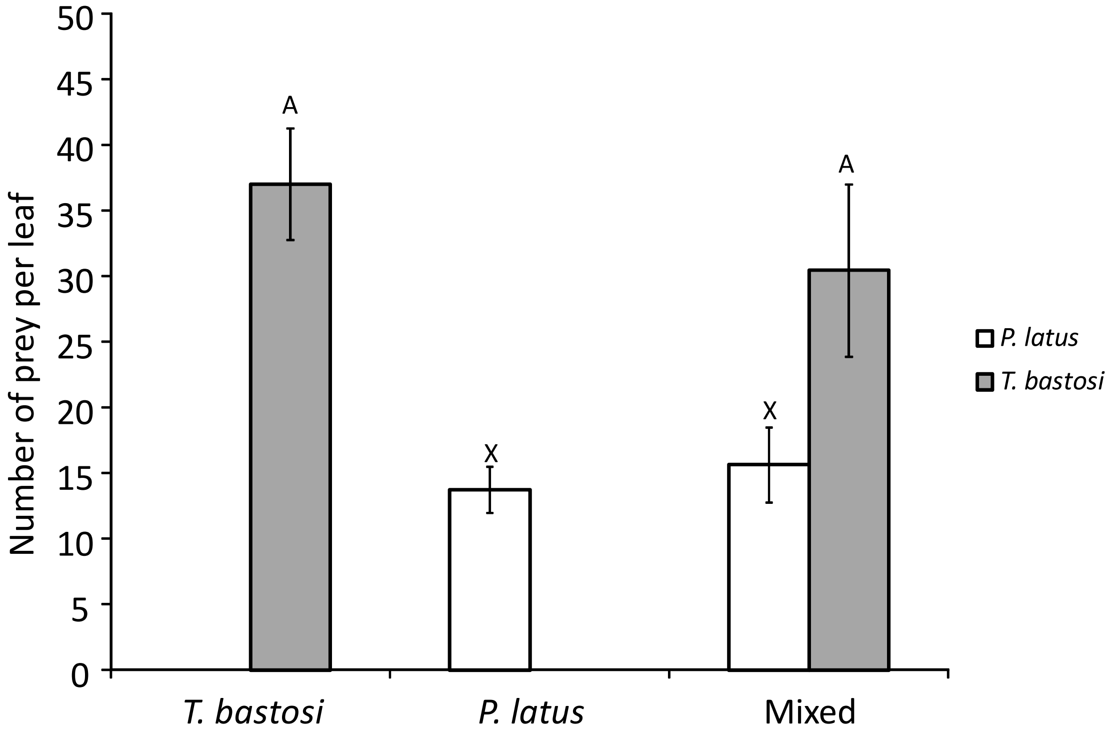


Figure 2. Numbers of prey recovered per leaf from plants with the predator *E. concordis* after four days. Plants harboured either the prey *T. bastosi* on the two lower leaves, the prey *P. latus* on the two apical leaves, or one leaf with each (Mixed). Grey bars give the numbers of *T. bastosi*, white bars numbers of *P. latus*. Per prey species, bars with different letters differ significantly (contrasts within prey species after GLM, $P < 0.05$).

CHAPTER IV

Reciprocal intraguild predation and predator coexistence

Renata Vieira Marques[1], Renato Almeida Sarmento[2], Adriana Gonçalves Oliveira[2], Diego de Macedo Rodrigues[2], Madelaine Venzon[3], Marçal Pedro-Neto[2], Angelo Pallini[1] & Arne Janssen[4]*

Ecology and Evolution (2018) 8: 6952–6964

DOI: 10.1002/ece3.4211

Address:

[1]Department of Entomology, Federal University of Viçosa, Viçosa, MG, Brazil;

[2]Federal University of Tocantins (UFT), Gurupi, TO, Brazil;

[3]Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), Viçosa, MG, Brazil;

[4]IBED, Department of Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

Abstract

Intraguild predation is a mix of competition and predation, and occurs when one species feeds on another species that uses similar resources. Theory predicts that intraguild predation hampers coexistence of species involved, but it is common in nature. It has been suggested that increasing habitat complexity and the presence of alternative food may promote coexistence. Reciprocal intraguild predation limits possibilities for coexistence even further. Habitat complexity and the presence of alternative food are believed to promote coexistence. We investigated this using two species of predatory mites, *Iphiseiodes zuluagai* and *Euseius concordis*, by assessing co-occurrence in the field and on arenas differing in spatial structure in the laboratory. The predators co-occurred on the same plants in the field. In the laboratory, adults of the two mites fed on juveniles of the other species, both in the presence and the absence of a shared food source, showing that the two species are involved in reciprocal intraguild predation. Adults of *I. zuluagai* also attacked adults of *E. concordis*. This suggests limited possibilities for coexistence of the two species. Indeed, *E. concordis* invariably went extinct extremely rapidly on arenas without spatial structure with populations consisting of all stages of the two predators and with a shared resource. Coexistence was prolonged on host plant leaves with extra food sources, but *E. concordis* still went extinct. On small, intact plants, coexistence of the two species was much longer, and ended with the other species, *I. zuluagai*, often going extinct. These results suggest that spatial structure and the presence of alternative food increase the coexistence period of intraguild predators.

Key words: population dynamics, bistability, extinction, predator-prey interactions, stage structure, biological control, *Jatropha curcas*

Introduction

Intraguild predation (IGP, hereafter) is a mix of competition and predation and occurs when one species feeds on another species that uses the same resources (Polis & Holt, 1992). In the last decades, IGP has received considerable attention because it commonly occurs in many ecosystems (Arim & Marquet, 2004; Holt & Polis, 1997; Morin, 1999; Polis & Holt, 1992; Polis, Myers, & Holt, 1989; Polis & Winemiller, 1996; Rosenheim, 2007; Rosenheim, Kaya, Ehler, Marois, & Jaffee, 1995). Theoretical models of intraguild predation usually consider three species: the shared resource, the IG-prey and the IG-predator, and predicts that all three species can coexist only if the IG-prey is the better competitor for the shared resource (Holt & Polis, 1997). Even then, however, the parameter space for coexistence is limited to intermediate levels of productivity (Diehl & Feissel, 2000; Holt & Polis, 1997; Mylius, Klumpers, de Roos, & Persson, 2001), hence, IGP is not predicted to be common. This discrepancy between theory and reality has resulted in a quest for factors that increase the probability of coexistence of species involved in intraguild predation, such as temporal variation, alternative prey and spatial structure (Amarasekare, 2007; Daugherty, Harmon, & Briggs, 2007; Holt & Huxel, 2007; Janssen, Sabelis, Magalhães, Montserrat, & van der Hammen, 2007; Mylius et al., 2001; Rosenheim, 2007; Rudolf, 2007; Vance-Chalcraft, Rosenheim, Vonesh, Osenberg, & Sih, 2007).

The stage or size of individuals often determine whether they are vulnerable or invulnerable to predation (Claessen, Van Oss, de Roos, & Persson, 2002; de Roos, Leonardson, Persson, & Mittelbach, 2002) and in the case of intraguild predation, whether they are predators or prey (Choh, Ignacio, Sabelis, & Janssen, 2012; Montserrat, Magalhaes, Sabelis, de Roos, & Janssen, 2012). In such size-structured systems, two predator species can attack each other’s vulnerable stages (Choh et al., 2012; Montserrat et al., 2012; Polis, 1984), thus engaging in reciprocal intraguild predation. This interaction occurs in natural systems but has not received much attention (Montserrat et al., 2012; Polis et al., 1989; van der Hammen, de Roos, Sabelis, & Janssen, 2010; Wissinger, 1992; Woodward & Hildrew, 2002). Modelling studies and experiments have shown that reciprocal IGP leads to mutual exclusion or alternative stable states, in which either one or the other competitor persists alone with the resource, depending on the initial densities (a so-called priority effect, HilleRisLambers & Dieckmann, 2003; Montserrat et al., 2012; Schellekens & van Kooten, 2012; van der Hammen et al., 2010).

Intraguild predation and reciprocal IGP is not only important in determining species coexistence, but also from an applied perspective. They occur frequently in biological control systems (Rosenheim et al., 1995), where, in theory, they would disrupt pest control. Although this disruption does not seem to occur very often, it does in some systems (Janssen et al., 2006; Rosenheim & Harmon, 2006). We therefore investigated the occurrence of IGP and reciprocal IGP between two predatory mite species that are considered for biological control of pests of the biodiesel plant *Jatropha curcas* L., on which both species occur (Marques et al., 2015; Sarmento et al., 2011).

Predatory mites are often used for biological control (Gould, 1977; Huffaker & Kennett, 1953; Janssen & Sabelis, 2015; Messelink, van Maanen, van Steenpaal, & Janssen, 2008; Nomikou, Janssen, Schraag, & Sabelis, 2001; Ramakers, 1980; van Lenteren, Bolckmans, Köhl, Ravensberg, & Urbaneja, 2017; Yaninek & Hanna, 2003). Intraguild predation and reciprocal IGP is common in phytoseiids (Ferreira, Cunha, Pallini, Sabelis, & Janssen, 2011; Guzmán, Sahún, & Montserrat, 2016; Hatherly, Bale, & Walters, 2005; Montserrat, Janssen, Magalhães, & Sabelis, 2006; Schausberger & Croft, 2000), and the use of multiple species of predatory mites can therefore disrupt biological control (Rosenheim & Harmon, 2006; Rosenheim et al., 1995). We therefore studied the interactions between two species of predatory mites: *Iphiseiodes zuluagai* Denmark & Muma and *Euseius concordis* Chant. These two phytoseiids attack the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the spider mite, *Tetranychus bastosi* Tuttle, Baker & Sales (Acari: Tetranychidae) in *Jatropha curcas* plantations in Brazil (Sarmento et al., 2011). We investigated whether the two predators are involved in IGP or reciprocal IGP. As explained above, if IGP would occur between the two predator species, theory predicts limited possibilities for coexistence, and no coexistence in the case of reciprocal IGP. In both cases, theory predicts that combining the two species would not result in better pest control (Janssen et al., 2006; Rosenheim & Harmon, 2006; Rosenheim et al., 1995).

We therefore first assessed the possible co-occurrence of *I. zuluagai* and *E. concordis* on the same *J. curcas* plants in the field, showing that the two species do co-occur on the same plants. This would not be expected when they engage in reciprocal IGP, and would be less likely to occur in the case of simple IGP. Subsequently, we show that the two species do engage in reciprocal IGP, and that one species indeed rapidly excludes the other species on small arenas without spatial structure in the laboratory. Coexistence between the two species was considerably prolonged, however, on plant leaves and on intact plants, suggesting that increased spatial structure promoted coexistence, explaining how the two species can coexist in the field while engaged in reciprocal IGP.

Material and Methods

The experimental system

The experimental system consisted of the two predatory mite species *I. zuluagai* and *E. concordis* and pollen of *Ricinus communis* L. as a shared resource. The life cycle of phytoseiid mites comprises five stages: egg, larva, protonymph, deutonymph and adult. The two phytoseiids can develop and reproduce on pollen as well as on *P. latus* and *T. bastosi*, the two predominant pest species occurring on *J. curcas* (Sarmento et al., 2011). This crop is becoming increasingly popular in the biodiesel industry in North Brazil. It is adapted to arid, stony and low fertility soils and can grow under a wide range of precipitation conditions (Gübitz, Mittelbach, & Trabi, 1999; Openshaw, 2000). For sustainable production of biodiesel, the main pests of this crop should be controlled without pesticides. Both predatory mites studied here have been considered for biological control of *P. latus* and *T. bastosi* in this crop.

Co-occurrence of I. zuluagai *and* E. concordis *in the field*

Because theory predicts that co-occurrence of the two species would not be likely when they would be engaged in IGP, we first assessed the co-occurrence of *I. zuluagai* and *E. concordis* on the same plants. Twelve surveys were carried out on 30 *J. curcas* plants located around the city of Gurupi, Tocantins State, Brazil (11°48'29"S, 48°56'39" W, 280 m altitude), during three periods (03 – 27 March, 08 – 27 May and 18 September – 24 October 2009). During each survey, nine leaves were collected from each plant, three from each canopy stratum (bottom third, medium third and top third), located from the fourth to eighth fully expanded leaf from the branches. Leaves were checked with a stereomicroscope (Tecnival SQF-F, Brazil) and mites were identified by Dr. Farid Faraj from the University of Amsterdam, The Netherlands, and Dr. Manoel Guedes of the Federal Rural University of Pernambuco, Brazil. Voucher species of mites were deposited in the collection of the Laboratory of Entomology at the Federal University of

Tocantins. Because *I. zuluagai* was not present in the field during the last period, only the first two periods could be analysed. Because predatory mites can only cover short distances when walking and mainly disperse passively on air currents (Johnson & Croft, 1981; Sabelis & Dicke, 1985), the presence of the species on a plant within each of the other two periods probably depended on its presence on this plant earlier during the same period (hence, observations per plant are probably not independent). We therefore first scored whether each of the two species had been present on a plant during this entire period. To further verify the observed co-occurrence patterns, we subsequently treated samples within a period as independent and analysed co-occurrence data per sampling date. The predators were often found not to co-occur with the prey, which were therefore excluded from the analysis. The probability of co-occurrence of *I. zuluagai* and *E. concordis* on the same plants was calculated following Griffith et al. (2016), assuming a hypergeometric distribution.

Plant material and mite rearing

Predatory mites used for experiments were collected from natural populations on *J. curcas* plants in Gurupi, Tocantins state, Brazil (11°45’47”S, 49°02’57”W). They were reared inside plastic boxes (11 x 11 cm) on flexible plastic discs (∅ = 6cm) floating on distilled water in a climate room at 28°C, 65-70% R.H. and a 12h L/12h D photoperiod. Small tent-like structures consisting of a folded piece of plastic with small pieces of cotton wool under it were supplied on the arenas, serving as oviposition substrate. A small quantity of castor bean (*Ricinus communis*) pollen was supplied daily on the arenas as food for the predatory mites (McMurtry & Scriven, 1964). The *I. zuluagai* colonies were also supplied with honey diluted to 50% with distilled water. Pollen was collected from native castor bean plants in the city of Gurupi, following the method described by Gravena, Benetoli, Moreira and Yamamoto (1994) and was conserved in glass recipients in a refrigerator (6 °C). The mites were manipulated with a small brush under a magnifying glass. Populations of predatory mites were regularly transferred to new arenas to prevent the development of undesired microorganisms. Cohorts of predators of the same age were obtained by incubating adult females from the rearing units on a new arena with ample pollen, allowing them to oviposit for 24h, after which they were removed and the eggs were reared until reaching the desired stage or age.

Intraguild predation in the presence and absence of a shared resource

Intraguild predation was measured both in the presence and absence of the shared resource (pollen, c. 1.5 x 10^{-4} g per day). Plastic arenas (∅ = 7.5cm) as described previously were used. To verify whether adults of the two species fed on the juveniles of the other species, one gravid adult female of either *I. zuluagai* or *E. concordis* (9 days old since egg stage) and 30 protonymphs of the other species, all from cohorts of similar aged individuals, were put on an arena, either without or with ample *R. communis* pollen. Adult females were not starved before the experiment. We used protonymphs and not larvae because larvae would develop into protonymphs during the experiment, and the first protonymphs might cannibalize the remaining larvae. Because predation events are not very frequent in predatory mites, it was not feasible to directly observe predation. We therefore assessed the numbers of protonymphs consumed and the numbers of eggs produced by the adult female predators after 48h. Arenas with 30 protonymphs of *E. concordis* or *I. zuluagai* without adult female predator were used to measure the natural mortality, and arenas with only an adult female predator and pollen were used to assess oviposition in the absence of intraguild predation. The experiment was replicated 10 times. The effect of the presence of an intraguild predator and the presence of alternative food on the proportion of dead juveniles of each species was analysed with a generalized linear model (GLM) with a quasi-Poisson error distribution (logit link) to correct for overdispersion, with the number of surviving and dead juveniles as dependent variable. The numbers of eggs produced by the adult female predators were analysed with a GLM with a Poisson (log link) error distribution with the treatments (with or without pollen) as main factors. Contrasts among treatments were assessed using the multcomp package with a Tukey test (Hothorn, Bretz, & Westfall, 2008). All analyses were done with the statistical software R (R Development Core Team, 2017).

Intraguild predation does not only involve killing individuals of the competing species, but also feeding on it (Polis et al., 1989), which can be observed directly, or can be inferred from increased survival, reproduction or development due to the feeding on the competitor (Fonseca et al., 2017). When this is not the case, the interaction should be classified as interspecific killing, an extreme form of interference competition (Fonseca et al., 2017). The previous experiment showed some indications that the IG-predators benefitted from feeding on IG-prey. To further confirm this, we measured oviposition rates of adults when feeding on potential intraguild prey (same numbers as above) or without food on experimental arenas as described above. Because the oviposition of the first day is affected by the diet of the previous day (Sabelis, 1990), we did not include it in the analysis. After 48h, the numbers of eggs produced by the adult female predators were assessed. The experiment was replicated 10 times. Differences in the numbers of eggs were analysed as explained above.

Population dynamics on an artificial arena with a shared resource

We evaluated the dynamics of mixed populations of the two predators in the presence or absence of pollen. Experiments were carried out on circular plastic arenas (∅ = 7.5 cm) inside plastic boxes (11 x 11 cm) filled with water. Ten adult females (9 days old since egg stage) of each predator species were placed on separate arenas and supplied with ample pollen. After six days, all life stages were present, and the number of adults, juveniles and eggs was assessed for each species. Subsequently, all individuals of both species were transferred to a clean arena, where the two species could interact. Arenas were supplied with either ample pollen or kept without pollen. The numbers of adults, juveniles and eggs of the two predators were assessed every two days until one of the species went extinct. The two species are easily recognized because *I. zuluagai* is dark, including its eggs, and all stages of *E. concordis* are light. In our cultures, each species persisted for longer than the duration of this experiment when supplied with pollen, and we therefore did not include a control in which the predator species were kept separately. Pollen was refreshed every day. The experiment was replicated 10 times.

Because one of the two species went extinct 4 days after the populations of the two species were joined (hence, on day ten), the data of this day were strongly zero-inflated and were not included in the statistical analysis. We therefore analysed differences in the number of mites on day eight with a GLM with a quasi-Poisson error distribution (log link), with the initial numbers on day six (when the two species were joined), and pollen and species as factors. Contrasts among treatments were assessed with general linear hypothesis testing with a Tukey correction for multiple comparisons (package lsmean, Lenth, 2016).

Intraguild predation on adults

Because rapid extinction of *E. concordis* was observed in the previous experiment, we suspected that adult females of *I. zuluagai* killed adults of *E. concordis*. To verify this, we quantified killing of the adults of *E. concordis* by adults of *I. zuluagai* in the presence of shared resource (pollen). Plastic arenas (∅ = 7.5cm) were used as described above. One adult female of *I. zuluagai* (9 days old since egg stage) and 5 adult females of *E. concordis* (9 days old since egg stage) were put on an arena with ample pollen. A treatment without *I. zuluagai* served as control. After 48 h, the numbers of adults of *E. concordis* eaten by the adult female of *I. zuluagai* were assessed. The experiment was replicated 10 times. Predation was analysed with a generalized linear model (GLM) as above.

Population dynamics on plants

Because we observed that *E. concordis* went extinct extremely rapidly in mixed populations of all stages of the two predators on artificial arenas and we found no evidence of exclusion on *J. curcas* plants in the field, we evaluated the dynamics of mixed populations of the two predators on 30-day-old *J. curcas* plants with four leaves. Populations were prepared on plastic arenas (∅ = 7.5 cm) inside a plastic tray filled with water as described above. Ten females (9 days old since egg stage) of each predatory mite were placed on separate arenas and supplied with ample pollen. After six days, all life stages were present, and the numbers of adults were quantified for each species. Subsequently, all individuals of both species were transferred to the plants, where the two species were allowed to interact. In the first treatment both *E. concordis* and *I. zuluagai* were placed on the newest leaf on the apical part of the plant. In the second treatment, *E. concordis* were placed on the newest leaf and *I. zuluagai* on the leaf below, and in the third treatment, *I. zuluagai* were placed on the newest leaf and *E.*

concordis on the leaf below. A control was added with only *I. zulugai* to verify whether it could persist on plants.

The plants were supplied with 1.5 x 10^{-4} g of *R. communis* pollen daily, weighed on a precision balance (Shimadzu, Kyoto-Japan) and placed on the leaves with the predators with a fine brush. The numbers of adults were quantified every two days until one of the species went extinct. It was impossible to count juveniles and eggs without destructive sampling. Each treatment was replicated 10 times. The numbers of mites were ln(x+1) transformed and were analysed with a linear mixed-effects model with treatment (i.e. release schedule) and time as fixed factors and time within plant as random factor. Contrasts among treatments were assessed as above. Differences in time to extinction of the populations were tested with a Cox proportional hazards model (Therneau, 2013).

Population dynamics on leaves with pollen and honey

Here, we evaluated the dynamics of the two predators on a leaf with leaf hairs and trichomes and in the presence of pollen and honey. The leaves of *J. curcas* are palmately veined, cordate to truncate at the base. Together, the venation and the trichomes form structures that potentially reduce encounters between the predators (Ferreira et al., 2011). Honey was added because the experiments above showed that *I. zuluagai* did not persist on plants with a diet of pollen alone. Leaves (diameter c. 5 cm) were placed inside plastic boxes (11 x 11 cm) filled with water. Ten females (9 days old since egg stage) of each predator species were placed on separate arenas and supplied with a large quantity of pollen and honey. After six days, all life stages were present, and the number of adults, juveniles and eggs was assessed for each species. Subsequently, all individuals of both species were transferred to a clean leaf, where the two species were allowed to interact. The leaves were supplied with 1.5 x 10^{-4} g of *R. communis* pollen daily and 3.7 x 10^{-2} g of honey once per week. The number of eggs, larvae, nymphs and adults of the two predators was assessed every two days until one of the species went extinct. Populations of only *I. zulugai* or *E. concordis* kept on the same arenas served as control. The experiment was replicated 10 times. The total numbers of mites (log(x+10) transformed) were analysed with a linear mixed-effects model with treatment (alone or together) and time as fixed factors and time within replicate as random factor.

Results

Co-occurrence of I. zuluagai *and* E. concordis *in the field*

Euseius concordis was found in 64.6 % of the samples and *I. zuluagai* occurred in 21.7 % of the samples. Analysis of the overall co-occurrence data of the two entire sampling periods in which both species occurred showed that the observed values were not significantly different from expected. Hence, there was no evidence that the two species excluded or avoided each other (Table 1). The number of plants on which the two species co-occurred was significantly higher than expected from an independent distribution in three out of the eight sampling dates, and it was not significantly higher on the other five days (Table 1). We conclude that the two species do co-occur at least during several generations on the same plants in nature. Because theory predicts that possibilities for co-occurrence are reduced when species are engaged in IGP, we subsequently tested the occurrence of IGP and reciprocal IGP in the laboratory.

Intraguild predation in the presence of a shared resource

The mortality of juveniles of *E. concordis* varied significantly among treatments (Fig. 1a, GLM, $F_{3,36} = 70.9$, $P < 0.001$). In the presence of large amounts of pollen, the mortality of protonymphs was higher in the presence than in the absence of adult females of *I. zuluagai* (Fig. 1a, 1st and 2nd bar). The mortality of juvenile *E. concordis* was higher without pollen than with pollen (Fig. 1a, 2st and 4th bar). The mortality did not differ between the two treatments without pollen, probably because it was very high in both cases (Fig. 1a, 3rd and 4th bar). Oviposition by *I. zuluagai* was significantly affected by treatment (Fig. 1b, GLM, $Chi^2 = 16.5$, *d.f.* = 3, $P = 0.0009$), and it was more than twice higher in the presence of pollen than in its absence (Fig. 1b, compare first two bars with last two bars). Feeding on juveniles of *E. concordis* resulted in an increase in oviposition, but this effect was not significant (Fig. 1b, compare 1st with 2nd bar and 3rd with 4th).

The mortality rate of juvenile *I. zuluagai* also differed significantly among treatments (Fig. 2a, GLM, $F_{3,36} = 22.4$, $P < 0{,}001$). In the presence of pollen, the mortality of juvenile *I. zuluagai* was significantly higher in the presence of adult female *E. concordis* than in its absence (Fig. 2a, cf. 1st with 2nd bar). Mortality of juvenile *I. zuluagai* was significantly higher without pollen than with it (Fig. 2a, cf. the 1st with the 3rd bar and the 2nd with the 4th bar). The mortality did not differ between the two treatments without pollen, but was not very high. Perhaps the adult *E. concordis* fed mainly on starving and dying protonymphs, which may be easier to capture. The type of diet affected the oviposition rate of *E. concordis* (Fig. 2b, GLM, $Chi^2 = 13.6$, *d.f.* = 3, $P = 0.003$). The addition of protonymphs of *I. zuluagai* increased the oviposition rate of *E. concordis*, but this effect was not significant (Fig. 2b, cf. the 1st with 2nd bar and 3rd with 4th bar). The oviposition rate of *E. concordis* was highest when the mites were feeding on pollen and protonymphs of *I. zuluagai* (Fig. 2b).

Because of the non-significant trends of an increase in oviposition by the IG predators in the presence of IG prey, we further tested oviposition in a separate experiment. The oviposition by *I. zuluagai* was significantly higher in the presence of juveniles of *E. concordis* than without food (Fig. 3a, GLM, d.f. = 1, 38, $Chi^2 = 9.0$, *d.f.* = 1, $P = 0.003$). Likewise, the oviposition by *E. concordis* was also significantly higher in the presence of juvenile *I. zuluagai* than without food (Fig. 3b, GLM, $Chi^2 = 4.3$, *d.f.* = 1, $P = 0.04$). Together, this shows that the two species are involved in reciprocal intraguild predation (Fonseca et al., 2017).

Population dynamics on a shared resource

The populations of both species increased in the period preceding the mixing of the populations from 10 individuals to 20-30 individuals on the 6th day, confirming our experience with rearing both species on a diet of pollen. After joining the two populations of predators on day 6, *E. concordis* invariably went extinct within 4 days, even in the presence of pollen (Fig. 4). The population of *I. zuluagai* persisted only in the presence of pollen. The densities of both species on day 8 was significantly affected by the numbers of predators present when the two species were joined (GLM, $F_{1,36} = 5.32$, $P = 0.027$) and by the interaction between the presence of pollen and the other species ($F_{1,35} = 419.2$, $P = 0.0001$). This interaction was caused by the densities of *I. zuluagai* being affected by the presence of pollen, but those of *E. concordis* not (Fig. 4, general linear hypothesis testing after GLM).

The rapid extinction of the populations of *E. concordis* suggests that *I. zuluagai* did not only target juveniles and perhaps eggs of the other species, but also adults. Indeed, the number of adult *E. concordis* decreased from 18.7 ± 2.26 (mean ± s.e.) to zero in the absence of pollen, and from 20.2 ± 2.39 to 3.6 ± 1.03 individuals in the presence of pollen. As intraguild predation on adult females is rare in Phytoseiidae, we further confirmed this in the following experiment.

Intraguild predation on adults

The mortality of adult *E. concordis* was much higher in the presence of an adult *I. zuluagai* than in its absence (average mortality with *I. zuluagai* ± s.e.: 0.42 ± 0.07; without: 0.04 ± 0.027; GLM, $Chi^2 = 23.0$, d.f. = 1, $P < 0.0001$). There was no mortality of adult *I. zuluagai*.

Population dynamics on plants

In contrast to the experiments on artificial arenas, the two populations now persisted for a much longer period (Fig. 5). There was a significant effect of the interaction between treatment and time on the average densities of *E. concordis* (LME, $Chi^2 = 10.4$, *d.f.* = 2, $P = 0.0054$): densities were significantly lower when it was released on the same leaf as *I. zuluagai* (Fig. 5a). Two and one populations of *E. concordis* went extinct before *I. zuluagai* when both species were released on the same leaf or when *I. zuluagai* was released on the highest leaf respectively. In all other replicates of all treatments, *I. zuluagai* went extinct first. There was no significant difference in densities of *I. zuluagai* among treatments (Fig. 5b, LME, $Chi^2 = 4.41$, *d.f.* = 2, $P = 0.11$). Time to extinction of populations of *I. zuluagai* did not differ significantly among treatments (Cox proportional hazards, *Likelihood ratio test* = 3.56, *d.f.* = 2, $P = 0.17$). This species eventually also went extinct when present alone on a plant (Fig. 5c), and although it took somewhat longer to go extinct than in the presence of *E. concordis*, this difference was not significant (Cox proportional hazards, *Likelihood ratio test* = 3.17, *d.f.* = 1, $P = 0.075$).

Population dynamics on leaves with pollen and honey

The populations of both predator species alone persisted on a plant leaf with pollen and honey for the entire experimental period (Figure 6a and c). After joining the two predator populations on day 6, *E. concordis* invariably went extinct before day 16, whereas *I. zuluagai* persisted (Fig. 6b and d). All stages went extinct at the same time, confirming that *I. zuluagai* attacked all stages of *E. concordis* (Figure 6b). For each species, there was a significant effect of the interaction between time and treatment (with or without the other species) on the total numbers of mites (LME, *E. concordis*: $Chi^2 = 19.7$, $d.f. = 1$, $P < 0.0001$; *I. zuluagai*: $Chi^2 = 5.85$, $d.f. = 1$, $P = 0.016$). Contrasts between the two time series showed significant differences between treatments for both species (Figure 6). For *I. zuluagai*, the two time series differed in the first part of the experiment, but this difference disappeared in the second half (Figure 6c and d).

Discussion

We show that the two species of predatory mites engaged in reciprocal intraguild predation, with adults of the two species feeding on each other's juveniles. The adult females of both species showed a significant increase in reproduction on a diet consisting of juveniles of the other species (Fig. 3), suggesting that they did not just kill the juveniles, but also fed on them. Furthermore, the adult females of *I. zuluagai* killed adults of *E. concordis*, causing *E. concordis* going extinct extremely rapidly in mixed populations of all stages of the two predators on arenas without spatial structure (Fig. 4). We do not know whether the adults of *I. zuluagai* benefited from feeding on adults of the other species, i.e. whether this qualified as intraguild predation. Without pollen, we encountered no eggs of *I. zuluagai* at the end of the experiment, suggesting that the population of *I. zuluagai* was also going extinct, and could perhaps not reproduce on a diet of adult *E. concordis* only. Possibly, adult *I. zuluagai* killed the adults of the other species to avoid that these would kill the offspring of the adult female *I. zuluagai*.

Intraguild predation is a common phenomenon in nature (Arim & Marquet, 2004; Polis et al., 1989). In general, the IG-predator feeds on the smaller stages of the IG-prey, and it is less common that adults of one species attack the adults of the other species (Polis et al., 1989). Adults of *I. zuluagai* (dorsal shield on average 343 x 278 µm) are bigger than adult of *E. concordis* (309 x 207 µm) (Lofego, 1998) and this might render adults of *E. concordis* vulnerable to predation by adults of the former species.

On plants, the period of coexistence of both populations was much longer than on artificial arenas and on leaves. This was perhaps caused by the increased spatial structure offered by plants compared to artificial arenas. It is known that plant structures may reduce the encounter and predation rates between predators (Ferreira et al., 2011; Pozzebon, Loeb, & Duso, 2015; Roda, Nyrop, Dicke, & English-Loeb, 2000; Schmidt, 2014). Habitat structure can reduce the effects of intraguild predation by reducing the strength of the interaction between intraguild predator and intraguild prey (Janssen et al., 2007). Probably, leaf structures such as trichomes may have affected the coexistence of the predators in our experiments. In contrast to the experiments on artificial arenas, *I. zuluagai* ultimately went extinct on the plants, and not *E. concordis*. Although the densities of *E. concordis* were significantly lower when it was released on the same leaf as *I. zuluagai* (Fig. 5a), *E. concordis* persisted and *I. zuluagai* went extinct. However, we found that *I. zuluagai* did not persist on plants with pollen only (Fig. 5c) and the time to extinction was not significantly affected by the presence of *E. concordis* (Fig. 5b,c). Perhaps the extinction of *I. zuluagai* was not caused by the interaction with *E. concordis*, but by the lack of a suitable diet. Indeed, when providing honey as a diet supplement on arenas consisting of single leaves, populations of *I. zuluagai* persisted, resulting in *E. concordis* again going extinct (Fig. 6), in line with theoretical predictions of the effects of alternative food for intraguild predators (Daugherty et al., 2007; Holt & Huxel, 2007). Together, these results show that an interplay between spatial structure and alternative food sources determine coexistence and exclusion of species involved in intraguild predation (Pozzebon et al., 2015). This may explain the co-occurrence of the two species on plants in the field. Possibly, the absence of *I. zuluagai* from the field in September – October, which is the end of the dry season in the study area, even from plants without *E. concordis* was also caused by the lack of a suitable diet.

In theory, reciprocal intraguild predation, as was found here, reduces possibilities for coexistence even more than simple IGP (HilleRisLambers & Dieckmann, 2003). Earlier experimental work on systems with reciprocal intraguild predation, but with adults being invulnerable to attacks by the other species, has shown the possibility of bistability in the dynamics (Montserrat, Magalhaes, Sabelis, de Roos, & Janssen, 2008; Montserrat et al., 2012). The mechanism behind this is that resident populations with a high density of adults can prevent the invasion of the other species by killing all its offspring. In the system studied here, this bistability is likely to occur less, because the adults of *E. concordis* are vulnerable for intraguild predation. Hence, *I. zuluagai* could probably even invade in populations with high densities of *E. concordis* adults. Hence, our experiments on plastic arenas suggest low probabilities for coexistence of these two predators in the field. In contrast, we found no evidence of exclusion of either of the species on *Jatropha* plants in the field, and prolonged periods of co-occurrence on single plants in the laboratory. We suggest that plants offer structural complexity that reduces the strength of intraguild predation, thus resulting in increased coexistence of the two species.

Acknowledgments

This research was supported by a scientific initiation scholarship from the National Counsel of Technological and Scientific Development, Brazil (CNPq) (projects 620028/2008-4 and 475408/2008-0) and by the Federal Agency for Support and Evaluation of Graduate Education (CAPES) for providing a scholarship from the National Postdoctoral Programme (CAPES-PNPD) to MP-N. AJ received a scholarship from FAPEMIG (CBB-30003 ⁄ 09) and AP from CNPq.

References

Amarasekare, P. (2007). Spatial dynamics of communities with intraguild predation: The role of dispersal strategies. *American Naturalist*, *170*, 819–831.

Arim, M., & Marquet, P. A. (2004). Intraguild predation: a widespread interaction related to species biology. *Ecology Letters*, *7*, 557–564.

Choh, Y., Ignacio, M., Sabelis, M. W., & Janssen, A. (2012). Predator-prey role reversals, juvenile experience and adult antipredator behaviour. *Scientific Reports*, *2 (728)*, 1–6. doi:10.1038/srep00728

Claessen, D., Van Oss, C., de Roos, A. M., & Persson, L. (2002). The impact of size-dependent predation on population dynamics and individual life history. *Ecology*, *83*, 1660–1675.

Daugherty, M. P., Harmon, J. P., & Briggs, C. J. (2007). Trophic supplements to intraguild predation. *Oikos*, *116*, 662–677.

de Roos, A. M., Leonardson, K., Persson, L., & Mittelbach, G. G. (2002). Ontogenetic niche shifts and flexible behavior in size-structured populations. *Ecological Monographs*, *72*, 271–292.

Diehl, S., & Feissel, M. (2000). Effects of enrichment on three-level food chains with omnivory. *American Naturalist*, *155*, 200–218.

Ferreira, J. A. M., Cunha, D. F. S., Pallini, A., Sabelis, M. W., & Janssen, A. (2011). Leaf domatia reduce intraguild predation among predatory mites. *Ecological Entomology*, *36*, 435–441.

Fonseca, M. M., Montserrat, M., Guzmán, C., Torres-Campos, I., Pallini, A., & Janssen, A. (2017). How to evaluate the potential occurrence of intraguild predation. *Experimental and Applied Acarology*, *72*, 103–114. doi:10.1007/s10493-017-0142-x

Gould, H. J. (1977). Biological control of glasshouse whitefly and red spider mite on tomatoes and cucumbers in England and Wales. *Plant Pathology*, *26*, 57–60.

Gravena, S., Benetoli, I., Moreira, P. H. R., & Yamamoto, P. T. (1994). *Euseius citrifolius* Denmark and Muma predation on citrus leprosis mite *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae). *Neotropical Entomology*, *23*, 209–218.

Griffith, D. M., Veech, J. A., & Marsh, C. J. (2016). COOCCUR: Probabilistic species co-occurrence analysis in R. *J Stat Softw*, *69*(2), 1–17.

Gübitz, G. M., Mittelbach, M., & Trabi, M. (1999). Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresource Technology*, *67*, 73–82. doi:http://dx.doi.org/10.1016/S0960-8524(99)00069-3

Guzmán, C., Sahún, R., & Montserrat, M. (2016). Intraguild predation between phytoseiid mite species might not be so common. *Experimental and Applied Acarology*, *68*(4), 441–453.

Hatherly, I. S., Bale, J. S., & Walters, K. F. A. (2005). Intraguild predation and feeding preferences in three species of phytoseiid mite used for biological control. *Experimental & Applied Acarology*, *37*, 43–55.

HilleRisLambers, R., & Dieckmann, U. (2003). Competition and predation in simple food webs: intermediately strong trade-offs maximize coexistence. *Proceedings of the Royal Society of London Series B-Biological Sciences*, *270*, 2591–2598.

Holt, R. D., & Huxel, G. R. (2007). Alternative prey and the dynamics of intraguild predation: theoretical perspectives. *Ecology*, *88*, 2706–2712.

Holt, R. D., & Polis, G. A. (1997). A theoretical framework for intraguild predation. *American Naturalist*, *149*, 745–764.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363.

Huffaker, C., & Kennett, C. (1953). Developments toward biological control of cyclamen mite on strawberries in California. *Journal of Economic Entomology*, *46*(5), 802–812.

Janssen, A., Montserrat, M., HilleRisLambers, R., de Roos, A. M., Pallini, A., & Sabelis, M. W. (2006). Intraguild predation usually does not disrupt biological control. In J. Brodeur & G. Boivin (Eds.), *Trophic and guild interactions in biological control* (Vol. 3, pp. 21–44). Dordrecht, The Netherlands: Springer.

Janssen, A., & Sabelis, M. W. (2015). Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. *Experimental and Applied Acarology*, *65*(4), 413–418. doi:10.1007/s10493-015-9901-8

Janssen, A., Sabelis, M. W., Magalhães, S., Montserrat, M., & van der Hammen, T. (2007). Habitat structure affects intraguild predation. *Ecology*, *88*, 2713–2719.

Johnson, D. T., & Croft, B. (1981). Dispersal of *Amblyseius fallacis* (Acarina: Phytoseiidae) in an apple ecosystem. *Environmental Entomology*, *10*(3), 313–319.

Lenth, R. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33. doi:10.18637/jss.v069.i01

Lofego, A. C. (1998). *Caracterizaçao morfológica e distribuiçao geográfica das espécies de Amblyseiinae (Acari: Phytoseiidae) no Brasil* (PhD Thesis). Institute of Biosciences, University of São Paulo, São Paulo.

Marques, R. V., Sarmento, R. A., Lemos, F., Pedro-Neto, M., Sabelis, M. W., Venzon, M., … Janssen, A. (2015). Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. *Functional Ecology*, *29*, 1317–1324. doi:10.1111/1365-2435.12439

McMurtry, J. A., & Scriven, G. T. (1964). Studies on the feeding, reproduction, and development of *Amblyseius hibisci* (Acarina: Phytoseiidae) on various food substances. *Annals of the Entomological Society of America*, *57*, 649–655.

Messelink, G. J., van Maanen, R., van Steenpaal, S. E. F., & Janssen, A. (2008). Biological control of thrips and whiteflies by a shared predator: two pests are better than one. *Biological Control*, *44*, 372–379. doi:10.1016/j.biocontrol.2007.10.017

Montserrat, M., Janssen, A., Magalhães, S., & Sabelis, M. W. (2006). To be an intra-guild predator or a cannibal: is prey quality decisive? *Ecological Entomology*, *31*, 430–436.

Montserrat, M., Magalhaes, S., Sabelis, M. W., de Roos, A. M., & Janssen, A. (2008). Patterns of exclusion in an intraguild predator-prey system depend on initial conditions. *Journal of Animal Ecology*, *77*, 624–630.

Montserrat, M., Magalhaes, S., Sabelis, M. W., de Roos, A. M., & Janssen, A. (2012). Invasion success in communities with reciprocal intraguild predation depends on the stage structure of the resident population. *Oikos*, *121*, 67–76. doi:10.1111/j.1600-0706.2011.19369.x

Morin, P. J. (1999). Productivity, intraguild predation, and population dynamics in experimental food webs. *Ecology*, *80*, 752–760.

Mylius, S. D., Klumpers, K., de Roos, A. M., & Persson, L. (2001). Impact of intraguild predation and stage structure on simple communities along a productivity gradient. *American Naturalist*, *158*, 259–276.

Nomikou, M., Janssen, A., Schraag, R., & Sabelis, M. W. (2001). Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Experimental and Applied Acarology*, *25*, 271–291. doi:10.1023/A:1017976725685

Openshaw, K. (2000). A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass and Bioenergy*, *19*, 1–15. doi:http://dx.doi.org/10.1016/S0961-9534(00)00019-2

Polis, G. A. (1984). Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? *The American Naturalist*, *123*, 541–564.

Polis, G. A., & Holt, R. D. (1992). Intraguild predation - the dynamics of complex trophic interactions. *Trends in Ecology & Evolution*, *7*, 151–154.

Polis, G. A., Myers, C. A., & Holt, R. D. (1989). The ecology and evolution of intraguild predation - potential competitors that eat each other. *Annual Review of Ecology and Systematics*, *20*, 297–330.

Polis, G. A., & Winemiller, K. O. (1996). *Food webs. Integration of patterns & dynamics*. New York, USA: Chapman & Hall.

Pozzebon, A., Loeb, G. M., & Duso, C. (2015). Role of supplemental foods and habitat structural complexity in persistence and coexistence of generalist predatory mites. *Scientific Reports*, *5*, 14997.

R Development Core Team. (2017). R: A language and environment for statistical computing (Version 3.3.3). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Ramakers, P. J. M. (1980). Biological control of *Thrips tabaci* (Thysanoptera: Thripidae) with *Amblyseius* spp. (Acari: Phytoseiidae). *Bulletin SROP/WPRS*, *1980*(III/3), 203–207.

Roda, A., Nyrop, J., Dicke, M., & English-Loeb, G. (2000). Trichomes and spider-mite webbing protect predatory mite eggs from intraguild predation. *Oecologia*, *125*, 428–435.

Rosenheim, J. A. (2007). Intraguild predation: new theoretical and empirical perspectives. *Ecology*, *88*, 2679–2680.

Rosenheim, J. A., & Harmon, J. P. (2006). The influence of intraguild predation on the suppression of a shared prey population: an empirical reassessment. In J. Brodeur & G. Boivin (Eds.), *Trophic and guild interactions in biological control* (Vol. 3, pp. 1–20). Dordrecht, The Netherlands: Springer.

Rosenheim, J. A., Kaya, H. K., Ehler, L. E., Marois, J. J., & Jaffee, B. A. (1995). Intraguild predation among biological control agents - Theory and evidence. *Biological Control*, *5*, 303–335. doi:10.1006/bcon.1995.1038

Rudolf, V. H. W. (2007). The interaction of cannibalism and omnivory: consequences for community dynamics. *Ecology*, *88*, 2697–2705.

Sabelis, M. W. (1990). How to analyze prey preference when prey density varies? A new method to discriminate between effects of gut fullness and prey type composition. *Oecologia*, *82*, 289–298.

Sabelis, M. W., & Dicke, M. (1985). Long-range dispersal and searching behaviour. In W. Helle & M. W. Sabelis (Eds.), *Spider mites: their biology, natural enemies and control* (Vol. 1B, pp. 141–160). Amsterdam: Elsevier.

Sarmento, R. A., Rodrigues, D. M., Faraji, F., Erasmo, E. A. L., Lemos, F., Teodoro, A. V., … Pallini, A. (2011). Suitability of the predatory mites *Iphiseiodes zuluagai* and *Euseius concordis* in controlling *Polyphagotarsonemus latus* and *Tetranychus bastosi* on *Jatropha curcas* plants in Brazil. *Experimental and Applied Acarology*, *53*, 203–214. doi:10.1007/s10493-010-9396-2

Schausberger, P., & Croft, B. A. (2000). Nutritional benefits of intraguild predation and cannibalism among generalist and specialist phytoseiid mites. *Ecological Entomology*, *25*, 473–480.

Schellekens, T., & van Kooten, T. (2012). Coexistence of two stage-structured intraguild predators. *Journal Of Theoretical Biology*, *308*, 36–44. doi:10.1016/j.jtbi.2012.05.017

Schmidt, R. A. (2014). Leaf structures affect predatory mites (Acari: Phytoseiidae) and biological control: a review. *Experimental and Applied Acarology*, *62*(1), 1–17.

Therneau, T. (2013). A package for survival analysis in S. (Version R package version 2.37-4).

van der Hammen, T., de Roos, A. M., Sabelis, M. W., & Janssen, A. (2010). Order of invasion affects the spatial distribution of a reciprocal intraguild predator (vol 163, pg 79, 2010). *Oecologia*, *164*, 851–851. doi:10.1007/s00442-010-1786-y

van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., & Urbaneja, A. (2017). Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl*, 1–21.

Vance-Chalcraft, H. D., Rosenheim, J. A., Vonesh, J. R., Osenberg, G. W., & Sih, A. (2007). The influence of intraguild predation on prey suppression and prey release: a meta-analysis. *Ecology*, *88*, 2689–2696. doi:10.1890/06-1869.1

Wissinger, S. A. (1992). Niche overlap and the potential for competition and intraguild predation between size-structured populations. *Ecology*, *73*, 1431–1444. doi:10.2307/1940688

Woodward, G., & Hildrew, A. G. (2002). Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, *71*, 1063–1074.

Yaninek, S., & Hanna, R. (2003). Cassava green mite in Africa–a unique example of successful classical biological control of a mite pest on a continental scale. In P. Neuenschwander, Borgemeister, C, & Langewald, L. (Eds.), *Biological control in IPM systems in Africa* (pp. 61–75). Wallingford UK: CABI Publishing.

Table 1. Co-occurrence of the predatory mites *E. concordis* and *I. zuluagai* on *Jatropha curcas* plants in the field.

Period[a]	*E. concordis*[b]	*I. zuluagai*[b]	Both	Exp(both)[c]	*P*[d]
March overall	28	6	6	5.6	0.634
3	9	3	3	0.9	**0.02**
10	25	3	2	3.5	0.43
18	17	4	3	2.3	0.41
27	10	0	0	0	
May overall	29	22	20	21.3	1
8	25	13	13	10.8	**0.043**
15	22	9	9	6.6	**0.035**
22	28	12	12	11.2	0.35
29	19	8	5	5.1	0.64

[a] Sampling was done in 3 periods, March, May and September-October 2009. Data from the last period are excluded because *I. zuluagai* was not encountered. Given are first overall co-occurrence data (i.e. presence/absence on plants during the entire month of March or May) and subsequently data for the four samples during the months with day of the month (*i.e.* March 3rd, 10th, etc.); [b] number of plants out of 30 sampled on which a species was encountered, including plants on which the other species was also encountered; [c] expected number of plants with co-occurrence; [d] probability of encountering both species the observed number of cases or more extreme, based on a hypergeometric distribution (Griffith et al. 2016). Significant values are given in bold.

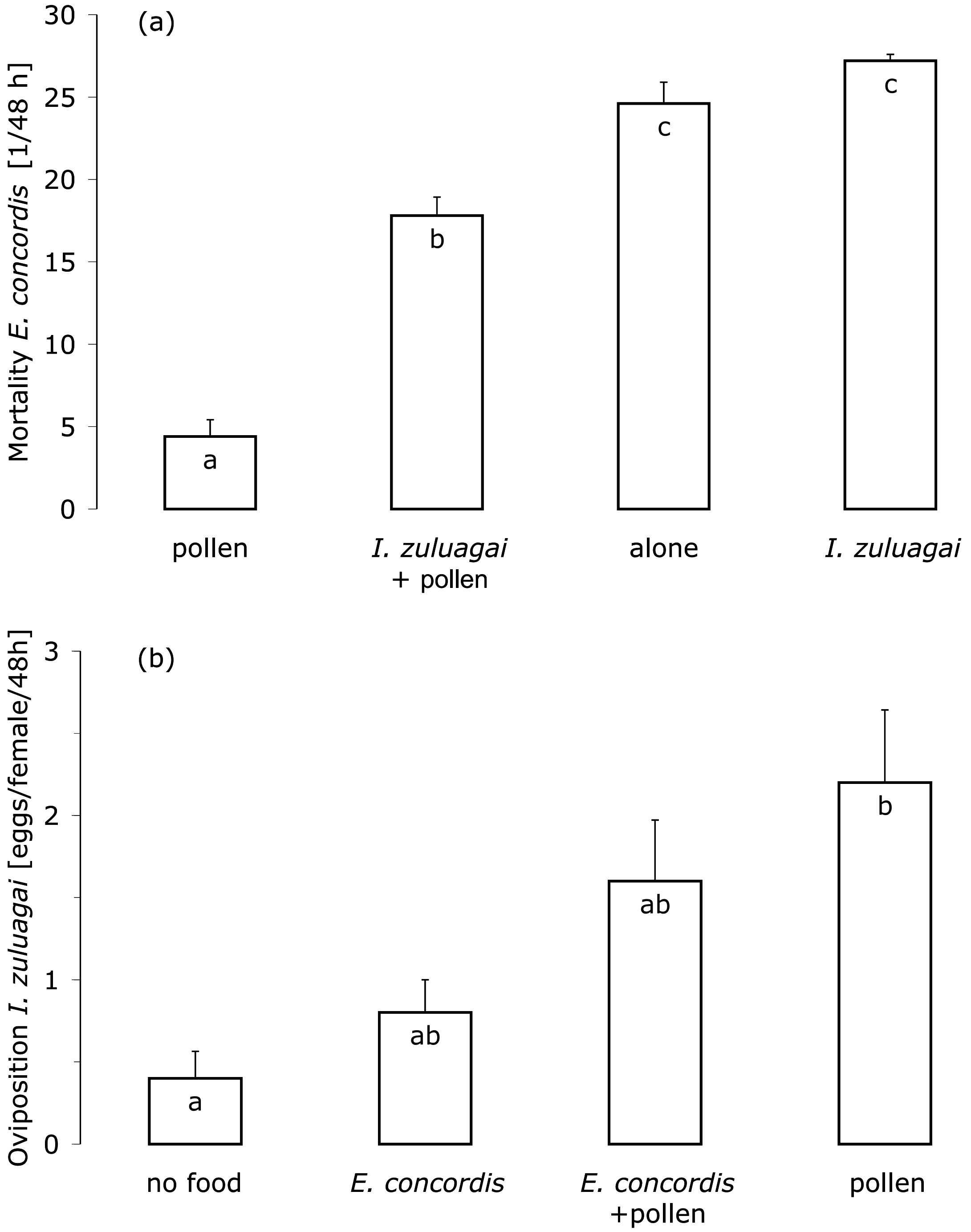


Figure 1 **(a)** Numbers of dead juvenile *E. concordis* (mean ± SE) after 48h in the presence or absence of ample pollen and in the presence or absence of adult *I. zuluagai*. **(b)** Oviposition rates (mean ± SE) after 48h of the adult *I. zuluagai* in the same experiments. Letters inside the bars indicate significant difference among treatments (contrasts after GLM).

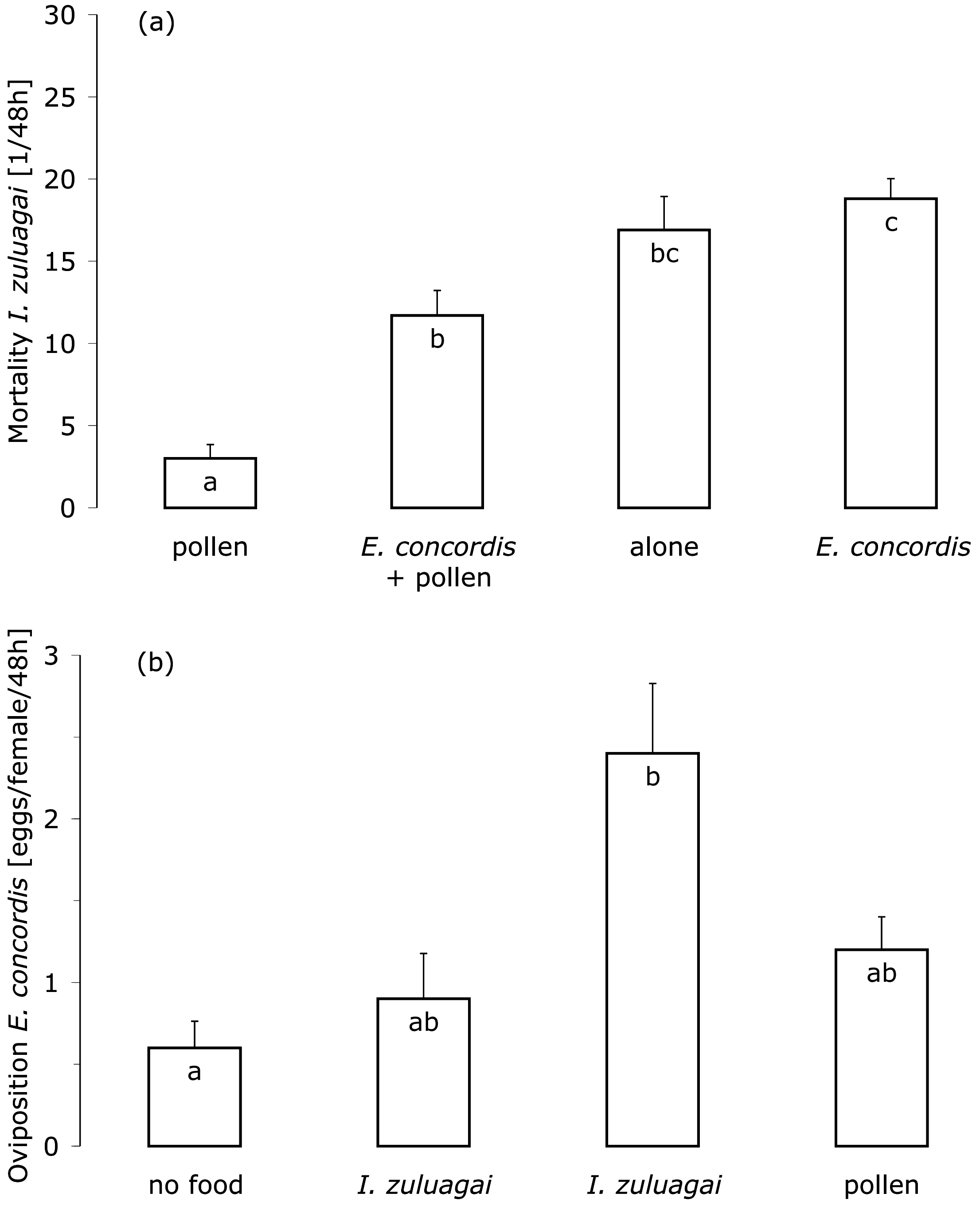


Figure 2(a) Numbers of dead juvenile *I. zuluagai* (mean ± SE) after 48h in the presence or absence of ample pollen and in the presence or absence of one adult female *E. concordis*. **(b)** Oviposition rates (mean ± SE) after 48h of the adult *E. concordis* in the same experiments. Letters inside the bars indicate significant difference among treatments (contrasts after GLM).

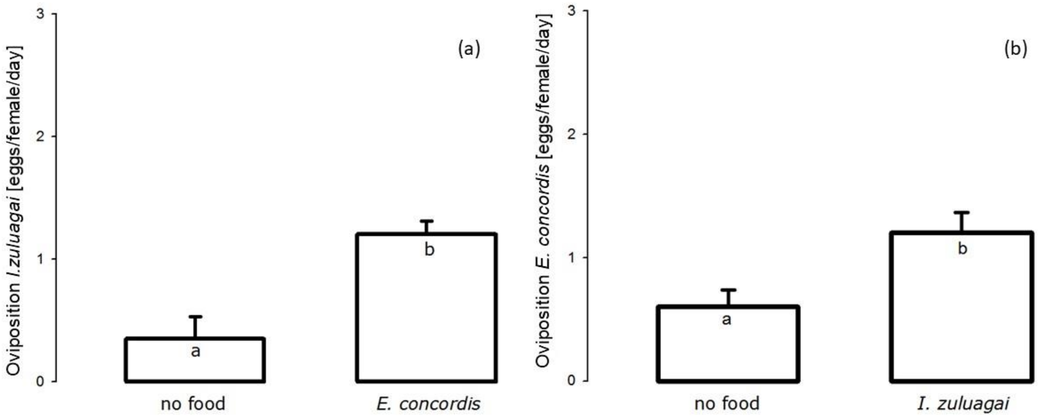


Figure 3 (a) Numbers of eggs (mean ± SE) produced by (a) adult female predatory mites *I. zuluagai* when feeding on juvenile *E. concordis*, and by **(b)** adult female predatory mites *E. concordis* when feeding on juvenile *I. zulugai*. Adult females of both species were allowed to feed on the juveniles of the other species for two days, but only oviposition rates of the second day were included to avoid effects of previous diet. Different letters above the bars indicate significant differences between treatments.

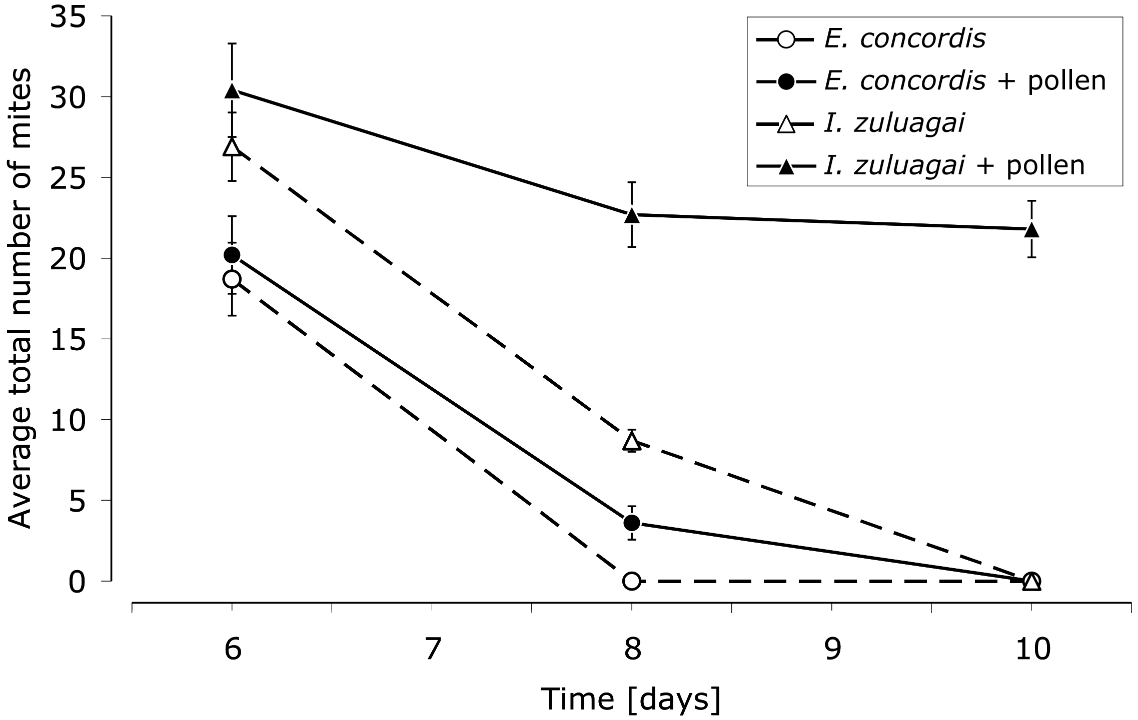


Figure 4 Mean total (all stages) numbers of mites (mean ± SE) of *I. zuluagai* (triangles) and *E. concordis* (circles). Populations of both species were started with 10 adult females and were allowed to grow on pollen for 6 days. Subsequently, populations of the two species were released on the same arena either with (drawn lines) or without (interrupted lines) pollen as a shared food source. See text for further explanation.

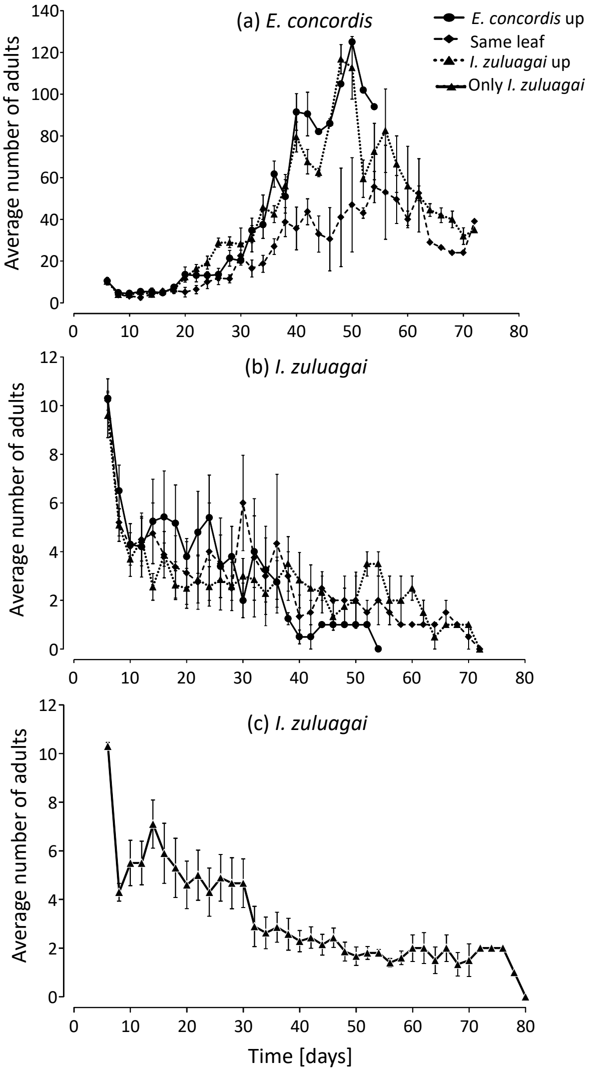


Figure 5 Mean (± s.e.) numbers of adults of *E. concordis* (**a**) and *I. zuluagai* (**b**) per plant. Populations of both species started with 10 adult females and were allowed to grow on pollen for 6 days. Both species were either released on the upper leaf (Same leaf, diamonds) or one of the species was released on the upper leaf and the other one leaf down (*E. concordis* up and *I. zuluagai* up). **(C)** Only individuals of *I. zuluagai* were released. See text for further explanation. The letters in the legend show significance of differences among treatments for *E. concordis* (contrasts after lme). Treatments did not differ significantly for *I. zuluagai*.

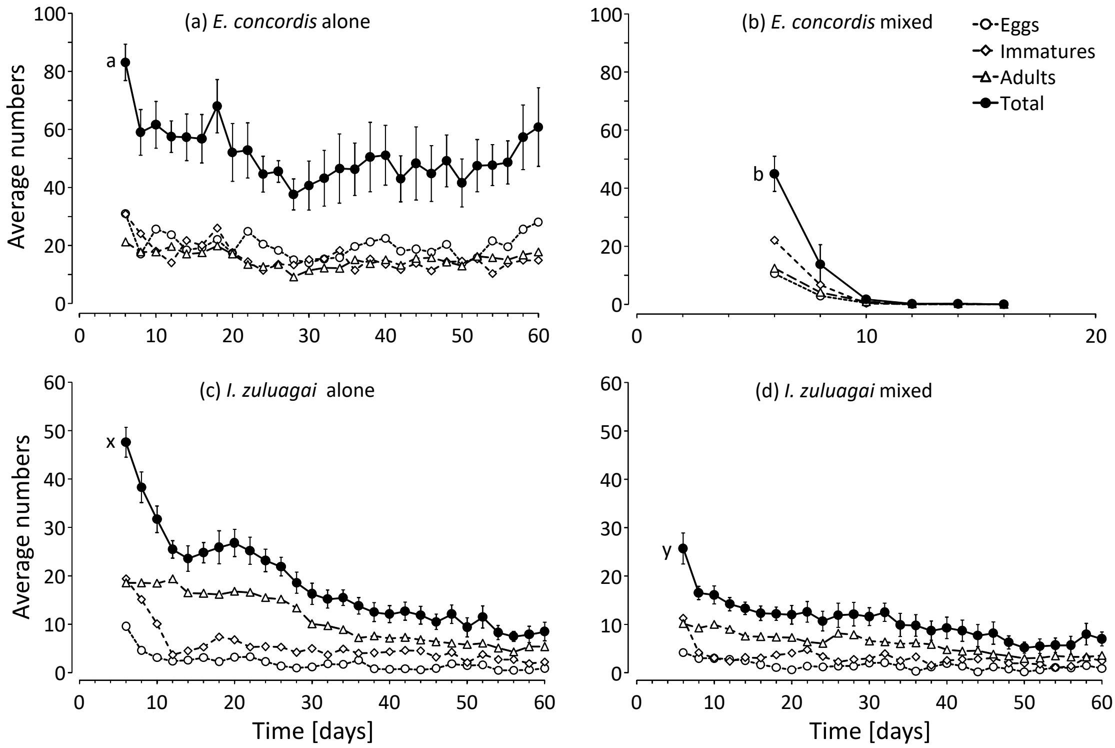


Figure 6 Mean numbers of eggs (open circles), immatures (open diamonds), adults (open triangles) and total mites (closed circles) of *E. concordis* (**a** and **b**) and *I. zuluagai* (**c** and **d**) per leaf, either alone (**a**, **c**) or together with the other species (**b**, **d**). Populations of both species started with 10 adult females and were allowed to grow on pollen and honey for 6 days. Subsequently, populations of the two species were released on the same leaf or on different leaves, in all cases with pollen and honey as food. Letters to the left of the total curves indicate a significant difference between the total numbers in the mixed and single populations. For reasons of clarity, s.e. are only shown for total numbers.

GENERAL CONCLUSIONS

In this thesis, I show that diet and habitat heterogeneity affect food web interactions and coexistence of predators. In Chapter 1, I show that diet choice affects the performance of a predatory mite. In the absence of spatial structure, the predatory mite *E. concordis* balanced its diet by feeding on mixtures of *T. bastosi* and *P. latus*. A diet that maximized the performance of the predator was composed of c. 1/3 *T. bastosi* and 2/3 *P. latus*. Hence, to ensure a higher fitness the predator seems to select for a mixed diet consisting of a higher proportion of *P. latus*.

In Chapter 2, I studied the effect of spatial structure on diet choice, showing that the predators no longer search for an optimal diet when they have to cover some distance to obtain such a diet. However, In Chapter 3, I used a more natural spatial structure, i.e. a plant, and show that the predators perform better on a mixed diet. Hence, *E. concordis* is able to cover long distances on a plant to mix its diet in order to find the two *natural* prey species.

The positive effects of mixed diets on predator performance is likely to result in better biocontrol of these two pests on *Jatropha curcas* when both pests are co-occurring on the same plants compared to plants where they occur singly, because the mixed diet results in an increase of predator densities and thus improves efficiency of the biological control. Further experiments are needed to demonstrate the relationships between pest levels and yield to enable the application of the results obtained here.

In Chapter 4, I show that diet and spatial structure affect co-occurrence of two predators that are engaged in IGP. The predatory mites *E. concordis and I. zuluagai* co-occur in the field but are involved in IGP or reciprocal IGP. However, spatial structure and alternative food sources promoted the coexistence of these two predators. For these experiments, the predators were supplied with pollen and honey as alternative food sources. An open question is whether the two naturally occurring prey could also promote the coexistence of the two predators. Although the predators co-occur in the field, it does not mean that they provide better control together. Future experiments should test the dynamics of the two pest mites in the presence of each predator separately and also with both predators together.

In conclusion, these results suggest that to improve the persistence of predators in the field it is important to increase the spatial structure of the habitat and the food items of the predator's diet.