Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control

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Summary

1. In modern agricultural landscapes, many organisms providing ecosystem services such as pollination and natural pest control are likely constrained by shortage of nectar and/or pollen required for adult nutrition. More and more flower-rich field margin strips and other habitats are created to eliminate these constraints. For most target organisms, however, it is not well known which (types of) flowers are effective in providing suitable pollen and nectar.

2. We studied the suitability of a wide range of flowers as a food source for zoophagous hoverflies (hoverflies with predatory larvae) at five different levels: nectar accessibility (based on flower morphology), realized adult fitness, flower choice (both based on cage experiments), flower visitation and hoverfly abundance in mixed vegetation (both based on field observations).

3. Realized survival of *Episyrphus balteatus* is related to effective flower depth by a sigmoid function. The critical flower depth is 1·6 mm, which is less than the proboscis size of the hoverfly. For Asteraceae, the critical floret depth is even less than 1·0 mm, which – in contrast to common knowledge – rules out most species within this family.

4. Both flower choice in the laboratory and flower visitation rates in the field are well correlated with nectar accessibility and realized adult survival.

5. In mixed floral vegetation, the number of zoophagous hoverflies is highly correlated with the abundance of only those flowers that have accessible nectar for these hoverflies.

6. *Synthesis and applications.* This comparative study demonstrates that nectar (and not pollen) accessibility is the main driver determining flower resource suitability, flower choice and abundance of zoophagous hoverflies in arable field margins. The study identifies the limited range of plant species that can effectively support these beneficial insects. Preserving the right flowers in and around agricultural fields could enhance local populations and the pest control and pollination services they provide.

Key-words: agro-environment schemes, conservation biological control, corolla depth, Diptera: Syrphidae, ecosystem services, extrafloral nectar, field margins, flower morphology, functional biodiversity, pollination

Introduction

Many insects that play a role in pest suppression are carnivorous during their larval stage, but require nectar, and sometimes pollen, during their adult life stage (Wäckers, Van Rijn & Bruin 2005). The scarcity of flowering plants in modern agricultural fields can therefore constrain the effective performance of these beneficial arthropods. Nectar and pollen-providing field margins are increasingly created to eliminate this constraint (Landis, Wratten & Gurr 2000; Marshall & Moonen 2002; Wäckers 2004; Bianchi, Booij & Tscharntke 2006; Van Rijn *et al.* 2008; Hogg *et al.* 2011; Tschumi *et al.* 2015). Knowledge on the suitability of field margin plants, however, is very limited (Wäckers & Van Rijn 2012).

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Field margin flowers may affect natural pest control by attracting natural enemies of pests, thereby affecting their spatial distribution. This does not necessarily enhance pest suppression, since the margins could also act as a sink (Landis, Wratten & Gurr 2000). The real benefit from the flowers needs to come from their impact on fitness components such as survival and reproduction and the resulting increase of the beneficial insect populations (Van Rijn & Sabelis 2005). Plants selected for the support of natural pest control should therefore not only attract the right natural enemies but also affect their fitness (Hogg *et al.* 2011; Wäckers & Van Rijn 2012).

Hoverflies (Diptera: Syrphidae) with zoophagous larvae (Syrphinae) are common natural enemies of aphids in arable crops, for example making up *c.* 50% of all predators found on potato leaves (Van Rijn *et al.* 2008). Hoverflies are also important as pollinators (Jauker *et al.* 2012). Adult hoverflies require both nectar and pollen as a source of energy and a source of protein for reproduction, respectively (Haslett 1989; Van Rijn, Kooijman & Wäckers 2013). Gilbert (1981, 1985a) and Branquart & Hemptinne (2000) showed a correlation between (relative) proboscis length and the average depth of the flowers the species visit. The relatively short proboscis of most zoophagous hoverflies compared to most saprophagous hoverflies and hymenopteran pollinators (bees and bumblebees), likely limits the range of flowers suitable as a nectar source for the former group (Gilbert 1985a).

Studies on the interactions between insects and flowers are generally based on recordings of flower visitation. Flower visitation rates, however, are relative measures, dependent on other flowering species present, competition with other flower visitors and previous experience (Wäckers & Van Rijn 2012). Moreover, flower preference does not necessarily indicate which flowers are most suitable in terms of realized fitness benefits. A direct test of flower suitability is to measure fitness components such as survival or reproduction of individuals that are provided a particular flowering species. While such tests had been performed for other groups of pest natural enemies, such as hymenopteran parasitoids (Wäckers, Björnsen & Dorn 1996; Begum *et al.* 2006; Winkler *et al.* 2009), lady beetles (Bertolaccini, Núñez-Pérez & Tizado 2008), lacewings (Venzon *et al.* 2006) and predatory mites (Van Rijn & Tanigoshi 1999), until very recently (Laubertie, Wratten & Hemptinne 2012) no such studies had been performed for hoverflies. Also within the guild of pollinators, flower suitability is generally concluded based on flower visitation or flower choice studies only (Olesen *et al.* 2007; Bosch *et al.* 2009; Stang *et al.* 2009; Campbell *et al.* 2012). For bees (both social and solitary), it is arguably very difficult to study the fitness impact of a range of floral resources, due to their social structure or the complex and long (univoltine) life cycle (but see Genissel *et al.* 2002; Praz, Müller & Dorn 2008). Zoophagous hoverflies, especially those with short life cycles, are much easier to study experimentally and are thus ideal model organisms

for studies on flower–pollinator interactions taking pollinator fitness into account. Here, we focus on the marmalade fly *Episyrphus balteatus*, one of the most common zoophagous hoverflies in Europe, while including all zoophagous hoverflies in our field observations.

To investigate which types of flowers are suitable as a food source for *E. balteatus*, we tested 32 flower species that are used as nectar and pollen plants in flowering field margins (see Appendix S1, Supporting Information). First, we estimated nectar accessibility, in terms of depth and width of nectar-protecting structure, in relation to proboscis length. Secondly, we assessed adult longevity for hoverfly individuals kept with single flowering plant species as a food source. Thirdly, we performed choice tests with flower-inexperienced adults to establish their (innate) flower preference. Fourthly, we measured flower visitation rates as a measure of flower preferences in the field. Fifth, we checked the validity of these results for more complex field conditions by sampling pollinator fauna in mixed field margin strips. In this way, we hoped to determine how these measures are related and to test whether flower morphology is sufficient to predict flower suitability for zoophagous hoverflies. In this way, we also hope to find a sound basis for the selection of plant species in and around agricultural fields for the support of natural pest control.

Materials and methods

STUDY SYSTEM

The laboratory studies were performed with adults from *Episyrphus balteatus* (De Geer, 1776) reared from pupae provided by Koppert B.V. (Berkel & Rodenrijs, NL). The plant species included in the tests were in the first instance selected on the basis of reported visitation by hoverflies in general (Colley & Luna 2000; Tooker, Hauser & Hanks 2006). Subsequently, more species were added with a corolla depth close to the preliminarily critical value. The plants were grown from seed (mostly annuals) or obtained from field margins or road sides (mostly perennials).

NECTAR ACCESSIBILITY

Nectar accessibility was determined by making high-resolution digital scans (1200 dots per inch) of vertically dissected flowers and flower heads (e.g. see Fig. 1a). The depth at which the nectar is available ('effective flower depth') is measured from digital images at a 20× magnification (see Appendix S2) and was defined as the distance between the opening that is still accessible by the insect's head and the top of the nectaries. Within the composites (Asteraceae), the depths of the disc florets are measured as the length of the upper part of the corolla that is wide enough for the insect mouth parts to enter (*c.f.* Gilbert 1981; Stang *et al.* 2009), so excluding the lower narrow part that is almost fully occupied by the style (see Fig. 1a). When the corolla opening is wide enough to be entered by the head of the hoverfly, the effective flower depth is defined as the minimum distance between head (in deepest position) and nectar source (see Appendix S3).
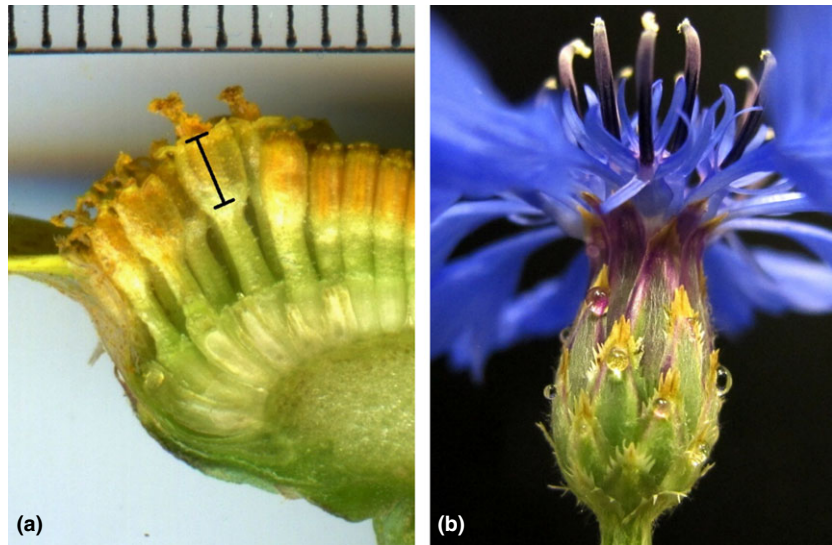
Fig. 1. (a) Cross section of flower head of *Glebionis segetum* (Asteraceae) indicating the effective depth of a tubular floret. Scale in mm. (b) Extrafloral nectar droplets on the bracts of *Centaurea cyanus* (Asteraceae).

In some species (*Phacelia tanacetifolia, Malva sylvatica*), the corolla opening is wide as well, but the nectaries are hidden in a chamber covered by fringes of hairs, presumably acting as barrier for the head and not for the proboscis. Accessibility can thus be defined by the distance between hairs and lower end of the nectar chamber. Finally, many legume flowers (Fabaceae) need to be opened first ('tripped') before the insect can reach nectar and pollen. This physical act requires insects (typically bees) much bigger than zoophagous hoverflies (Bosch *et al.* 2009). The effective depth of these flowers has therefore been measured in a closed position from the nearest entrance point. The presence of nectar droplets indicating extrafloral nectaries (EFN) is checked by direct observations under high humidity conditions in the laboratory (see Fig. 1b).

ADULT SURVIVAL TIME

Adult survival was measured in gauze cages ($48 \times 48 \times 93$ cm) under controlled conditions (23 ± 1 °C, 80% RH, 16L : 8D). Each cage contained a 12-mL bottle with wet cotton wool for water supply and a number of flowers of a single plant species. Whenever possible, full potted plants were offered inside the cage. When this was not possible, for example with perennial plants, flowers were cut and provided in a small bottle, the opening plugged with cotton wool to prevent accidental drowning by insects. Wade & Wratten (2007) showed that parasitoids performed equally well when provided with excised flowers as with intact flowering plants from the same source. Flowers obtained from field sites were put for at least 1 day in the laboratory before testing. As a positive control, a 1-M sucrose solution (342 g L^{-1}) was offered and replenished daily in a small vial featuring a yellow lid with a 1-cm round hole.

In each cage, one or two males and one or two females of *E. balteatus* were released within 24 h after emergence. The survival of the hoverflies was checked daily or every second day, during which times water was added, and if necessary, flowers were replaced. Based on the fact that in the control experiments (with water only) hoverflies survived for about 2 days, all hoverflies that died within the first day were omitted from data analysis as these were assumed to represent mortalities not related to the food source. Plant species were only included when tested ten times during at least three different years over the period 2006–2013. This resulted in an average of 25·5 hoverflies tested and 24 events (observed mortalities) per plant species.

FLOWER CHOICE IN EXPERIMENTAL SET-UP

Flower choice was tested in large square gauze cages ($1 \times 1 \times 1\cdot2$ m) in climate-controlled greenhouses. In each cage, plants or cuttings from four different species were placed in the respective corners. Each flowering species was represented by *c.* 1 dm^2 of fresh flowers. In each cage, two young inexperienced hoverflies (one male and one female) were released and observed for 30 min. For each individual, we recorded the time spent on specific flowers, on the cage, or flying. Only individuals that spent at least 8 min on flowers were included in the analysis (in total 118). Individual plant species were included in the analysis only when performing in at least ten choice tests as well as in the survival experiment. The same combination of plant species was never tested more than twice. When a hoverfly spent more than ¼ of its flower-visiting time on one species (which in practice was always 5 min or more), this was counted as a selection event for this species.

VISITATION OF FLOWER SPECIES IN THE FIELD

Flower choice under field conditions was observed on quiet and sunny days between early July and mid-August in the years 2009, 2011, 2013 and 2014. Observations were performed in 3-m wide mixed flower strips, bordering arable fields in the Hoeksche Waard (province Zuid-Holland, the Netherlands). All selected flower strips contained at least five different species that each contributed at least 5% to the flowering vegetation. An aggregation of flowers from a single species adding up to 1 m^2 was considered as one patch. Patches were observed for 5 min, recording all main groups of flower-visiting insects. The number of hoverflies was recorded to the genus level, differentiating between zoophagous hoverflies and hoverfly species with other larval feeding habits. The number of zoophagous hoverflies was expressed relative to the total number of nectar-feeding visitors, including other hoverflies as well as bees (honeybees, bumblebees and solitary

bees). By taking a relative measure, we partly corrected for variation in the number of active insects due to season, time, weather conditions, etc. Plant species were only included in the analysis when at least six patches were sampled in at least two different years with at least 24 insects recorded.

HOVERFLY DENSITIES IN MIXED FLOWER STRIPS IN THE FIELD

Flower-rich field margin strips (n = 35) in the Hoeksche Waard, partly designed to support pest natural enemies, were sampled once or twice between 15 and 22 July 2009, 2010 and 2011. On quiet and sunny days representative quadrants of 3 × 1·5 m were observed for 4 min each, to estimate the number of flower-visiting insects within different functional groups listed above. Additionally, in the same period, net samples (from 20 sweeps each) were taken and stored in 70% ethanol for full identification of hoverfly species present. Abundance of all flowering species was estimated (as percentage cover) for each quadrant. Flowers were categorized in three groups by flower depth on the basis of the hoverfly survival study. Regression analysis was used to determine the contribution of each group in predicting hoverfly numbers, and if necessary to correct for year and hour of sampling.

STATISTICS

The effect of plant species on hoverfly longevity was tested by survival analysis using Cox proportional hazards model including censored data (in case hoverflies escaped or were injured before they died naturally) (R function 'coxph'), with cage as random effect (frailty) (R Core Team 2014). Other factors, that is sex, year (six levels) and season (three levels), were sequentially added selecting the most parsimonious model (lowest AIC). The estimated Cox coefficient for each plant species was subsequently related to its nectar accessibility by linear and logistic functions, which, when significant, allow us to estimate the critical flower depth. Since nectar accessibility can also be affected by flower width, which is very different for Asteraceae compared to all other plant families, we included this as a two-level factor in the analysis. Using Solver (in MS Excel 2010) for a nonlinear least-square model fitting procedure, we selected the most parsimonious model (lowest AICc) by sequentially adding more parameters.

Laboratory and field choice data were analysed by generalized linear models (R function 'glm') for binomial data, relating them to flower depth and survival time with a logit link function. Hoverfly numbers in mixed flower strips were also analysed by generalized linear models (GLM). Since the data were overdispersed relative to a Poisson distribution, a negative binomial distribution function was selected (R function 'glm.nb'). The effect of the abundances of three functional groups of flowers, the year and time of the day were studied, selecting the model with the lowest AICc.

Results

EFFECTIVE FLOWER DEPTH

The selected plant species show clear differences in nectar accessibility, which is partly related to plant family (see Appendix S1 for data and Appendix S2 for images).

Flowers of Apiaceae all produce nectar from an unprotected disc at the basis of the style ('stylopodium') which can easily be accessed by flies. In only a few other species, the corolla is so wide that it composes no barriers to access the nectar. This most clearly applies to buckwheat *Fagopyrum esculentum* where nectar is produced in five shallow holes surrounding the stamen. Nectaries are slightly more protected in *Gypsophila elegans*, *Borago officinalis* and *Ranunculus acris*, resulting in effective flower depth values of around 1·5 mm. In *Phacelia tanacetifolia,* this value is 2·1 mm due to fringes of hairs limiting closer access.

Within the Asteraceae, nectar-producing florets come mainly in three types: narrow tubular florets on the disc of the flower head (subfamily Asteroideae), wider conical florets with long lobes (Carduoideae) and ligulate florets (Cichorioideae). In the first two types, nectar accessibility is characterized by the depth of the tubular part of the corolla (see Appendix S1). In the third type, the accessibility is likely limited by the close packing of the florets. The depth of the tubular florets in our study varies from 0·9 mm for *Matricaria recutita* to 4·5 mm for *Cosmos bipinnatus*. Among the selected plant species, extrafloral nectaries are present in *Vicia sativa*, *Centaurea cyanus* and *Helianthus annuus*. In the first species, they are located on the stipules. In the two Asteraceae species, they are inconspicuously located on the involucre of the flower head (see Fig. 1b). Pollen was easily accessible for hoverflies in all flower species with the exception of the Fabaceae.

ADULT SURVIVAL TIME

In the absence of any food (water only), both sexes of the adult hoverflies survive for 2·0 (±0·2) days. In the presence of a 1-M sucrose solution, survival time was on average 11·3 days. With flowers, the average longevity varies between 2·3 and 17·1 days (see Appendix S1). Survival is clearly affected by plant species (Cox PH, d.f. = 31, X^2 = 448, $P < 10^{-15}$), as well as by sex (X^2 = 11·9, P = 0·0006), year and season (see Table S4.1). The latter two effects suggest variability in plant and insect material between years and seasons. Most plant species allow the hoverflies to live longer than on water only (Cox PH, $X^2 > 5$, $P < 0·05$) with the exception of *Calendula officinalis, Cosmos bipinnatus, Crepis capillaris* and *Cichorium intybus* (see Table S4.2). For ten plant species, survival is not different from 1-M sucrose: *Pastinaca sativa, Heracleum sphondylium, Ammi majus, Daucus carota, Borago officinalis, Fagopyrum esculentum, Achillea millefolium, Gypsophila elegans*, as well as the two species with EFNs: *Centaurea cyanus* and *Vicia sativa*. With all other plant species, the hoverflies have shorter lives than in the positive control (Cox PH, $X^2 > 3·9$, $P < 0·05$).

Survival time is to some extent related to plant family (see Fig. 2 and Appendix S1). All six umbelliferous species (Apiaceae) yield survival times of more than 8 days
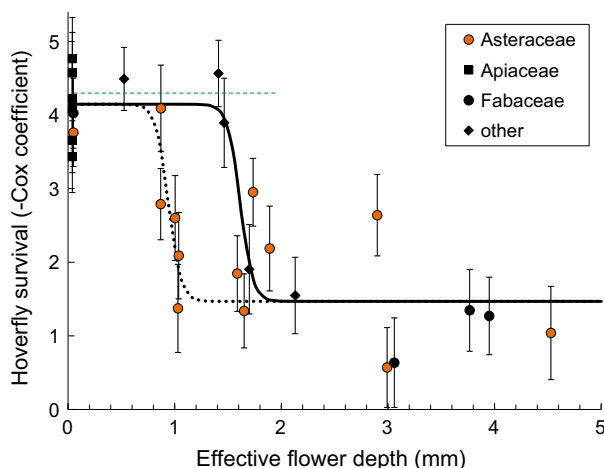
Fig. 2. Survival of *Episyrphus balteatus* (species-specific coefficient of Cox PH model, (negative value ± C.I) in relation to the effective flower depth (EFD) of the plant species provided. For plants with extrafloral nectar, EFD was put to zero. Symbols indicate plant family. Horizontal line represents positive control (sucrose: 4·30 ± 0·45). Curved lines represent parsimonious logistic model, with different critical flower depth values for Asteraceae (0·93 mm, dotted line) compared to plants from other families (1·63 mm, drawn line). Other model parameters are identical for both groups (max = 4·14, min = 1·49, slope = −19·3 mm^{-1}, see Table S4.3).

(Cox coefficient (CC) > 3·4). On the other hand, flowers of the legume family (Fabaceae) only allow female hoverflies to live for 3·4 days on average (CC < 1·5). *Vicia faba,* having extrafloral nectaries (EFN), was the clear exception (CC = 4·0). Among the 15 species tested within the composite family (Asteraceae), however, large differences in survival time are observed.

The differences between species (represented by the Cox coefficient) can, as expected, largely be explained by the accessibility of their nectar. A logistic function describes this relationship better than a linear one (Table S4.3). When taking into account differences between composite and noncomposite flowers, the most parsimonious model is a full logistic model where only the (shift) parameter for critical flower depth is different between the two taxonomic groups (see Fig. 2 and Table S4.3). This indicates that for composites, the critical floret depth is 0·93 mm, while for other flowers the critical depth is around 1·6 mm. Some composites with florets between 1 and 2 mm deep do not completely fit with the model, showing intermediate CC values. However, with flower depths beyond 2 mm survival is always low (CC < 1·6), except for *Jacobaea vulgaris* or plants with EFNs (*Centaurea cyanus, Vicia sativa*). The two composite species with only ligulate florets (Cichorioideae), for which the flower depth is not well defined, also yield CC values indicative of deeper flowers.

FLOWER CHOICE TEST

Based on all replicates, the average chance for one of the four plant species in our set-up to be selected by the hover-fly was 0·321. This figure is higher than 0·25, since in 27% of the trials hoverflies select two species to feed on. Four species are selected significantly more often than this average: *Fagopyrum esculentum, Coriandrum sativum, Foeniculum vulgare* and *Gypsophila elegans* (GLM, $z > 2$, $P < 0.05$). Flower choice by flower-inexperienced hoverflies is positively correlated with the mean longevity in the non-choice tests (Fig. 3b, GLM, $z = 3.92$, $P < 0.0001$, Table S4.4). Flower choice is also negatively related to flower depth (Fig. 3a, GLM, $z = -3.90$, $P < 0.0001$). Figure 3a shows a triangular cloud of data points, indicating that for plants with deep corollas (>2 mm) the preference is always low, whereas for plants with more shallow corollas the preference can vary, depending on other features.

VISITATION OF FLOWER SPECIES IN THE FIELD

Direct observations (in on average nine patches per species) were performed on 18 flowering species in field margin strips. Among the 700 pollinating insects recorded, 57·1% were zoophagous hoverflies, 19% other hoverflies and 24% bees. *Episyrphus balteatus* made up 33% of the zoophagous hoverflies, whereas *Sphaerophoria* spp. (32%) were almost equally numerous. The proportion of zoophagous hoverflies is significantly affected by plant species, with *Gypsophila elegans, Matricaria recutita* and five other species showing a higher than average proportion (>70%, $z > 2.1$, $P < 0.05$, Table S4.5), and *Phacelia tanacetifolia, Centaurea jacea* and four other species a lower proportion (<30%, $z < -2.6$, $P < 0.01$). The proportion of zoophagous hoverflies on each plant species is positively related to the realized survival time (see Fig. 3d, GLM, $z = 8.2$, $P < 0.001$, Table S4.5) and declines with increasing flower depth (see Fig. 3c, GLM, $z = -8.7$, $P < 0.001$). This indicates that the trend in flower choice observed for *E. balteatus* in the laboratory can be extended to the guild of zoophagous hoverflies as observed in the field. Similarly to the survival of *E. balteatus,* the proportion of zoophagous hoverflies visiting flowers shows a stepwise decline when corolla depth increases beyond c. 1·6 mm.

HOVERFLY DENSITIES IN MIXED FLOWER STRIPS IN THE FIELD

In the field margin strips designed to support pest natural enemies, zoophagous hoverflies make up the majority of the flower-visiting insects (on average 8·1 per observation against 1·6 for saprophagous hoverflies and 2·5 for bees). Among zoophagous hoverflies, *Episyrphus* is the most commonly observed genus (making up 42%), followed by *Sphaerophoria* (16%), the *Melanostoma/Platycheirus* morphs (13%), *Eupeodes* (13%) and *Syrphus* (9%) (see Appendix S5 for a full species list). The number of zoo-phagous hoverflies is significantly affected by the hour of the day as well as by the coverage of flowers, but not by the year of sampling (see Fig. 4a and Table S4.6). Based
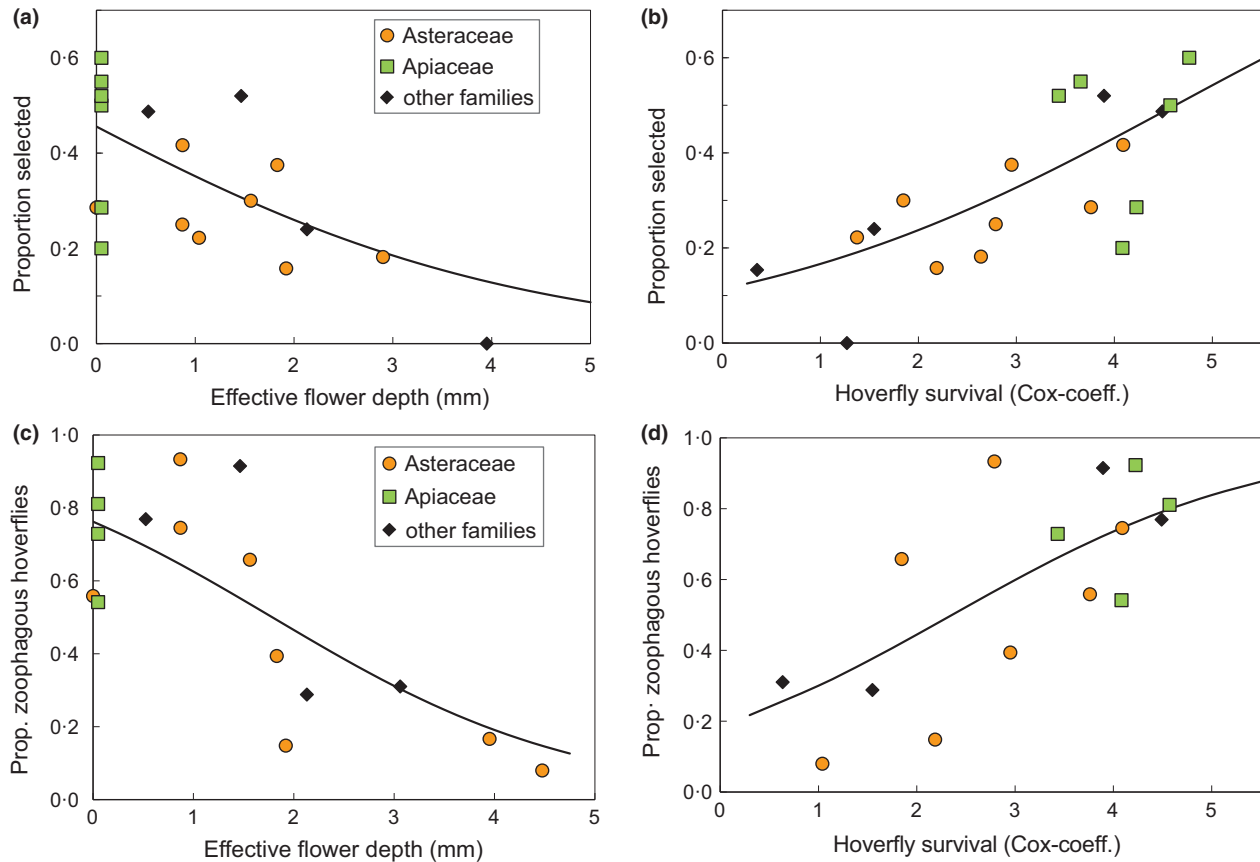
Fig. 3. Flower choice of *Episyrphus balteatus* in experimental set-up (a and b) and proportion of zoophagous hoverflies within pollinating insects (bees and hoverflies) in field (c and d) plotted against hoverfly survival (*n* = 19 and 15) and effective flower depth for each flower species (*n* = 18 and 17). Symbols indicate plant family. Lines indicate fitted GLM (logit-model, |*z*| > 4, *P* < 0·0001 in all cases).

on the adult survival test, the flowers could be subdivided into three groups with respect to nectar accessibility: group A: well-accessible nectar (depth < 1·0 mm), group B: composites with floret depth between 1·0 and 2·0 mm) and group C: inaccessible nectar (depth > 2 mm) (for species see Appendix S1). The coverage of flowers with deeper corollas (group C) has a negative coefficient and does not contribute to predicting hoverfly numbers (Table S4.6). The coefficients of groups A and B are almost equal, which means that the unweighted sum of these flowers can explain most of the variation in hoverfly numbers after correction for the hour of sampling (GLM-nb, *z* > 3·2, *P* < 0·0012), as illustrated by Fig. 4. These results indicate that zoophagous hoverflies are attracted to vegetation based on the amount of flowers with nectar accessibility at 2 mm or less, and that the presence of deeper flowers does not play a role in this attraction. For comparison, the number of bees and saprophagous hoverflies is most strongly predicted by the amount of flowers from group C as well as B (Table S4.7).

Discussion

This study shows that the suitability of flowers as a food source for the hoverfly *E. balteatus* –based of adult fitness

– is largely determined by the accessibility of the nectar, quantified as effective flower depth. It also shows that the choice for specific flower species is strongly related to its suitability in terms of nectar accessibility, when flower choice is assessed in the field as well as under controlled laboratory conditions. Finally, it shows that the number of hoverflies in mixed field margin strips can largely be explained by the abundance of flowers with accessible nectar.

Most studies on flower–insect interactions (including hundreds of pollination studies that are published each year) are based on foraging activities, either directly observed in the field (Olesen *et al.* 2007; Stang *et al.* 2009) or inferred from the identity of pollen found in or on the insect (e.g. Bosch *et al.* 2009). One of the patterns that emerges from these studies is that pollinators on average visit flowers that are slightly less deep than their proboscis size (Stang *et al.* 2009; see also Gilbert 1981). Our study is likely the first to measure suitability of flowers for a pollinating insects based on a fitness measure (survival time) for a sufficient range of plant species to detect significant patterns. The study gives strong evidence that fitness, as well as flower choice, is resulting from the accessibility of the nectar as defined by the morphology of flowers.
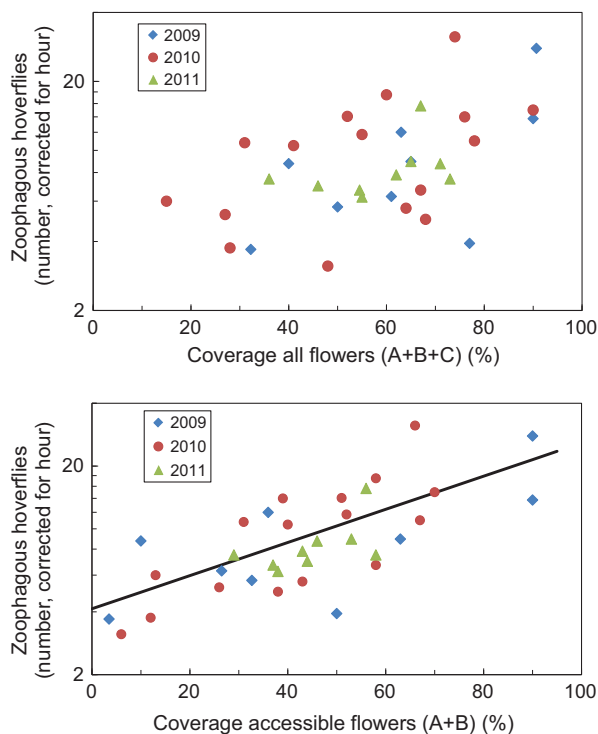
Fig. 4. Number of zoophagous hoverflies (log scale, corrected for time by $0.11\ h^{-1}$) in mixed field margin strips in mid-July 2009, 2010 and 2011, in relation to (a) total coverage of flowers and (b) coverage of flowers with nectar accessible within 2 mm (group A and B, see Table S4.6). Solid line represents regression line for all years combined.

The proboscis of *E. balteatus* is about 2·1 mm for the female and 2·6 mm for the male (P.C.J. Van Rijn, personal observation). For noncomposite flowers, the critical flower depth appears to be about 1·6 mm deep (Fig. 2) which is, as was expected, just below the length of the proboscis. Within composites (Asteraceae), however, the critical flower depth appears to be even less than 1·0 mm (Fig. 2). A possible explanation is that in composite flowers, the pistil in the centre of the narrow tubular floret and the staminal tube surrounding it (see Fig. 1a) can prevent the hoverfly reaching further down the floret.

Recently, two other studies focussed on the fitness of *E. balteatus* in relation to (a limited range of) flower species. Pinheiro *et al.* (2013) found increased longevity with two out of five flower species: *Anchusa azurea* and *Malva sylvestris*. Interestingly, they found increased fructose levels in hoverflies that had been provided these two floral resources, and only in males. They were, however, not able to explain why these two species were more suitable than the others. In the other study, Laubertie, Wratten & Hemptinne (2012) compared the effect of six different flower species on hoverfly longevity and fecundity. To trigger oviposition, they provided bean plants with aphids. The much higher longevity values compared to our study indicate that in their study, aphid honeydew was used as an additional carbohydrate source (see also Van Rijn, Kooijman & Wäckers 2013). The availability of honeydew

also explains why in the presence of *Phacelia tanacetifolia* the hoverflies do not feed on nectar, but still survived for 30 days on average (while only 4 days in our study). This study is therefore mainly indicative of the value of flowers as pollen sources (when a sugar source is already available), and not on their value as nectar source.

For nonpollinating insects, a much wider range of studies of fitness effects are available. This includes many hymenopteran parasitoids (see Jervis *et al.* 1993; Wäckers, Van Rijn & Bruin 2005; Wäckers & Van Rijn 2012 for reviews). Studies that included eight or more plant species generally (with Kehrli & Bacher (2008) as the exception) indicated some relationship between parasitoid fitness and flower morphology, either corolla opening diameter (Idris & Grafius 1995) or flower depth (Orr & Pleasants 1996; Wäckers, Björnsen & Dorn 1996), although statistical tests were either not significant or not included. In addition to flower morphology, nectar composition (sugar and nonsugar components) is also shown to affect parasitoid fitness (Wäckers 2001), and sugar suitability appears to correlate with innate sugar acceptance (Wäckers 1999; Makatiani *et al.* 2014). However, the contribution of nectar sugar composition in explaining the suitability of flowers as a food source is less conclusive (Vattala *et al.* 2006; Pinheiro *et al.* 2013).

Flower choice measures, both in the laboratory and in the field, are both positively related with realized fitness (adult survival). Flower choice appears to be affected by nectar accessibility in quite a similar way as survival, in the sense that their values are clearly higher for flowers that are less than 2-mm deep. The importance of nectar accessibility seemingly contrasts with the seminal results by Gilbert (1981, 1985a) that *E. balteatus* and related short-tongued hoverflies spend most of their time feeding on pollen, whereas only longer tongued hoverflies are mainly feeding on nectar (see also Reemer *et al.* 2009). In the light of our results, a possible explanation for Gilbert's results could be that the gardens he used for his observations had limited flowers with accessible nectar. The hoverflies observed may have had little alternative than to use the flowers as a source of pollen and obtain their sugars elsewhere, for example from flowering weeds or honeydew from aphids (Van Rijn, Kooijman & Wäckers 2013). For hoverflies with longer tongues, more flowers will have been available as a nectar source, which has resulted in a higher incidence of nectar feeding.

The monitoring of mixed flower strips suggests that at this level, the abundance of zoophagous hoverflies is not only largely determined by the availability of nectar, it is also affected by the hour of sampling (see Gilbert 1985b). When subdividing flowers into three groups with respect to nectar accessibility, hoverfly numbers show the highest correlation with the amount of flowers from group A (featuring fully accessible nectar). By adding flowers from group B (Asteraceae with florets between 1 and 2 mm deep), the explained variation further increases (from 45 to 60%, Table S4.6). Group C of plants with inaccessible

nectar (>2 mm deep) does not show any contribution in attracting these hoverflies, even though most of the flowers in this group still have well-exposed pollen (Appendix S1). The fact that group B still explains some of the variation in hoverfly numbers may have two reasons: (i) some of these flowers still attract some hoverflies in choice experiments (Fig 3a,c) and still have some benefit to *E. balteatus*, as they slightly enhance survival relative to water only (Fig. 2) and (ii) in the field other zoophagous hoverflies also are present (see Appendix S6), with some featuring slightly longer proboscises (Gilbert 1985a).

The results at all five levels of observation show that apart from most Apiaceae, only a few flower species are suited to support zoophagous hoverflies; especially, within Asteraceae, which are widely applied in fields margins, most species have tubular florets that are too deep (*Achillea* and *Matricaria* being exceptions). Those species that have only ligulate florets (Cichorioideae) seem to be unsuitable as well (see also Pinheiro *et al.* 2013), although more species would need to be tested. This result urges us to re-evaluate various experiments (reviewed by Marshall & Moonen (2002) and Bianchi, Booij & Tscharntke (2006)) in which flower mixtures have been used to enhance biological pest control, without underpinning evidence that the flowers used are actually suitable for the target natural enemies such as zoophagous hoverflies. When landscape management aims to support natural pest control, we suggest that vegetation in and around the agricultural fields should specifically contain plants with accessible nectar, as defined in this paper, or with EFNs. At the same time, care should be taken that the plants are not supportive for pests or diseases as well (Géneau *et al.* 2012). Optimal field margin strips can most easily be created by sowing annual seed mixtures (Balzan & Moonen 2014; Tschumi *et al.* 2015), containing, for example *Fagopyrum esculentum, Centaurea cyanus*, annual Apiaceae or other species with open or shallow flowers. Also perennial field margins can be created with the right flowers (such as *Achillea millefolium* and various Apiaceae), but competition and succession make them more difficult to design and to maintain (Van Rijn 2014). Even (semi-) natural vegetation could be preserved and managed for this purpose (Fiedler, Landis & Wratten 2008). Since several weeds have shallow flowers, for example *Matricaria recutita*, *Cirsium arvense* and *Persicaria maculosa*, selective weeding (c.f. Barberi *et al.* 2010), could be an alternative or additional strategy for providing zoophagous hoverflies with the resources they need.

Acknowledgements

Data accessibility

The data set supporting this article is deposited in Dryad Digital Repository doi: 10.5061/dryad.d37n0 (van Rijn & Wäckers 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Summary of data from all plant-specific tests: flower depth, adult survival, flower choice, and flower visitation rate.

Appendix S2. Flower dissection scans of 27 species showing effective flower depth.

Appendix S3. Defining effective flower depth, special case.

Appendix S4. Results from statistical tests.

Appendix S5. Species composition of hoverflies (Syrphidae) in field margin strips.