

**Pollination ecology of**  
***Scabiosa columbaria***  
**a comparison between small and large populations**



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**april 1995**

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Doctoral report of  
Laboratory of Plant Ecology  
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## Summary

We studied the pollination ecology of *Scabiosa columbaria* (Dipsaceae) in relation to population size. We observed visitors in 9 populations in the central and southern part of the Netherlands.

We found a positive relationship between population size of *S. columbaria* and insect species richness. *S. columbaria* was frequently visited in the field, about 25 visits per head per day was found. Only a small number (12 of 33) of the insect species visiting *S. columbaria* were common found (>10 sights of 2200). The syrphid *Eristalis tenax* and the nightmoth *Autographa gamma*, which flies at day-time, were clearly the most common visitors.

*Bombus pascuorum* had the highest foraging speed of the flower visitors, as well in number of flowerheads ( $7.6 \pm 0.1$ ) as number of flowers ( $85 \pm 8.4$ ) visited per minute. In the field however *B. pascuorum* rarely visited *S. columbaria*, but it was a common visitor on *Centaurea jacea*. *Pieris rapae* had a higher foraging speed than *E. tenax*,  $4.4 \pm 0.5$  heads per minute vs  $3.3 \pm 0.6$  heads and  $31 \pm 3.3$  flowers per minute. *E. tenax*, *A. gamma* and *P. rapae* switched the same times from plant species, every fifth visited flower(head) was of an other species. The amount of visits on *S. columbaria* was significant larger for *E. tenax* (80%) than for *A. gamma* and *P. rapae*. In a population where *S. columbaria* occupied a smaller part of the vegetation for *E. tenax* a relatively lower number of visits on *S. columbaria* was found.

*B. pascuorum* carried the largest number of pollen on its body (mainly males were seen), on average  $1765 \pm 860$  pollen grains. Butterflies carried a very low number of pollen, *A. gamma* and *P. rapae* carried on average  $19 \pm 7$  and  $72 \pm 33$  grains.

The amount of *S. columbaria* pollen in the pollen load of insects foraging on *S. columbaria* was for all species low, about 25 %. This was reflected in the amount of pollen of *S. columbaria* on the stigma's, about 20%. Species with many pollen on stigma's of *S. columbaria* were *Centaurea ssp.*, *Origanum vulgare*, *Daucus carota* and *Compositae*. The pollen of *Knautia arvensis*, a related species, were not distinguishable from pollen of *S. columbaria*, their part was probably underestimated.

The mean deposition rate was for *E. tenax*  $8.5 \pm 3.0$  and *B. pascuorum*  $3.8 \pm 0.6$  grains per stigma per minute. The number of deposited pollen in the field increased steadily from about 09.00 am during the rest of the day, till in the mid of the afternoon about 9 pollen per stigma (only stigma's which receive pollen were included) were deposited. Then the number of pollen remains the same.

*E. tenax* and *P. rapae* only flew short distances, more than 90 % less than 2 meter. *P. rapae* made 15 % of its flights over distances larger than 10 meter.

With fluorescent dye powder the possible transport distance of pollen was traced. Most of this powder was distributed within 10 meter of the donor plant. A small amount however was distributed over a distance more than 100 meter.

The number of visits and the number of pollen deposited on the stigma's of *S. columbaria* seems to be sufficient for seedset, but the quality is a question. Qualitative aspects of pollination seem to be more important than quantitative aspects for *S. columbaria*. This is caused by the generalistic behaviour of the flower visitors. Differences in quality of pollination were not related to population size of *S. columbaria*, but could be more related to density of flower heads of *S. columbaria* in relation to other flowering plants.

## Samenvatting

We hebben de bestuivings ecologie van Duifkruid (*Scabiosa columbaria*, *Dipsacaceae*) in relatie tot populatie grootte onderzocht. We hebben bloem bezoekers bekeken in 9 populaties in het midden en zuiden van Nederland. In dit onderzoek wordt een positief verband gevonden tussen populatie grootte van Duifkruid in Nederland en de soorten rijkdom aan bloembezoekers. Duifkruid wordt vrij druk bezocht, gemiddeld zo'n 25 bezoeken per hoofdje per dag. Van de insecten soorten die Duifkruid bezoeken zijn slechts enkele (12) redelijk talrijk te noemen (>10 waarnemingen van totaal 2200). De zweefvlieg *Eristalis tenax*, de Blinde bij, en de nachtvlinder *Autographa gamma*, het Gamma uiltje, dat overdag vliegt, zijn veruit de algemeenste bezoekers.

Van de bezoekers haalt *Bombus pascuorum*, de Akkerhommel, de hoogste fourageer snelheid zowel in hoofdjes ( $7.6 \pm 0.1$ ) als bloemen per minuut ( $85 \pm 8.4$ ). In het veld bezochten Akkerhommels Duifkruid nauwelijks, maar het waren algemene bezoekers van Knoopkruid (*Centaurea jacea*). *Pieris rapae*, het Klein koolwitje, fourageerde wat sneller dan *E. tenax*, resp.  $4.4 \pm 0.5$  hoofdjes per minuut en  $3.3 \pm 0.6$  hoofdjes en  $31 \pm 3.3$  bloemen per minuut. *E. tenax*, *A. gamma* en *P. rapae* wisselden even vaak van plantesoort, 1 keer per 5 bezoeken. Het aandeel bezoeken op Duifkruid was echter significant hoger (80%) voor *E. tenax* dan voor *A. gamma* en *P. rapae*. In een populatie waar Duifkruid een kleiner deel van de vegetatie uitmaakte bezocht *E. tenax* relatief minder Duifkruid. *B. pascuorum* droeg ruim de meeste pollen op zijn lichaam (vooral mannetjes zijn waargenomen), gemiddeld  $1765 \pm 860$  korrels. Vlinders droegen erg weinig pollen op hun lichaam, *A. gamma* en *P. rapae* droegen respectievelijk gemiddeld  $19 \pm 7$  en  $72 \pm 33$  pollen.

Het aandeel Duifkruid in de pollenlading van de insecten die op Duifkruid fourageerden was bij alle soorten laag, gemiddeld zo'n 25%. Dit werd teruggevonden op de stigma's van Duifkruid, waar het aandeel Duifkruid pollen ook laag was, zo'n 20%. Soorten waarvan veel pollen gevonden werden op stigma's van Duifkruid waren *Centaurea* ssp., Marjolein (*Origanum vulgare*), Wilde peen (*Daucus carota*) en *Compositae*. De pollen van Beemdtkroon (*Knautia arvensis*) zijn niet goed te onderscheiden van die van Duifkruid, hun aandeel is waarschijnlijk onderschat.

De gemiddelde depositie snelheid was voor *E. tenax* en *B. pascuorum*  $8.5 \pm 3.0$  en  $3.8 \pm 0.6$  korrels per stigma per minuut. Het aantal korrels dat afgezet wordt op de stempels neemt in het veld vanaf ongeveer 9 uur 's ochtends snel toe tot midden op de middag zo'n 9 korrels per stempel zijn afgezet. Daarna blijft het aantal hetzelfde.

*E. tenax* en *A. gamma* vlogen vooral korte afstanden, meer dan 90 % minder dan 2 meter. *P. rapae* maakte 15 % van zijn vluchten over afstanden van meer dan 10 meter.

Met behulp van fluorescerend poeder is de mogelijke transport afstand van pollen nagegaan. Het meeste poeder werd binnen tien meter van de donorplant verspreid. Een klein deel werd echter over een afstand van meer dan 100 meter verspreid.

De hoeveelheid bezoek en het aantal afgezette pollen per stigma lijkt toereikend te zijn voor de zaadzetting, maar het is de vraag of de kwaliteit van de pollen goed genoeg is.

Kwalitatieve aspecten van de bestuiving lijken voor een Duifkruid populatie belangrijker te zijn dan kwantitatieve aspecten. Dit wordt veroorzaakt door het generalistische gedrag van de bloem bezoekers. Verschillen in kwaliteit van bestuiving waren niet gerelateerd aan populatie grootte, maar zouden meer gerelateerd kunnen zijn aan de dichtheid van bloemhoofdjes van Duifkruid in relatie tot andere bloeiende planten.

## Introduction

Today loss of habitat and fragmentation may be viewed in natural populations all over the world (Caughley, 1994). The original populations split up in different subpopulations which become isolated from each other. Problems with dispersion and local extinction will start the decrease of many species. An example of such in the Netherlands is the plant species *Scabiosa columbaria* (in Dutch: Duifkruid).

*S. columbaria* grows on calcareous dry grasslands along rivers and in the limestone area in the southeastern part of the Netherlands. During the last decades the abundance of *S. columbaria* has decreased enormously (Ouborg, 1993). *S. columbaria* is indicated on the Red List of the Dutch flora as most vulnerable (Weeda *et al.*, 1990). The occurrence nowadays is restricted to several places along the river IJssel, one along the Oude Rijn and several isolated populations in Zuid-Limburg (fig.1). Most populations are situated in nature reserves. These nature reserves in Zuid-Limburg often includes vegetations of species-rich chalk grasslands.

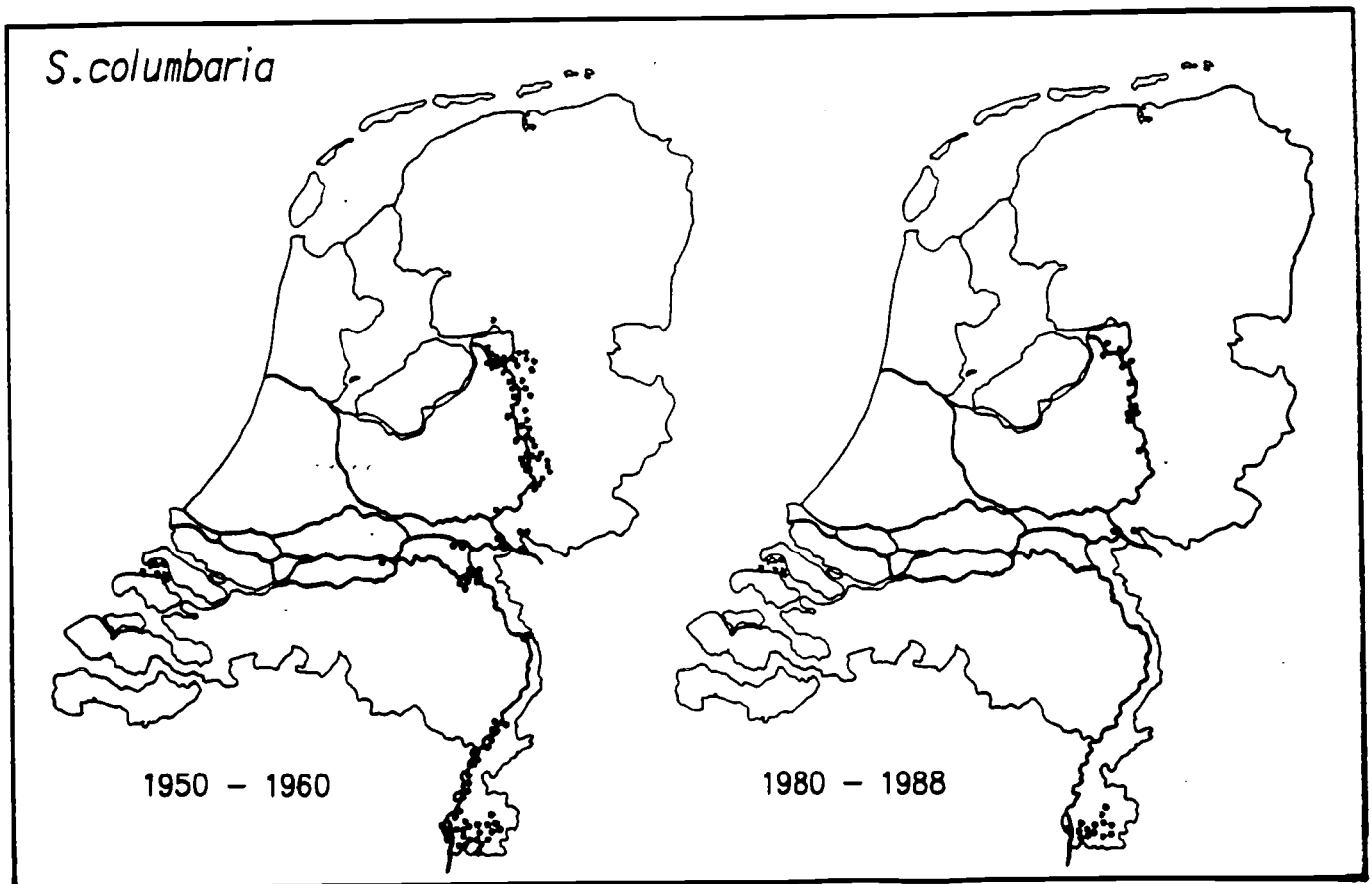


Figure 1: Distribution of *S. columbaria* in the Netherlands during the years 1950-1960 (left) and 1980-1988. Each dot represents a sight in which the species was recorded to exist (after Van Treuren, 1993).

The causes of local extinction are diverse. Human influence by changing habitat and changed management of grasslands turns out to be a major factor in eliminating small populations. Enrichment of the nutrient-poor chalk grassland vegetation by deposition of N from the air forms a serious problem. But also more natural factors influence the occurrence. Genetic erosion and environmental stochasticity are such processes, often operating together. Ouborg (1993) and Van Treuren (1993) showed that small populations contain less genetic variation than the larger ones. This so-called genetic erosion has a negative influence on several vitality parameters, increasing the chance of extinction of small populations.

Population dynamics of one species often influences the whole local ecosystem. Changes in this local ecosystem can affect all species, since often complex relations between the different components of an ecosystem exist. The inbreeding rate and seed set of a plant species for example depends on the presence and behaviour of its pollinators. The fate of *S. columbaria* populations is related to that of their pollinators, and partly the reverse.

In plant populations of different size a shift of the species composition of pollinating insects may occur (Jennersten, 1988). The size of a population and its rate of isolation may influence the chance that individuals from polylectic insect species specialize on a certain plant species. This so-called flower constancy is less likely to occur if population size is small. An area may become so unattractive (few flowering plants) that it remains unvisited by insects.

The shift of species and behaviour of visitors influences the pollination. Flower visitors may differ in their pollination efficiency and mean flight distance (Herrera, 1987). Oligolectic insects and temporally specialized insects are probably pollinators with a higher efficiency than non-specialized insects, by the fact that they deposit more conspecific pollen.

Thus small populations may have a pollination problem. Due to a possible shift from specialistic to generalistic insects species and the disappearance of temporally specialized foraging behaviour of generalistic insects, pollination may be influenced both in a quantitative and qualitative way. This report compares small and large populations in respect to pollination.

## Questions

What is the effect of population size of *Scabiosa columbaria* on the pollination by insects.

- 1 a. What is the influence of the population size of *Scabiosa columbaria* on the species composition of the flower visitors?
- b. Is there a relation between population size and visitation frequency?
2. What is the influence of the species composition of insects on the pollination of *S. columbaria*?
  - a. Does pollination limitation occur in populations of *S. columbaria*?
  - b. Do insect species differ in foraging speed?
  - c. Do insect species differ in flower constancy?
  - d. Do insect species differ in the number of pollen they carry and in pollen deposition?
  - e. Do insect species differ in flight distance?
  - f. What is the transport distance of *S. columbaria* pollen?



## Methods

### *Scabiosa columbaria*

*Scabiosa columbaria* is a perennial, tall herb belonging to the Dipsacaceae (fig. 2). The violet flowers are arranged in flower heads (each 50-80 flowers, fig. 3). In the field up to 50 flower heads per plant occur, with a mean value of 6 (fig. 4). The flowers are protandric, which means that flowers are first in a male stage (6 days) and later female (1 day). This prevents self pollination within a flowerhead. *S. columbaria* is visited by a scale of insects, foraging for nectar and pollen.



Figure 2: *Scabiosa columbaria*

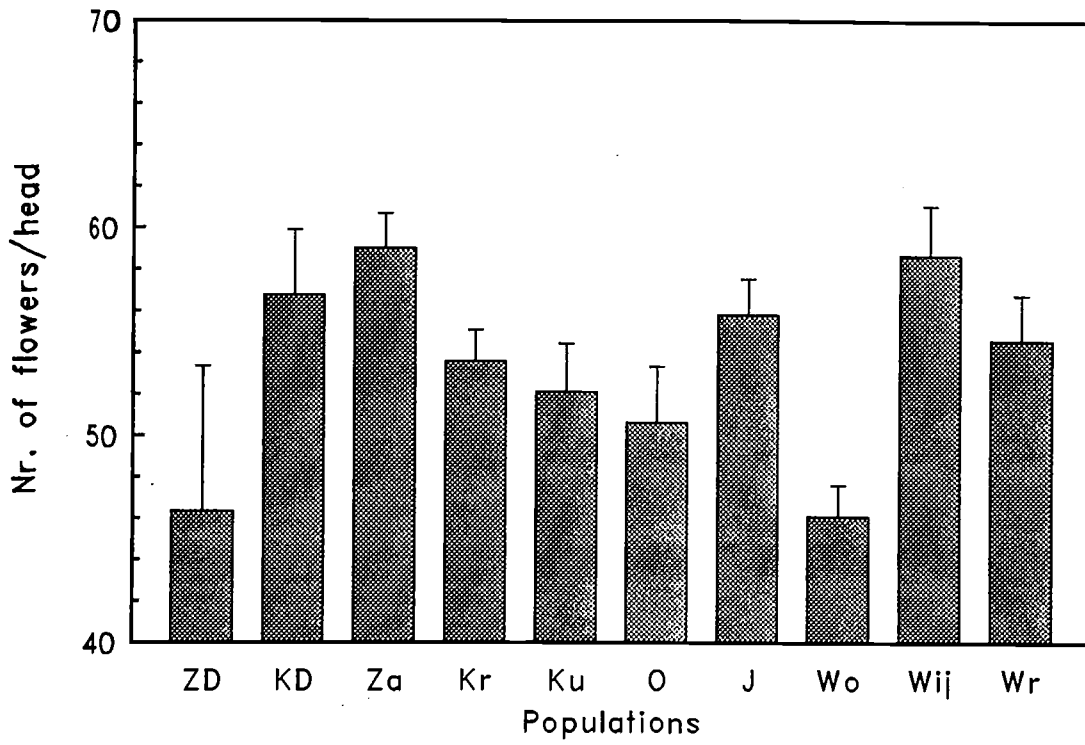


Figure 3: Number of flowers per head in the studied populations.

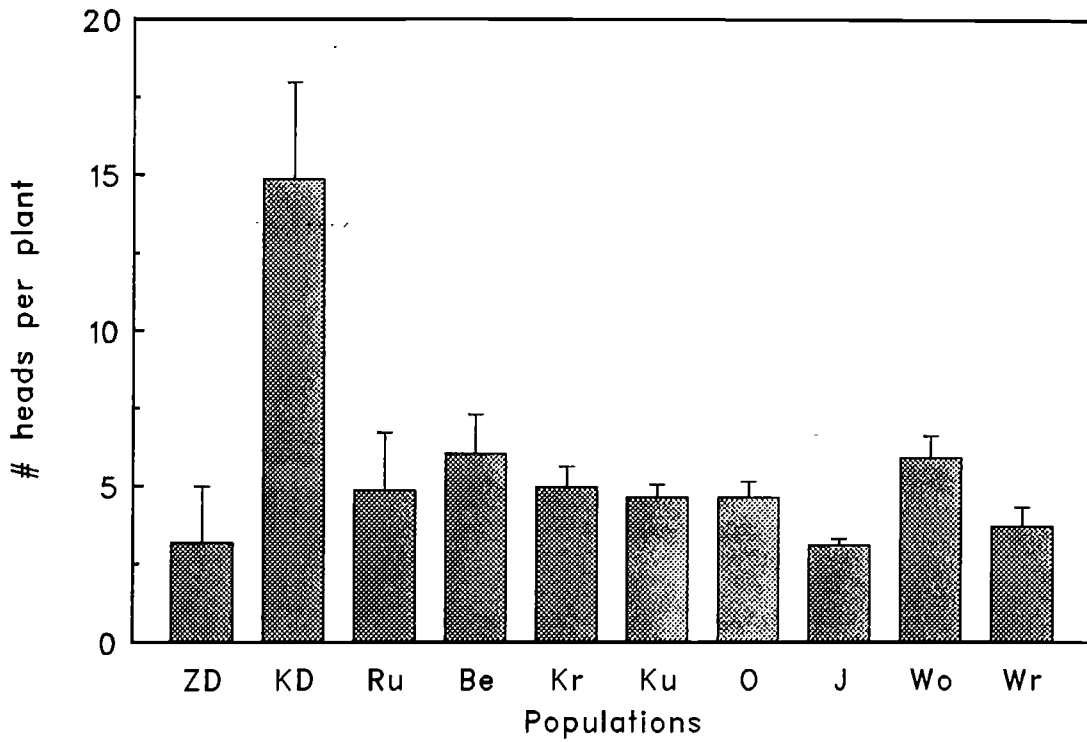


Figure 4: Number of heads per plant in the studied populations.

## Populations

Twelve populations of *S. columbaria* in the Netherlands have been visited (Figure 5) in August-September 1994 and used for the examination of one or more questions. They varied in size from 7 up to 40,000 flowering heads (Appendix 1). All the populations form smaller or larger isolated islands in an agricultural landscape. The smallest distance between two populations is about 5 kilometers, so direct contact between them is unlikely. From these twelve populations only nine are visited to observe insects. In the other three the number of insects was too low due to a very small population size or bad weather conditions.

## Species composition of visitors on *S. columbaria*

In populations of *S. columbaria* transects were described with respect to plant species and number of insect visitors on *S. columbaria*. To get representative data of the species composition the transects were minimal 10x2 meter if possible. Within this area the number of flowerheads of *S. columbaria* and other attractive plant species for insects were counted. These numbers changed during the season (figure 6). Observations on the species and numbers of insect visitors of *S. columbaria* were made along the transect walking with a constant speed.

## Visitation frequency

With the transect method no information about the number of visits per flowerhead in a certain time interval can be obtained. For this purpose observations were made in a plot with a known number of flowerheads. During a time interval from at least 30 minutes all visits on flowerheads of *S. columbaria* were scored per insect species (table 1).

Visitation frequency is the number of visits per flowerhead per day. A day for pollination is taken as 6 hours, the time that at least all insect species were active. It is the total number of visits which may lead to pollination, since a flowerhead is only one day in the female stage. Note that a visit does not imply pollination.

Table 1:  
Date, total observation time for transects and visitation frequency and weather during observations per population.

Population	date	time (min.)	temp. (°C)	clouds (%)
Zure Dries	230894	30	20-25	40
Kwart. Dijk	220894	30	20-25	40
Zalk	220894	30	20-25	100
Kruisberg	110894	225	25-30	50
Olst	230894	30	20-25	40
Juliana Gr.	220894	30	20-25	50
Wolfskop	250894	60	20-25	10
Wijlre	100894	150	25-30	10
Wrakelberg	090894	225	25-30	0



Figure 5: Populations of *S. columbaria* in the Netherlands, where fielddata were collected. Abrevations are explained in appendix 1.

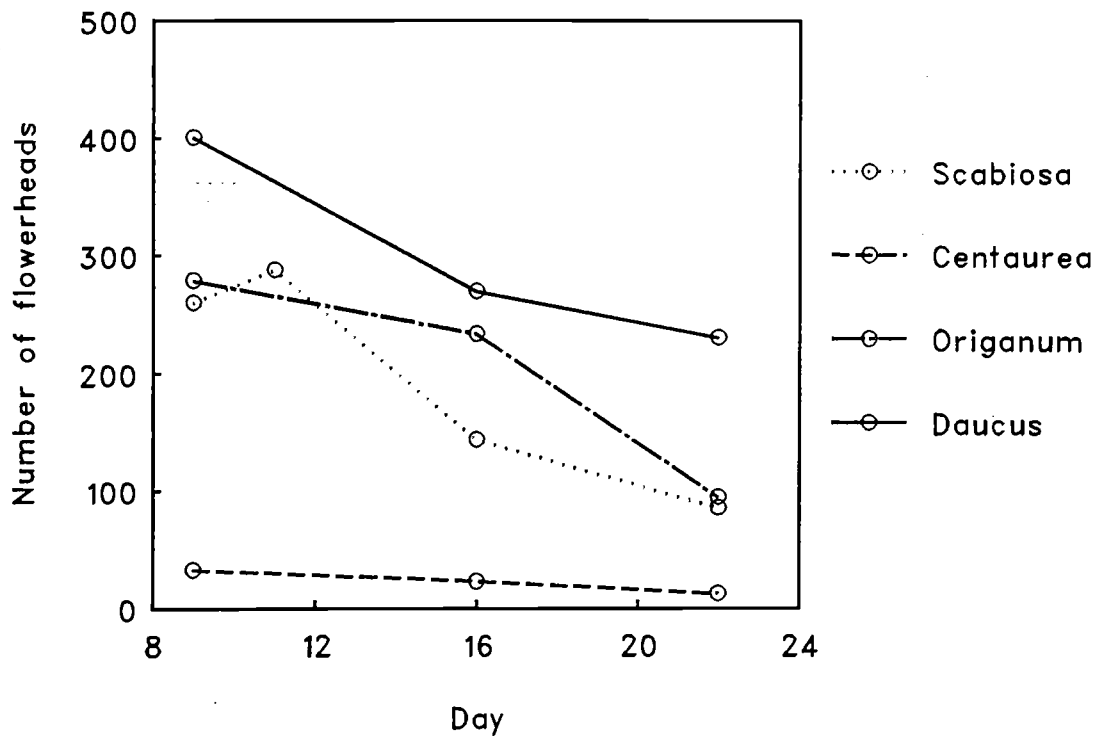


Figure 6: Changes in number of flowering heads per plant species in the Wrakelberg population in August 1994.

### **Pollination limitation**

Pollination limitation is here defined as stigma's receiving too few conspecific pollen to induce seed set. A method to control whether pollen limitation occurs is to compare seed set after an extra hand pollination and after natural pollination. If there is pollination limitation seed set in the natural pollinated heads will be less than in the hand-pollinated heads.

One flowerhead is hand-pollinated by pulling it through a small box with ripe, burst-open anthers. The anthers are taken from plants more than 10m away. The hand pollination is checked by controlling the number of pollen grains on the stigma, using a loupe. The other head is left untreated. Number of flowerheads sampled per population are shown in table 2.

Three variants of this method, depending on the field conditions, were used (see table 2).

- A. Two female heads of the same plant were used.
- B. Two female heads of neighbouring plants were used (one on each plant) when they could not be found on a single plant.
- C. Hand-pollination is done by selecting a well-pollinated female head as hand-pollinated.

It is preferable to have female heads on the same plant since genetic variation between the heads is then excluded. Also difference in environmental factors is severely reduced. Both genetic and environmental factors are involved when the heads come from two different plants. When they are neighbouring plants the influences of these factors are reduced as good as possible.

To replace hand-pollination by selecting well-pollinated female heads has an advantage. Pollination by insects seem to be more successful than by hand (pers. obs.). More pollen per stigma are deposited and better in the middle of the stigmatic surface than in hand-pollination. A disadvantage is that the female heads are no longer random chosen. The best pollinated are chosen which may influence the outcome of the experiment.

When the seeds have ripened (that is when the seeds fall out if the head is touched) they were collected. This was after a period between one and two weeks.

Van Treuren (1993) distinguished developed seeds (with endosperm development) and non-developed seeds (without endosperm development) considering their size. Thick ones are well developed and thin ones bad. The discrimination between thick and thin seeds appeared useless in the field. In the field the whole range from thick to thin was represented. So seeds were germinated, 20 seeds per petridish, on a filterpaper with demiwater in a 12/12 light/dark and 25/15°C regime. Per plant two, three or four petridishes were used. All seeds produced by a single flowerhead were germinated (see fig. 3 for mean number of flowers per head in the field populations).

Table 2:

Method used to examine pollination limitation. Number of heads is the total number of heads germinated. Methods are mentioned in text.

Population	Number of heads	Method
Kruisberg	18	A
Wolfskop	45	BC
Wijlre	34	AB
Wrakelberg	50	AB

### Foraging speed

In the experimental garden of the Biological Centre in Haren a large amount of *S.columbaria* grewed in trays outside. In September, when foraging speed was measured, they were in full flower. The observations were done on 20, 23, 26 and 27 September 1994 (table 5). Observations on a day started when the anthers of the plants burst open, because pollen deposition (see below) was measured together with foraging speed. The anthers opened around 11.00 am. The opening of the anthers depended strongly on the weather conditions, especially the amount of sunshine. Observations ended when most pollen had disappeared from the anthers. They were partly distributed and partly eaten by syrphid flies.

Only data of 26 September (in sunny wheather) were included in the analysis. On the other data pollen were soon removed by pollen-eating insects after opening of the anthers.

Foraging speed in the field was measured by making behavioural protocols of the flower visitors. An insect is followed and foraging time per head of *S.columbaria* was noted. Protocols were made on seven days and two places. Only data of the Wrakelberg on 9 August 1994 and the Kruisberg on 20 August and 21 August 1994 were included in the analysis. Number of observations is represented in table 5.

### Flower constancy and feeding behaviour

Individual insects were followed during their foraging trip. The protocol always started with an insect visiting *S. columbaria*. After its first flower visit the plant species of the next 10 visited flowerheads was noted. Individual insects which were lost before 10 visits were ignored in the analysis. During the protocols notes were made on the feeding behaviour on *S. columbaria*: consumption of nectar or pollen. Protocols were made in patches in two populations: they differed in density of flowerheads of *S. columbaria* and the related species *Knautia arvensis* (table 3). Observations were made in the period 9-25 August 1994 from 12.00 - 16.00 pm during sunny weather with temperatures from 20-25 °C.

Table 3:

Characteristics of the vegetation of the two patches where the protocols are made.

heads/m <sup>2</sup>	Wrakelberg	Kruisberg
<i>S. columbaria</i>	10.0	1.9
<i>Knautia arvensis</i>	0.0	0.6

In addition to fieldwork in natural populations we did on 6 September an experiment with the syrphid fly *Eristalis* ssp. in the experimental garden in Assen. Here we offered *S. columbaria* and *Centaurea jacea* in three patches with a different ratio between the two species (table 4). *C. jacea* is an abundant and attractive species in chalk grasslands. The patches were made on 5 September so the syrphid flies could get used on the new situation. The distance between the patches was about 50 meters. Protocols of the flower visiting syrphid flies were made in the same way as in the natural populations.

Table 4:

Composition of the patches used in the experimental garden in Assen for a flower constancy experiment.

patch	A	B	C
<i>S. columbaria</i> flowerheads (%)	90	50	10
Number of <i>S. columbaria</i> flowerheads	150	15	15
Number of <i>C. jacea</i> flowerheads	15	15	150

### Pollenload on insect body

Pollen on the bodies of insects visiting *S. columbaria* were removed from the insect body with basic fuchsin gel (Beattie, 1971). This was done in the Wrakelberg population in the same patch where the protocols were made. Only the underside and the head of the insect was cleaned, because this part of the insect makes contact with the stigmas. After cleaning, the piece of gel was put on an object glass and was melted. The number of pollen grains per plant species was determined with a microscope under a 40x magnification. A reference collection of pollen from the anthers of all flowering species, prepared in the same way, was used.

### Pollination effectiveness indices

The different aspects of the pollination effectiveness are compared for the most abundant species. Pollinator effectiveness is used here as an overall measurement. Both quantitative (total visitation frequency) and qualitative aspects (per visit efficiency) are included. In order to get a relative measurement index-values are used. Effectiveness indices are calculated by multiplying the index values of visitation frequency, total pollenload and percentage of *S. columbaria* pollen. This effectiveness index gives a relative value per insect species for the number of conspecific pollen per time interval deposited on *S. columbaria* stigmas.

### Rate of pollen deposition

In the experimental garden in Haren pollen deposition was measured. Observation days and time are the same as for foraging speed measurements (see table 5). Only data of 26 September were included in the analysis. Pollen deposition per insect species was measured by offering an insect a virgin female head. The total time an insect forages on this head is scored as well as the number of visited flowers. Afterwards the number of pollen per stigma was counted with a 10x loupe.

**Table 5.** Number of observations of foraging speed and pollen deposition per insect species per day.

Date	Observ.time	Nr.indiv. <i>E. tenax</i>	Nr.indiv. <i>B. pascuorum</i>	Nr.indiv. <i>A. mellifera</i>
190994	15.00-16.00	4	8	-
200994	10.30-11.30	4	6	-
230994	11.30-12.30	1	2	-
260994	10.30-12.30	17	13	13

In the field the increase in number of pollen per stigma during the day is observed in the Juliana groeve on 23 August 1994 and on 16 and 23 August 1994 on the Wrakelberg. Per female head five stigma's were sampled on the Wrakelberg (8 heads on 16 August, 9 heads 23 August). In the Juliana-groeve 10 stigma's per head were sampled on 8 heads. About each hour, on 16 August every second hour, the number of pollen grains per stigma was counted with a loupe.

#### **Pollenload on stigmas**

During the fieldwork it appeared that the stigmas possibly received many heterospecific pollengrains. To test this we cleaned stigmas with gel in the same way as the insect bodies. A piece of the gel was thoroughly polished over the stigmas of the whole flowerhead. With a loupe was checked if the stigmas were clean. Three populations (Wrakelberg, Julianagroeve and Kruisberg) were sampled at the end of a day with high foraging activity. Per population 10 flower heads were cleaned. Due to limited time it was not possible to clean the 3 populations on the same day.

#### **Flight distance**

A direct measure of the flight distance was obtained by protocols of individual insects. During the foraging trip the flight distance between two flower visits was measured. In the analyses of the data all the protocols were used, including protocols from individuals which were lost before 10 visits.

The flight distance over a longer time interval was measured with colour marked insects on the Wrakelberg on 16 August 1994 and on the Kruisberg on 11 and 25 August 1994. On 15 August insects on the Wrakelberg were marked in the afternoon on two places about 200 meter from each other with a mark on the thorax. On 16 August is looked for the marked insects the whole day by laying a transect from the place where they were captured downhill (about 150 m). There is also searched for marked insects by walking over the whole hill especially in the neighbourhood of the marking places. On the Kruisberg insects were marked in the early afternoon on 11 August in three distinct patches of *S. columbaria*. Late in the afternoon was looked in the patches for marked insects. On 24 August insects were marked in the three patches in the morning on the Kruisberg. On 25 August the whole population was divided in three parts. On each part an observer looked in the morning for marked insects by walking his part. For the number and species of insects marked see table 17 (results).



### **Transport of fluorescent dye powder**

An overall impression of the transport distance of pollen was obtained with fluorescent dye powder. Fluorescent dye powder and pollen movement follow similar patterns, so the powder can be used as pollen analogue for qualitative aspects of pollen dispersal (Kearns & Inouye, 1993). Fluorescent dye powder of different colours (red, green, blue) was applied on three flowerheads on 24 august 1994 10.00 am in the linear population Kruisberg separated by 42 and 60 m respectively. On 17.00 pm stigmas were collected on different distances from the donor flowers. Stigmas were collected from 49 flowerheads and per flower about 10 stigmas were collected. The number of dye particles of each colour was counted under 400 magnification with UV-light.

### **Seed set and germination**

It was tried to determine seed set for all populations. In the field at least 30 flowerheads with ripe seeds were collected per population. Van Treuren (1993) distinguished developed seeds with endosperm development and non-developed seeds without endosperm development on account of their size. It became clear that the differences were less clear in the field. So we decided to take the percentage of germination as an indication for the seed quality. For all populations seeds were put in petridishes in a climatroom at a changing temperature from 25°C and 15°C and 12/12 light/dark. After six weeks when no new germination occurred the percentage of germinated seeds was determined.

### **Statistics**

When the data were normal or log-normal distributed a Student t-test was applied for two sample hypotheses and an one factor ANOVA was performed for multisample hypotheses. Otherwise the Mann-Whitney U test and the Kruskal-Wallis test were applied respectively. For multiple comparisons the Student-Newman-Keuls test is used for normal distributed data (Zar, 1984).

## Results

### Activity during the day

Before answering this question a methodical problem should be solved. Insects are sensitive to weather conditions as sunshine and temperature (Gilbert, 1985; Grösser & Klapperstück, 1977 and Unwin & Corbet 1991). It was impossible to make observations in all 12 populations at the same day. So influences of daytime are investigated in the largest population to analyze the error margins (fig. 7). Influence of weather was much more difficult to analyze because weather parameters were not very accurate measured. However no strange observations can be subscribed to unusual weather conditions (and no really deviate conditions occurred) as far as we can see.

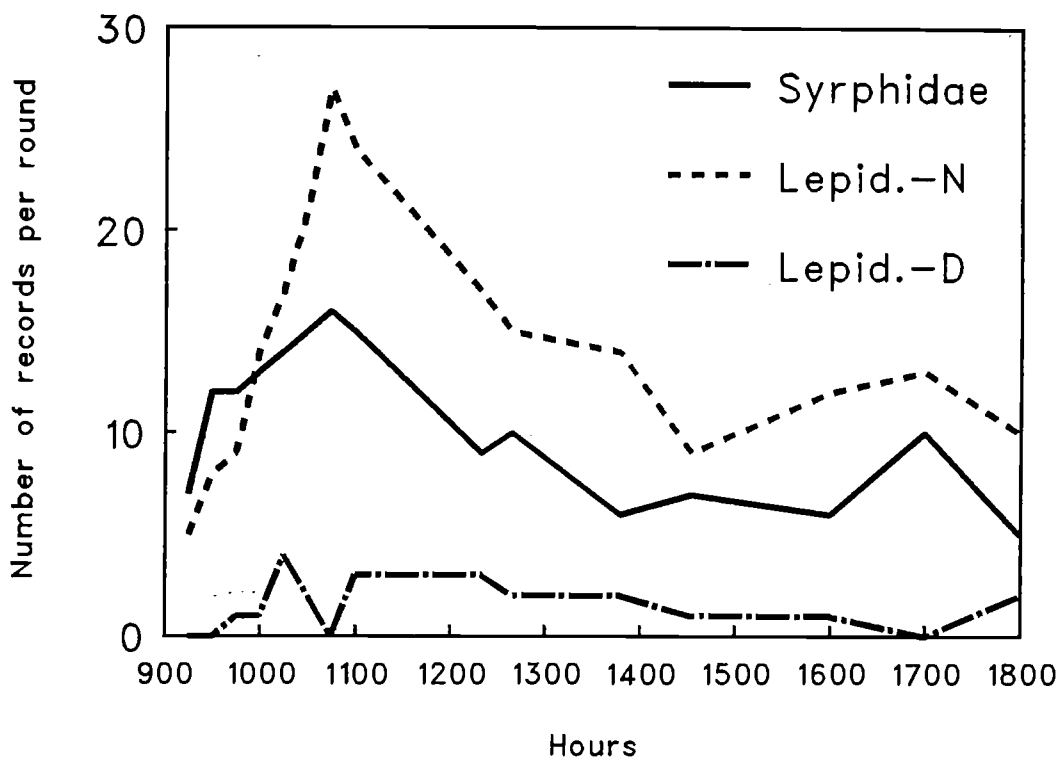


Figure 7: Insect activity during the day on the Wrakelberg, 9 August 1994. Syrphidae consists of syrphid flies mainly from the genera *Eristalis* and *Helophilus*, Lepidoptera-Night of *Autographa gamma* (it belongs to the nightmoths, but flies at daytime) and a few *Zygaena filipendula*, Lepidoptera-Day of *Pieris rapae*.

During the day the number of insects varied. Most insects were observed at the end of the morning. The ratio between the number of records of the insect groups remained more or less constant. This made comparison between populations in number of insect species possible when the data were not collected exactly at the same time of the day. Most data in other populations are collected around 11 am.

## Relation population size and insect species richness

A positive relation between population size of *S. columbaria* and number of insect species existed (fig. 8). There was also a positive relationship between population size and number of plant species, but the relation is weak (fig. 9). Only flowering plants were concerned which are attractive for flower visiting insects. This means dicotyls, most herbs, with striking flowers (see appendix 2 for a species list). Table 6 shows the correlation matrix with insect species richness as dependent variable of population size of *S. columbaria* and number of plant species.

Table 6: The correlation matrix of regression analysis with population size and plant species as independent variable and insect species as dependent variable.  $R^2 = 0.881$ . Significance: plant species  $p=0.083$ , population size  $p=0.013$ .

	Populationsize	Plant species	Insect species
Population size	1.000	0.644	0.891
Plant species	0.644	1.000	0.798
Insect species	0.891	0.798	1.000

From the figures 8 and 9 and table 6 can be seen that population size is better correlated with insect species richness than plant species richness is. Insect species richness can for 89,1% be explained by population size of *S. columbaria*. Plant species richness does not explain an extra part of the variation which is not explained by population size ( $p=0.083$ , not significant). A large *S. columbaria* population often means that more other flowering species were present ( $r^2=0.414$ ), although no significant relationship existed ( $p=0.061$ ).

In figure 10 the insect species per group, visiting *S. columbaria*, are shown for three Dutch populations. The larger the population, the more insect groups were represented and more species per group are present. Which groups were not represented in a small population seems not to be strictly regulated as in the smallest population two groups occur which are absent in the largest.

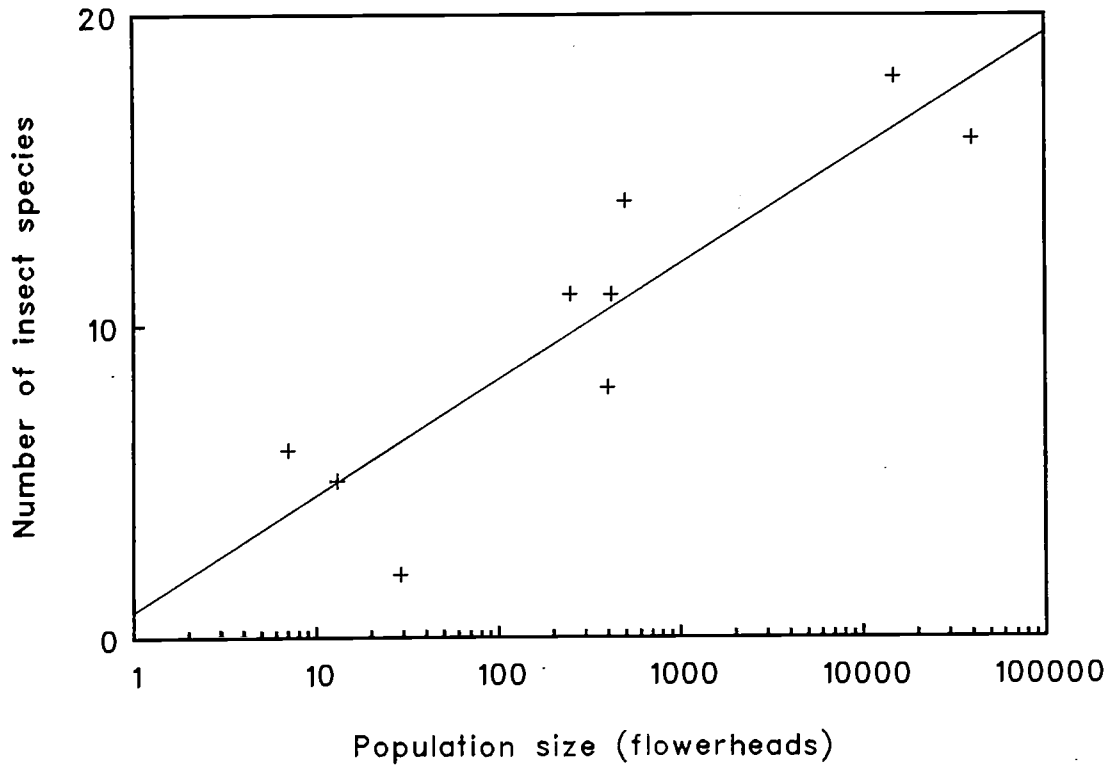


Figure 8: Population size of *S. columbaria* (number of flowerheads) and number of insect species.  $R^2=0.891$ ,  $p=0.001$ .

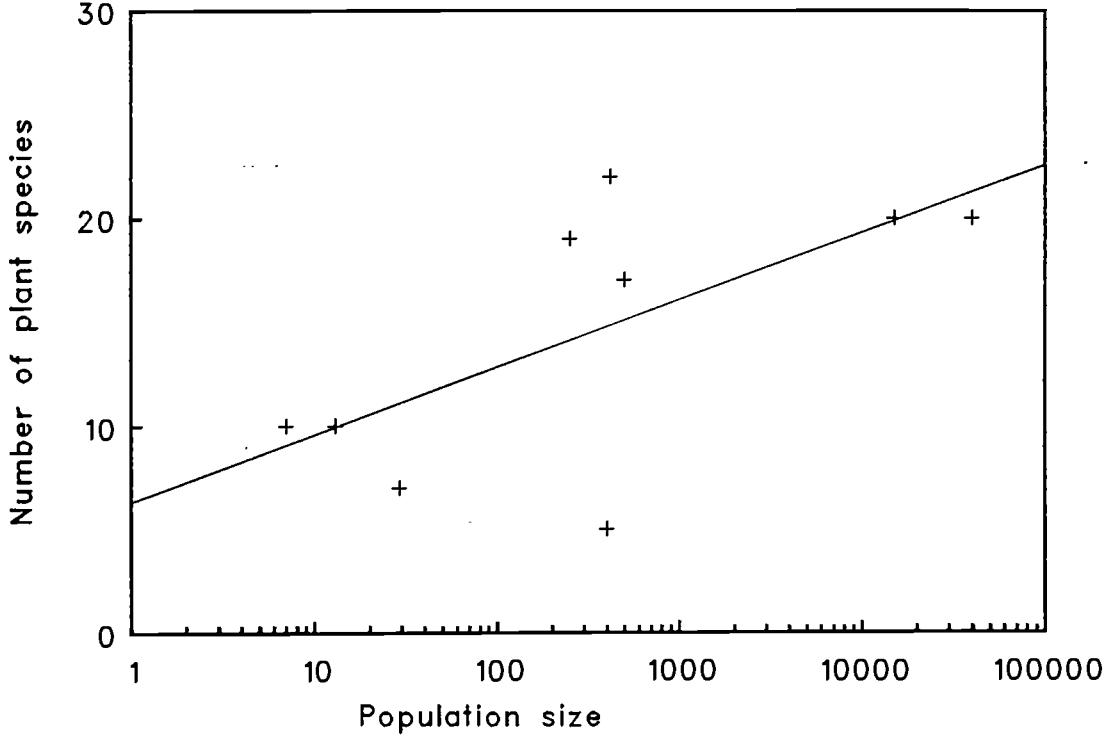


Figure 9: Population size of *S. columbaria* (number of flowerheads) and number of flowering plant species, attractive for insects.  $R^2=0.644$ ,  $p=0.061$ .

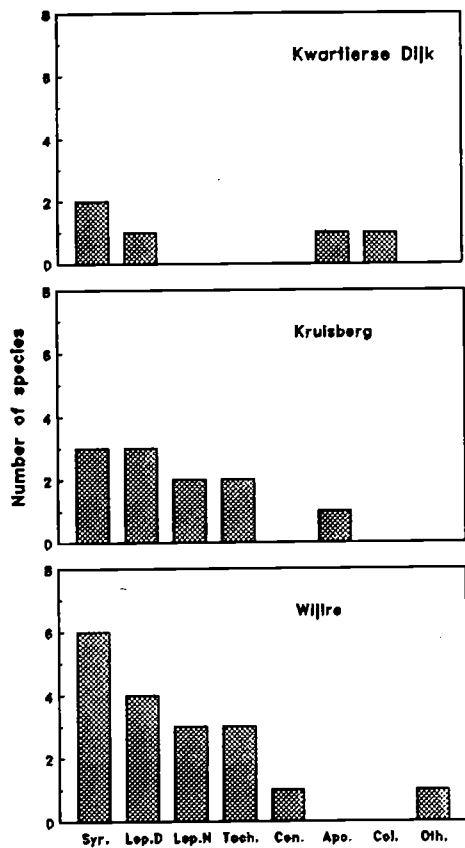


Figure 10: Number of species visiting *S. columbaria* per insect group per population. On the x-axis: Syrphidae, Lepidoptera-Day, Lepidoptera-Night, Tachinidae, Conopidae, Apoidae, Coleoptera, Other. The number of insect species is counted using the transect method (see Methods).

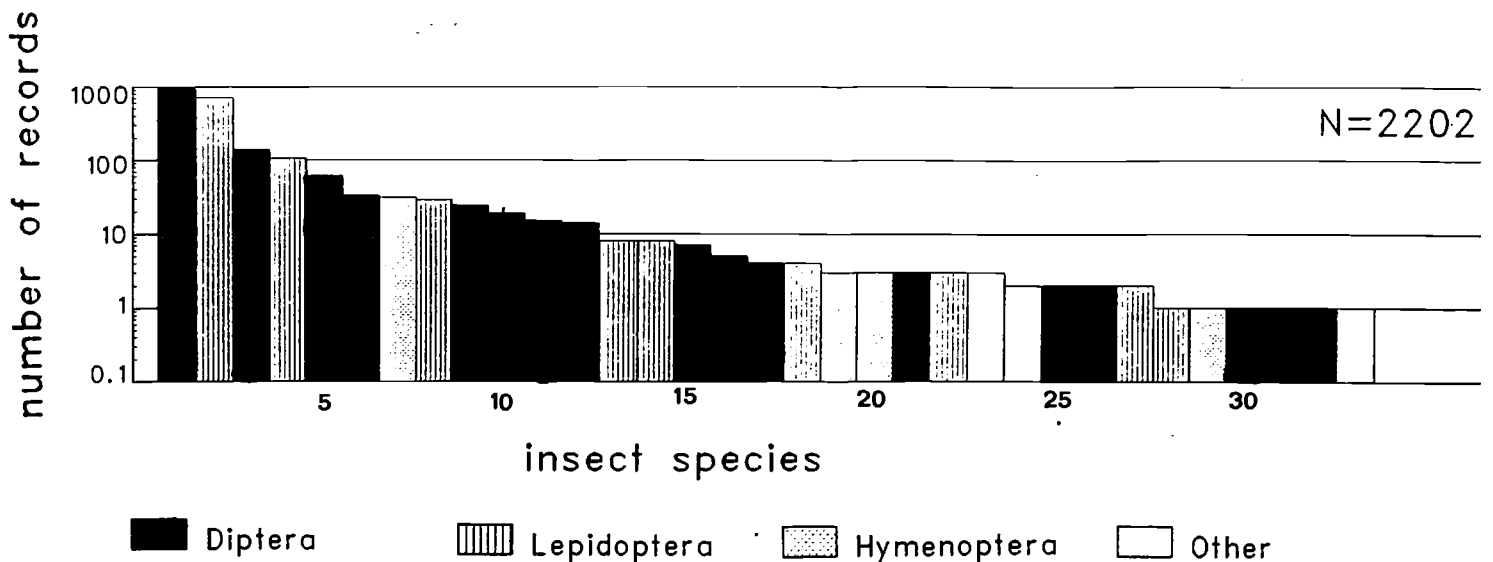


Figure 11: The distribution of the number of records of insect species, visiting *S. columbaria*.

### Species composition of insects

Not only insect species but also the number of insects is important for pollination. About 5 species were numerous visitors of *S. columbaria*, about 7 were regularly seen and the other 21 species were only observed 1-10 times (fig. 11 and appendix 3).

Syrphid flies of the genus *Eristalis* and *A. gamma* were the most common visitors (fig. 12). No relation between abundance of insect groups and population size occurred (fig. 13 and appendix 5). In all populations *E. tenax* and *A. gamma* were the most common species. They made 75% of all visits. In the very small population Zure Dries the most deviate frequency distribution is observed. This is due to the small number of records and the neighbourhood and not to population size. This population is situated in a woodland area and a typical woodland species as the syrphid *Volucella pellucens* was recorded. One bumblebee was seen foraging on *S. columbaria*, perhaps because there was no alternative due to the lack of other flowering herbs. Except Zure Dries also Zalk had a low number of records, other populations had about hundred or more (see table 7).

Table 7: Summary of records of insects visiting *S. columbaria* per population. One record means a visit of a flowering head by one individual. Total observation time is in minutes.

Population	Observation date	Total obs.time	Number of species	Number of records
Zure Dries	230894	30	6	15
Kwartierse Dijk	220894	30	5	95
Zalk	220894	30	1	2
Kruisberg	110894	225	11	110
Julianagroeve	230894	30	11	391
Olst	220894	30	8	195
Wolfskop	250894	60	14	543
Wijlre	100894	150	18	440
Wrakelberg	090894	240	16	411

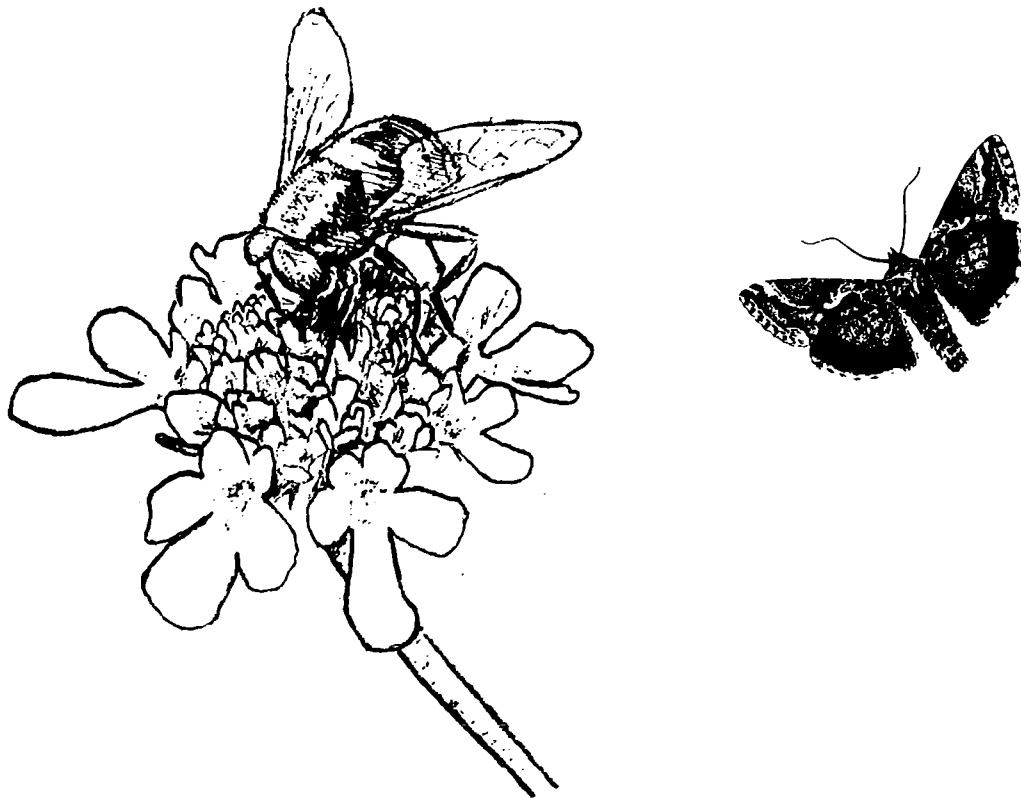


Figure 12: *E. tenax* and *A. gamma*

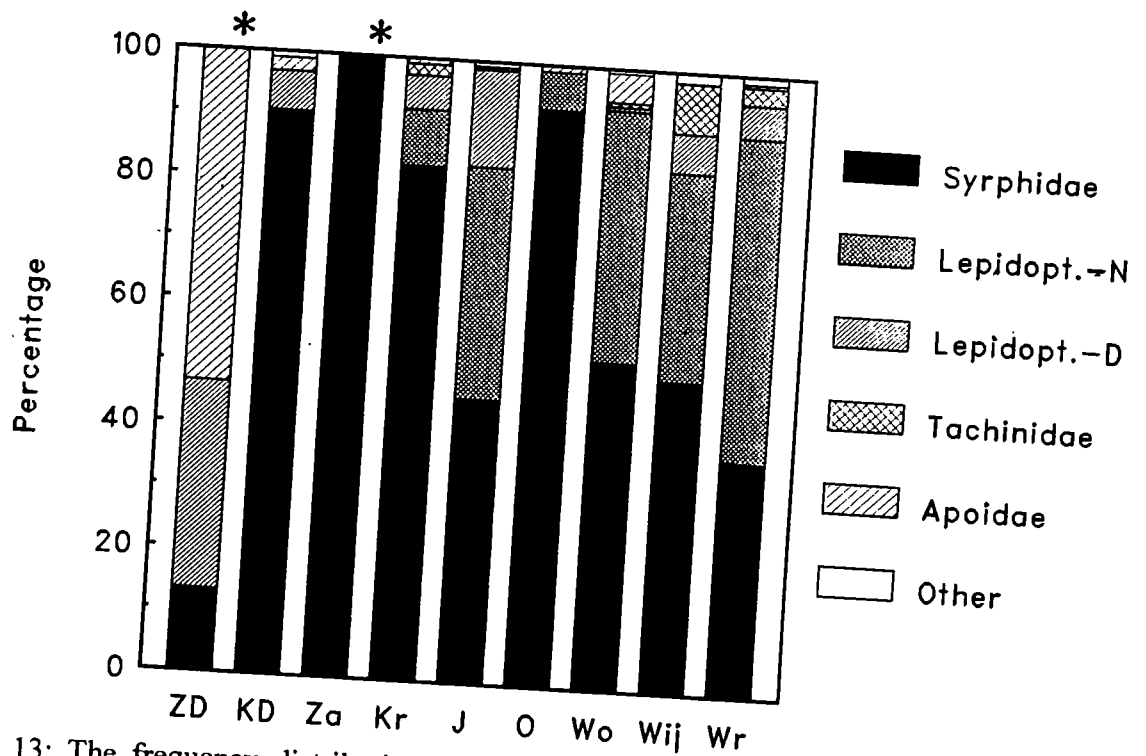


Figure 13: The frequency distribution of insect groups in populations of *S. columbaria*. Populations are ranked according increasing number of flowerheads. For explanation of used abbreviations see appendix 1.

### Visitation frequency

Visitation frequency was averaged if data of different patches were collected. No relation between population size and visitation frequency was found (figure 14).

Visitation frequency may be dependent on density (table 8, figure 15), but the number of patches with different densities within a population was low. In most cases the number of visits per head is lower in high density than in low density. Number of visits per head per day was about 20 up to 100.

Table 8 Visitation frequency in number of visits per head per day in different densities in eight populations.

Population	Date	Observation time (min.)	Number of heads	Density (heads/m <sup>2</sup> )	Number of visits
Zure Dries	230894	90	5	0.18	12
Kwartierse Dijk	220894	30	12	2.67	95
Zalk	220894	30	12	2	2
Kruisberg	240894	70	10	6.67	12.85
Julianagroeve	230894	120	25	12.5	58.65
	250894	30	33	6.88	32
Olst	220894	30	11	1.76	73.09
	220894	30	8	2	40.5
	220894	30	18	9	48.66
Wolfskop	170894	45	94	15.67	16.67
	170894	50	108	15.4	5.13
	250894	30	34	10.1	65.29
	250894	30	31	15.5	32.51
Wrakelberg	230894	90	16	10	29



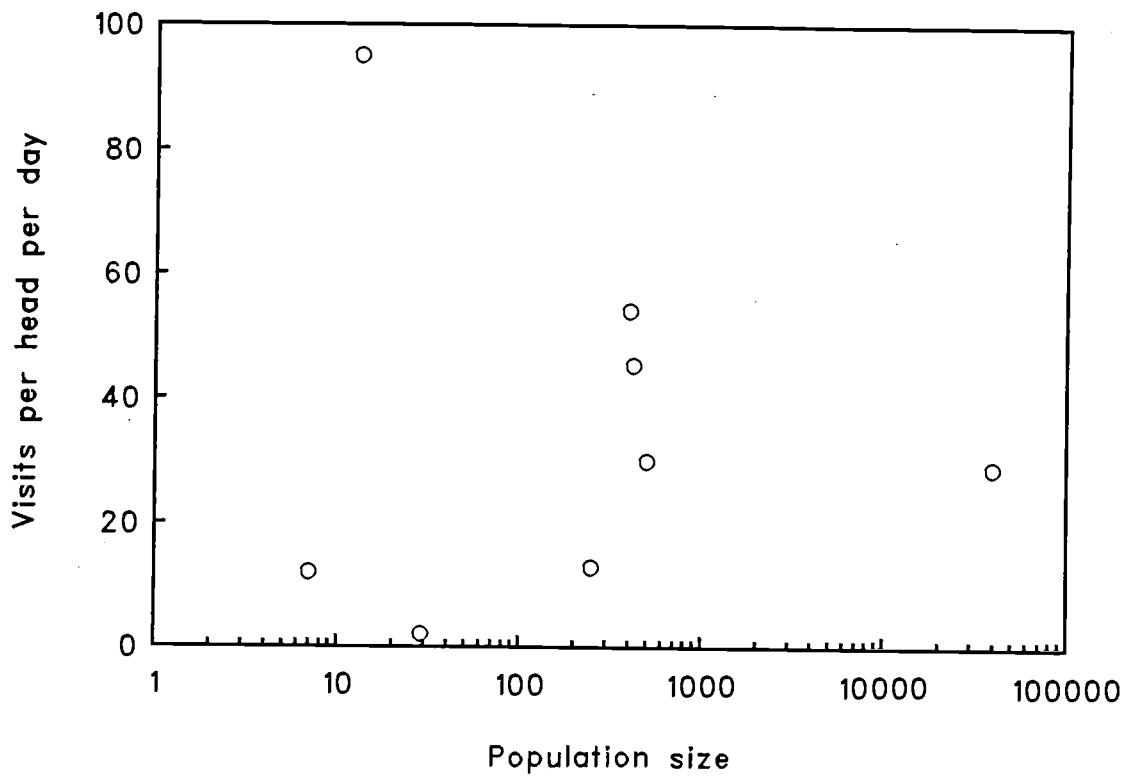


Figure 14: Relation between population size and visitation frequency per head per day of *S. columbaria*.

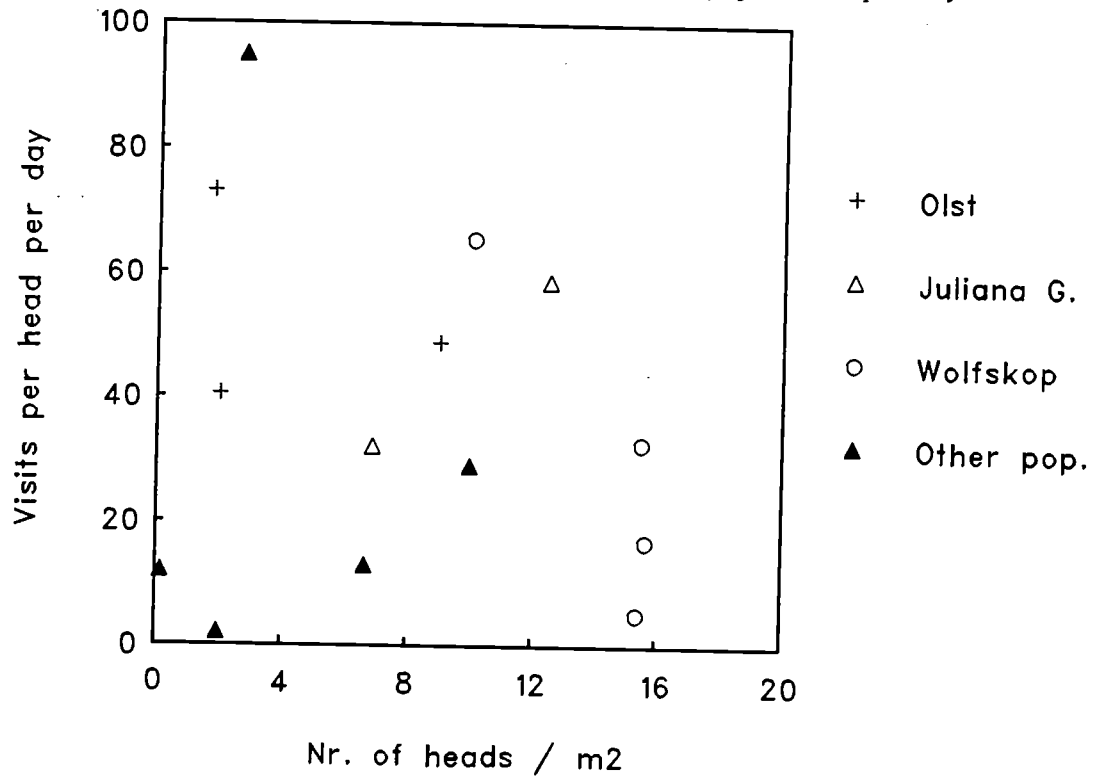


Figure 15: Relation between patch density and visitation frequency per head of *S. columbaria*.

### Foraging speed

Foraging speed was for all three species significant different (SNK-test after ln-transformation). *B. pascuorum* had the highest foraging speed, 85 flowers per minute, *A. mellifera* visited 50 flowers per minute and *E. tenax* 31 flowers per minute (table 8).

In the field foraging speed differed between the species. Foraging speed is expressed as number of heads visited per minute. On the Wrakelberg differences between the species were not significant. On the Kruisberg on 20 August 1994 foraging speed differed significant (SNK-test after ln-transformation) between *B. pascuorum*, 7.6 heads per minute and *P. rapae*, 4.4 heads per minute and *E. tenax*, 3.3. On 21 August 1994 *E. tenax* foraged significant (SNK-test after ln-transformation) slower, 2.9 heads per minute than *P. rapae*, 4.6 heads per minute on the Kruisberg (table 10).

Table 9. Foraging speed (mean  $\pm$  SE) measured in Haren for three insect species. Foraging speed is expressed as number of flowers per minute. N is number of individuals. For statistic significance see text.

Species	N	Foraging speed (mean $\pm$ SE)
<i>E. tenax</i>	17	31 $\pm$ 3.3 <sup>a</sup>
<i>B. pascuorum</i>	13	85 $\pm$ 8.4 <sup>b</sup>
<i>A. mellifera</i>	13	50 $\pm$ 3.4 <sup>c</sup>

Table 10. Foraging speed (mean  $\pm$  SE) measured on the Wrakelberg and the Kruisberg. Foraging speed is measured as number of flower heads visited per minute. N is number of scored visits. Only species within one site and date were tested. For statistic significance see text.

Soort	N	Wrakelberg 090894		Kruisberg 200894		Kruisberg 210894	
		N	Foraging speed mean $\pm$ SE	N	Foraging speed mean $\pm$ SE	N	Foraging speed mean $\pm$ SE
<i>E. tenax</i>	100	3.6 $\pm$ 0.6 <sup>a</sup>	79	3.3 $\pm$ 0.6 <sup>a</sup>	221	2.9 $\pm$ 0.5 <sup>a</sup>	
<i>A. gamma</i>	129	4.1 $\pm$ 0.3 <sup>a</sup>	-	-	34	3.5 $\pm$ 0.6 <sup>ab</sup>	
<i>P. rapae</i>	22	4.6 $\pm$ 0.6 <sup>a</sup>	30	4.4 $\pm$ 0.5 <sup>a</sup>	86	4.6 $\pm$ 0.3 <sup>b</sup>	
<i>B. pasc.</i>	-	-	94	7.6 $\pm$ 0.1 <sup>b</sup>	-	-	

### Feeding behaviour

In the natural populations all the abundant visitors on *S. columbaria* fed nearly only on nectar in August. At the end of the season (second half of September) large numbers of females of *E. pertinax*, *E. tenax* and *E. horticola* were seen feeding on pollen directly from the anthers and stigmas of *S. columbaria* in the experimental garden in Haren (table 11). For male *E. tenax* no pollen consumption was observed in both

August (N=46) and September (N=16). In August only 2.6 % of female *E. tenax* foraged on pollen (N=38; ♀:♂≈1:1) in the natural populations. During this period only some smaller syrphid flies of the *Syrphinae* group (*Episyrphus balteatus*, *Syrphus ribesii*, *Melanostoma mellinum*) and *Eumerus strigatus* fed mainly on pollen, but these species visited rarely *S. columbaria*.

Table 11:

Pollen consumption from anthers or stigmas of *S. columbaria* by female syrphid flies in the experimental garden in Haren on 24 September 1994.

species	N	♀:♂	♀ foraging for pollen (%)
<i>E. tenax</i>	48	3:1	27.1
<i>E. pertinax</i>	153	no♂	83.3
<i>H. pendulus</i>	31	>10:1	22.6
<i>M. mellinum</i>	10	-	100.0

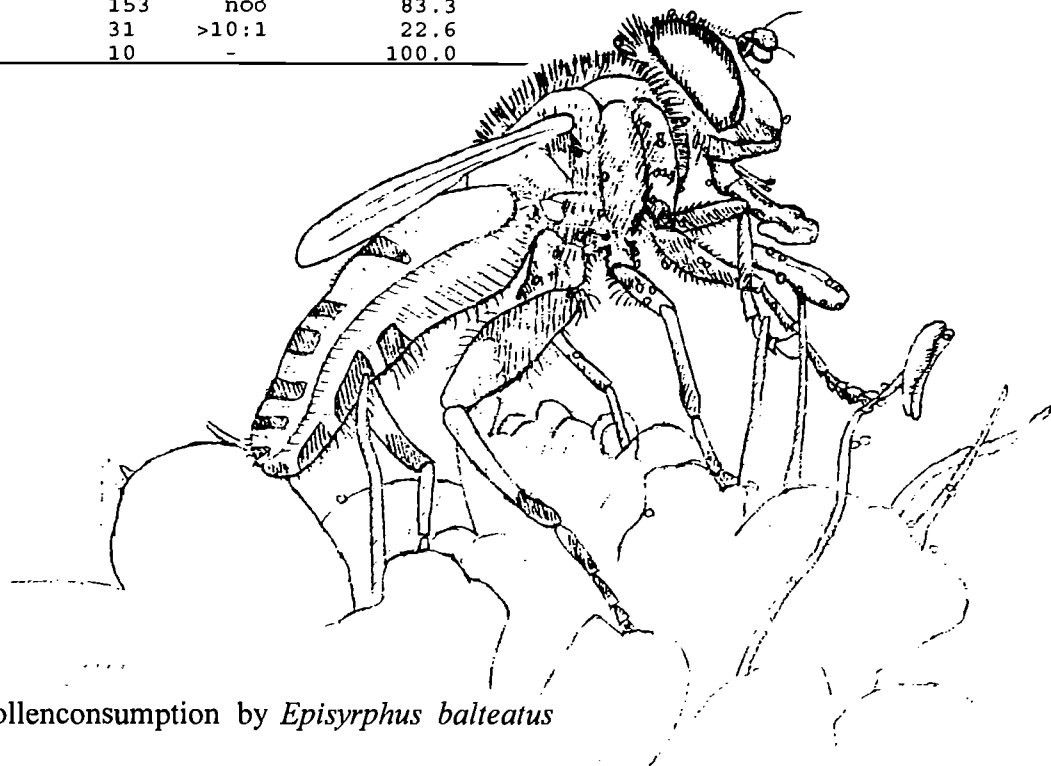


Figure 16: Pollenconsumption by *Episyrphus balteatus*

### Flower constancy

Insects switched between plant species. The percentage of visits on *S. columbaria* within a population differed between insect species (fig. 17). The percentage of visits on *S. columbaria* flowers per foraging bout was significantly higher for *E. tenax* than for *A. gamma* and *P. rapae* (SNK,  $p < 0.05$ ). In fig 19 the percentage of visits from *E. tenax* and *P. rapae* between the populations Wrakelberg and Kruisberg is compared. In the Kruisberg population flowers from *S. columbaria* had a smaller part in the vegetation (fig 18). For *E. tenax* the proportion of visits to *S. columbaria* was significant lower (MWU,  $p = 0.0002$ ) in the Kruisberg population compared to the Wrakelberg population (fig. 19). For *P. rapae* the percentage of visits was not significantly different between the populations. Bumblebees (mainly *Bombus pascuorum*) visited *S. columbaria* only rarely ( $< 2\%$ , appendix 7). They foraged mainly on *Centaurea* ssp. and *Origanum vulgare*; the percentage of bumblebees visiting *Knautia arvensis* in appendix 7 seems overestimated due to the small sample size (N=2).

Besides the percentage of visits on *S. columbaria* the number of changes of plant species during foraging is used as an index for flower constancy. This changing-index is calculated by dividing the number of changes from plant species by the total number of visits made by an insect in a protocol. Index values are for *E. tenax*, *A. gamma* and *P. rapae* the same, 0.2 changes from plant species per visit (table 12). No significant differences occurred between the three species on the same place (SNK-test) or for one species between the Wrakelberg and the Kruisberg (T-test).

Table 12: Changing-index (number of changes of an insect from plant species divided by the total number of visited flowerheads in a protocol). Measured on the Kruisberg on 20 and 21 August 1994.

Species	Number individuals	Changing-index (Mean $\pm$ SE)
<i>E. tenax</i>	21	0.17 $\pm$ 0.03
<i>A. gamma</i>	7	0.23 $\pm$ 0.05
<i>P. rapae</i>	5	0.21 $\pm$ 0.09

In the experimental garden the percentage of visits of *Eristalis* spp. was proportional to the ratio of *S. columbaria* and *Centaurea jacea* flowerheads (table 13).

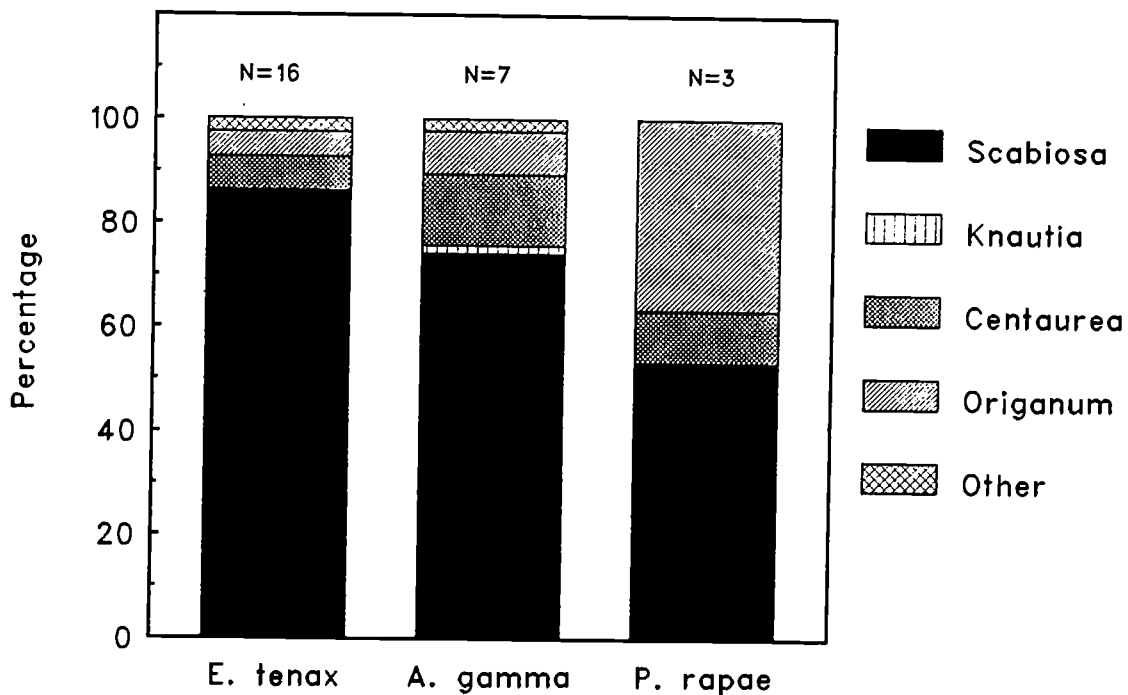


Fig. 17: Flower constancy of insect visitors on *S. columbaria* in the Wrakelberg population. Visited plant species per insect species

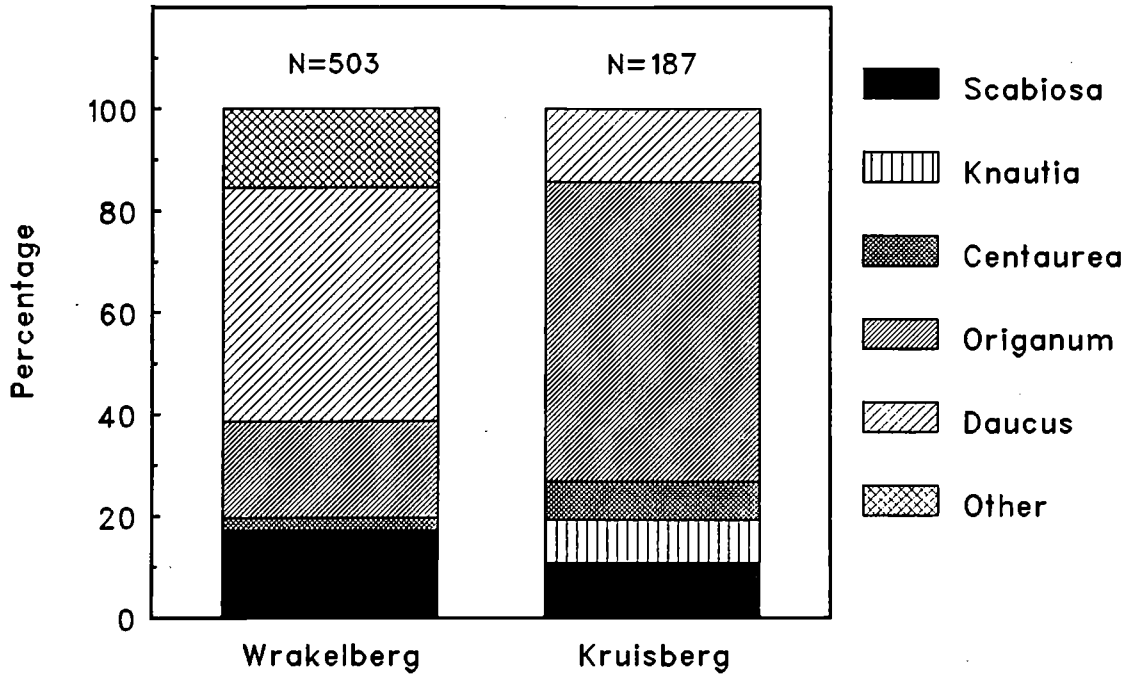


Fig. 18: Number of flowerheads from different plant species in the Wrakelberg and the Kruisberg population.

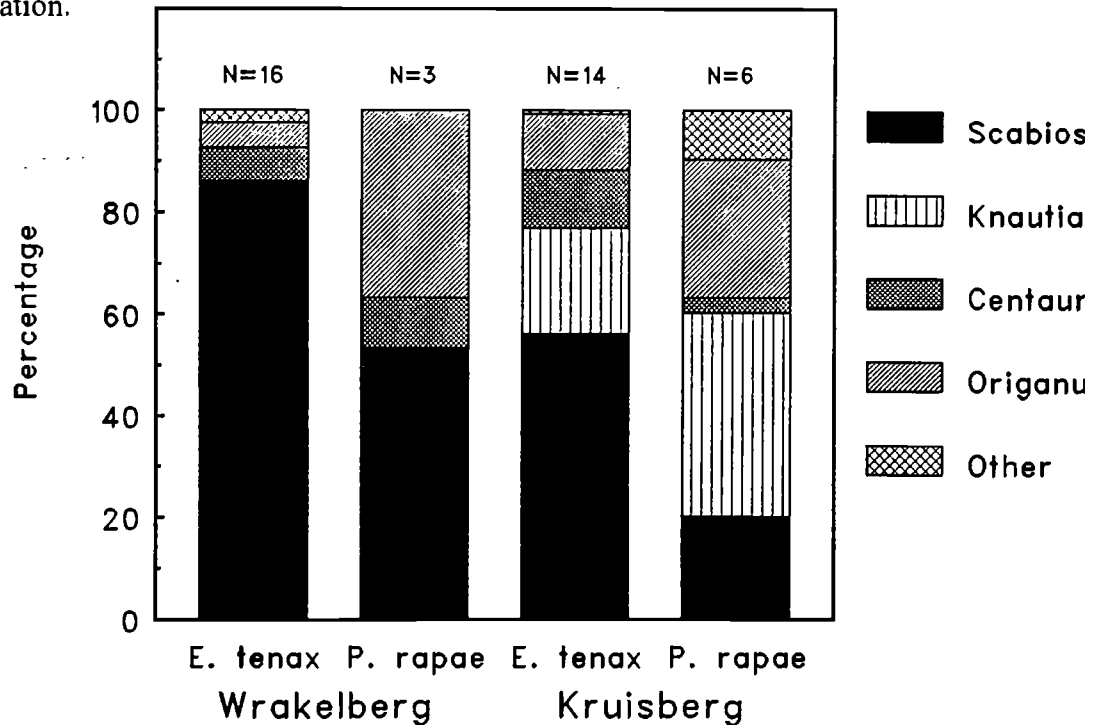


Fig. 19: Flower constancy of insects in the populations Wrakelberg and Kruisberg. Visited plant species per insect species

Table 13:

Visitation on *S. columbaria* by *Eristalis* ssp. in three patches. The patches had a different ratio of *S. columbaria* and *C. jacea* flowers.

patch	A	B	C
<i>S. columbaria</i> flowers (%)	90	50	10
visits on <i>S. columbaria</i> (%)	95.0	64.8	16.7
N	18	5	9

### Pollenload on insect bodies

Insect species differed in their mean total number of pollen grains on their bodies (fig. 20 and appendix 8). *B. pascuorum* contained the highest number of pollen grains on its body (mean  $\pm$  SE =  $1765 \pm 860$ ), although the difference with *E. tenax* was not significant, probably because the small number of individuals of *B. pascuorum*. Differences between the other species were all significant (SNK,  $p < 0.05$ ). The pollenload of butterflies was very small compared to *E. tenax* and *B. pascuorum*; for *A. gamma* and *P. rapae* respectively  $19 \pm 7$  and  $72 \pm 33$ .

The composition of plant species in the pollen load on insect bodies is shown in figure 21. All insects carried only a very low percentage (maximum 38%) of *Scabiosa* / *Knautia* pollen. It was not possible to distinguish between pollen of *S. columbaria* and the closely related *Knautia arvensis* due to large overlap in size and morphology. However in the Wrakelberg population only very small numbers of flowerheads of *K. arvensis* occurred. All the insect species carried many heterospecific pollen on their bodies, especially from *Centaurea* ssp. The percentage of *S. columbaria* pollen was comparable between the species, only *B. pascuorum* had a significant lower fraction of *S. columbaria* pollen than *E. tenax*. For the percentage of *Centaurea* pollen no significant differences were detected (SNK,  $p > 0.05$ ).

In absolute numbers *E. tenax* carried most pollen of *S. columbaria* (table 14).

Table 14:

Absolute number of pollen grains of *S. columbaria* in the pollen load of different insect visitors on *S. columbaria* in the Wrakelberg population. Different letters represent significant differences (SNK,  $p < 0.05$ ).

species	N	mean $\pm$ SE
<i>E. tenax</i>	14	$407.1 \pm 82.8^a$
<i>A. gamma</i>	13	$3.7 \pm 1.3^b$
<i>P. rapae</i>	6	$23.5 \pm 7.4^c$
<i>B. pasc.</i>	4	$96.3 \pm 85.3^c$

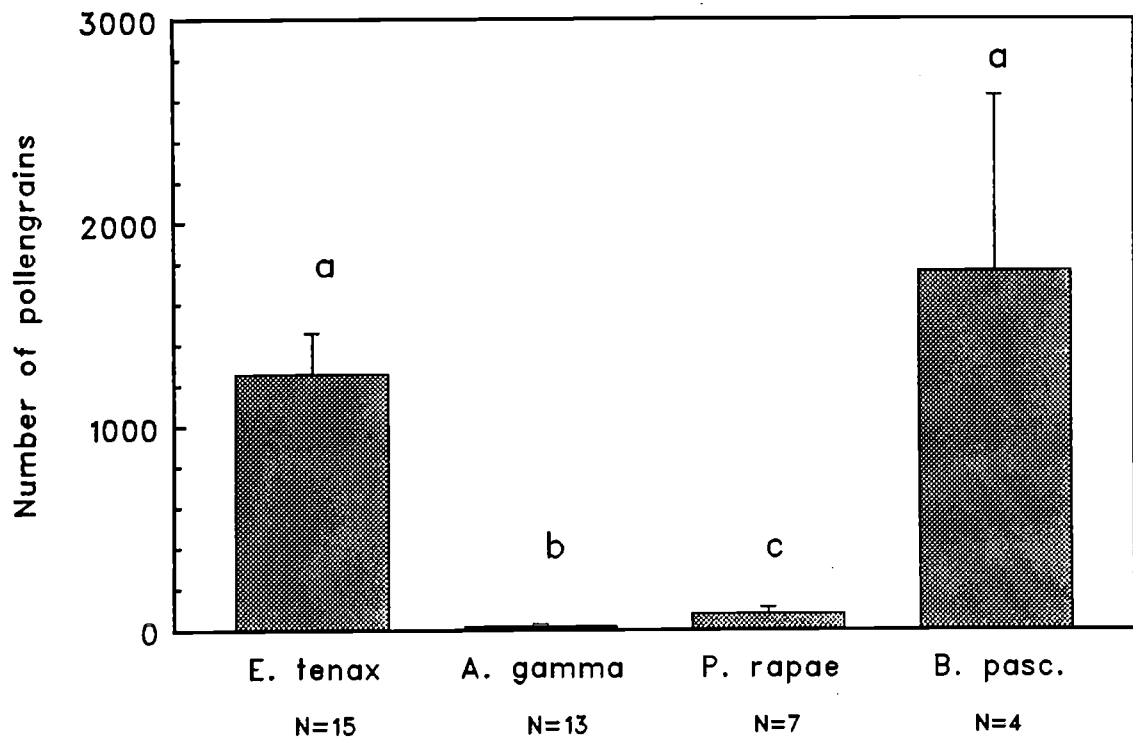


Fig. 20:  
Total number of pollen grains (mean  $\pm$  SE) on bodies of different insect species. Different letters represent significant differences (SNK,  $p < 0.05$ ).

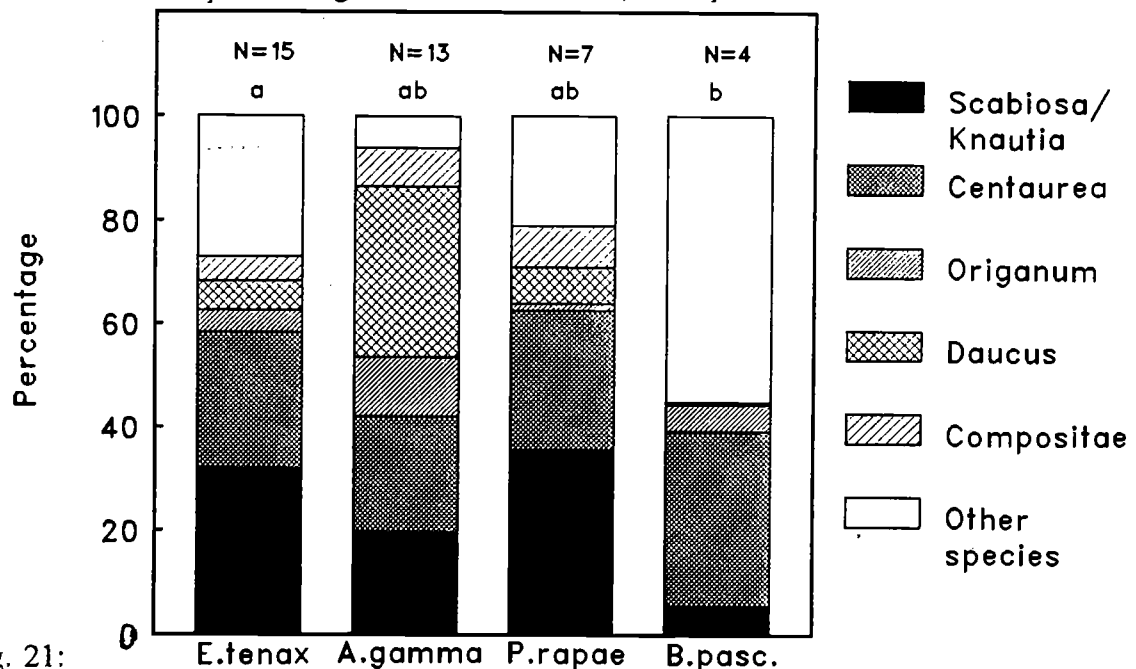


Fig. 21:  
Pollen grains (in percentages) of various plant species in the pollen load of different insect visitors on *S. columbaria* in the Wrakelberg population. Different letters represent significant differences in percentage of *S. columbaria* pollen (SNK,  $p < 0.05$ ).

### Pollen deposition

In the analysis of number of grains deposited per stigma in the experimental garden the stigma's without pollen deposition were ignored. For *E. tenax* number of grains deposited per stigma was 3.1, for *B. pascuorum* 2.2 and for *A. mellifera* 2.3 (table 15). No significant differences occurred between the species (Kruskal-Wallis test after ln-transformation).

The mean deposition rate for *E. tenax* was 8.5 grains per stigma per minute for *B. pascuorum* 3.8 and for *A. mellifera* 11.3. The difference in deposition rate was not significant different for the species (SNK-test after ln-transformation).

Table 15: Number of pollen grains deposited per stigma and pollen deposition rate for three insect species. Stigma's without pollen deposition were ignored in the analysis of number of pollen grains deposited per stigma and deposition rate. Deposition rate is expressed as number of pollen grains per stigma per minute. N is number of individuals. For statistic significance see text.

Species	N	Nr.grains /stigma (mean $\pm$ SE)	Deposition rate (mean $\pm$ SE)
<i>E.tenax</i>	17	3.1 $\pm$ 0.6	8.5 $\pm$ 3.0
<i>B.pascuorum</i>	13	2.2 $\pm$ 0.2	3.8 $\pm$ 0.6
<i>A.mellifera</i>	13	2.3 $\pm$ 0.2	11.3 $\pm$ 5.5

In the field we measured the increase in number of pollen grains on stigmas during the day. The number of deposited pollen grains on a stigma increased during the day (table 16, fig. 22). After three hours observation in all cases at least 8 pollen grains per stigma were deposited. In the mid of the afternoon the number of pollen grains per stigma stabilizes around 9 grains per stigma. The pattern of increase of pollen grains for the two populations is rather similar.



Table 16: Number of pollen per stigma during the day. Time of observation and mean values with SE per stigma per population.

Population	Time	Pollen/stigma (mean $\pm$ SE)
Wrakelberg 160894 8 heads sampled, 5 stigma's/head	10.45	3.9 $\pm$ 1.5
	13.00	5.3 $\pm$ 1.5
	15.00	9.2 $\pm$ 1.6
	17.00	11.3 $\pm$ 1.8
	18.15	11.6 $\pm$ 1.7
Wrakelberg 230894 9 heads sampled, 5 stigma's/head	09.30	1.4 $\pm$ 0.6
	10.45	4.1 $\pm$ 1.2
	11.45	7.3 $\pm$ 1.9
	12.45	9.5 $\pm$ 2.2
Juliana-groeve 230894 8 heads sampled, 10 stigma's/head	09.30	0.1 $\pm$ 0.0
	10.00	0.6 $\pm$ 0.4
	11.30	1.7 $\pm$ 0.5
	12.15	5.5 $\pm$ 1.9
	13.15	8.3 $\pm$ 1.9

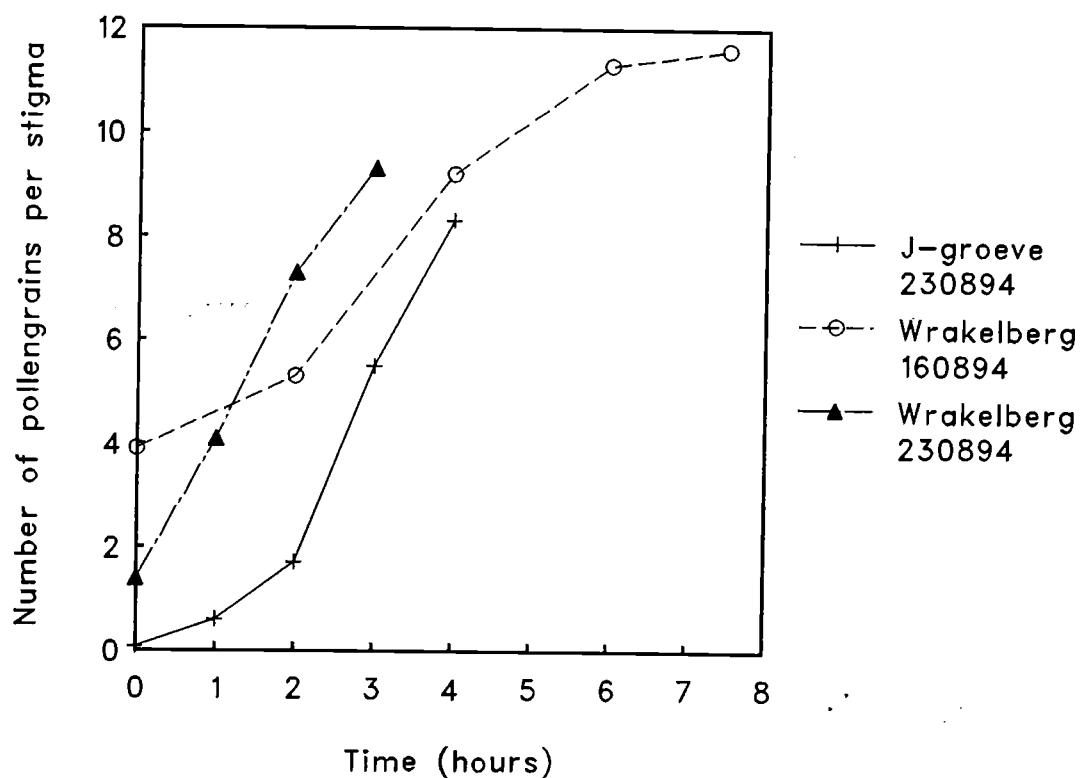


Figure 22: Number of pollen per stigma during the day in population Wrakelberg on 16 and 23 August and Juliana-groeve on 23 August.

### Pollination effectiveness indices

Index values for different aspects of the pollination effectiveness are summarized in table 17 in such a way that the importance of the insect species can be compared for the different aspects of pollination.

Table 17:

Index for different aspects of pollination effectiveness on *S. columbaria*. For each aspect the maximum value is set 1.00. The overall effectiveness index is calculated by multiplying the index values: visitation frequency x total pollenload x % *Scabiosa* pollen.

	visitation frequency	total pollenload	% <i>Scabiosa</i> pollen	effectiveness index
<i>E. tenax</i>	1.00	0.71	0.98	0.6958
<i>A. gamma</i>	0.76	0.01	0.50	0.0038
<i>P. rapae</i>	0.11	0.04	1.00	0.0044
<i>B. pascuorum</i>	0.03	1.00	0.09	0.0027

In number of visits on *S. columbaria* *E. tenax* and *A. gamma* are quantitative the most important pollinators. But the pollenload of *A. gamma* is very small, so for the quality component the value of the pollination service of this species is low. For *B. pascuorum* its the other way around: the pollenload is very large, but consists mainly of pollen from other species because due to their low percentage of visits on *S. columbaria*. The effectiveness index (= visitation frequency x total pollenload x % *Scabiosa* pollen) is for *E. tenax* much higher than for the other species. However this effectiveness index not includes the flight distance, which may be an important qualitative aspect.

### Pollenload on stigmas

The composition of pollen grains from different plant species on *S. columbaria* stigmas as depicted in fig. 23 shows that in all 3 populations the amount of conspecific pollen is very low. Other important plant species found as pollen grains on stigmas of *S. columbaria* were *Centaurea* ssp., *Origanum vulgare*, *Daucus carota* and *Compositae*. For the Kruisberg population it must be noted that flowering *Knautia arvensis* was quite abundant in the vegetation, so in this population the real fraction of *S. columbaria* was probably lower than suggested in the figure.

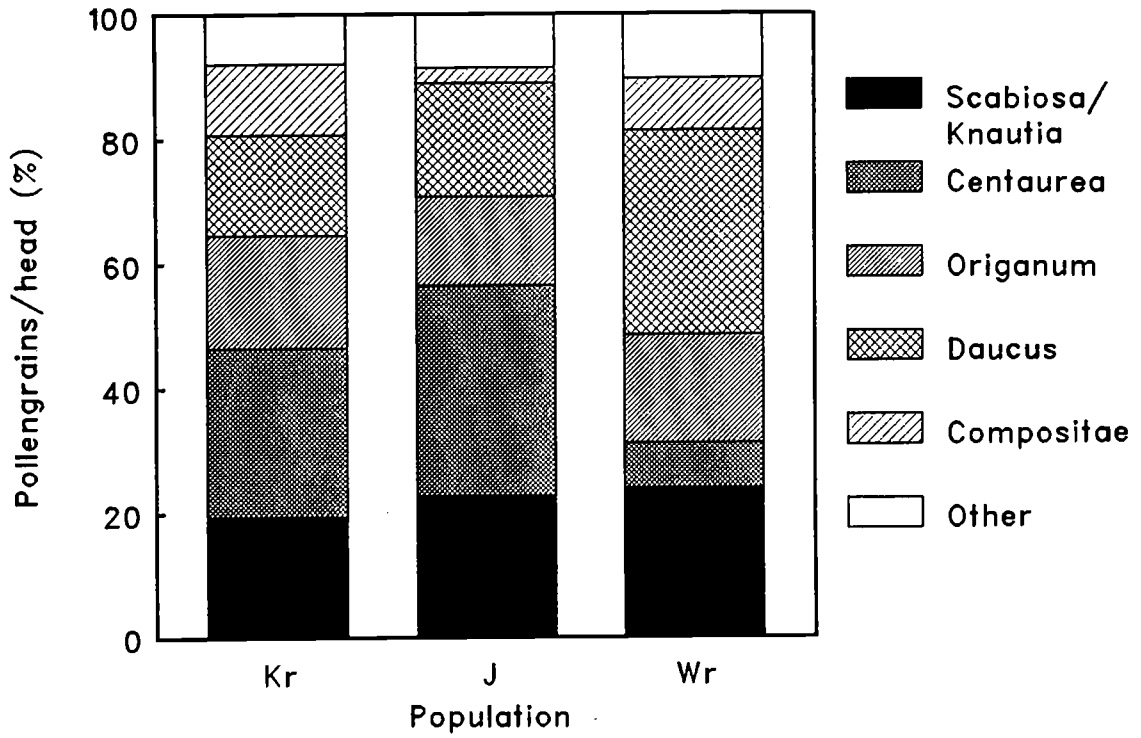


Fig. 23:  
Composition of plant species in the pollen grains on stigmas of *S. columbaria* in different populations (10 flowerheads per population). Mean number of pollengrains per stigma: N=500 for the Kruisberg population and N=1200 for the Wrakelberg and Juliana Groeve populations. Differences in composition are not significant (SNK,  $p > 0.05$ ).

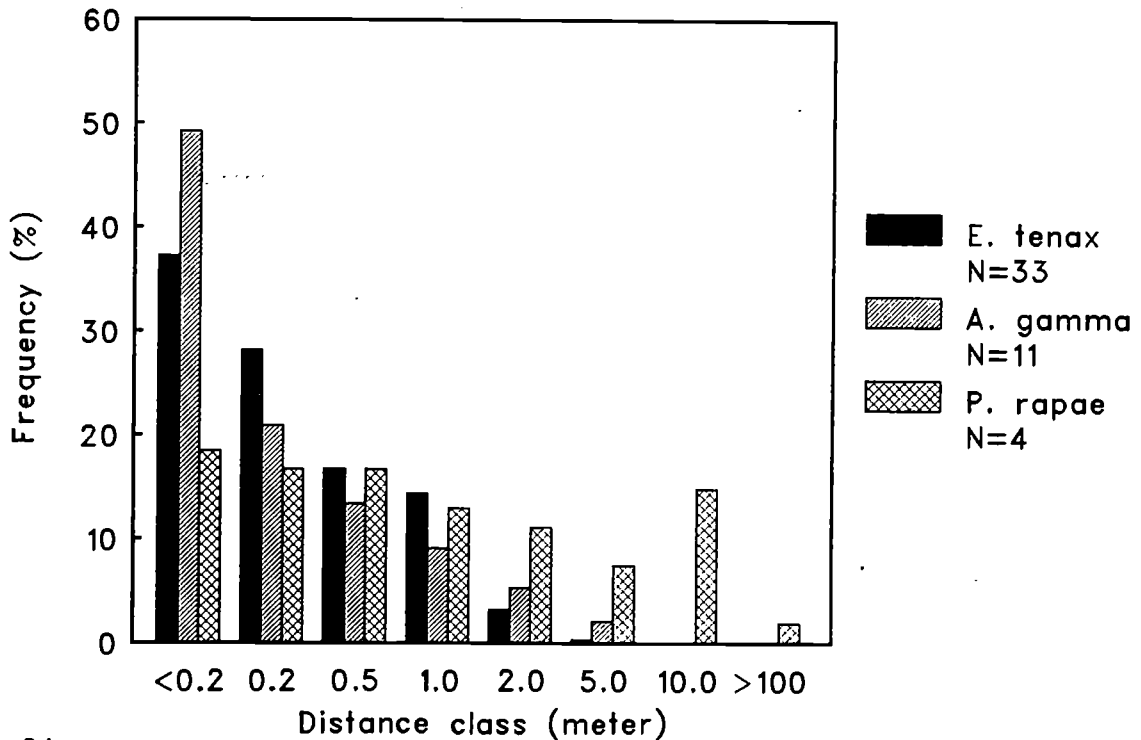


Fig. 24:  
Flight distances of insect visitors on *S. columbaria* in the Wrakelberg population. Percentage of flights per distance class (0.2 means from 0.2 meter till 0.5 meter).

## Flight distance

The insect visitors on *S. columbaria* differed markedly in their flight distance between two flowerheads (fig. 24). *E. tenax* and *A. gamma* mainly flew short distances: more than 90 % less than 2 meter. From both species no flights over distances more than 10 meter were recorded. In contrast, the butterfly *P. rapae* made 15 % of its flights over distances more than 10 meter and even some flights over more than 100 meter. The mean flight distances are given in table 18. But because of the skewed distribution the mean flight distance is less accurate measurement as the percentage of flights in the different distance classes.

Table 18:

Flight distance (mean  $\pm$  SE) of insectvisitors on *S. columbaria* in the Wrakelberg and Kruisberg population. Different letters represent significant differences (SNK,  $p < 0.05$ ).

species	N(flights)	mean $\pm$ SE
<i>E. tenax</i>	699	0.65 $\pm$ 0.04 <sup>a</sup>
<i>A. gamma</i>	184	0.73 $\pm$ 0.11 <sup>a</sup>
<i>P. rapae</i>	182	3.53 $\pm$ 0.63 <sup>b</sup>
<i>B. pascuorum</i>	93	1.17 $\pm$ 0.29 <sup>a</sup>

A low number of the marked insects is re-seen (table 19). The *Bombus* species are relatively often re-seen. The low reobservation percentage of syrphid individuals may indicate that they disappeared from the *S. columbaria* or were not active the whole day. Distances between recaptures were within 50 meter, but some were over 100 meter (table 20).

Table 19: Number of insects marked on the Wrakelberg on 15 August 1994 and re-seen on 16 August and marked on the Kruisberg on 11 and 24 August 1994 and re-seen on 11 and 25 August. The number of insects which were re-seen is a minimum number.

	Wrakelberg		Kruisberg		marked 240894	resight 250894
	marked 150894	resight 16089	marked 110894	resight 110894		
<i>E. tenax</i>	173	3	63	3	72	3
<i>E. nemorum</i>	5	-	-	-	2	1
<i>E. arbustorum</i>	7	-	21	1	3	-
<i>H. trivittatus</i>	7	1	-	-	-	-
<i>B. pascuorum</i>	-	-	-	-	3	1
<i>B. soroensis</i>	-	-	-	-	3	2
<i>B. lapidarius</i>	-	-	-	-	1	1
<i>A. gamma</i>	14	-	2	-	-	-
Lepidoptera sp.	16	-	-	-	11	-

Table 20. Distance between two resights of marked insects. Total of re-sights on Wrakelberg (160894) and Kruisberg (110894 and 250894). The number between brackets indicates the minimum number of individuals which are seen. Observations of >70 m consist of two *E. tenax* on 102m and a *H. trivittatus* on 150m. The *E. tenax* marked with \* includes an individual which is seen three days after marking.

Distance (m)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	>70
<i>E. tenax</i> (9)	16	1	5	1	3	1	3*	2
<i>E. nemorum</i> (1)	-	1	-	-	-	-	-	-
<i>E. arbustorum</i> (1)	1	-	-	-	-	-	-	-
<i>H. trivittatus</i> (1)	-	-	-	-	-	-	-	-
<i>B. pascuorum</i> (1)	-	-	-	1	-	-	-	-
<i>B. soroensis</i> (2)	-	-	2	7	2	3	-	-
<i>B. lapidarius</i> (1)	2	1	-	-	-	-	-	-

### Transport of fluorescent dye powder

Most fluorescent dye powder is transported over distances less than 10 meter from the donor flowerhead of *S. columbaria* (fig 25). About 4% of the dye powder however is transported over distances more than 100 meter (maximum 160 m).

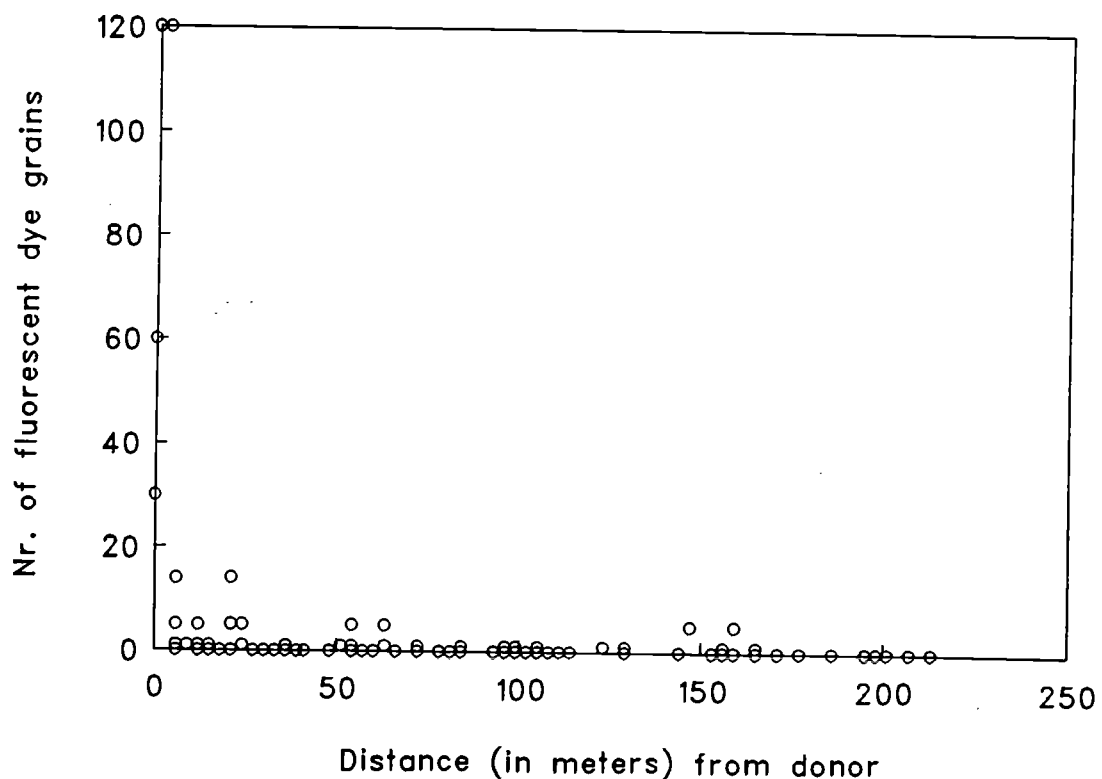


Fig. 25: Transport distance from fluorescent dye powder in the Kruisberg population from the three colours combined.

## Germination

In all populations the percentage of germination was rather low, less than 45% (fig 26 and appendix 10). In the population Wolfskop the seeds were not ripe enough at the time of collecting; they did not germinate. The percentage of germination was not related to the population size of *S. columbaria* (fig 27).

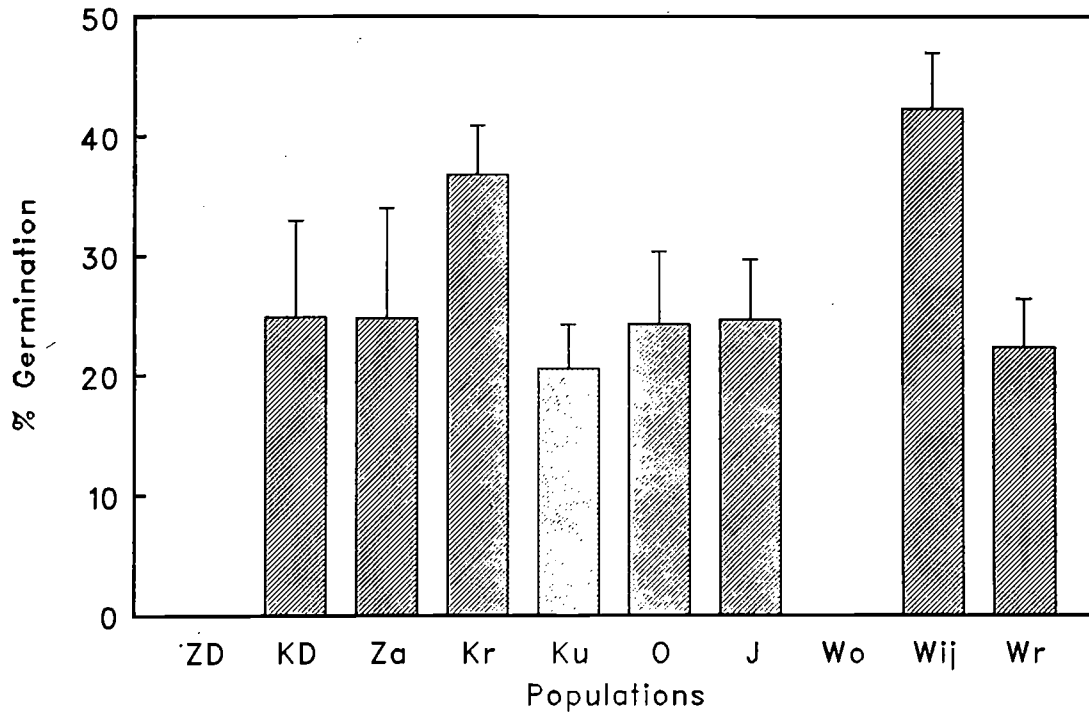


Figure 26:  
Germination percentage of *S. columbaria* seeds per population (mean  $\pm$ SE). Different letters represent significant differences (SNK,  $p < 0.05$ ).

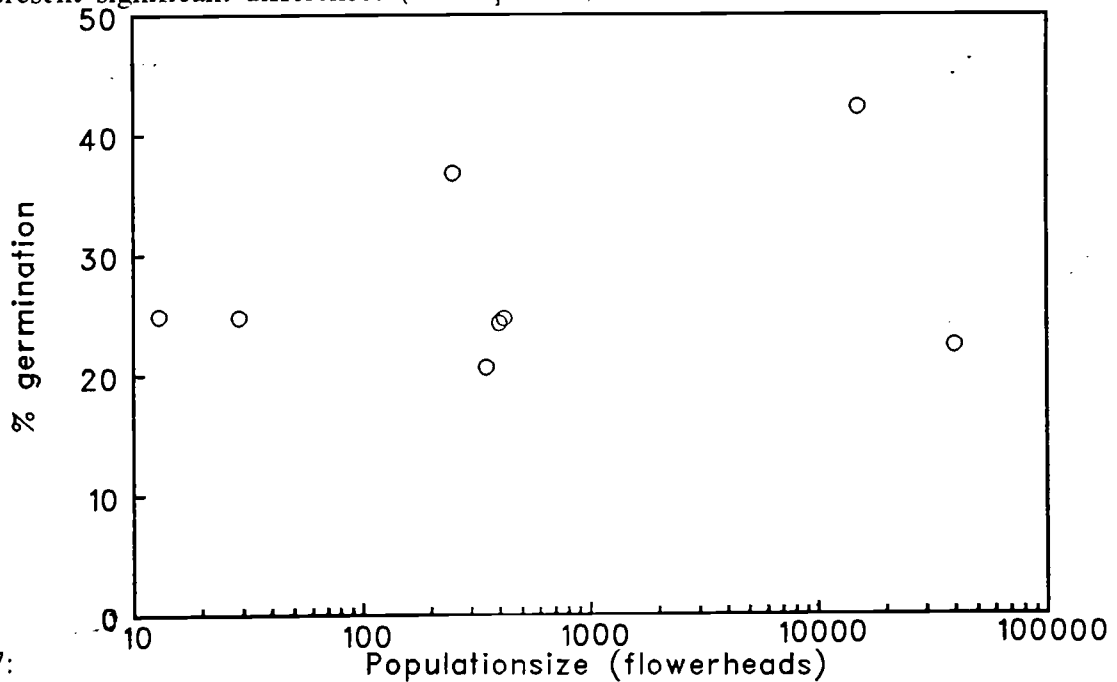


Figure 27:  
Population size and percentage of germination per population.

## Discussion

### Activity during the day

The peak in insect activity at the end of the morning and a small one late in the afternoon is found in several studies in temperated areas of north-western Europe (Gilbert 1985). Insects have to warm up in the morning, because the muscles need to be wam enough to generate the power to fly (Unwin & Corbet 1991). Small insects have the same body temperature as the air temperature. Larger insects can maintain a thoracic temperature above the air temperature. They can fly when the air temperature is for others below flight temperature. Butterflies have to dry up from the dew before they start foraging. With increasing temperature insects increase forage activity. In the morning most nectar and pollen (after the anthers burst open, which takes some time) is available. These are the main food sources for the flower visiting insects. Some plants produce nectar the whole day, but the largest volumes are present early in the morning. In the afternoon it can become too hot for part of the insect species to fly. Body temperature can get too high and heat is avoided by moving into the shade (Unwin & Corbet 1991). At the end of the day when temperature drops they start foraging again.

### Relation population size and insect species richness

Population size of *S. columbaria* gives a rather good explanation of insect species number. This is a correlation, no direct causal relation between the population size and *S. columbaria* is proven. Population size is probably related with several other environmental factors and size of an area. A larger area means often more variation in micro habitats. All visitors of *S. columbaria* in the Netherlands are generalists. A relation between total number of flowering herbs, attractive for insects, and insect species richness is expected more than a relation between insect species number and population size of *S. columbaria*. For generalistic insect species the number of plant species is not most important. Other factors, for example total number of flowers and variation in flowering plants or availability during a long season, may give a better relation.

Time of observation per population influences the number of insect species observed. If observation time is proportional to population size the number of insect species in a small population can easy be underestimated. Observation of insects in a small population is more influenced by chance because of the low numbers than in large populations. Compensation for this chance effects by a longer observation period in small populations is a possibility to solve this problem. In a larger population more time is needed to include all species.

### Insect species composition

A comparison is made between the species composition of insects visiting *S. columbaria* in different countries (figure 28). The great amount of Syrphidae seems to be a typical Dutch situation. In stead of these syrphids in Great-Brittain a large amount of the visitors consists of butterflies (day). Also Hymenoptera take an important part which becomes nearly hundred percent in the French Alps. In Great-Brittain the Hymenoptera consist mainly of bumblebees, in the French Pyrenees of honeybees. Both

are together with most butterflies and syrphids generalists in regard of foraging behaviour. In the French Alps the specialistic bee *Dasypoda argentata* occurs; the visits shift nearly completely to this oligolectic species.

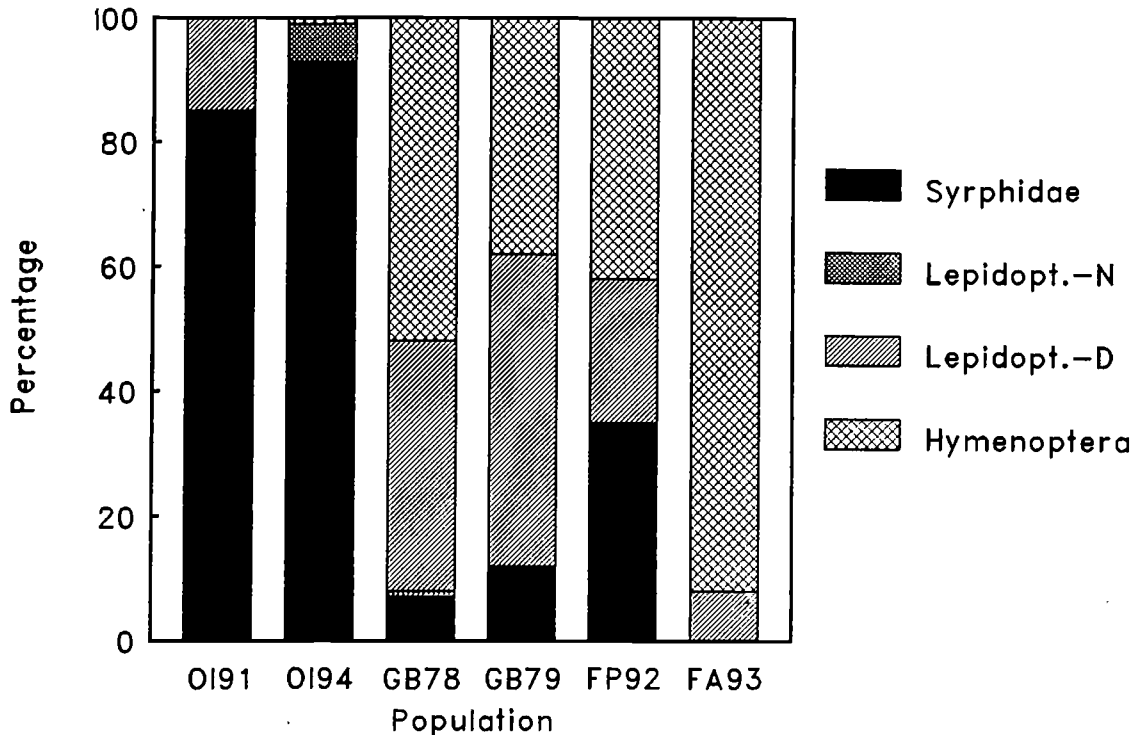


Figure 28: The contribution of insect groups visiting *S. columbaria* in different populations. Olst (Netherlands) 1991 and 1994, Great-Brittain 1978 and 1979 (Lack, 1982, French Pyrenees 1992 and French Alps 1993 (Kwak, 1994).

*S. columbaria* is pollinated in the Netherlands only by polylectic insect species. In Germany and the French Alps *S. columbaria* populations are partly pollinated by the oligolectic bees *Andrena marginata* or *Dasypoda argentata* (Westrich, 1990a,b and Kwak, 1994). This difference between oligo- and polylectic visitors may have consequences for the purity of pollen deposition. In the case of polylectic insects heterospecific pollendeposition can be expected. This was indeed observed. However, we were not able to test the other possibility: conspecific pollendeposition when oligolectic bees are pollinators.

The most abundant insect visitor on *S. columbaria* in the Netherlands was *E. tenax*. It is one of the most common syrphid flies in NW Europe, with a strong increase in antropogenic environments in this century (Bańkowska, 1980). The larvae occur in eutrophic water (Bańkowska, 1980 and Verlinden, 1991). Its occurrence in nutrient poor nature reserves can be seen as an indication of eutrophication of the environment at landscape scale. It seems therefore unlikely that *E. tenax* was in the past the most abundant visitor.



It is unknown if *S. columbaria* was pollinated in the past by the oligolectic *A. marginata* or *D. argentata* due to a lack of data from Hymenoptera from the beginning of this century. The oligolectic *D. argentata* is unlikely to have occurred in the Netherlands in the recent past since its areal reaches its northern border in Baden Württemberg (Westrich, 1990b) and it is not known to have occurred in Belgium (Rasmont *et al.*, 1993). The oligolectic bee *A. marginata* may have occurred in the Netherlands. This species has declined very strongly in Belgium (Rasmont *et al.*, 1993) and Germany (Westrich, 1990b). Therefore it may already be too late to find a shift in species composition on *S. columbaria* in the Netherlands from oligolectic to polylectic species. If *A. marginata* has become extinct in the Netherlands its place is occupied by species with a generalistic feeding behaviour. The other possibility is that the Netherlands already in the past layed beyond the border of the areal of *A. marginata* so *S. columbaria* is here naturally pollinated by polylectic species. Geographical ranges of oligolectic bees and the plant species they depend on rarely coincide precisely (Feinsinger, 1983).

On 6 July 1994 we observed in several populations the oligolectic bee species *Andrena hattorfiana* on *Knautia arvensis*. This was before the flowering season of *S. columbaria*, but in Germany it visits also *S. columbaria* early in the flowering period (Westrich, 1990b).

The local occurrence of insect species in a population of *S. columbaria* is determined partly by the spatial distribution of habitats in the surroundings of the chalk grasslands. Many syrphid flies occur as a larvae in rotten wood or depend on relative high humidity (Bańkowska, 1980). So their occurrence depends on forest in the surroundings of the chalk grasslands. In the small population Zure Dries the occurrence of *Volucella pellucens* can be explained by the surrounding of forest. In chalk grasslands in the Gaume (Belgium) which are surrounded by old forests *S. columbaria* is visited by many other syrphid species characteristic for forests or with a preference for relatively high humidity (Ozinga, pers. obs. 1991).

### Visitation frequency

Differences in visitation frequency were not related with population size. Visitation frequency in Dutch *Scabiosa* populations seems to be quite high, when compared with the visitation rate in the French Alps. Between 2-100 visits per head per day were observed in the Dutch populations, roughly 30 visits per head per day, mainly spent by the oligolectic bee *D. argentata* are observed in the French Alps (Kwak 1994). If a female head is ripe, one visit can be enough to have a succesful pollination. Therefore the quantitative aspect of pollination in *S. columbaria* seems not to be a problem in the Dutch *S. columbaria* populations. From the relation between flower density and visitation rate is clear that it is difficult to give one value for visitation rate for a whole population.

The weather conditions during the fieldwork were good with high temperatures (many days >25°C) and nearly no rain. In colder years with more rain the frequency of the insect species will change. The large syrphid fly *E. tenax* becomes active at lower temperatures than smaller species (Gilbert, 1985). With rainy weather however there is nearly no flight activity from syrphid flies (own observations and Grosser & Klapperstück, 1977) while bumblebees can still be active with rainy weather or with

high wind velocity (Lundberg, 1980 and Kwak, 1993). During bad weather conditions, which are quite normal in the Netherlands, it can be expected that bumblebees get a relative larger part in the visitation frequency. *Succisa pratensis*, a species very similar to *S. columbaria* was visited and pollinated by only a very few bumblebee individuals during cloudy rainy weather (Kwak, pers. obs.). So the large number of visiting species can have a positive impact on the resistance of the pollination system of *S. columbaria* populations to extreme weather conditions.

### **Pollination limitation**

To investigate if pollination limitation occurred, seeds of four populations were germinated after hand- and natural pollination. No germination occurred. Seeds of the same populations did germinate in the experiment for comparing populations on percentage germination. They were collected on other date and selected on ripeness. The seeds for the pollination limitation experiment were per population collected on the same day from specific, marked, plants. Seeds are ripe in one or two weeks. Due to this variation not all flowerheads are ripe at the same moment. When the seeds are ripe they start to fall out, so the flowerheads need to be collected then. Normally seeds can ripe in a dry paperbag, but the lack of germination of *Scabiosa* indicated that this did not occur.

If seeds are so sensitive to an early harvest, management by cutting should be carefully done at the right moment. Also grazing, which was the case in at least 3 of the 9 studied populations, can have a great influence on the amount of ripe seeds reaching the soil.

### **Feeding behaviour**

In the natural populations all the abundant visitors on *S. columbaria* fed nearly only for nectar in August. *S. columbaria* is probable an attractive flower for polylectic insects because the nectar is easy reach. Some rare visitors of the *Syrphinae* group (*Episyrphus balteatus*, *Syrphus ribesii*, *Melanostoma mellinum*) however foraged mainly on pollen. At the end of the season (second half of September) in the experimental garden large numbers of females of *Eristalis* species (especially *E. pertinax*) were seen feeding on pollen from the anthers and stigmas of *S. columbaria*, probably to obtain extra proteins for overwintering and for the development of their eggs. Flower visiting syrphid flies from the genus *Eristalis* are known to feed mainly on nectar (Gilbert, 1981). Pollen consumption by *Eristalis* species was observed rarely in the natural populations earlier in the season. When this feeding behaviour on *S. columbaria* also occurs in the natural populations this may lead to a large pollen loss (decrease in male reproductive success) and in some cases quantitative problems with pollination at the end of the flowering season may occur.

Besides this direct pollen consumption we also observed indirect pollen consumption. *Eristalis*- and to a lesser extend *Helophilus*-species spend between flower visits a lot of time combing pollen from their body with their front and hind legs and bringing it to the mouthparts. This method is described by Holloway (1976) for large and hairy syrphid flies which almost exclusively foraged on nectar-producing plants. He found evidence that pollen on the thorax and abdomen may be left for several days before it has been removed. In respect to pollination one can wonder whether these pollen are still viable.

### **Flower constancy**

All visitors of *S. columbaria* were generalists visiting several other plant species. For bumblebee species it is known that as an individual workers can behave as specialist. On *S. columbaria* this behaviour is not observed, but it was mainly visited by male bumblebees. On the flower constancy of male bumblebees little is known (Jennersten *et al.*, 1991). The observed bumblebee species on *S. columbaria* had a preference for *Centaurea* ssp. and *Origanum vulgare*. In the experimental garden in Haren *Bombus pascuorum* was a regular visitor of *S. columbaria* but here were almost no other flowers in the surrounding available. Also the honeybee (*Apis mellifera*) which was a frequent visitor in the experimental garden had no choice. In the natural populations honeybees occurred only in small numbers and visited *S. columbaria* rarely. Most syrphid species in NW-Europe forage on many plant species (De Buck, 1990). *E. tenax* visited a smaller proportion of flowers from *S. columbaria* when this plant species had a smaller part in the vegetation. So for the visitors on *S. columbaria* the percentage of visits is influenced to a large extend by the availability of flowers in the surrounding vegetation.

### **Foraging speed**

Foraging speed differed for the flower visiting species. Measured foraging speed with a higher value for *B. pascuorum* than *E. tenax*, is in accordance with Kwak, who found the same difference between *B. pascuorum* and *E. tenax* on *S. columbaria* (Kwak, 1993). The values we found are both somewhat higher, 7.6 and 3.3 visited heads per minute for *B. pascuorum* and *E. tenax* vs 5.1 and 1.5 (Kwak, 1993). These differences are during active foraging, but *E. tenax* spends more time on resting between periods of active foraging (pers. obs.) in the mean while ceaping flowerheads occupied. Also in number of flowers *B. pascuorum* visited by far most flowers per minute. Comparing these two values gives 11.2 visited flowers per head per minute for *B. pascuorum* and 9.4 for *E. tenax*. For *S. columbaria* *B. pascuorum* seems an advantageous visitor, because he can pick up more pollen per minute and distribute them over more heads than *E. tenax*. This depends strongly on the amount of pollen on the body and the deposition of these pollen.

### **Pollen deposition**

In Haren *E. tenax* deposited most pollen per stigma, 3.1, but differences with *B. pascuorum* and *A. mellifera* were not significant. The value of 3.1 grains per stigma is in accordance with Kwak, who found for syrphids a deposition of 3.7 grains per stigma on *S. columbaria* (Kwak, 1993). For *B. pascuorum* she found a higher value, 6.1 grains per stigma.

### **Pollination efficiency**

All the species had only a small percentage pollen from *S. columbaria* on their body compared to the percentage of visits to flowerheads of this plant. This is probably caused by the relatively small number of pollengrains produced by the anthers ( $\pm 200$  per anther). The grains are large ( $\phi \approx 100\mu$ ; Erdtman, 1969) compared to other plants from

chalk grasslands. In the same visitation time the body of insects probably takes up much more pollen from other plant species compared to *S. columbaria*. The percentage of *S. columbaria* pollen was in the same order of magnitude for *E. tenax*, *A. gamma* and *P. rapae* so differences in pollen deposition on the stigmas between these species will depend on the total pollenload and the visitation frequency. Furthermore differences in rate of pollendeposition can influence the overall effectiveness of a pollinator. *B. pascuorum* and *E. tenax* had the largest total pollenload on their body but *B. pascuorum* had a smaller percentage of *S. columbaria* pollen. So in combination *E. tenax* carries the largest absolute number of *S. columbaria* pollen. With regard to the number of conspecific pollengrains deposited on *S. columbaria* flowers *E. tenax* seems the most effective pollinator. But for an honest comparison between the insect species the differences in genetic quality of the conspecific pollen they deliver to *S. columbaria* should also be considered.

### **Distance of pollen transport**

In general one can expect pollen transported over a larger distance are genetic more different from the recipient plant than pollen from shorter distances. The flight distance of an insect species will provide an indication for genetic similarity of the pollen transported.

The low number of resights of marked insects can be due to the enormous number of insects in the area. The relatively high amount of resights of *Bombus* species is probably caused by the restricted foraging area of bumblebees. They have to return to their nest to deliver the stored pollen. An individual often uses its own foraging route. Males don't have to return to the nest but fly patrolling routes. Not so much is known about the foraging of males. Jennersten *et al.* (1991) found that both males and workers flew similar distances. We saw relatively many males.

The two most abundant visitors on *S. columbaria*, *E. tenax* and *A. gamma*, made more than 90% of their flights over distances shorter than 2 meter. About 40% of the flights were within 0.2 meter of the last visited flower. This causes a relatively large chance of pollination between flowerheads from the same individual plant. This chance increases with increasing number of simultaneously flowering heads per plant.

In this study flights over distances more than 10 meter were only made by the butterfly *P. rapae*. This species made some long distance flights between patches separated by more than 150 meter and may therefore be responsible for genetic exchange between these patches although its total pollenload is small and the rate of pollendeposition on stigmas is low. The amount of long distance flights by butterflies is underestimated in this study because of the greater chance of being loosing it during long distance flights. The butterfly *Cynthia cardui* which is known to migrate over hundreds of kilometres (Bink, 1992) may even transport pollen between populations. Although interpopulation pollen transfer may be accidental and is very difficult to prove, it may have a positive impact on genetic variation in the Dutch populations. In respect to pollination one may wonder whether these pollen are still viable.

Butterflies have strongly declined in the Dutch chalk grasslands this century both in number of species and in number of individuals (Swaay, 1990). This may imply that the frequency of pollen transport over large distances has decreased during this century. This could lead to more genetic differentiation between patches.

Campbell (1985) showed in computer simulations based on field experiments, evidence that generalistic feeding behavior can lead to a decrease in distance of gene dispersal. Visitation of another plant species reduced the distance of conspecific pollen transport and also the number of pollen transported to conspecific stigmas. A shift from oligolectic to polylectic insects on *S. columbaria* could therefore result in reduction of the distance of conspecific pollen transport.

### **Pollenload on stigmas**

The stigmas of *S. columbaria* contained large amounts of heterospecific pollen grains. It was not possible to discriminate between pollen of *S. columbaria* and *K. arvensis*. The percentage of these two species in the total pollenload on the stigmas was in the same order of magnitude as the percentage in the pollenload on the bodies of *E. tenax*, *A. gamma* and *P. rapae*. Differences between the three sampled populations in percentage were not significant. It should however be noted that only in the Kruisberg population *K. arvensis* was abundant so the percentage of *S. columbaria* pollen will be lower here. The large amount of heterospecific pollen is a result of the generalistic feeding behaviour of the insects visiting *S. columbaria*. *S. columbaria* populations pollinated by the oligolectic *A. marginata* and *Dasyptoda argentata* are expected to receive less heterospecific pollengrains. Therefore a shift in insect species composition from specialists to generalists will result in an increase in improper pollen transfer.

The effect on the seed set of the large fraction of heterospecific pollen grains on the *S. columbaria* stigmas is unknown. Negative effects of heterospecific pollen grains on germination, pollen tube growth and ovule development are found by Sukhada & Jayachandra (1980) and Thomson *et al.* (1981). They explained this effect by allelopathic properties of the heterospecific pollen. To clarify possible effects of heterospecific pollen grains on seed set of *S. columbaria* further study will be done.

### **Germination**

Germination is used as a measure of reproductive success. Germination is not only influenced by the quantity and quality of delivered pollen (Waser & Price, 1991) but also by environmental factors such as nutrient supply. Verkaar & Schenkeveld (1984) showed that seeds from *S. columbaria* produced in treatments with the highest nutrient supply were most numerous and heaviest. They found a positive linear relationship between seed weight and germination.

In an experiment with standardized environmental conditions Van Treuren *et al.* (1994) found for *S. columbaria* in two densities a decrease in fraction of developed seeds at a lower plant density, while the outcrossing rate was 100% for both densities. Artificial self- and cross pollination of *S. columbaria* showed that the fraction of developed seeds after cross pollination was higher. They suggest that the difference in success between self and cross pollen causes an equalization of the outcrossing rates.

In conclusion qualitative aspects of pollination effectiveness seem to be more important in determining differences between populations of *S. columbaria* in the Netherlands than quantitative aspects, as a consequence of the generalistic feeding behaviour of the insect visitors. Differences in quality of the pollination of *S. columbaria* were not related to population size but may be more related to the density of flowerheads from *S. columbaria* in relation to the amount of other flowering species. This is in accordance with Kunin (1993) who found in an experimental design the strongest negative density effect on seed set when the pollinators behaved as generalists and suggested that qualitative aspects are more important than parallel declines in the quantity of visits.

### **Acknowledgements**

We would like to thank Manja Kwak for the enthusiastic supervision, Odilia Velterop for the stimulating coöperation in the field and Kasper Reinink for showing us suitable *S. columbaria* populations in the field.

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**Appendix 1 : Populations of *S. columbaria* visited to collect fielddata in 1994**

<b>Population</b>	<b>Abbr</b>	<b>Position</b>	<b>Nr.flower heads <i>S.col.</i></b>	<b>Flower. surround.</b>
Zure Dries	ZD	Zuid-Limburg	7	-
Kwartierse Dijk	KD	Oude Rijn	13	-
Ruitenbergr	R	IJssel	14	-
Zalk	Za	IJssel	29	-
Bemelerbergr	Be	Zuid-Limburg	35	+
Kruisbergr	Kr	Zuid-Limburg	250	+
Kunderbergr	Ku	Zuid-Limburg	350	+
Olst	O	IJssel	400	+/-
Julianagroevr	J	Zuid-Limburg	420	+
Wolfskop	Wo	Zuid-Limburg	500	++
Wijlre	Wij	Zuid-Limburg	15,000	+++
Wrakelbergr	Wr	Zuid-Limburg	40,000	++

Abbreviation: abbreviation of the names of the population as used in figures and tables.

Number of flowering heads of *S. columbaria*: measure to express populationsize in an insect relevant way. The number of flowering heads is the estimated number of flowering heads at the moment of insect countings, not the total in a season.

Flowering surrounding: indication of the flowering of the surrounding

- : a small amount of other flowers in the neighbourhood and/or in a low density
- + : a larger amount of other flowers in the neighbourhood and/or in a high density

This index is an estimate for the attractiveness of the vegetation for insects.

Appendix 2:  
Flowering plant species in *S. columbaria* populations. Populations arranged according increasing population size, abbreviations see appendix 1.

Plant species	ZD	KD	Za	Kr	O	J	Wo	Wij	Wr	Dutch name plant species
Scabiosa columbaria	x	x	x	x	x	x	x	x	x	Duifkruid
Knautia arvensis	-	x	-	x	x	x	x	x	x	Beemdkroon
Centaura jacea	x	-	x	x	x	x	x	x	x	Knoopkruid
Organum vulgare	x	x	x	x	x	x	x	x	x	Marjolein
Daucus carota	x	x	x	x	x	x	x	x	x	Wilde peen
Pimpinella saxifraga	x	x	x	x	x	x	x	x	x	Kleine bevernel
Carlina vulgaris	x	x	x	x	x	x	x	x	x	Driedistel
Campanula ssp.	x	x	x	x	x	x	x	x	x	Klokje (geslacht)
Ononis repens	x	x	x	x	x	x	x	x	x	Kattedoorn
Lotus corniculatus	x	x	x	x	x	x	x	x	x	Gewone rolklaver
Trifolium pratense	x	x	x	x	x	x	x	x	x	Rode klaver
Genista tinctoria	x	x	x	x	x	x	x	x	x	Verfbrem
Centaura scabiosa	x	x	x	x	x	x	x	x	x	Grootbloem centaurie
Picris hieracioides	x	x	x	x	x	x	x	x	x	Bitterkruid
Leontodon hispidus	x	x	x	x	x	x	x	x	x	Ruige leeuwetand
Agrimonia eupatoria	x	x	x	x	x	x	x	x	x	Gewone agrimonie
Cirsium acaule	x	x	x	x	x	x	x	x	x	Aarddistel
Centaurium erythrae	x	x	x	x	x	x	x	x	x	Echt duizendguldenkruid
Thymus ssp.	x	x	x	x	x	x	x	x	x	Tijm (geslacht)
Saturea vulgaris	x	x	x	x	x	x	x	x	x	Borstelkrans
Rubus fruticosus sl	x	x	x	x	x	x	x	x	x	Braam s.l.
Tanacetum vulgare	x	x	x	x	x	x	x	x	x	Boerenwoonkruid
Galium verum	x	x	x	x	x	x	x	x	x	Echt walstro
Senecio ssp.	x	x	x	x	x	x	x	x	x	Kruiskruid (geslacht)
Succisa pratensis	x	x	x	x	x	x	x	x	x	Blauwe knoop
Cirsium arvense	x	x	x	x	x	x	x	x	x	Akkerdistel
Cirsium vulgare	x	x	x	x	x	x	x	x	x	Speerdistel
Cirsium palustre	x	x	x	x	x	x	x	x	x	Kale jonker
Mentha suaveolens	x	x	x	x	x	x	x	x	x	Witte munt
Echium vulgare	x	x	x	x	x	x	x	x	x	Slangekruid
Prunella vulgaris	x	x	x	x	x	x	x	x	x	Gewone brunel
Trifolium repens	x	x	x	x	x	x	x	x	x	Witte klaver
Cichorium intybus	x	x	x	x	x	x	x	x	x	Wilde cichorei
Hypochoeris radicata	x	x	x	x	x	x	x	x	x	Gewoon biggekruid
Silene vulgaris	x	x	x	x	x	x	x	x	x	Blaassilene
Achillea millefolium	x	x	x	x	x	x	x	x	x	Duizendblad
total n° of species	10	10	7	19	5	22	17	20	20	

Appendix 3:  
Visitation frequency on *S. columbaria* per insect species in the transects

population date	observation time (in minutes)	population size (number of flowerheads)	ZD	KD	Za	Kr	O	J	Wo	Wij	Wl	tot. #pop.
			2308	2208	2208	1108	2208	2308	2508	1008	908	
			30	30	30	225	30	30	60	150	225	
			7	13	29	250	400	420	500	15000	40000	
<b>Species</b>	<b>orde (family)</b>											
Eristalis tenax	Diptera (Syrphidae)	1	85	0	0	73	116	118	259	176	117	945
Autographa gamma	Lepidoptera	0	0	0	8	12	146	220	128	207	721	8
Eristalis arbustorum+memorum	Diptera (Syrphidae)	0	0	2	16	14	24	18	38	27	139	7
Pieris rapae	Lepid. (Pieridae)	4	6	0	3	0	59	0	18	16	106	6
Helophilus pendulus	Diptera (Syrphidae)	0	1	0	0	0	31	26	0	1	2	61
Lucilia spec.	Diptera (Tachinidae)	0	0	0	1	0	0	2	3	20	7	33
Bombus pascuorum	Hymenoptera (Apidae)	6	0	0	1	0	1	0	23	0	1	31
Zygaena filipendula	Lepid. (Zygaenidae)	0	0	0	2	0	0	0	0	19	8	29
Helophilus trivittatus	Diptera (Syrphidae)	0	0	0	2	5	0	6	2	9	24	3
Sarcophaga carnaria	Diptera (Tachinidae)	0	0	0	0	0	0	0	1	14	4	19
Eristalis intricarius	Diptera (Syrphidae)	0	0	0	0	0	15	0	0	0	0	15
Conops flavipes	Diptera (Conopidae)	0	0	0	0	0	2	2	5	5	14	4
Cynthia cardui	Lepid. (Nymphalidae)	0	0	0	2	0	0	0	0	3	3	8
Lasiommata megera	Lepid. (Nymphalidae)	0	0	0	1	0	0	0	5	2	8	3
Syrphus ribesii	Diptera (Syrphidae)	0	0	0	0	0	6	1	0	0	7	2
Epi-syrphus baltheatus	Diptera (Syrphidae)	0	0	0	0	0	0	5	0	0	0	5
Tachinidae indet.	Diptera (Tachinidae)	0	0	0	1	0	0	0	0	2	1	4
Pieris napi	Lepid. (Pieridae)	1	0	0	0	0	0	0	3	0	0	4
Bombus soroseensis	Hymenoptera (Apidae)	0	2	0	0	0	0	0	0	0	1	3
Bombus terrestris	Hymenoptera (Apidae)	0	0	0	0	0	0	0	3	0	0	3
Eristalis horticola	Diptera (Syrphidae)	0	0	0	0	0	0	0	0	3	0	3
Aglais urticae	Lepid. (Nymphalidae)	0	0	0	0	0	0	0	0	2	1	3
Halictus spec.	Hymenoptera (Apidae)	2	0	0	1	0	0	0	0	0	0	3
Coccinella septempunctata	Coleopt. (Coccinell.)	0	0	0	0	0	1	1	1	0	0	2
Diptera indet.	Diptera	0	0	0	0	0	0	0	0	2	0	2
Eristalis pertinax	Diptera (Syrphidae)	0	0	0	0	0	0	0	2	0	0	2
Maniola jurtina	Lepid. (Nymphalidae)	0	0	0	0	0	0	2	0	0	0	2
Nemophora spec.	Lepid. (Incurvari.)	0	0	0	0	0	0	0	0	1	0	1
Apis mellifera	Hymenoptera (Apidae)	0	0	0	0	0	1	0	0	0	0	1
Volucella pellucens	Diptera (Syrphidae)	1	0	0	0	0	0	0	0	0	0	1
Rhingia campestris	Diptera (Syrphidae)	0	0	0	0	0	0	0	1	0	0	1
Syrphid pipiens	Diptera (Syrphidae)	0	0	0	0	0	0	0	0	0	0	1
Cerambycidae indet.	Coleoptera (Ceram.)	0	1	0	0	0	0	0	0	0	0	1

Visitors on *S. columbaria* not included in the transects

Species	orde (family)	population
Melanostoma mellinum	Diptera (Syrphidae)	Olst, Haren
Sphaerophoria scripta	Diptera (Syrphidae)	Wrakelberg
Syrphus torvus	Diptera (Syrphidae)	Haren
Sericomyia silentis	Diptera (Syrphidae)	Assen, Haren
Ocyptera brassicaria	Diptera (Tachinidae)	Wrakelberg
Dufaurea spec.	Hymenoptera (Apidae)	Wrakelberg
Bombus lapidarius	Hymenoptera (Apidae)	Kruisberg
Pieris brassicae	Lepid. (Pieridae)	Wrakelberg

Appendix 4:  
Visitation frequency per insect group

POPULATION	ZD	KD	Za	Kr	O	J	MO	WJ	Wr	tot.	#POP.
Syrphidae	2	86	2	91	181	179	287	221	155	1204	9
Lepidoptera-Night	0	0	0	10	12	146	220	148	215	754	6
Lepidoptera-Day	5	6	0	6	0	61	3	28	22	131	7
Tachinidae	0	0	0	2	0	2	4	36	12	56	5
Hymenoptera	8	2	0	1	2	0	26	0	2	41	6
Conopidae	0	0	0	0	0	2	2	5	5	14	4
Coleoptera	0	1	0	0	0	1	1	0	0	3	3
other	0	0	0	0	0	0	0	2	0	2	1
total	15	95	2	110	195	391	543	440	411	2202	9

Appendix 5:  
Percentage of visitation per insect group

POPULATION	ZD	KD	Za	Kr	O	J	MO	WJ	Wr	tot.
Syrphidae	13.3	90.5	100.0	82.7	92.8	45.8	52.9	50.2	37.7	54.7
Lepidoptera-Night	0.0	0.0	0.0	9.1	6.2	37.3	40.5	53.6	52.3	34.1
Lepidoptera-Day	33.3	6.3	0.0	5.5	0.0	15.6	0.6	6.4	5.4	6.0
Tachinidae	0.0	0.0	0.0	1.8	0.0	0.5	0.7	8.2	2.9	2.5
Hymenoptera	53.3	2.1	0.0	0.9	1.0	0.0	4.8	0.0	0.5	1.9
Conopidae	0.0	0.0	0.0	0.0	0.0	0.5	0.4	2.3	1.2	0.6
Coleoptera	0.0	1.1	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.1
Other	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.1

Appendix 6:  
Number of species per insect group

POPULATION	ZD	KD	Za	Kr	O	J	MO	WJ	Wr	tot.
Syrphidae	2	2	2	3	5	5	6	6	4	12
Lepidoptera-Night	0	0	0	2	1	1	1	1	3	3
Lepidoptera-Day	2	1	0	3	0	2	1	4	4	6
Tachinidae	0	0	0	2	0	1	2	3	3	3
Hymenoptera	2	1	0	1	2	0	2	0	2	5
Conopidae	0	0	0	0	0	1	1	1	1	1
Coleoptera	0	1	0	0	0	1	1	0	0	2
other	0	0	0	0	0	0	0	1	0	1
total	6	5	2	11	8	11	14	18	16	33

**Appendix 7:**

Flower constancy of insect visitors on *S. columbaria*. Percentage of visits per plant species. Wr=Wrakelberg, Kr=Kruisberg. N=number of individuals (per individual > 10 flowervisits)

species population N	E.tenax		A.gamma	P.rapae		B.pasc.
	Wr 16	Kr 14	Wr 7	Wr 3	Kr 6	Kr 2
Scabiosa	85.7	56.2	74.1	53.3	20.0	1.4
Centaurea	6.6	11.3	13.7	10.0	3.0	22.3
Knautia	0.3	20.8	1.6	0.0	40.3	59.8
Origanum	4.8	11.0	8.3	36.7	27.1	16.6
Daucus	1.3	0.0	0.0	0.0	0.0	0.0
Compositae	1.3	0.0	1.4	0.0	0.7	0.0
other	0.0	0.7	1.0	0.0	8.9	0.0

**Appendix 8:**

Total number of pollen grains on the body of different insect species.

species	N	mean	SE	95% confid.int. for mean		
E. tenax	14	1283.1	213.3	822.4	To	1743.9
A. gamma	13	18.8	6.9	3.9	To	33.8
P. rapae	6	71.8	33.3	0.0	To	157.5
B. pascuorum	4	1764.5	860.6	0.0	To	4503.4

**Appendix 9:**

Pollen grains of *Scabiosa* / *Knautia* (%) on the body of different insect species.

species	N	mean	SE	95% confid.int. for mean		
E. tenax	14	37.0	6.6	22.8	To	51.3
A. gamma	13	18.8	6.9	3.6	To	33.9
P. rapae	6	37.8	9.6	13.1	To	62.6
B. pascuorum	4	3.3	1.7	0.0	To	8.8

**Appendix 10:**

Germination (%) per population.

population	N	mean	SE	95% confid.int. for mean		
Zure Dries	6	0.0	0.0	0.0	To	0.0
Kwart. Dijk	3	24.9	8.1	7.3	To	42.5
Zalk	12	24.8	9.1	4.6	To	44.9
Kruisberg	56	36.8	4.1	28.5	To	45.0
Kunderberg	35	20.6	3.7	13.1	To	28.0
Olst	22	24.3	6.1	11.6	To	36.9
Juliana Gr.	32	24.6	5.1	14.3	To	34.9
Wolfskop	45	0.0	0.0	0.0	To	0.0
Wijlre	30	42.3	4.7	32.7	To	51.9
Wrakelberg	31	22.4	4.1	14.1	To	30.6