Journal of Apicultural Research and Bee World 48(3): 149-155 (2009)
DOI 10.3896/IBRA.1.48.3.01

ORIGINAL RESEARCH ARTICLE

Larval food composition and food plants of the solitary bee *Colletes halophilus* (Hymenoptera: Colletidae)

Marinus J. Sommeijer[1*], Eveline F. Rooijakkers[1], Chiel Jacobusse[2] and Jacob D. Kerkvliet[1]

[1]Behavioural Biology, Utrecht University, P.O. Box 80.086, 3508 TB, Utrecht, the Netherlands.
[2]Stichting Het Zeeuwse Landschap, the Netherlands.

Received 10 July 2008, accepted subject to revision 24 January 2009, accepted for publication 2 April 2009.

*Corresponding author: Email: m.j.sommeijer@uu.nl

Summary

The pollen component of the larval food of *Colletes halophilus,* studied in four nature reserves in the Netherlands, was strongly dominated by *Aster tripolium*. The liquid component of the larval food contained high concentrations of sugars (glucose and fructose), far more than could be supplied by *A. tripolium* pollen present in the food. This indicates that in addition to pollen, considerable amounts of nectar were collected from this plant. The larval food showed hydrogen peroxide production. We did not, however, find this in pollen collected directly from *A. tripolium* flowers. We conclude that females of *C. halophilus* produce the enzyme glucose oxidase and add this to larval food. This is the first such finding for solitary bees. The apparent dependency of *C. halophilus* on *A. tripolium* has implications for the conservation management of this solitary bee.

Composición del alimento larval y plantas alimenticias de la abeja solitaria *Colletes halophilus* (Hymenoptera: Colletidae)

Resumen

Se estudió la composición polínica del alimento larval de *Colletes halophilus*, en cuatro reservas naturales de los Países Bajos, encontrándose una fuerte dominancia de *Aster tripolium*. El líquido que compone el alimento larval contiene altas concentraciones de azúcares (glucosa y fructosa), mucho más de lo que podría ser suministrado por el polen de *A. tripolium* presente en el alimento. Esto indica que además de polen, una considerable cantidad de néctar fue recolectado de ésta planta. Además, el alimento larval demostró producción de peróxido de hidrógeno. Sin embargo, este componente no lo encontramos en el polen recolectado directamente de las flores de *A. tripolium*. Concluimos que las hembras de *C. halophilus* producen la enzima glucosa oxidasa y la adicionan al alimento larval, siendo éste el primer hallazgo en abejas solitarias. La aparente dependencia de *C. halophilus* por *A. tripolium* tiene implicaciones para el manejo y conservación de esta abeja solitaria.

Keywords: food plants, palynological analysis, Colletidae

Introduction

The solitary bee *Colletes halophilus* Verhoeff 1943*,* (Apidae, Colletinae) occurs along the coasts of England, Ireland, Germany, the Netherlands, Belgium, and Atlantic France. In France, it has recently been found as far south as Hossegor in Aquitaine (Genould and Dittlo, 2007). Coastal dunes and salt marshes are the typical habitat of this species (Westrich, 1990).

Verhoeff described the species as *C. succincta halophila* in 1943. At that time, it was considered to be oligolectic on *Calluna* (heather). Apart from the larger body size of the specimens separated by Verhoeff, it was notable that these were collected in areas where *Calluna* was not present. In 1937, Van Lith had already reported that in certain areas *C. succinctus* was observed visiting *Aster tripolium* (sea aster) in large numbers. It was concluded that the newly described *C. halophilus,* together with some other (sub-) species such

as *C. succinctus* s.str. and *C. hederae*, belong to the *C. succinctus* group. Recently, Kuhlmann *et al*. (2007) clarified the status of these and other related subgroups. Obviously, food plant relations of the respective *Colletes* species are of importance in relation to speciation within this group (Westrich, 1990; Kuhlmann, personal communication).

One of the special features of most colletid bees is the fact that their larval food is liquid instead of being solid (Almeida, 2008; Michener, 2000). The nature of the liquid component of the larval food is still unknown. Michener (2000) called it “nectar”, while O’Toole and Raw (1999) referred to it as being “honey” (which is in fact a hive product made through the processing of nectar by eusocial bees such as honey bees, Apini, and stingless bees, Meliponini).

C. halophilus builds nests in the soil, sometimes in large aggregations. In the Netherlands aggregations occur in the province of Zeeland in coastal areas. e.g. in nature reserves managed by “Stichting Het Zeeuwse Landschap”. These aggregations are located in tidal areas with an abundance of *A. tripolium*. One of the largest aggregation sites in the Netherlands is located in “Het Verdronken Land van Saeftinghe”. In recent years the *A. tripolium* population has been declining there as well as all over the Dutch Delta area (Lensink *et al*., 2008). Improved knowledge on the foraging behaviour of *C. halophilus* and its food plant selection thus has relevance for conservation.

In this paper we report on analyses of the larval food of *C. halophilus*. Through the application of a palynological method, we first answer the question whether populations of *C. halophilus* forage for pollen on plants other than *Aster tripolium,* if available. We further analyse whether individual females show differences in their food plant selection, and finally we study the nature of the liquid component of the larval food.

Materials and methods

Sites and Sampling

For the study of population level foraging behaviour of *C. halophilus,* three sites were sampled in 2005 and one additional site in 2006. These sites had to some extent different food plant availability. The sites in 2005 were the nature reserves ‘Het Verdronken Land van Saeftinghe’, ‘Hooge Platen’ and ‘Paulina Schor’ (Fig. 1). The food plant complex at Saeftinghe was dominated by *Aster tripolium* with some *Cirsium arvense, Glaux maritima,* and *Leontodon sp*. Hooge Platen had mainly *A. tripolium* but also some *Diplotaxis tenuifolia*, *Sonchus arvensis*, *Cakile maritima, Glaux maritima, Leontodon autumnalis, Limonium vulgare* and *Cirsium arvense* as potential food plants. The flora of Paulina Schor had other flowering plants, including *Sonchus arvensis*, *Cirsium arvense*, *Diplotaxis tenuifolia, Cakile maritima* and *Lapsana communis*, apart from the most common *A. tripolium*.

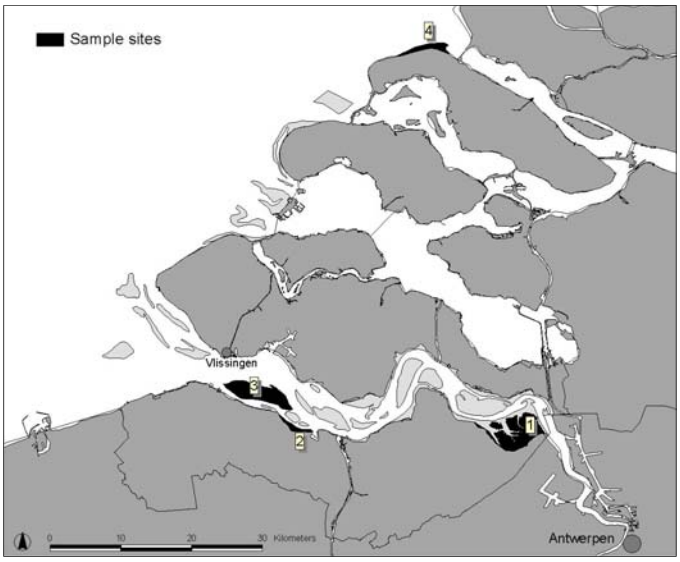

Fig. 1. Map of the province of Zeeland, the Netherlands, showing the four sampling sites: 1. Het Verdronken Land van Saeftinghe; 2. Paulina Schor; 3. Hooge Platen; 4. Kwade Hoek.

Concerning the abundance of available food plants, we estimated (considering a flight range of 600 m.) that in Saeftinghe *A. tripolium* constituted 80% of the total available food plants for *C. halophilus*. For Paulina Schor this was 50%, and for Hooge Platen 70%. For Kwade Hoek (additionally sampled in 2006), where *Leontodon saxatile, Crepis capillaris, Pulicaria dysenterica, Odontites verna, Eupatorium cannabinum, Senecio jacobaea* and *Senecio inaequidens* were also present, this was estimated to be 70%.

In 2005, for an analysis of seasonal effects, three sites were visited at three moments during the reproductive period: at the beginning; in the middle; and at the end of the flight season. The successive sampling at these sites was always done within a period of a few days (Table 1). During each visit, 55 to 75 brood cells were excavated (at Paulina Schor, only 25 to 35 cells were sampled because the *C. halophilus* population there was significantly smaller). Only brood cells that contained eggs or just-hatched larvae were used. At the end of the flight period, fewer cells with eggs and just-hatched eggs were available. Collected cells were kept frozen for analysis in the laboratory.

In 2006, we sampled brood cells at Saeftinge, Hooge Platen and also at "Kwade Hoek". For this palynological analysis the larval food of a number of cells from each of these sites was pooled.

Between 14 September 2005 and 10 October 2005 we studied the individual foraging of *C. halophilus* females at Saeftinghe. For this, we filled the tunnels of nests of active females with plaster of Paris which allowed us to trace the individual cells.

Four samples of larval food and samples of fresh pollen from *A. tripolium* were collected to determine reference values for water-insoluble dry matter (gravimetric), water-soluble matter expressed as sucrose content (by refraction measurement) and water content (by

Table 1. Results of the palynological analysis of the larval food samples collected in 2005 and 2006 at the nesting sites of *Colletes halophilus*. Indicated is the relative contribution of pollen of *Aster tripolium*, *Sonchus arvensis* and other plants. In 2005 three sites were visited on three occasions during the reproductive period. These were at the beginning, in the middle and at the end of the flight season. The successive sampling at the three sites was always done within a period of a few days.

Site	Sampling	Date	No of cells in analysis	Percentage pollen		
				Aster tripolium	*Sonchus arvensis*	Misc. pollen
Saeftinghe	1	5 September 2005	38	94.8	4.1	1.1
Hooge Platen	1	7 September 2005	38	96.2	0.6	3.2
Paulina Schor	1	8 September 2005	12	92.6	3.0	4.4
Saeftinghe	2	19 September 2005	45	99.0	0.0	1.0
Hooge Platen	2	21 September 2005	50	95.8	0.5	3.7
Paulina Schor	2	20 September 2005	19	95.6	0.8	3.6
Saeftinghe	3	3 October 2005	7	97.7	0.8	1.5
Hooge Platen	3	6 October 2005	14	94.1	1.1	4.8
Paulina Schor	3	4 October 2005	4	92.0	0.8	7.2
	Sample					
Saeftinghe	1	9 September 2006	8	96.2	0.7	3.1
Saeftinghe	2	14 September 2006	12	98.2	0.5	1.3
Hooge Platen	4	14 September 2006	18	96.3	0.2	3.5
Kwade Hoek	5	21 September 2006	15	96.8	0.2	3.0
Saeftinghe	3	22 September 2006	30	97.9	0.3	1.8
Average Value (± SD)				95.94 (± 2.04)	1.05 (± 1.16)	3.08 (±1.71)

difference). In order to collect the fresh pollen, large bunches of cut flowering plants were placed in vases filled with water and these were placed on a clean surface.

Palynological analysis

From each sample, the larval food of the brood cells was suspended in water and thoroughly mixed. From this mixture about 50 µl was applied in duplicate to a microscope slide on a surface of 1.8 cm x 1.8cm. The applied suspension was dried at about 40˚C and treated with Kaiser's glycerine-gelatine, with and without basic fuchsin (Von der Ohe *et al.*, 2004). From each sample, at least 500 pollen grains were counted and identified.

Chemical analysis of larval food, pollen and nectar

Gravimetric analysis and refraction measurements

For each sample of larval food (taken from 4-6 pooled cells) about 1 g (exactly weighted - S g), was put into a centrifuge tube of known weight. In total this resulted in nine samples for 2005 and four for 2006. To each of the thirteen tubes about 0.5 g (exactly weighted - W g) demineralised water was added and mixed with the food. The samples were then rotated in a centrifuge for 20 min. at *c.* 2000 rpm. The supernatant in each tube was used for refraction measurement. The refraction value stands for the water-soluble matter (water extract), which mainly contains carbohydrates. Results are expressed as % sucrose value (in g / 100 g).

The remaining sample in the tubes was washed with demineralised water and centrifuged for 10 min. at *c.* 2000 rpm. This procedure was repeated two more times. The sample was dried inside the tubes at 37˚C until stable dry weight. The water-insoluble dry matter, D (g) is calculated as the difference between the weight of the dried tube and the initial weight of the centrifuge tube.

For reference values, the same procedure was carried out with fresh *A. tripolium* pollen. As more water is needed in this case to obtain enough liquid phase for refraction measurement, 250 mg pollen was used, instead of 1 g of larval food, and 0.75 g demineralised water, instead of 0.5 g.

Hydrogen peroxide measurement

Larval food and fresh *A. tripolium* pollen sampled in 2006, were screened for accumulation of hydrogen peroxide (H_2O_2). This is reported to originate from the enzyme glucose oxidase which is present in the hypopharyngeal glands of the honey bee (Gauhe, 1941), in honey bee honey (White *et al.*, 1962; 1963) and in honey from other bee species like *Apis cerana* and *Melipona* spp. (Shrestha, 1997; de Bruijn and Sommeijer, 1997). As far as known, accumulation of H_2O_2 does not occur in pollen (Weston, 2000). The screening method was carried out according to the method for honey described by Kerkvliet (1996). Demineralised water was added to 1.0 g larval food and, for control purposes, to *A. tripolium* pollen directly from the plant (for larval food 1.5 ml; for *A. tripolium* pollen 4.0 ml plus 1.0 g glucose). The suspension was incubated at 20°C for 10 to 48 hours.

Table 2. Results of the palynological analysis of individual brood cells from nests of female *Colletes halophilus* collected at Seaftinghe. Shown are the relative contributions of *Aster tripolium*, *Sonchus arvensis* and other plants.

Nest	Cell	Percentage pollen		
		Aster trapezium	***Sonchus arvensis***	**Misc. Pollen**
1	1	95.7	0	4.3
2	1	99.4	0	0.6
2	2	100	0	0
2	3	100	0	0
6	1	97.1	0.2	2.7
7	1	98.7	1.3	0
7	2	99.2	0	0.8
8	1	99.8	0	0.2
9	1	99.8	0	0.2
9	2	100	0	0
10	1	99.4	0.2	0.4
10	2	99.4	0	0.6
11	1	97.7	1.7	0.6
12	1	100	0	0
12	2	99.4	0.2	0.4
12	3	99.8	0	0.2
12	4	99.2	0	0.8
13	1	99	0.6	0.4
13	2	99.4	0.4	0.2
13	3	99.8	0.2	0
13	4	98.3	1.1	0.6

After various time intervals H_2O_2 production was measured with Merckoquant peroxide test strips (Merck art. Nr. 1.10011.0001: range 0.5-25 mg / l H_2O_2).

Sugar spectrum of the extract

In order to determine the type of sugars present in the water-soluble matter of the larval food samples of 2006 and in the *A. tripolium* reference pollen, the sugar spectrum was determined after silylation of the sugars (Bogdanov *et al.*, 1997) by a gas chromatographic method with flame ionisation detector.

Calculation of the results

The % water-insoluble dry matter of the larval food and pollen is calculated by dividing the amount of dry matter D (g) by the amount of sample S (g), multiplied by 100, using the formula: (D/S) x 100%. The % water-soluble matter (extract) of the larval food and pollen is expressed as % sucrose and calculated by multiplying the refraction value R by the dilution factor, using the formula:-

$$\frac{W + (S - D)}{S} \bullet R$$

in which: W = the amount of water (g) added to the sample; S = sample weight (g); D = weight of dry matter (g) of the sample; R = refraction value of the liquid layer of the diluted sample, expressed as g sucrose / 100 g. The % moisture (in g / 100 g) of the larval food and pollen is found by subtracting the sum of the above percentage from 100%. The percentage of *A. tripolium* pollen in the samples of larval food is calculated by dividing the water-insoluble dry matter of the samples by that of the reference *A. tripolium* pollen and multiplying the result by 100 (P_{At} %). The remaining part (100 % - P_{At} %) consists of water and water soluble matter (sugars) from the nectar of the plant.

Also calculated are the % of water and extract which correspond to the % *A. tripolium* pollen in the samples of larval food. The surplus % of water-soluble matter and moisture belong to the liquid part of the larval food. By dividing the surplus % of water-soluble matter by the sum of the surplus % water-soluble matter and moisture and multiplying the results by 100, the % of sugars in the liquid part is found.

Results

Pollen types

All samples contained large amounts of *A. tripolium* pollen, size 30 µm. Minor amounts of other Asteraceae were present, especially three species of the T -(*Taraxacum*)-type: *Sonchus arvensis* (size 40-42 µm); *Leontodon* sp. (size 28-30 µm), and *Hieracium* sp. or *Lapsana* sp. (size 25 µm). Unfortunately it could not be established which of these two species was present.

There were two types of *Brassicaceae*. One was of size 28 µm and strongly reticulate. Considering both structure and size of the pollen and flowering-time of the plant, the origin of this pollen can only have been *Diplotaxis tenuifolia*. The other pollen type was 25 µm in size and also strongly reticulate, and was probably *Cakile maritima*. In addition to these, we found minimal amounts of: *Glaux maritima; Limonium vulgare; Eupatorium* sp.; *Plantago* sp.; Gramineae and small amounts of other pollen.

Food plant selection

In all samples, *A. tripolium* pollen contributed most to the pollen content of the larval food. In all of the nine cases in 2005 at least 92% was *A. tripolium* pollen, and in five cases it was greater than 95% (Table 1).

The sites in 2005 did not differ in the proportions in which *A. tripolium* was collected (Two-way ANOVA between subjects: $F(2, 0) = 4.607$; $P = 0.092$; eta squared = 0.697) although there was a trend that *A. tripolium* was less dominant in Paulina Schor than in

Table 3. Results of the chemical analysis of larval food of *Colletes halophilus*, and *Aster tripolium* pollen. From Saeftinghe only in 2005, and from Saeftinghe, Hooge Platen and Kwade Hoek in 2006. Hydrogen peroxide production was measured in 2006 from larval food from Saeftinghe and Hooge Platen and in pollen directly collected from flowering plants in pots.

No.	Year	Site / Material	Hydrogen peroxide production	Water- in-soluble dry matter (%)	Water-soluble matter (extract) (%)	Moisture (%)	Pollen in larval food (%)	Sugar in nectar part of larval food (%)
1	2005	Saeftinghe		23.9	46.3	29.8	53.1	48.6
2	2005	Saeftinghe		22.5	46.0	31.5	50.0	47.8
3	2005	Saeftinghe		24.7	44.0	31.3	54.9	43.7
4	2005	Saeftinghe		20.5	43.3	36.2	45.6	42.5
5	2005	Saeftinghe		20.5	44.0	35.5	45.6	43.0
6	2005	Saeftinghe		28.7	43.5	27.8	63.8	42.9
7	2005	Saeftinghe		26.4	44.7	28.9	58.9	45.5
8	2005	Saeftinghe		28.7	42.9	28.4	63.8	40.6
9	2005	Saeftinghe		29.1	43.7	27.2	64.7	42.8
2	2006	Saeftinghe		23.6	49.3	27.1	52.4	54.9
3	2006	Saeftinghe	positive	22.5	51.6	25.9	50.0	59.0
4	2006	Hooge Platen	positive	29.6	34.1	36.3	65.8	14.6
5	2006	Kwade Hoek		33.3	37.7	29.0	74.0	19.2
7	2006	*Aster tripolium*	negative	45.0	44.2	10.8		

Saeftinghe. During the whole season the same proportions of *A. tripolium* were collected (Two-way ANOVA between subjects: $F(2, 0) = 1.977$; $P = 0.253$; eta squared = 0.497).

Sonchus arvensis pollen contributed less than 4.1% of the pollen content, in six cases being even less than 1.2%. There were no differences between the different sites ($F(2, 0) = 0.532$; $P = 0.624$; eta squared = 0.210) or during the season ($F(2, 0) = 2.990$; $P = 0.161$; eta squared = 0.599).

All of the brood cells of individual *C. halophilus* females contained at least 95.7% *A. tripolium* pollen (Table 2). On average *A. tripolium* pollen contributed 99.1% (SD = 1.1), while on average only 0.3% (SD = 0.5) *S. arvensis* was present. On average, other pollen types made up only 0.6% (SD = 1.0) of the total.

Chemical analysis of larval food and pollen

The results of the analyses of larval food and of the fresh *A. tripolium* pollen are presented in Table 3. For the nine larval food samples from 2005, the weight of the contents of a known number of larval food cells was determined. A brood cell contained on average 88 mg (± 22) pollen and 69 mg (± 7) liquid component.

The ratio of pollen: liquid component in the larval food calculated for all 13 larval food samples was 1.44 (± 0.57). By omitting the two extreme values from 2006 (sample 4 from Hooge Platen and sample 5 from Kwade Hoek) the ratio pollen: liquid was 1.26 (± 0.37).

The average sugar concentration of the liquid component of the 13 samples was 41.9% (± 12.3) or, by omitting the above two extreme values, 46.5% (± 5.8), expressed as g per 100g sucrose. Additional gas chromatographic analysis of the different sugars in this liquid part and in the *A. tripolium* reference pollen revealed that mainly glucose and fructose were present in nearly similar amounts, only minor quantities of sucrose and other sugars being found.

As far as was investigated (sample 3 from Saeftinghe and sample 4 from Hooge Platen, both from 2006), the larval food showed hydrogen peroxide production, originating from the enzyme glucose oxidase. *A. tripolium* pollen itself showed, as expected, a negative reaction (sample 7 from 2006).

Discussion

The results of the palynological analysis showed that in all four locations *A. tripolium* was by far the dominant pollen source for the larval food of *C. halophilus*. In the present study most brood cells contained more than 95% *A. tripolium* pollen. In 2006 Cane and Sipes presented a revision of the lexicon of pollen specialization. They distinguished between “monolecty”, “narrow oligolecty”, “oligolecty”, “eclectic specialization” and “mesolecty” and accounted for the species richness of the selected plant families. Following their views, we state that *C. halophilus* is indeed "oligolectic" on *Aster tripolium*, or even "narrow oligolectic" (see also Westrich, 1990 and Linsley, 1960).

Bischoff and coworkers found for *C. cunicularis* that the use of only one host plant may be dependent on the abundance of this plant (Bischoff *et al.* 2003; see also Müller *et al*, 2006). We however found no significant difference in the high *A. tripolium* pollen content (average 96%) in larval food from the various sites, including those

where other potential food plants were more available than at Saeftinghe. We also did not find a difference between samples collected at different times in the flight season, although it is likely that the available plant composition also varies between these times.

The analysis of the pollen content of the various cells from a single nest confirmed on an individual level the nearly exclusive foraging for pollen on *A. tripolium*. There were no differences between the different individual females and within a single nest the cells were all similar.

The considerable range of food plants on which females and males of *C. halophilus* were found as flower visitors by other observers was clearly not reflected in the proportions of pollen in the larval food of this study. We did find some of the pollen types mentioned by other authors (Guichard, 1974; Manning, 1955; Westrich, 1990), as well as other pollen types, but the low proportions of all of these types suggest that they could be treated as contamination (Cane and Sipes, 2006). Further confirmation for the presumed "oligolecty" of *C. halophilus* on *A. tripolium* could be obtained by conducting experiments with varying, controlled availability (including absence) of the food plant species.

The analysis of the chemical composition of the larval food showed that the liquid component of the larval food had a high Refractive Index, indicating a rather high sugar concentration (46.5% w / w). Further analysis by gas chromatography showed that the sugars present were mainly glucose and fructose. In addition some unidentified components were found in small amounts. This finding suggests that the liquid part of the larval food is derived from plant nectar. As virtually no other pollen than *A. tripolium* was found in the larval food, this is a strong indication that *C. halophilus* also collects nectar from *A. tripolium.* This plant is known as a very rich nectar source for honey bees, *Apis mellifera.*

In fresh *A. tripolium* pollen we found a sugar concentration of 44.2%. Large amounts of carbohydrates in pollen are not uncommon. In South Africa, Human and Nicolson (2006) found for fresh pollen of *Aloe greatheadii* var. *davyana* (water content 13.1% (± 1.4) a value of 34.7% (± 3.1) carbohydrate in dry matter. Linskens and Jorde (1997) found 36% total carbohydrates in pollen, calculated as invert sugar on dry weight (water content 17%).

The fact that the larval food showed hydrogen peroxide production whilst *A. tripolium* pollen itself did not, indicates that *C. halophilus* females produce the enzyme glucose oxidase and add this to the larval food. This is the first indication of production and use of this enzyme in solitary bees, and could be related to the typically liquid nature of the larval food, which requires specific preservation.

It remains possible that *C. halophilus* females do not only collect nectar from *A. tripolium* but also from other food plants. Nectar collection from other plants could also explain the presence of pollen of these plants in small quantities in the larval food pollen spectrum. Greenleaf *et al*. (2007) studied the foraging ranges of various bee species, using existing data sets, and Gathmann and Tscharntke (2002) studied the foraging ranges of 16 solitary bees. In both studies it appeared that body length and maximum foraging distance are positively correlated. *C. halophilus* was not included in these studies, but using their criteria, the female body length of 12-14 mm (Verhoeff, 1943) would suggest a foraging reach of 600 m. *C. halophilus* is different from many other solitary bees such as *C. succinctus* in that its actual nesting site is generally in a different place from where the major food plant occurs. Soil requirements for the nesting sites are different from those of marshland suitable for *A. tripolium*.

Acknowledgements

We thank Stichting Het Zeeuwse Landschap for supporting this study. We are grateful to Robert Bijlsma for his assistance in parts of the chemical analyses in 2005 at the Utrecht University Endocrinology & Metabolism laboratory. Dr. Henri Groeneveld, Plant Ecophysiology, Utrecht University, allowed us to use chemical analysis equipment and personally measured specific chemical components. Members of the Zeeland Entomological Study Group, Mr. Jos Arens, beekeeper (Hulst), and Jaap van Poperingen, beekeeper (Sint Annaland), are thanked for their cooperation. Maria Schipper, Utrecht University, provided statistical assistance. Great thanks are due to Jos and Marjan Neve for their essential assistance. An anonymous reviewer is thanked for important suggestions.

References

ALMEIDA, E A B (2008) Colletidae nesting biology (Hymenoptera: Apoidea), *Apidologie* 39: 16-29.

BISCHOFF, I; FELTGEN, K; BRECKNER, D (2003) Foraging strategy and pollen preferences of *Andrena vaga* and *Colletes cunicularius* (Hymenoptera, Apidae). *Journal of Hymenoptera Research* 12: 220-237.

BOGDANOV, S; MARTIN, P; LÜLLMANN, C (1997). Harmonised methods of the European Honey Commission. *Apidologie*, extra issue: 45-47. Also at: http://www.alp.admin.ch/themen/00502/00555/00564/index.html?lang=de.

DE BRUIJN, L L M; SOMMEIJER, M J (1997) The composition and properties of honey of stingless bees (*Melipona*). In Sommeijer, M J; Beetsma, J; Boot, W J; Robberts, E-J; de Vries, R (Eds). *Perspectives for honey production in the tropics.* NECTAR; Utrecht, The Netherlands. 149-168.

CANE, J H; SIPES, S (2006) Characterizing floral specialization by bees: Analytical methods and a revised lexicon of oligolecty. In: Waser, N M; Ollerton, J (Eds). *Plant-pollinator interactions: from specialization to generalization*. University of Chicago Press; USA. 99-122.

GATHMANN, A; TSCHARNTKE, T (2002) Foraging ranges of solitary bees. *Journal of Animal Ecology* 71: 757-764.

GAUHE, A (1941) Über ein glukoseoxydierendes Enzym in der Pharynxdrüse der Honingbiene. *Zeitschrift für vergleichende Physiologie* 28(3): 211-253.

GENOULD, D; DITTLO, F (2007) Contribution à la connaissande de *Colletes halophilus* Verhoeff (Hym., Colletidae) en France et confirmation de sa présence en Aquitaine. *Osmia* 1: 3-4.

GREENLEAF, S S; WILLIAMS, N M; WINFREE, R; KREMEN, C (2007) Bee foraging ranges and their relationship to body size. *Oecologia* 153: 589-596.

GUICHARD, K M (1974) *Colletes halophila* Verhoeff (Hym., Apidae) and its *Epeolus* parasite at Swanscombe in Kent, with a key to the British species of *Colletes* Latreille. *Entomologists Gazette* 25: 195 -199.

HUMAN, H; NICOLSON, S W (2006) Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var. *davyana* (Asphodelaceae). *Phytochemistry* 67: 1486-1492.

KERKVLIET, J D (1996) Screening method for the determination of peroxide accumulation in honey and relation with HMF content. *Journal of Apicultural Research* 35(3/4): 110-117.

KUHLMANN, M; ELSE, G R; DAWSON, A; QUICKE, D L J (2007) Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae). *Organisms, Diversity and Evolution* 7: 155–165.

LENSINK, R; REITSMA, J M; MEIJER, A J M (2008) *Beheerplan Verdronken Land van Saeftinghe 2009-2020. deel 1 Inventarisatie*. Stichting Het Zeeuwse Landschap/ Bureau Waardenburg rapport nr 08-038.1. 185 pp.

LINSKENS, H F; JORDE, W (1997) Pollen as food and medicine – a review. *Economic Botany* 51(1): 78-87.

LINSLEY, E G (1960) The role of flower specificity in the evolution of solitary bees. Verhandlungen XI. Internationaler Kongreß für Entomologie Wien, Band I (sektion I-VI): 593-596.

MANNING, F J (1955) Note on *Colletes halophila* Verhoeff. *Journal of the Society for British Entomology* 5: 130-131.

MICHENER, C D (2000) *The bees of the world*. The John Hopkins University press; USA. 913 pp.

MÜLLER, A S; DIENER, S; SCHNYDER, K; STUTZ, K; C; SEDIVY, C; DORN, S (2006) Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee-flower relationships. *Biological Conservation* 130: 604-615.

O'TOOLE, C; RAW, A (1999) *Bees of the world*. Blandford Press; London, UK. 192 pp.

SHRESTHA, M (1997) Laboratory experience in Nepal: Honey analysis and honey composition. In Sommeijer, M J; Beetsma, J; Boot, W J; Robberts, E-J; de Vries, R (Eds). *Perspectives for honey production in the tropics.* NECTAR; Utrecht, the Netherlands. 127-138.

VAN LITH, J P (1937) Het zijdebijtje en haar nest. *De levende natuur* 41(10): 305-308.

VERHOEFF, P M F (1943). Opmerkingen over Hymenoptera aculeata. in: Verslag van de Zes-en-zeventigste Wintervergadering der Nederlandse Entomologische Vereniging. *Tijdschrift voor Entomologie* 86: XLII.

VON DER OHE, W; PERSANO ODDO, L; PIANA, M L; MORLOT, M; MARTIN, P (2004) Harmonized methods of melissopalynology. *Apidologie* 35: S18-S25.

WESTON, R J (2000) The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chemistry* 71: 235-239.

WESTRICH, P (1990): *Die Wildbienen Baden-Württembergs*. 2. Aufl. Ulmer Verlag; Stuttgart, Germany. (Two volumes: Allgemeiner Teil und Spezieller Teil). 972 pp.

WHITE, J W Jr; SUBERS, M H; SCHEPARTZ, A I (1962) The identification of inhibine. *American Bee Journal* 102(11): 430-431.

WHITE, J W Jr; SUBERS, M H; SCHEPARTZ, A I (1963) The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta* 73: 57-70.