Pollination Ecology of Succisa pratensis



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A comparison between populations differing in size and flower diversity

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Summary

In this study, the effects of population structure on the pollination success of *Succisa pratensis* Moench (Dipsacaceae) were investigated. Pollination services and their effects on plants' reproductive success are compared for four (natural) populations of *S. pratensis* that differ in size and flower diversity. Pollination services include quantity (total visitation frequency) as well as quality (per visit efficiency) components of pollination.

In all populations, Succisa was visited by a large variety of insect species (22 species in total), belonging to the Syrphidae, other Diptera, Hymenoptera and Lepidoptera. Syrphid flies were the main visitors of Succisa during the whole flowering period, especially members of the Eristalinae (a sub-family of the Syrphidae). Helophilus trivittatus, Eristalis horticola, Helophilus pendulus, Eristalis tenax/pertinax and Eristalis arbustorum/nemorum (all members of the Eristalinae, arranged according to their abundance) showed by far the highest numbers of individuals at the sites. These syrphid fly species are (very) common in The Netherlands.

On average, a flower head of *Succisa* received between 24 and 83 insect visits a day. Such a visitation rate is quite high in comparison with other flowering plant species and this means that *Succisa* is an attractive plant species for insects. The visitation rates (quantity component of pollination) did not differ between populations varying in size and flower diversity.

The most frequent Succisa visitors carried a high proportion of heterospecific pollen on their bodies (ranging from 22 to 80%) due to their generalistic feeding behaviour. Heterospecific pollen consisted mainly of Asteraceae pollen grains. The composition of the pollen loads of the visiting insects is reflected very well in the pollen deposited on Succisa stigmas. A small fraction of conspecific pollen was deposited on the stigmas of Succisa in all populations. In contrast to the quantity component of pollination, this quality component differed between the studied populations. Large populations received a higher proportion of conspecific pollen (45% and 64%) than small ones (17% and 18%). Presumably, this difference is the result of passive flower constancy of the most frequent Succisa visitors.

Therefore, in The Netherlands, qualitative aspects of pollination seem to be more important in determining differences between populations of *Succisa* than quantitative aspects. No difference in the proportion of conspecific pollen deposited was found between populations differing in flower diversity. This is probably caused by large flight distances of the visiting insects. Long foraging distances of the pollinators of *Succisa* may lead to substantial gene flow by pollen between *Succisa* populations. This reduces the threat of genetic erosion to *Succisa* populations. The difference in proportion of conspecific pollen deposited does not lead to differences in seed germination rates between populations. The germination rate was low in all populations (<50%). Probably, this is (partly) caused by the deposition of large amounts of heterospecific pollen, even in large *Succisa* populations. Thus, *Succisa* plants suffer from competition through interspecific pollen transfer. Artificial hand pollinations are needed to determine the susceptability of *Succisa* to the deposition of heterospecific pollen, unambiguously.

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1 Introduction

Insects are of great importance for the pollination of both cultivated and wild plants (Allen-Wardel *et al.* 1998). For example, in Northwest Europe insects pollinate eighty percent of the flora (entomophilous plants) (Kwak 1994). Although few data exist, many plant-pollinator relationships are considered to be disrupted due to destruction, deterioration or fragmentation of habitats (Allen-Wardel *et al.* 1998; Parra-Tabla *et al.* 2000; Schulke and Waser 2001). Furthermore, the IUCN predicts a global loss of 20,000 flowering plant species within the next few decades and this will undoubtedly lead to the decline of the co-dependent pollinators that need them for survival (Allen-Wardel *et al.* 1998). Pollinators that specialize on particular plant taxa (mono- and oligolectic species) may be at greater risk than "generalist" pollinators (polylectic species) or plants that are pollinated by a large variety of insects. Most entomophilous plant species are visited by a large variety of insects (Proctor *et al.* 1996), but their pollination efficiency differs. Basic data about which insect species serve as native pollinators for wild and cultivated plant species have not been available for a long time. Conservation measures were not taken so far (Allen-Wardel *et al.* 1998).

Because of the long-term decline of pollinators and the potential consequences of these losses on the conservation of biodiversity (Allen-Wardel *et al.* 1998) and ecosystem functioning (Parra-Tabla *et al.* 2000), conservation biologists increase their attention to plant-pollinator relationships (Allen-Wardel *et al.* 1998). Furthermore, from a restoration ecology perspective more information is needed about the relative importance of insect species for plant populations. It is necessary to predict possible re-establishment of plants in the presence of available pollinators. Some important pollinating species could become extinct in the case of environmental change (Kwak 1994). Despite this increased attention, even today little is known about the long-term ecological implications of diminished pollinator populations and plant declines. Information about habitat alterations that may lead to a loss of biodiversity, initially of pollinators and followed sconer or later by a decline in flowering plant diversity is also lacking for the greater part (Allen-Wardel *et al.* 1998). This information might be useful for adequate habitat management and restoration plans and therefore further research in this area is needed.

Studies in both natural and experimental plant populations suggest that the field situation of populations and the presence of other plant species that are attractive for insects may influence the species composition and abundance of pollinator guilds and change the foraging behaviour of individual insects (Kwak 1988; Kwak and Jennersten 1991; Petanidou *et al.* 1995a; Kunin 1997a,b; Steffan-Dewenter and Tscharntke 1999; Parra-Tabla *et al.* 2000; Utelli and Roy 2000; Chittka and Schürkens 2001; Mustajärvi *et al.* 2001; Schulke and Waser 2001).

The success of animal-pollinated plants depends on the quantity (total visitation frequency) and quality of pollinator visits (per visit efficiency) it can attract (Kunin 1997a; Utelli and Roy 2000). Kunin (1997a) assumes that both of these factors are likely to depend on local flower abundance (the three most important components: population size, population density and population purity). The quantity of pollinator visits may vary in a complex way with local abundance. In populations where flowers are rare, pollination facilitation can occur: simultaneously flowering plant species may help each other to attract insects. However, relatively abundant flowers are assumed to attract pollinators away from competitors (competition for pollination, Kunin 1997a). The quality of pollinator visits is determined by a number of factors, such as the species composition of pollinators, their behaviour when visiting a flower (pollen uptake and deposition) and their movement patterns between flowers (flower constancy and flight distance) (Kunin 1997a; De Vlas 2002). From a plant's point of view, the value of insect species that are flower inconstant (generalists) depends greatly on the relative density of the plant population (purity) (Kunin 1997a). Generalists visit practically all simultaneously flowering plant species on a particular site and are not effective pollinators at low relative density (deposition of more heterospecific pollen due to interspecific pollen transfer). Flower-constant pollinators, however, remain efficient pollinators even if conspecific plants are widely scattered and mixed with other plant species (Kunin 1997a). But from the insects' perspective small and sparse plant populations are unprofitable for flower constancy and this may lead to shifts in the behaviour of pollinators (Ozinga and Bakker 1995; Kunin 1997a).

A few studies provide (experimental) evidence for the above stated assumptions. Nearly all studies show that pollination problems are more likely at low density. Plants at low density receive fewer or less effective pollinator visits than plants at high density (Kunin 1993, 1997a). Both shifts in the species composition of pollinators and changes in the foraging behaviour of individual insects determine the quality of the visits. The reduced quantity and quality of the visits leads to a decline in reproductive success of the plant species, especially in self-incompatible species (Kunin 1993, 1997a,b). The effects of population size on pollination are less clear (Kunin 1997a). Some studies show population size effects (Sih and Baltus 1987; Ågren 1996; Kunin 1997a; Mustajärvi et al. 2001), others do not (Kunin 1997a,b). Observations in natural systems suggest higher visitation rates and therefore higher pollination success in large populations compared with small populations (lower attractiveness) (Sih and Baltus 1987; Mustajärvi et al. 2001). However, in natural populations population size and population density are often highly correlated (Kunin 1997b; Mustajärvi et al. 2001). Experimental data show no effect of population size on plants' reproductive success (Kunin 1997a). Studies of the effects of population purity suggest that competing flowers sometimes increase the number of visits, but almost always decrease the quality of the visits (due to lower visitor constancy) (Petanidou et al. 1995a,b) and reproductive success (Kwak and Jennersten 1991; Kunin 1997a).

1.1 Research Questions

This study deals with the effects of local abundance on quantity and quality components of the pollination of the insect-pollinated, mainly outcrossing *Succisa pratensis* Moench (Dipsacaceae). The species composition of the pollinator guild is investigated and effectiveness measures are calculated for the most frequent visitors. Furthermore, pollination services and their effects on plants' reproductive success are compared for (four) populations of *S. pratensis* that differ in population size and flower diversity (as measure of population purity)¹. The aim of this study is a better understanding of the influence of the field situation on various aspects of the pollination ecology of *S. pratensis*. This information might be useful for appropriate habitat management and conservation measures for this and other plant species that are threatened by ongoing effects of (anthropogenic) disturbance.

The main question addressed in this project is: what are the effects of different population parameters on the reproductive success of *S. pratensis*? This main question is divided into four sub-questions:

- 1. Do the composition and abundance of pollinator guilds differ between *Succisa* populations that vary in size and flower diversity?
- 2. Do the visitation rates of pollinator guilds differ between *Succisa* populations that vary in size and flower diversity?
- 3. Does the behaviour of pollinators differ between *Succisa* populations that vary in size and flower diversity?
- 4. What are the consequences of the three above stated questions for the reproductive success of S. pratensis, measured as seed set and seed germination?

1.2 Hypothesis

I hypothesize that small populations of the target species are likely to suffer reproductive loss. Small populations are less attractive to pollinators, resulting in lower visitation rates and therefore lower pollination success compared to large populations.

Furthermore, I expect that in small populations the co-occurrence of other flowering plant species is of crucial importance for target plant species' reproductive success. Both competition and facilitation for pollination can occur in small *Succisa* populations with high flower diversity. Depending on the fact whether competition or facilitation occurs, the magnitude and direction of the effects on the plants' reproductive success are different. Competition for pollination has strong negative effects on the plants' reproductive success due to a reduction in quantity and quality of pollinator visits. Pollination facilitation, however, increases visitation rates and therefore pollination success. Because large

¹ In strict sense, populations cannot differ in flower diversity, but sites can. Population size of *Succisa* is the number of *Succisa* flower heads at a particular site. Flower diversity is the number of simultaneously flowering plant species that are attractive to insects at a particular site. For reasons of simplicity, I will use the term population in this context.

populations are considered to be able to attract sufficient numbers of pollinators, the presence of other flowering plant species is of minor importance in large populations.

Herrera (1988) states that abundance and visitation rates of pollinators can vary markedly among and within populations. Earlier research on *Scabiosa columbaria*, a species very similar to *S. pratensis*, showed that small *Scabiosa* populations are able to attract sufficient pollinators (Ozinga and Bakker 1995). In The Netherlands, *S. columbaria* is pollinated by common polylectic syrphid fly species (Ozinga and Bakker 1995). Ozinga and Bakker (1995) conclude that the qualitative aspects of pollination seem to be more important in determining differences between populations of *S. columbaria* in The Netherlands than quantitative aspects, because of the generalistic feeding behaviour of the insect visitors. They suggest that the differences in quality of the pollination of *S. columbaria* may be related to the density of flower heads of *S. columbaria* in relation to the amount of other flowering plant species (Ozinga and Bakker 1995).

2 Materials and Methods

2.1 The Plant

Succisa pratensis Moench (Devilsbit Scabious, Dipsacaceae) is a perennial herb that grows in borders of canals and ditches, verges, mires, (calcareous) fens and wet meadows (Pegtel 1986). Although S. pratensis can be locally abundant, the species is on decline (Weeda et al. 1999). Since 1935 the area of distribution of Succisa decreased by 74% in The Netherlands, due to changes in land use, habitat fragmentation and habitat deterioration (Van der Meijden et al. 2000). The remaining populations are isolated from each other and many are small (Vergeer et al. 2003a). The main flowering season starts in the middle of August and continues till half October. The plant forms one or two (sometimes four) flower branches that bear one or several heads with 30-100 small blue flowers per head (3 mm long) (Bühler and Schmid 2001). The flowers are protandrous, which means that the anthers have dehisced by the time the stigma becomes receptive. In all phases the flowers contain nectar (Kołodziejska 2002). Each flower produces only one seed (Bühler and Schmid 2001).

2.2 The Populations

Eight populations differing in size of *Succisa* (defined as number of *Succisa* flower heads) and flower diversity (defined as number of simultaneously flowering plant species that are attractive to insects) were chosen. The investigated populations are situated in verges (near Assen in the province of Drenthe: Annen, De Haar, Ekehaar, Eleveld and Gasteren) or nature reserves (in the province of Friesland: Rotstergaast and Wijnjewoude) in the northern part of The Netherlands. Observations were also made in one artificial population in Assen (garden of M.M. Kwak) (figure 1). At the beginning of observations (week 35 and week 36, 2002), populations varied in size from 135 up to more than 27,000 flower heads and plant species richness ranged from 2 to 32. In all populations, data about species composition (both insect and plant species composition) and visitation rates were recorded, but only in four populations additional observations were performed (table 1). These four populations are representative for the in table 1 distinguished population types.

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Figure 1 Studied populations of *Succisa pratensis* in the northern part of The Netherlands: (1) De Haar, (2) Gasteren, (3) Ekehaar, (4) Eleveld, (5) Rotstergaast, (6) Wijnjewoude, (7) Annen and (8) Assen.

Table 1 Studied *Succisa* populations and the methods used there. Population size is defined as the number of *Succisa* flower heads at the beginning of observations (week 35 and week 36, 2002) and is given between brackets. Flower diversity is measured as the number of simultaneously flowering plant species that are attractive to insects (between brackets), also at the beginning of observations. The numbers in the column "methods" refer to the methods used: (1) transect observations, (2) plot observations, (3) following insects, (4) pollen loads on insect bodies, (5) pollen deposition and (6) seed set and germination.

Number in		Dutch grid	Populat	ion type	
figure 1	figure 1 Population (Am coö		Population size of Succisa	Flower diversity	Methods
1	De Haar	231.8-554.6	Small (135)	Low (11)	1, 2
2	Gasteren	240.4-562.8	Small (250)	Low (11)	1-6
3	Ekehaar	237.8-552.3	Small (350)	High (17)	1-6
4	Eleveld	235.0-552.5	Small (615)	High (15)	1, 2
5	Rotstergaast	191.4-547.2	Large (27,000)	Low (3)	1, 2
6	Wijnjewoude	207.3-564.1	Large (10,000s)	Low (2)	1-6
7	Annen	242.9-565.0	Large (1,600)	High (16)	1-6
8	Assen	235.3-555.6	Large (1,750)	High (32)	1, 2

2.3 Observations

2.3.1 Transect Observations

Transect observations were carried out in order to collect data about the overall composition of the visitor (and pollinator) guild (research question 1). Flower-visiting insects were observed while walking slowly. The weather conditions (temperature, cloudiness and wind-force), duration of the walks, species and numbers of insect visitors per plant species and the available number of inflorescences or umbels were recorded per plant species. The dimensions of the transect were 50 m x 3 m. On average, the duration of a transect observation was about 25 minutes.

Transect observation data of flowers and insects were used to calculate diversity. This measure incorporates both species richness and abundance. This diversity index was calculated as follows:

$$\mathbf{H} = -\sum_{i=1}^{s} p_i \ln p_i$$

where p_i is the proportion of species *i* in a sample.

2.3.2 Plot Observations

In plots with a known number of flower heads, all visits on flower heads of *Succisa* were scored per insect species during ten minutes. In general, a plot measured 1m x 1m. Plot observations were made 1-4 times a day. Recordings were: weather conditions (temperature, cloudiness and wind-force), insect species, number of visited flower heads, number of male and female flower heads in the plot and dimensions of the plot. From those recordings the visitation rate (defined as number of visits per flower head in a certain time interval) was calculated. By comparing the visitation rates of *S. pratensis* in different populations, research question 2 can be answered.

2.3.3 Following Insects

Individuals of the most frequent flower-visiting insect species were followed during their foraging trip in three populations. In Gasteren (very small population size and only a few other flowering plant species in the surrounding), the number of insects was too low to get representative data. By following the movements of individual insects, information can be obtained about quantity and quality components of pollination. On the one side, residence time (time spent on a flower head with active foraging behaviour) is a measure for the quantity component of pollination. A long residence time may result in the uptake of more pollen on a male flower head and in the deposition of more pollen on a female flower head (Velterop 2000). On the other hand, flower constancy (*sensu* Waser 1986) and foraging speed (number of flower heads visited per unit time) are quality components of pollination. Flower constancy of individual insects plays a role in the amount of heterospecific pollen deposition (De Vlas 2002). Heterospecific pollen can negatively affect ovule fertilization in various ways (Utelli and Roy 2000). A high foraging speed is supposed to promote cross-pollination (Velterop 2000).

The observations always started with an insect visiting a flower head of *Succisa*. The minimum bout length was five visits (equals four transitions). Insect bouts with less than five visits were ignored in the analysis. Other recordings were: weather conditions (temperature, cloudiness and wind-force), insect species, observation time, total residence time on *Succisa* and available number of inflorescences and umbels. From those recordings the average flower constancy, foraging speed and residence time per insect species can be calculated.

Changing index values per insect species were used as measures of flower constancy. The changing index was calculated by dividing the number of intraspecific transitions by the total number of transitions. This index has a range from 0 to 1 and the outcome is the proportion of intraspecific transitions (Slaa 2003). The changing index values were analysed in two different ways. Changing index values of insect species within a population were compared as well as changing index values for a particular insect species between populations.

During the observations it became clear that individual insects visited not only inflorescences and umbels in succession during their foraging trip. They also visited leaves and seed capsules of plants, occasionally. Probably, the insects have to rest now and then during their foraging trip. Sometimes insects cleaned their body on a leaf between two visits. These visits to leaves and seed capsules led to problems by the calculation of flower constancy and foraging speed of insects. Therefore, the foraging bout was split up into different parts, when a leaf or seed capsule was visited during the observations. Each part of the foraging bout was analysed separately. To avoid pseudoreplication, for each individual insect, mean values of flower constancy were calculated. Parts with less than five visits were ignored in the analysis.

In order to determine if this behaviour of visiting leaves/seed capsules (hereafter called "resting behaviour") was equal between insect species, the average time between two *Succisa* visits and resting behaviour per species were calculated. The time between two *Succisa* visits was calculated as follows: (observation time – residence time) / number of transitions. Resting behaviour is defined as number of leaves/seed capsules visited per minute. In this respect, it is assumed that a higher number of visits to leaves and seed capsules increases the chance of pollen loss from the bodies of visiting insects. For the calculation of these measures the foraging bout was analysed as a whole.

The populations where the observations of behaviour were performed differ in population size of *Succisa* and flower diversity. If possible, ten or more individuals per species were followed on one day per population. Only in population Wijnjewoude the observations were performed on two days with comparable weather.

2.3.4 Pollen Loads on Insect Bodies

Another method to determine the effectiveness of flower visitors is to analyze the presence, size and composition of pollen loads on the bodies of flower-visiting insects. In four *Succisa* populations, the pollen loads of the most frequent visitors were sampled. After having visited a *Succisa* flower head, insects were captured, lightly anaesthetized and pollen on the bodies was removed by using small pieces of gel (Beattie 1972). Only the ventral side of the body and the head of the insect were cleaned, because these parts of the insect make contact with stigmas. After cleaning, the piece of gel was put on a microscope slide and was melted. Pollen grains were could under a light microscope (10 x 10 or 10 x 40 magnification) and identified by using a reference collection. In this way, data about flower constancy (measured as the proportion of target pollen and number of pollen species) and size of pollen loads (measured as total number of pollen grains) per insect species were obtained. If possible, ten individuals per species and per population were sampled.

2.3.5 Pollen Deposition

Pollen deposition during the day is determined by allowing insects to visit virgin female flower heads. Early in the morning, virgin female flower heads were collected in the research areas and offered in test tubes filled with water attached to wooden sticks. At the end of a day, the number of target pollen grains deposited per stigma was counted with a loupe (10 x magnification; *S. pratensis* grains are about 90 μ in diameter (Adams 1954)). Every time, the sample consisted of 15 stigmas per flower head. Then, the stigmas were cleaned with gel in the same way as the insect bodies. A small piece of gel was polished over the stigmas of the whole flower head (n=7 per population) and a microscope slide of this gel was prepared. With a loupe was checked if the stigmas were clean. Number of pollen grains of *Succisa* and other plant species in the preparations were counted under the microscope. The proportion of *Succisa* pollen was determined from a sample of at least 300 pollen grains.

2.3.6 Seed Set and Germination

Seed set and germination were used as measures of reproductive success. Seed set indicates the potential offspring of a particular plant, but for plants' reproductive success seed set only is not enough: seeds have to be viable. Therefore, the percentage of germinated seeds was used as an indication for seed quality (Ozinga and Bakker 1995). By determining seed quality you can see if different pollination regimes (during the season and in different populations) influence reproductive

success of *Succisa* (research question 4). Per population at least 15 flower heads in the female phase were marked and bagged to prevent seeds from falling off. All bags were collected when the seeds were fully ripe. For all populations seeds were put in petridishes in a climate room at changing temperature from 25°C and 15°C and 12/12 hours light/dark. After six weeks when no germination occurred the percentage of germinated seeds was determined.

2.4 Statistical Analysis

Analyses of variance (One-Way Anova and Kruskal-Wallis) and (linear) regression statistics were computed using the Statistical Package for the Social Sciences (SPSS version 10.0 for Windows) and Microsoft Excel (version 2000). Homogeneous groups were seperated using Tukey's HSD multiple comparison tests. The Mann-Whitney test (in SPSS) was applied when two samples hypotheses were tested. For all statistical tests a significance level of 5% (α =0.05) was used.



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3 Results

3.1 Species Composition of Insects on Succisa

Flower visitors on *Succisa* were, in order of abundance, members of the Syrphidae, Diptera other than Syrphidae (hereafter called other Diptera), Hymenoptera and Lepidoptera. Syrphid flies formed the greatest part (60% till 100%) of *Succisa* visitors in all populations during the whole flowering period. Members of the other Diptera, Hymenoptera and Lepidoptera visited *Succisa* flower heads mainly early in the flowering season.



Figure 2 The number of individuals per insect species visiting *Succisa* arranged according to their abundance (summed over all transect observations, n=16; September 6th till October 9th). Total number of observed individuals is 740. The figure shows that only five members of the *Eristalinae* were abundant. The species numbers refer to the numbers used in appendix V.

Five syrphid fly species were frequent visitors of S. pratensis, in order of abundance: Helophilus trivittatus, Eristalis horticola, Helophilus pendulus, Eristalis tenax/pertinax and Eristalis arbustorum/nemorum. Four species (belonging to other Diptera, Hymenoptera and Syrphidae), were regularly observed. Regularly observed species appeared in low numbers at the sites, but visited a moderate number of populations (see appendix V). The other 13 insect species (belonging to Lepidoptera, Syrphidae and Hymenoptera) were seen occasionally (1-10 times) (figure 2).

Nearly all observations of syrphid flies refer to members of the *Eristalinae* (a sub-family of the Syrphidae), even all frequently visiting insects belong to the *Eristalinae*. The proportion of *Eristalinae* visitors increased during the flowering season (table 2). In population De Haar, only one *E. tenax/pertinax* as a member of *Eristalinae* was observed at the end of the flowering season (4-10-2002). *Eristalis intricaria* and *Myathropa florea* are members of the *Eristalinae* that visited *S. pratensis*, but were not abundant at the sites. The other observed syrphid flies belong to the sub-families *Syrphinae* and *Milesiinae*. The most common *Succisa* visitors of these taxonomic groups were *Melanostoma* sp. (*Syrphinae*), *Sericomyia silentis* and *Rhingia campestris* (both *Milesiinae*) (arranged according to their abundance).

Table 2 Percentage of *Eristalinae* visitors per population during the flowering season. In general, the proportion of *Eristalinae* visitors increases in the course of the flowering season. The exact dates can be obtained from appendix I.

Percentage of Eristalinae visitors					
	Date 1	Date 2	Date 3		
De Haar	87	20			
Gasteren	64	100	100		
Ekehaar	64	80			
Eleveld	95	90			
Wijnjewoude	90	86	100		
Assen	96	100	-		

In figure 3 the number of insect species per taxon, visiting *S. pratensis*, is shown for four populations. Much more insect species were observed in a large population of *Succisa* than in a small one at sites with low flower diversity. The insect species also represented more taxonomic groups in large populations compared with small populations. The population size of *Succisa* seems to be of minor importance at sites with high flower diversity, since Ekehaar (small population size) and Annen (large population size) showed the same number of insect groups and species present. Which taxonomic groups were not represented at a particular site is not fixed, as some groups were present in other populations of the same type.



Figure 3 Number of insect species visiting *Succisa* per taxon in different populations. The numbers of insect species were counted during transect observations at the sites early in the flowering season: Gasteren (September 13th), Wijnjewoude (September 6th), Ekehaar (September 17th) and Annen (September 13th). From the figure, flower diversity appears to be of crucial importance in attracting different insect visitors to small *Succisa* populations.



Figure 4 The relative abundance of insect groups visiting *Succisa* per population. Populations are arranged according to their number of flower heads and flower diversity in the following way: small-low (2 populations), small-high (2 populations), large-low (2 populations) and large-high (2 populations). The figure shows that *Eristalinae* visitors formed the greatest part of *Succisa* visitors in all populations. Moreover, the proportion of *Eristalinae* visitors shows a positive relationship with population size of *Succisa*.

Figure 4 shows the relative abundance of insect groups, visiting *S. pratensis*, per population. The data were averaged over all observation days per population. In general, populations of *Succisa* had the same visiting insect species (and potential pollinator species). A relation between the population size of *Succisa* and the insect species composition exists (figure 4). The larger the population of *Succisa* (populations are arranged according to increasing number of flower heads), the larger the proportion of *Eristalinae* visitors is and consequently the smaller the proportion of visitors belonging to other taxonomic groups.

Flower diversity at the sites, expressed in the Shannon index, ranged from 0.25 (population Wijnjewoude) till 2.12 (population Ekehaar). Shannon indices of insect diversity varied from 0.00 (population Gasteren) to 1.92 (population Wijnjewoude). No relation between flower diversity and insect species composition on *Succisa* was found (R square=0.0037; figure 5). Insect diversity (both overall insect diversity and insect diversity on *Succisa*) declined in the course of the flowering season (figures 6 and 7).



Figure 5 The relation between insect diversity on *Succisa* (Shannon index, H) and flower diversity (Shannon index, H') in populations De Haar (\diamond), Gasteren (\Box), Ekehaar (\diamond), Eleveld (\times), Rotstergaast (-), Wijnjewoude (*), Annen (\circ) and Assen (+). Based on transect observation data of all populations (September 6th till October 9th), a trendline was estimated that makes clear that no significant relation between insect diversity on *Succisa* and flower diversity at sites exists (R square=0.00).



Figure 6 Overall (insect species on all flowering plant species) insect diversity (Shannon index, H) at the sites in the course of the flowering season. The figure shows that, in general, overall insect diversity declines during the season.



Figure 7 Insect diversity on Succisa (Shannon index, H) per population during the flowering season. In general, the insect diversity on Succisa decreases in the course of the flowering season.

3.2 Visitation Rate

Per population, visitation rates of different patches on the same observation day were averaged, and then averaged over all observation days. Visitation rates did not differ significantly between populations (not shown, Kruskal-Wallis, p=0.269) and population types (Kruskal-Wallis, p=0.092; figure 8). In small populations of *Succisa* with only a few other flowering plant species in the surrounding, the average visitation rate was the lowest, however not significantly different from visitation rates in the other populations. The number of visits per flower head during a ten minutes observation period ranged from 0.80 to 2.76. If we take into account that most insects species were active five hours a day on average (personal observations), then a flower head receives between 24 and 83 insect visits per day. It is this total number of visits that may lead to pollination, since a flower head is one day in the female stage.

The way visitation rates change during the season differs between populations (figure 9). Visitation rates in populations Gasteren and Annen seem to increase during the season, whereas the visitation rates in populations Ekehaar and Wijnjewoude appear to decrease. However, the data should be interpreted with some caution. Plot observation data in populations Gasteren and Annen were only sampled in September. In populations Ekehaar and Wijnjewoude, plot observations were performed until October.





Figure 8 Average visitation rates per population type based on plot observation data. Visitation rates do not differ between the population types (Kruskal-Wallis, p=0.092).



Figure 9 Average visitation rates per population during the flowering season. Visitation rates in populations Gasteren and Annen seem to increase during the season, whereas the visitation rates in populations Ekehaar and Wijnjewoude appear to decrease. However, the data should be interpreted with some caution. Plot observation data in populations Gasteren and Annen were only sampled in September. In populations Ekehaar and Wijnjewoude, plot observations were performed until October.

3.3 Effectiveness of Visitors

3.3.1 Behaviour of Individual Insects

Flower Constancy

In table 3, changing index values as measures of flower constancy are given per insect species for three populations. Helophilus pendulus shows lower changing index values than H. trivittatus and E. horticola in all studied populations. However, only in population Ekehaar the changing index of H. pendulus is significantly different from the changing indices of the other syrphid fly species (One-Way Anova, p=0.004). In this population, 75% of the transitions of H. pendulus were transitions between the same plant species. For H. trivittatus and E. horticola 95% of the transitions were intraspecific. This percentage of intraspecific transitions of these two species was comparable for all studied populations (H. trivittatus, One-Way Anova, p=0.581; E. horticola, One-Way Anova, p=0.723). Helophilus trivittatus and E. horticola can therefore be considered as very flower constant visitors, independent of population size of Succisa and flower diversity at the sites. The changing index values of H. pendulus differ significantly between the populations Ekehaar and Wijnjewoude (Tukey, p=0.001). These Succisa populations differ in size and flower diversity. The changing index of H. pendulus in population Annen differs not significantly from the values found in the populations Ekehaar (Tukey, p=0.254) and Wijnjewoude (Tukey, p=0.062). Actual data about the number of flower heads of Succisa and flower diversity at sites were not gathered on the day of observations. This made a clear comparison between the different populations impossible.

Table 3 Flower constancy of three frequent Succisa visitors. Changing index values (mean \pm S.E.) per insect species were used as measures of flower constancy. The changing index was calculated by dividing the number of intraspecific transitions by the total number of transitions between flowers. This measure ranges from 0 to 1 and the outcome is the proportion of intraspecific transitions. The high changing indices indicate a high degree of flower constancy to Succisa for the observed insect species. Changing index values of insect species within a population were compared as well as changing index values for a particular insect species between populations. Significant differences between the species and populations (One-Way Anova, Tukey) are indicated by different capital and normal letters, respectively. N.a. means value not available.

The Lore hards			
	Ekehaar	Wijnjewoude	Annen
H. pendulus	0.74 ± 0.09 (n=7) ^{Bb}	0.99 ± 0.01 (n=18) ^a	0.86 ± 0.06 (n=9) ^{ab}
H. trivittatus	$0.95 \pm 0.03 (n=17)^{A}$	1.00 ± 0.00 (n=4)	0.94 ± 0.03 (n=11)
E. horticola	$0.95 \pm 0.02 (n=14)^{A}$	n.a.	0.94 ± 0.03 (n=11)

Foraging Speed

Foraging speeds (number of flower heads visited per minute) differed not significantly between insect species in all populations where observations of behaviour were performed (Ekehaar, One-Way Anova, p=0.088; Wijnjewoude, One-Way Anova, p=0.408; Annen, One-Way Anova, p=0.948; table 4). Individuals of the syrphid fly species that were followed, visited between 1 and 3 flower heads per minute on average. Great differences between individuals of the same species existed. In population Ekehaar, the foraging speed of *H. pendulus* was considerably lower than the foraging speeds of *H. trivittatus* and *E. horticola*. But, probably due to the low sample size of *H. pendulus* (n=3) this difference between the species is not significant (One-Way Anova, p=0.088). A comparison between the populations is difficult, because the observations in the three populations were performed on different days with different weather conditions. The activity of individual insects depends strongly on weather conditions.

Table 4 Foraging speeds of three frequent *Succisa* visitors. Foraging speed (mean \pm S.E.) is measured as the number of flower heads visited per minute. Foraging speeds differed not significantly between the insect species in the studied populations (One-Way Anova). N.a. means value not available.

	Foraging speed (mean ± S.E.)				
	Ekehaar	Wijnjewoude	Annen		
H. pendulus	1.00 ± 0.20 (n=3)	1.43 ± 0.18 (n=17)	2.63 ± 0.41 (n=4)		
H. trivittatus	3.08 ± 0.46 (n=17)	1.10 ± 0.22 (n=4)	2.44 ± 0.40 (n=10)		
E. horticola	3.19 ± 0.34 (n=13)	n.a.	2.59 ± 0.39 (n=10)		

Residence Time

Residence time is expressed in table 5 as the time spent on a flower head with active foraging behaviour (in seconds). The residence times were not significantly different between the insect species in the three populations (Ekehaar, One-Way Anova, p=0.212; Wijnjewoude, One-Way Anova, p=0.845; Annen, One-Way Anova, p=0.510). The individual insects spent between 19 and 33 seconds on a flower head in the populations Ekehaar and Annen on average, but the residence times in population Wijnjewoude were much longer. Great differences between individuals of the same species existed. These long residence times in population Wijnjewoude were probably caused by less favourable weather conditions for insects in this population during the observations.

Table 5 Residence times of three frequent *Succisa* visitors. Residence time (mean \pm S.E.) is defined as the time spent on a flower head with active foraging behaviour (in sec) by a particular insect species. The residence times differed not significantly between the insect species in the studied populations (One-Way Anova). N.a. means value not available.

Residence time (mean ± S.E.)					
	Ekehaar	Wijnjewoude	Annen		
H. pendulus	27.69 ± 4.67 (n=7)	53.61 ± 9.93 (n=18)	22.00 ± 2.42 (n=9)		
H. trivittatus	20.48 ± 3.02 (n=17)	57.96 ± 10.53 (n=4)	33.40 ± 8.48 (n=11)		
E. horticola	18.75 ± 2.09 (n=14)	n.a.	31.98 ± 7.81 (n=11)		

Time between Two Succisa Visits and Resting Behaviour

As mentioned in section 2.3.3, individual insects visited leaves and seed capsules of plants now and then during their foraging trip. The time between two *Succisa* visits and the frequency of resting behaviour (number of leaves/seed capsules visited per minute) were calculated in order to compare this behaviour for different insect species. Note that the time between two *Succisa* visits not only includes the flight time of an insect, but also time spent on inflorescences and umbels of other plant species than *Succisa* and time spent on leaves/seed capsules.

Table 6 The time between two visits to *Succisa* flower heads (in sec) given for three frequent *Succisa* visitors. The time between two *Succisa* visits (mean \pm S.E.) was calculated as follows: (observation time – residence time) / number of transitions. Significant differences between the species (One-Way Anova, Tukey) are indicated by different capital letters. N.a. means value not available.

Time between two Succisa visits (mean ± S.E.)					
	Ekehaar	Wijnjewoude	Annen		
H. pendulus	$20.43 \pm 6.53 (n=7)^{A}$	4.77 ± 0.80 (n=18)	5.86 ± 1.28 (n=9)		
H. trivittatus	4.79 ± 1.30 (n=17) ^B	3.38 ± 0.34 (n=4)	2.73 ± 0.94 (n=11)		
E. horticola	$2.04 \pm 0.20 (n=14)^{B}$	n.a.	4.90 ± 2.60 (n=11)		

In table 6, the time between two visits to Succisa flower heads (in seconds) is given per insect species for three populations. Helophilus pendulus had longer time intervals between two visits to Succisa flower heads than H. trivittatus and E. horticola in all studied populations. However, only in population Ekehaar the time between two Succisa visits of H. pendulus was significantly different from the time intervals of the other syrphid fly species (One-Way Anova, p<0.001). Individuals of H. pendulus spent 20 seconds between two Succisa visits in this population on average, whereas

individuals of *H. trivittatus* and *E. horticola* spent 5 and 2 seconds, respectively. The time intervals of *H. trivittatus* and *E. horticola* were comparable for all studied populations. Great differences between individuals of the same species existed. In population Ekehaar, the long time interval of *H. pendulus* was caused by a relative high number of visits to leaves and seed capsules (see table 7). Here, *H. pendulus* visited significantly more leaves/seed capsules than *H. trivittatus* and *E. horticola* (One-Way Anova, p=0.002). This was also the case in population Annen (One-Way Anova, p<0.001), but here the time between two *Succisa* visits of *H. pendulus* was not significantly different from the other species (One-Way Anova, p=0.474). *Helophilus pendulus* visited also more leaves/seed capsules than *H. trivittatus* in population Wijnjewoude, but this difference is not significant (One-Way Anova, p=0.529).

The high values of resting behaviour of *H. pendulus* are supported by the fact that 32% of the foraging bouts of this species were skipped in the analysis. These foraging bouts were broken down by visits to leaves/seed capsules. They consisted of less than five visits to inflorescences and umbels.

Table 7 Resting behaviour of three frequent *Succisa* visitors. Resting behaviour (mean \pm S.E.) is measured as the number of leaves/seed capsules visited per minute. Significant differences between the species (One-Way Anova, Tukey) are indicated by different capital letters. N.a. means value not available.

Resting behaviour (mean ± S.E.)					
	Ekehaar	Wijnjewoude	Annen		
H. pendulus	0.31 ± 0.11 (n=6) ^A	0.03 ± 0.02 (n=18)	0.19 ± 0.06 (n=9) ^A		
H. trivittatus	$0.08 \pm 0.04 (n=17)^{B}$	0.00 ± 0.00 (n=4)	$0.00 \pm 0.00 (n=11)^{B}$		
E. horticola	0.01 ± 0.01 (n=14) ^B	n.a.	$0.01 \pm 0.01 (n=11)^{B}$		

3.3.2 Pollen Loads on Insect Bodies

In tables 8, 9 and 10, some characteristics of the pollen loads of four syrphid fly species are given per population. It was not possible to sample all syrphid fly species in all populations. It was also not possible to analyse pollen loads of species that belong to other taxonomic groups than the Syrphidae, because of the low numbers of these insects at the sites. In population Gasteren, only *H. pendulus* appeared in sufficient numbers to get a representative sample. This makes a comparison between insect species for this population impossible.

Pollen Load Size

The pollen loads of *H. pendulus* were smaller than the pollen loads of the other insect species in all studied populations (table 8). The loads of *H. pendulus* consisted of 78 till 399 pollen grains on average (all plant species), while the size of loads of the other insect species ranged from 193 to 1847 pollen grains on average. Great differences between individuals of the same species existed. Only in population Ekehaar this difference in pollen load size between insect species is significant (One-Way Anova, p=0.042). Here, the bodies of *E. horticola* individuals contained significantly more pollen grains than the bodies of *H. pendulus* individuals.

Table 8 The total number of pollen grains (mean \pm S.E.) in the pollen loads of four frequent *Succisa* visitors. Pollen loads of all insect species tend to be larger in small *Succisa* populations with the same flower diversity, and likewise at sites with a high flower diversity with the same population size. Significant differences between the species and populations (One-Way Anova, Tukey) are indicated by different capital and normal letters, respectively. N.a. means value not available.

	F			
	Gasteren	Ekehaar	Wijnjewoude	Annen
H. pendulus	399 ± 110 (n=9) ^a	287 ± 42 (n=10) ^{Bab}	78 ± 20 (n=10) ^b	257 ± 90 (n=10) ^{ab}
H. trivittatus	n.a.	n.a.	325 ± 171 (n=10)	497 ± 249 (n=11)
E. horticola	n.a.	1847 ± 713 (n=10) ^{Aa}	193 ± 49 (n=10) ^b	853 ± 141 (n=11) ^{ab}
E. tenax	n.a.	n.a.	380 ± 113 (n=10)	n.a.

The insect species showed differences in pollen load size between populations. The pollen load of H. *pendulus* was significantly smaller in population Wijnjewoude compared with population Gasteren (Tukey, p=0.020). These Succisa populations differ in size. The pollen loads of H. *pendulus* were of comparable size in populations Ekehaar and Annen (Tukey, p=0.991). The number of pollen grains in loads of E. *horticola* differed significantly between populations Ekehaar and Wijnjewoude (Tukey, p=0.023). The bodies of E. *horticola* individuals in population Ekehaar contained 1847 pollen grains on average, whereas the loads in population Wijnjewoude consisted of only 193 pollen grains on average. These Succisa populations differ in size and flower diversity.

Pollen loads tend to be larger in small *Succisa* populations with the same flower diversity, and likewise at sites with a high flower diversity with the same population size.

Composition of Pollen Loads

In general, all insect species that were sampled carried a high proportion of heterospecific pollen on pollen consisted mainly of pollen grains of Heterospecific their bodies. Hieracium/Hypochaeris/Leontodon, Calluna/Erica and Achillea/Tanacetum. The ratio of conspecific/heterospecific pollen in the pollen loads differed between insect species and populations. In population Wijnjewoude, the load of H. pendulus contained a significantly higher proportion of Succisa pollen (78%) than the pollen loads of the other insect species (H. trivitattus: 38%, Tukey, p=0.011; E. horticola: 45%, Tukey, p=0.045; E. tenax: 25%, Tukey, p<0.001; figure 10). Thus, even in a large Succisa population with only Potentilla as other flowering plant species in the vegetation, individuals of H. trivittatus, E. horticola and E. tenax showed a high proportion of heterospecific pollen. In the other populations, the proportion of Succisa pollen in the pollen loads differed not significantly between insect species (Ekehaar, One-Way Anova, p=0.074; Annen, One-Way Anova, p=0.105; see appendix VIII).



Insect species

Figure 10 Composition of pollen loads of four frequent *Succisa* visitors in population Wijnjewoude (September 12th). As the figure shows, the load of *H. pendulus* contained a significantly higher proportion of *Succisa* pollen than the pollen loads of the other insect species (One-Way Anova, Tukey; indicated by the different capital letters). Moreover, the figure makes clear that, even in a large *Succisa* population with low flower diversity, individuals of *H. trivittatus*, *E. horticola* and *E. tenax* showed a high proportion of heterospecific pollen.



Figure 11 Composition of pollen loads of *H. pendulus* in different populations. The figure shows that, independent of flower diversity at sites, *H. pendulus* carried a significantly higher proportion of *Succisa* pollen in large populations compared with small populations (One-Way Anova, Tukey; indicated by the different capital letters).

The proportion of Succisa pollen (and consequently the proportion of heterospecific pollen) in the pollen loads of the insect species depends on the population size of Succisa. Insects captured in small Succisa populations had a lower proportion of Succisa pollen in their pollen loads (ranging from 20% to 37%) compared with insects captured in large Succisa populations (ranging from 25% to 78%), independent of flower diversity at the sites (see appendix VIII). However, this difference in proportion of Succisa pollen on insect bodies between small and large populations is only significant for H. pendulus (One-Way Anova, p<0.001; figure 11).

Eristalis horticola had the highest (absolute) numbers of *Succisa* pollen grains in all populations (pollen load size and proportion of *Succisa* combined; table 9). The loads of *E. horticola* contained between 96 and 508 *Succisa* pollen grains on average, while the number of *Succisa* pollen grains in the loads of the other insect species ranged from 27 to 105 on average. This difference in number of *Succisa* pollen grains between insect species is significant for the populations Ekehaar (One-Way Anova, p=0.003) and Annen (One-Way Anova, p<0.001). The number of *Succisa* pollen grains in the pollen loads of *H. trivitatus* and *E. horticola* was significantly higher in population Annen compared with population Wijnjewoude (*H. trivitatus*, One-Way Anova, p=0.007; *E. horticola*, Tukey, p=0.002). These *Succisa* populations differ in flower diversity.

Table 9 Absolute number of Succisa pollen grains (mean \pm S.E.) in the pollen loads of four frequent Succisa visitors. Significant differences between the species and populations (One-Way Anova, Tukey) are indicated by different capital and normal letters, respectively. N.a. means value not available.

Number of Succisa pollen grains in pollen load (mean ± S.E.)						
	Gasteren	Ekehaar	Wijnjewoude	Annen		
H. pendulus	95 ± 51 (n=9)	49 ± 11 (n=10) ^B	66 ± 19 (n=10)	102 ± 28 (n=10) ^B		
H. trivittatus	n.a.	n.a.	27 ± 6 (n=10) ^b	$105 \pm 24 (n=11)^{Ba}$		
E. horticola	n.a.	341 ± 84 (n=10) ^{Aab}	96 ± 51 (n=10) ^b	508 ± 85 (n=11) ^{Aa}		
E. tenax	n.a.	n.a.	39 ± 9 (n=10)	n.a.		

The number of pollen species found in the pollen loads was more or less the same for all insect species and populations (table 10). The pollen loads of the different insect species contained between 6 and 9 pollen species on average, independent of population size of *Succisa* and flower diversity at the sites. Only in population Wijnjewoude the pollen load of *E. tenax* contained significantly more pollen species than the pollen load of *H. pendulus* (Tukey, p=0.027). For *H. trivittatus* and *E. horticola* species richness of the pollen loads was comparable in population Wijnjewoude (Tukey, p=1.000).

Table 10 Number of pollen species (mean \pm S.E.) in the pollen loads of four frequent *Succisa* visitors. The number of pollen species found in the pollen loads was more or less the same for all insect species and populations. Significant differences between the species (One-Way Anova, Tukey) are indicated by different capital letters. N.a. means value not available.

Number of pollen species in pollen load (mean ± S.E.)					
	Gasteren	Ekehaar	Wijnjewoude	Annen	
H. pendulus	8 ± 0.38 (n=9)	8 ± 0.61 (n=10)	6 ± 0.40 (n=10) ^B	7 ± 0.67 (n=10)	
H. trivittatus	n.a.	n.a.	8 ± 0.96 (n=10) ^{AB}	6 ± 0.37 (n=11)	
E. horticola	n.a.	8 ± 0.31 (n=10)	8 ± 0.63 (n=10) ^{AB}	6 ± 0.34 (n=11)	
E. tenax	n.a.	n.a.	$9 \pm 0.66 (n=10)^{A}$	n.a.	

3.3.3 Pollen Deposition

The fraction of conspecific pollen deposited on stigmas of *Succisa* was low for all populations (figure 12). This means that the deposition of heterospecific pollen was quite high. Heterospecific pollen consisted mainly of pollen grains of Asteraceae, like *Hieracium*, *Hypochaeris*, *Leontodon*, *Achillea* and *Tanacetum*. The pollen loads on stigmas at high flower diversity sites contained 10 pollen species on average. The number of pollen species in the pollen loads on stigmas at low flower diversity sites was significantly lower (between 6 and 7 pollen species on average) (One-Way Anova, p<0.001; see appendix IX). The stigmas of *Succisa* flower heads in large populations received a significantly higher fraction of conspecific pollen (45% and 64%) than the stigmas of *Succisa* flower heads in small populations (17% and 18%) (One-Way Anova, p<0.001). No significant differences between sites that differ in flower diversity were found.

Data about the number of *Succisa* pollen grains per stigma provide information about the potential seed set. Potential seed set is defined as the fraction of stigmas (within a flower head) that receives four or more *Succisa* pollen grains, since four pollen grains are needed for ovule fertilization. The values found for potential seed set were low (less than 21%) and differed not significantly between populations (One-Way Anova, p=0.178; figure 13). On average, a stigma received between 1 and 2 *Succisa* pollen grains during a day (five and a half hours; see appendix IX). It is this number of *Succisa* pollen grains that may lead to seed set, since a flower head is in the female stage for only one day.



Figure 12 Fraction of *Succisa* pollen deposited on the stigmas of *Succisa* (within a flower head) in different populations. The stigmas of *Succisa* flower heads in large populations received a significantly higher fraction of conspecific pollen than those in small populations (One-Way Anova, Tukey; indicated by the different capital letters).





3.3.4 Seed Set and Germination

It was the aim to study seed set and germination in the populations Gasteren, Ekehaar, Wijnjewoude and Annen. However, because of mowing activities in the road verges of Ekehaar and Annen, it was not possible to study seed set and germination in these populations. Population Assen was chosen as alternative for population Annen. The time of mowing in population Ekehaar was too late to look for alternatives.

Seed Set

In general, the number of seeds produced per flower head of *Succisa* was comparable between populations (figure 14). Each flower head of *Succisa* produced between 39 and 56 seeds on average. In two cases values of seed production differed between populations, when populations with comparable times of seed set were compared (the date of marking the flower heads). Flower heads of *Succisa* in population Annen (24 September 2002) produced significantly more seeds than flower heads of *Succisa* in population Gasteren (24 September 2002) (Tukey, p=0.020). These *Succisa* populations differ in size and flower diversity. Furthermore, seed production per flower head of *Succisa* was significantly higher in population Assen (9 October 2002) compared with population Wijnjewoude (3 October 2002) (Mann-Whitney, p<0.001). These *Succisa* populations differ in flower diversity and were not examined on the same day.

The number of seeds produced per flower head of *Succisa* was more or less constant during the flowering season. Remarkable is that the flower heads of *Succisa* with the latest time of seed set (Assen, 9 October 2002), showed the highest numbers of seeds per flower head.

Germination

The percentage of seed germination per flower head of *Succisa* was low in all populations: less than 50% (figure 15). This percentage differed not significantly between populations, when populations with comparable times of seed set were compared.

The proportion of germinated seeds per flower head of *Succisa* declined towards the end of the flowering season. Seed set at 9 October in population Assen did not contribute to plants' reproductive success, since the average percentage of germination was 0.





Figure 14 Number of seeds produced per flower head of *Succisa* in different populations at different times in the flowering season. The figure shows that, in general, the number of seeds produced per flower head was comparable between populations. Values of seed production were compared between populations with comparable times of seed set (the date the flower heads were marked) as indicated by differences in font. Significant differences (One-Way Anova, Tukey, Mann-Whitney; within these groups) are indicated by different letters.



Figure 15 Percentage of germinated seeds per flower head of *Succisa* in different populations at different times in the flowering season. The percentage of germination was low for all populations. This percentage differed not significantly between populations, when populations with comparable times of seed set were compared (the date the flower heads were marked). Significant differences (One-Way Anova, Tukey) are indicated by different capital letters.

4 Discussion and Conclusion

4.1 Species Composition of Insects

In all populations, flower heads of Succisa are visited by a large variety of insect species (22 species in total), belonging to the Syrphidae, other Diptera, Hymenoptera and Lepidoptera. Kwak and Olff (manuscript) mention the same taxonomic groups as visitors of Succisa. Therefore, Succisa can be considered as a generalist plant species: it is not only visited by more than one insect species, but the insect species also belong to different taxonomic groups. Syrphid flies formed the greatest part of Succisa visitors, especially members of the Eristalinae (a sub-family of the Syrphidae). Actually, only five species belonging to the Eristalinae were abundant. Helophilus trivittatus, E. horticola, H. pendulus, E. tenax/pertinax and E. arbustorum/nemorum showed by far the highest numbers of individuals (figure 2). Previous research in the same populations also showed that members of the genera Eristalis and Helophilus were the most abundant visitors (Kołodziejska 2002). It must be said, however, that the transect observations were done during favourable weather conditions with high temperatures and no rain. Syrphid flies are not active with rainy weather (Ozinga and Bakker 1995), so the composition of visiting insects may change with the weather conditions. Bumble bees are still active during rainy and windy conditions (Kwak 1993; Ozinga and Bakker 1995; Peeters et al. 1999), and therefore they may play an important role in the pollination of Succisa in the case of less favourable weather conditions.

The proportion of *Eristalinae* visitors shows a positive relationship with the population size of *Succisa*. The larger the population of *Succisa*, the larger the proportion of *Eristalinae* visitors and consequently the smaller the proportion of visitors belonging to other taxonomic groups (figure 4). A higher proportion of *Eristalinae* visitors may be the result of a greater number of visitors belonging to the *Eristalinae*, a smaller number of visitors of other taxonomic groups or a combination of both.





Figure 16 clearly shows that a positive relationship between population size of Succisa and the number of Eristalinae visitors to Succisa exists. The number of individuals of other taxonomic groups does not depend on the size of the population and their numbers remain more or less constant with increasing population size. This implies that the members of the Eristalinae are responsible for the greater number of visitors in larger populations. Sih and Baltus (1987) found a similar relation between visitor abundance and patch size. They argue that plants in large patches may exhibit mutual attraction of visitors, which leads to a greater number of insect visitors in the plant population. However, a greater number of visitors does not necessarily mean a higher visitation rate from a plant's perspective. This is dependent on whether the number of visiting insects increases proportionally with the population size, viz. the change in visitor density (Sih and Baltus 1987). If visitor density is defined as the average number of visitors per flower head, the Eristalinae species show by far the highest density in all populations. This density also increases with the population size of Succisa. Thus, the number of Eristalinae visitors increases more than proportionally with the number of Succisa flower heads. Moreover, the proportion of flower heads visited by a forager is of importance with respect to the visitation rate per flower head. Goulson (2000) suggests that pollinators visit a smaller proportion of the available flower heads in large populations. He argues that from an insect's point of view this is an optimal strategy, because in large populations searching for the remaining unvisited inflorescences is more difficult than in small populations. Possible differences in visitation rates between the populations will be discussed in the next section.

No relation between flower diversity and the species composition of Succisa visitors was found (figure 5). Possibly, this is caused by the flowering time of Succisa. Since Succisa flowers from late summer till autumn, many insects do not fly anymore and insect diversity is expected to be low in all populations. The diversity of Succisa visitors declines as the flowering season makes progress due to the natural phenology of syrphids (figure 7). Species richness differs between sites early in the flowering season (figure 3). At sites with a low flower diversity, population size matters with respect to insect species richness. The highest number of insect species was observed in the large population of Succisa. In small populations of Succisa, the presence of other flowering plant species is crucial to attract a higher number of insect species. It is generally accepted that a high diversity of insect species at sites is beneficial for pollination of a particular plant species (Velterop 2000). The more pollinator species available, the greater the chance the appropriate pollinator is among them. Furthermore, the greater the number of pollinator species present, the greater the diversity of behavioural characteristics is. For example, butterflies are good long distance travellers and bees are very flower constant. Syrphid flies are pretty good travellers and moderate flower constant. Since the Eristalinae formed the greatest part of the visitors in all populations, it seems reasonable to expect that they play an important role in the pollination of Succisa. However, this depends on the pollination effectiveness of the members of the Eristalinae, which is discussed in section 4.3.

The proportion of *Eristalinae* visitors increased during the flowering season (table 2), while members of the other Diptera, Hymenoptera and Lepidoptera visited *Succisa* flower heads mainly early in the flowering season. This is due to the natural phenology: individuals of *H. trivittatus*, *E. horticola*, *H. pendulus* and *E. arbustorum* can be seen till October, while most bumble bees, solitary bees, butterflies and day-active moths do not fly anymore at that time. *Eristalis tenax* overwinters as an adult and is still active in the late autumn.

The most frequent visitors of Succisa are (very) common syrphid fly species in The Netherlands (Verlinden 1991). The larvae of these species occur in (organically rich and) polluted ditches, pools and ponds (Stubbs and Falk 2002). The presence of these syrphid fly species in nutrient poor nature reserves, like Wijnjewoude and Rotstergaast, indicates the euthrophication of the environment on a landscape scale. Recently, the number of organically enriched environments has increased due to human activities. So, it is uncertain if these syrphid fly species were the most frequent visitors (and potential pollinators) in the past. Peeters et al. (1999) mention the oligolectic bee Andrena marginata as a frequent visitor of Succisa in the past. In the sixties, this species became extinct in The Netherlands (Peeters et al. 1999). According to this study, the place of A. marginata as pollinator of Succisa seems to be occupied by common large syrphid fly species. In contrast to A. marginata, these species all show generalistic feeding behaviour. This leads to the deposition of heterospecific pollen,

which may be detrimental to Succisa. In Belgium and Germany, A. marginata is still present (Ozinga and Bakker 1995). So, it is interesting to compare the situation in The Netherlands with that in the surrounding countries. Because of a strong decline of A. marginata in these countries (Ozinga and Bakker 1995), a similar shift from oligolectic to polylectic species should be expected. It may be of interest to investigate if this shift in pollinator guild goes along with the deposition of more heterospecific pollen on the stigmas of Succisa. If this is the case, offspring performance of Succisa is expected to decline, which may at least partly explain the recent decline of Succisa in The Netherlands.

4.2 Visitation Rate

On average, a flower head of *Succisa* receives between 24 and 83 insect visits a day (5h). This is quite high in comparison with other flowering plant species (see table 11). However, visitation rates of different plant species are difficult to compare, because they are usually calculated for different flower units (per flower, umbel or for the whole plant), pollinator guilds and time intervals. Furthermore, flower longevity differs between plant species. I assume the visitation rates of *Succisa* and *S. columbaria* to be comparable, because they have almost the same flower architecture, a comparable pollinator guild and the female stage of both species lasts one day. Ozinga and Bakker (1995) report for *S. columbaria*, a close relative of *Succisa*, 20 up to 100 visits per head a day. These visitation rates correspond with those of *Succisa*. Such high visitation rates indicate a high attractiveness of these plant species to insects. This high attractiveness may be the consequence of the odour and/or colour of the flowers, the easy accessability of nectar and pollen (flower architecture), the high quantity and/or high quality of nectar and pollen or a combination of these factors (Jennersten and Kwak 1991). Kołodziejska (2002) found that the nectar volume of *Succisa* flowers is very low compared to other flowering plant species. Further research is needed to provide insight in the role the different factors play with respect to the attractiveness of *Succisa* in combination with other co-flowering plant species.

Plant family	Plant species	VR (10 min)	Flower	Visitors	Reference
Ranunculaceae	Aconitum lycoctonum s.l.	0.01-0.25	Flower	Bumble bees	Utelli and Roy 2000
	Ranunculus acris	0.08-0.24	Flower	Flies	Totland 1993
	Cerastium alpinum	0.06-0.15	Flower	Flies	Totland 1993
Caryophyllaceae	Lychnis viscaria	0.67-1.00	Plant	Bumble bees, honey bees, nectar robbing bees, syrphid flies and others	Mustajärvi et al. 2001
	Silene acaulis	0.01	Flower	Flies	Totland 1993
Brassicaceae	Brassica kaber	0.20-0.69	Flower	Bees and syrphid flies	Kunin 1997
Saxifragaceae	Parnassia palustris	0.09-0.15	Flower	Flies	Totland 1993
Rosaceae	Potentilla crantzii	0.03-0.12	Flower	Flies	Totland 1993
Geraniaceae	Geranium sylvaticum	0.31	Flower	Flies	Totland 1993
Balsaminaceae	Impatiens glandulifera.	1.00-4.00	Flower	(Bumble) bees	Chittka and Schürkens 2001
	Gentiana pneumonanthe	0.70-1.19	Flower	Bumble bees	Petanidou <i>et al.</i> 1995a
Gentianaceae	Gentiana cruciata	0.00-2.29	Flower	Bumble bees	Petanidou et al. 1995b
Lamiaceae	Stachys palustris	0.37-0.64	Flower	(Bumble) bees	Chittka and Schürkens 2001
Scrophulariaceae	Melampyrum pratense	0.00-0.14	Flower	Bumble bees	Jennersten and Kwak 1991
	Succisa pratensis	0.80-2.76	Flower head	(Syrphid) flies, (bumble) bees, wasps, butterflies and moths	This study
Dipsacaceae	Scabiosa columbaria	0.56-2.78	Flower head	(Syrphid) flies, (bumble) bees, beetles, butterflies and moths	Ozinga and Bakker 1995
Asteraceae	Leontodon autumnalis	0.03-0.47	Flower	Flies	Totland 1993
	Taraxacum croceum	0.18-0.86	Flower	Flies	Totland 1993

Table 11 Visitation rates (VR) of different plant species, derived from the literature on pollination.

The visitation rates are comparable for all populations and population types (figure 8). Nevertheless, flower heads of *Succisa* at sites with a high flower diversity receive more visits than flower heads at sites with a few flowering plant species. Thus, maybe there is a small influence of the presence of other flowering plant species in the surrounding on the visitation rate of *Succisa*. This might be evidence for mutual attraction of visitors by different flowering plant species, in the introduction called (interspecific) pollination facilitation.

4.3 Effectiveness of Visitors

In the previous section, we found no significant differences in visitation rates (quantity component of pollination) between different types of *Succisa* populations. This raises the question whether the quality component of pollination differs between the studied populations. The quality component of pollination will be addressed in the current section.

4.3.1 Behaviour of Individual Insects

Pollen Load Size

The body size of syrphid flies determines the number of pollen grains a fly can transport. This number differs between species. The amount of pollen syrphid fly species carried, ranged from 78 to 1847 pollen grains on average. *Helophilus pendulus* has the smallest body size and this may explain why this species contained the lowest number of pollen grains in all populations. Considerable variation between individuals of the same species exists. This variation between conspecific individuals is the result of the sequence of acceptable flowers each individual has encountered during its foraging trip. Flowers of different plant species produce different amounts of pollen grains, which also differ in size. For example, pollen grains of the Asteraceae are small and stick together (De Buck 1990). Therefore, it is possible that large amounts of those pollen grains are picked up, even if the number of visits to Asteraceae is not very large (Kołodziejska 2002). On the contrary, pollen grains of *Succisa* are relatively large and syrphid fly species can carry only a small amount of them on their bodies. This difference in pollen size and pollen production rate between plant species leads to differences in pollen load size between populations. The total pollen loads of the syrphid fly species are relatively large in small *Succisa* populations and at sites with high flower diversity (table 8).

Flower Constancy

From the observations of behaviour, *H. pendulus*, *H. trivittatus* and *E. horticola* appear to be (very) flower constant. On average, nothing less than 75% of the transitions during their foraging bout were consecutive transitions between *Succisa* flower heads. *Helophilus trivittatus* and *E. horticola* are always very flower constant visitors, independent of population size of *Succisa* and flower diversity at the sites (table 3). Flower constancy of *H. pendulus* depends on the population size of *Succisa*. This species shows higher flower constancy in the large populations compared with the small population. The largest difference in flower constancy was found between the population types smallhigh and large-low, respectively. These findings are not surprising, because for insect species it is easier to be flower constant in large populations and sites with low flower diversity compared to small populations and sites with high flower diversity. In general, *H. pendulus* is less flower constant than *H. trivittatus* and *E. horticola*.

In comparison with the observations of behaviour, the analysis of pollen loads shows another picture with respect to flower constancy. All insect species had large amounts of heterospecific pollen on their bodies, an indication of low flower constancy. The number of pollen species in the pollen loads was more or less the same for all insect species and populations (table 10). Probably, this is the consequence of learning and/or memory constraints of visiting insects with respect to flower handling. The proportion of heterospecific pollen mainly consisted of Asteraceae pollen grains. De Buck (1990) mentions that flowers of some Asteraceae species are regularly visited by syrphid fly species. Even in a large *Succisa* population with low flower diversity (Wijnjewoude), individuals of *H. trivittatus, E. horticola* and *E. tenax* carried a high proportion of heterospecific pollen (figures 10 and 17). A lot of

□ Succisa pratensis □ Potentilla erecta ■ Other



Figure 17 Flower constancy of *H. trivittatus* in population Wijnjewoude. The first bar (choice) represents the relative number of visits of *H. trivittatus* to a particular flowering plant species, based on transect observations (september 6^{th}). In the second bar (supply) the composition of available flowering plant species in population Wijnjewoude is given (september 6^{th}). From both measures a preference measure (choice/supply) was calculated, which is illustrated in the preference bar. Bar four gives the pollen load composition for population Wijnjewoude (september 12^{th}). The figure makes clear that flower preference observed during transect observations does not necessarily reflect the pollen load composition (as measure of flower constancy) on the body of *H. trivittatus*.

pollen species found in the pollen loads of these insect species were not present in the area of this Succisa population. For example, in population Wijnjewoude, the loads of E. horticola contained a proportion of 16% Calluna pollen, on average. In Wijnjewoude, the distance between the Succisa population and the nearest Calluna population is about 300m. Therefore, it seems reasonable that the observed insect species obtain pollen from flowering plant species in the surrounding of the Succisa population and flew at least 300m. Eristalis tenax and H. trivittatus are known as migrant species (Verlinden 1991; Stubbs and Falk 2000) and thus able to fly large distances. Helophilus pendulus also is a notable wanderer (Stubbs and Falk 2000). It remains, however, questionable whether these species fly such large distances during their foraging trip. The syrphid fly species of which flower constancy was investigated, do all have large body sizes. Maybe there is a correlation between foraging distance and body size of syrphid flies. A (positive) relationship between foraging distance and body size exists for butterflies, bees and carabid beetles (Gathmann and Tscharntke 2002). Long foraging distances of the pollinators of Succisa may lead to substantial gene flow by pollen between Succisa populations. I argue that gene flow by pollen between fragments of formerly large Succisa populations, seperated by a few kilometres, might be expected. Other Succisa populations are highly fragmented, so gene exchange between these populations is not plausible. Gene exchange between Succisa populations increases the effective size of these populations and reduces the threat of genetic erosion (Ellstrand 1992).

The proportion of Succisa pollen in the pollen loads of the insect species differs between populations of Succisa differing in size. Insects in small populations had a lower proportion of Succisa pollen in their pollen loads compared with insects in large populations. This is an indication of passive flower constancy: flower constancy of the captured insects shows a positive relationship with the population size of Succisa. Thus, individual insects show temporarily flower constancy, dependent on the availability of Succisa flower heads compared to other flowers. The difference in proportion of Succisa pollen on insect bodies between small and large populations is only significant for H. pendulus (figure 11). Although differences in body size are small, H. pendulus has the smallest body size of the four species for which flower constancy was investigated. Therefore, the foraging range of H. pendulus in large populations of Succisa may largely be restricted to these populations. Pollinators

should broaden their diet and switch hosts more frequently as patch size declines relative to foraging range (Levin 1978).

In population Wijnjewoude, *H. pendulus* showed a significantly higher proportion of conspecific pollen than the other species (figure 10). This is in contradiction with the results of the observations of behaviour. This supports the idea of passive flower constancy. According to De Buck (1990), flower constancy of syrphid flies depends on the number of available flowers of the same species. Kunin (1993) observed that flower constancy behaviour of *E. tengx* and *E. cf. arbustorum* indeed depends on population density and the presence of other flowering plant species. The discrepant results of both methods used may also be explained by their difference in time interval with which flower constancy is defined. Observations of behaviour provide information about the flower constancy of the followed insects at the time the observations are performed (short term). Pollen loads, however, remain on the body of an insect for a given time (long term). The results of both analyses together suggest that once within a *Succisa* population, the individual insects are indeed flower constant (observations of behaviour), but outside the population they visit many other flowering plant species (analysis of pollen loads). For the pollination of *Succisa*, purity of the pollen load may be most important.

Pollen Deposition

Both the visitation rate and the amount of pollen transferred per visit influences pollination success. The number of deposited *Succisa* pollen grains per visit depends on the preference of individual insects for the male or female stage of a flower, the residence time and deposition rate of insects visiting *Succisa*. In the above calculated mean number of visits per flower head (section 4.2), the possible preference of individual insects for the male or female stage is not taken into account. According to Kołodziejska (2002), *E. tenax* and *Helophilus* sp. have a preference for female flower heads. This may be beneficial to the pollination of *Succisa* plants, because one visit to a male flower head is enough for the uptake of many pollen, that can be deposited on many stigmas. Therefore, future research should account for this sex preference.

If the deposition rates (number of deposited pollen grains per stigma per minute) and the residence times of the visitors of Succisa are known, something can be said about the number of deposited Succisa pollen grains on a Succisa flower head during a day. It is this total number of visits that may lead to pollination, since a flower head is one day in the female stage. From this study, only data about the residence times of Succisa visitors are available. Ozinga and Bakker (1995) mention a deposition rate of 8.5 ± 3.0 pollen grains per stigma per minute (mean \pm S.E.) for *E. tenax* on stigmas of *S. columbaria* is comparable with that of Succisa. Therefore, I will use the deposition rate of *E. tenax* on *S. columbaria* here. The number of received Succisa pollen grains on a Succisa flower head is calculated as follows:

#SP = VR * RT * DR, in numbers:

$$0.17 * 0.53 * 8.5 = 0.77$$

where,

#SP = mean number of received Succisa pollen grains per minute,

VR = mean visitation rate per flower head per minute,

RT = mean residence time of three Eristalinae syrphid fly species in minutes,

DR = mean deposition rate of *E. tenax* per minute.

This means that a flower head of *Succisa* receives on average 230 (= 0.77*60min*5h) *Succisa* pollen grains a day. If we take into account that a flower head of *Succisa* consists of 65 flowers on average (ranging from 30-100 flowers), one flower receives a mean of 4 *Succisa* pollen grains if these are evenly distributed between flowers within a flower head. This number of pollen grains per stigma is enough for ovule fertilization (Kwak and Olff, *manuscript*). On the basis of this information, you may assume that potential seed set should be quite high. However, in this study, the values found for potential seed set were low for all populations (figure 13). This discrepancy could be the result of the use of a wrong approximation of the deposition rate of the *Succisa* visitors. The pollen may also be deposited in clogs, which means that the distribution of pollen on the stigmas of *Succisa* is not uniform. Moreover, the stigmas of *Succisa* could be occupied by heterospecific pollen, instead of

conspecific pollen. This depends largely on the feeding behaviour of the individual insects visiting *Succisa*. Non-flower constant behaviour by flower visiting insects may lead to the deposition of heterospecific pollen.

The composition of the pollen deposited on *Succisa* stigmas reflects that of the pollen loads on the bodies very well. A high fraction of heterospecific pollen was deposited on the stigmas of *Succisa* in all populations. Again, heterospecific pollen consisted mainly of pollen grains of Asteraceae. The proportion of heterospecific pollen deposited, depends on the population size of *Succisa*. In large populations, flower heads of *Succisa* received a significantly higher fraction of conspecific pollen than those in small populations (figure 12). It is unknown if the deposition of heterospecific pollen is detrimental to *Succisa* (Kwak and Olff, *manuscript*). According to Waser (1986), interspecific pollen transfer might lead to disruption by foreign pollen or the loss of pollen deposited on foreign flowers, each of which could reduce fitness. This may have great consequences for the survival abilities of *Succisa* plants. In section 4.4, seed set and germination of *Succisa* come up for discussion.

Foraging Speed, Residence Time and Resting Behaviour

In all populations, the residence times differed not significantly between insect species (table 5). Individuals of the followed syrphid fly species spent between 19 and 33 seconds on a flower head on average. Great differences between individuals of the same species existed. No distinction was made between residence times on female (with only nectar) and male flower heads (with nectar and pollen). Hence, the foraging aim of the species (nectar or pollen) may influence residence time (Velterop 2000). The time of the day and changing weather conditions during the day also result in large intraspecific differences in residence time. Longer residence times in population Wijnjewoude are due to less favourable weather conditions during the observations. Temperature, humidity, and the amount of sunshine are of great importance in determining the activity patterns of syrphid fly species (Stubbs and Falk 2000).

A long residence time makes a high foraging speed impossible. Consequently, in population Wijnjewoude, foraging speeds of syrphid fly species are the lowest. In all populations, foraging speed differed not significantly between insect species (table 4). Individuals of the observed syrphid fly species visited between 1 and 3 flower heads per minute on average. Again, time of the day and weather conditions strongly influence foraging behaviour. Kołodziejska (2002) reports lower foraging speeds of *Eristalis* sp. and *Helophilus* sp.. Presumably, this is caused by the less favourable weather conditions during her research. The number of flower heads visited per minute is also influenced by the number and duration of visits to other flowering plant species and time spent on resting between periods of active foraging (not included in the foraging speed).

Helophilus pendulus pays more visits to other flowering plant species compared with the other insect species. In addition, in all populations, *H. pendulus* spends more time on resting between periods of active foraging than the other insect species. Individuals of this species frequently visit leaves and seed capsules of plants during their foraging trip (table 7). Stubbs and Falk (2000) report comparable behaviour of *H. pendulus*: "Though frequently found at flowers, this species is commonly seen sitting on leaves and often emits a buzzling sound whilst resting in this fashion.". The higher number of visits of *H. pendulus* to other flowering plants and leaves/seed capsules did not result in significant differences in foraging speed between the insect species. So, the rate of cross pollination is expected to be comparable for the syrphid fly species.

4.4 Seed Set and Germination

Seed Set

Flower heads of *Succisa* consist of 30-100 flowers. Each flower produces only one seed (Bühler and Schmid 2001). This means that maximum (full) seed set may range from 30 till 100 seeds per flower head, dependent on the number of flowers within a flower head. The actual number of seeds per flower head was comparable for all populations and ranged from 39 till 56 (figure 14). The number of seeds per flower head (seed quantity) remains more or less constant during the flowering season. From this you may argue that the potential offspring of plants is the same during the flowering season, independent of pollinator visitation and, for example, weather conditions. However, seed quality is

also of importance. Kwak and Olff (*manuscript*) found that the proportion of seeds with endosperm development (so-called full seeds) decreases in the course of the flowering season. Similar results were found by Kołodziejska (2002). This may have negative consequences for the germination of seeds later in the flowering season.

Germination

The percentage of seed germination per flower head of Succisa was low in all populations (<50%). No significant differences in the proportion of germinated seeds between populations were found (figure 15). Probably, these low seed germination rates are (partly) the result of the small number of deposited Succisa pollen grains per stigma. On average, a stigma received between 1 and 2 Succisa pollen grains during a day (five and a half hours). This is caused by the low effectiveness of pollinators in depositing conspecific pollen. The bodies of the most frequent visitors and the stigmas of Succisa contained large amounts of heterospecific pollen. It is unclear if the deposition of heterospecific pollen is detrimental to Succisa, but it undoubtedly results in the loss of receptive stigma surface on female flower heads. Moreover, it leads to a reduction in male reproductive success, because of the deposition of Succisa pollen on foreign flowering plant species. These lost pollen and stigma surfaces otherwise would have contributed to fertilization events (Rathcke 1983). However, seed germination is not only influenced by the quantity and quality of delivered pollen, but also by demographic, environmental and genetic factors like inbreeding (for examples, see Hooftman 2001; Billeter et al. 2002; Soons and Heil 2002; Hooftman et al. 2003; Vergeer et al. 2003a,b). These factors influence the performance of S. pratensis in a complex way. Due to interaction effects, unambiguous relationships between pollen limitation and reproductive success of Succisa may not be found in the field situation. Therefore, the influence of heterospecific artificial pollination on seed set should be compared with conspecific pollination (Dafni 1992; Brown and Mitchell 2001). For example, the following three hand pollination treatments could be used: conspecific (excess of Succisa pollen), mixed (a mixture of Succisa and Asteraceae pollen grains), heterospecific (excess of Asteraceae pollen). Additionaly, in future research, potted Succisa plants with the same soil should be used to ensure that the effects of resource limitation are the same for all plants. Moreover, the use of populations of potted plants is a practical response to the loss of target flowering plants due to mowing activities. This (at that time) inappropriate management for Succisa also led to significant reductions in reproductive success. But this is the fate of the Dutch Succisa populations, especially in road verges.

The proportion of germinated seeds per flower head of *Succisa* declined towards the end of the flowering season. Probably, this is the result of the declining proportion of full seeds produced during the flowering season. However, in this study, no distinction was made between developed (full) and undeveloped (empty) seeds. This lower proportion of germinated seeds later in the season may be caused by resource limitation (pollen, light, nutrients and water), unfavourable weather conditions influencing the quality of seeds or a combination of both. However, this was not the subject of this study. Further research is needed to clearify the possible effects of these factors on the temporal differences in reproductive succes.

4.5 Final Conclusion

In conclusion, Succisa is a generalist plant species and mainly pollinated by a few, common, large polylectic syrphid fly species. In all populations, Eristalinae species were the most frequent visitors of Succisa. Visitation rates (quantity component of pollination) to Succisa flower heads were quite high and did not differ between populations varying in size and flower diversity. The generalistic feeding behaviour of the most frequent visitors led to the deposition of heterospecific pollen on the stigmas of Succisa. In line with my expectations, large populations received a higher proportion of conspecific pollen (quality component of pollination) than small ones. Probably, this difference is the result of passive flower constancy of the visiting insect species. Therefore, in The Netherlands, qualitative aspects of pollination seem to be more important in determining differences between populations of Succisa than quantitative aspects. This is in agreement with previous research on S. columbaria (Ozinga and Bakker 1995), Gentiana cruciata (Petanidou et al. 1995b) and Brassica kaber (Kunin 1993). No difference in proportion of conspecific pollen deposited was found between populations differing in flower diversity. This is probably caused by large flight distances of the visiting insects.

Their long flights have a strong potential to increase neighbourhood size (Levin 1978; Velterop 2000). According to my study, the difference in proportion of conspecific pollen deposited does not lead to differences in seed germination rates between populations. The proportion of germinated seeds was low in all populations. Likely, this is (partly) caused by the deposition of large amounts of heterospecific pollen, even in large *Succisa* populations. Thus, *Succisa* plants suffer from competition through interspecific pollen transfer, also called interference competition (Rathcke 1983; Waser 1983). The smaller the population size, the stronger the competition.

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Appendices

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Captions

Appendix I: Weather conditions at the time the transect observations were performed (sessions).

- Appendix II: Flower diversity and insect diversity of the different transect observations. Session numbers refer to the numbers used in appendix I. H represents the Shannon index of diversity.
- Appendix III: Data from transect observations. Plant taxa are sorted in alphabetic order with the number of inflorescences given per transect observation. For each plant taxon the total number of visiting insect taxa is mentioned. Session numbers refer to the numbers used in appendix I.
- Appendix IV: Data from transect observations. Insect taxa are arranged according to their abundance (on all plant taxa). The number of individuals per insect taxon per transect observation is given. For each insect taxon the total number of plant taxa that were visited are mentioned. Session numbers refer to the numbers used in appendix I.
- Appendix V: Data from transect observations. Insect taxa are arranged according to their abundance (on *Succisa* only). The number of individuals per insect taxon per transect observation is given. Session numbers refer to the numbers used in appendix I.
- Appendix VI: Weather conditions and visitation rates on Succisa for the different plot observations.
- Appendix VII: Weather conditions at the time the observations of behaviour were performed.
- Appendix VIII: Fraction of pollen grains of different plant taxa on the bodies of different insect taxa in four populations (Gasteren, Ekehaar, Wijnjewoude and Annen). Furthermore, the total number of pollen grains per insect body is given.
- Appendix IX: Fraction of *Succisa* pollen deposited on the stigmas within a *Succisa* flower head in four populations (Gasteren, Ekehaar, Wijnjewoude and Annen). Furthermore, the number of *Succisa* pollen grains per stigma and potential seed set are given. Potential seed set denotes the fraction of stigmas within a *Succisa* flower head that receives four or more *Succisa* pollen grains (initially virgin female flower heads after five and a half hours of visitation).
- Appendix X: Seed set and germination per *Succisa* flower head in four populations (Gasteren, Wijnjewoude, Annen and Assen) at different times in the season.

		Dutch	arid			Tempera-	Cloudi-	Wind-	Humi-
Session Population	Date	×	- -	Start	Finish	ture (°C)	ness (7/8)	force (Bft)	dity (%)
1 Wijnjewoude	6-9-02	207.3	564.1	13:32	13:59	19	2	4	×
2 De Haar	11-9-02	231.8	554.6	16:10	16:33	18	ω	n	×
3 Assen	11-9-02	235.3	555.6	12:32	13:05	18	8	9	×
4 Annen	13-9-02	242.9	565.0	10:56	11:37	21	0	1	73
5 Gasteren	13-9-02	240.4	562.8	15:04	15:18	21	1	2	64
6 Rotstergaast	15-9-02	191.4	547.2	16:52	17:15	20	2	1	×
7 Ekehaar	16-9-02	237.8	552.3	13:08	13:40	17	8	n	×
8 Ekehaar	17-9-02	237.8	552.3	11:25	11:52	18	7	2	×
9 Eleveld	17-9-02	235.0	552.5	13:30	14:04	18	8	n	×
10 Gasteren	24-9-02	240.4	562.8	10:50	11:00	16	0	1	×
11 Eleveld	30-9-02	235.0	552.5	13:53	14:17	18	0	1	×
12 Gasteren	2-10-02	240.4	562.8	11:45	12:00	18	F	0	×
13 Wijnjewoude	3-10-02	207.3	564.1	12:30	12:45	18	80	2	×
14 De Haar	4-10-02	231.8	554.6	10:50	11:18	17	2	4	×
15 Wijnjewoude	8-10-02	207.3	564.1	11:40	11:55	12	0	-	×
16 Assen	9-10-02	235.3	555.6	11:12	11:30	12	1	4	×

Appendix 1 Transect Observation Data - weather Conditions

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Sessi	ion Population	Date	# inflore- scences	# flowering plant species	Flower diversity (H)	# insects	# visiting insect species	Overall insect diversity (H')	# Succisa visiting insect species	Insect diversity on Succisa (H")
	1 Wijnjewoude	6-9-02	7190) 2	0.25	175	17	1.95	17	1.92
	2 De Haar	11-9-02	1822	2 10	1.32	50	10	1.74	2 .	1.71
	3 Assen	11-9-02	2791	32	1.18	230	10	1.81	10	1.77
	4 Annen	13-9-02	2635	5 16	1.99	192	12	1.96	თ	1.78
	5 Gasteren	13-9-02	324	11 11	1.22	14	7	1.73	5	1.47
	6 Rotstergaast	15-9-02	1095	3	1.01	31	9	1.11	9	1.08
	7 Ekehaar	16-9-02	3186	3 18	2.1	44	10	1.47	4	1.12
	8 Ekehaar	17-9-02	292	7 18	2.12	75	15	2.09	0	1.57
	9 Eleveld	17-9-02	202	7 15	2.07	127	10	1.88	80	1.67
	10 Gasteren	24-9-02	112	4	1.09	4)	3	1.05	2	0.69
	11 Eleveld	30-9-02	1073	3 15	1.88	21	80	1.68	7	1.49
	12 Gasteren	2-10-02	5:	3	1.39	-	1	0	-	0
	13 Wijnjewoude	3-10-02	74(2	0.66	16	5	1.13	5	1.22
	14 De Haar	4-10-02	124	7 6	0.78	J	5	1.56	4	1.33
	15 Wijnjewoude	8-10-02	17:	2 2	0.67	1	5	0.5	2	0.5
	16 Assen	9-10-02	46	9 17	1.95	27	5	1.3	2	0.6

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Appendix II Transect Observation Data – Flower Diversity and Insect Diversity

Appendix III Plant Taxa per Population

Plant taxon							S	iess	ion				1	terin .			# visitir insect	ng t
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	taxa	
Achillea millefolium		50	180	100	45				16	16	3	9		85				8
Amaranthus sp.			350															
Anthriscus sylvestris									1							~~		-
Aster/Erigeron sp.			225													90		5
Bellis perennis							7	10										2
Calluna vulgaris			40	375		335	600	570								11		2
Calvstegia sepium			4															
Carduus sp.											1							
Centaurea jacea			15													1		1
Cerastium fontanum ssp																		
vulgare				10	5		3	5	60		13							
Chamerion angustifolium			7						25		12							2
Circium nalustre							5	5										
Echium vulgare			400													130		
			10	6														
Encatetraix			10	0												3		
Euphoroia sp.			1	20			250	220								0		
Euphrasia stricta				30			250	220	-									
Galeopsis tetrahit									3									
Galinsoga quadriradiata					6							2						
Galium verum			1															
Hieracium aurantiacum		1																
Hieracium laevigatum		300	100	5 20	2		720	600	185		75			85		12		12
Hieracium umbellatum									20		10							2
Hieracium vulgatum									290		145							5
Hypericum dubium			15															
			10		4		2	1		2		1						
Hypericum perioratum					-		60	50			5	1						4
Hypochaeris radicata			40	05			440	70	220	7	445	2				32		7
Leontodon autumnalis			10	20	5		110	70	220	'	115	2				52		'
Lotus sp.				6.1					13									
Lotus uliginosus			5	10														1
Lythrum sp.			10															
Matricaria discoidea							170	190										
Mentha aquatica			15															
Oenothera biennis			2													1		
Plantago lanceolata		25	3	250	38		55	60	225	7	40	6		10				2
Polygonum aviculare							13	15			2							
Polygonum hydroniner		1100					8	8						1000				4
Polygonum mito		1100			5		0	0										
Polygonum mile	400	125	4		5	100	700	650	70		35		465	35	104	1		6
Potentilla erecta	490	125	1	-		190	700	050	10		55		400	55	104			0
Prunella vulgaris			_	5														
Ranunculus acris			5															
Ranunculus flammula			2	1														
Satureja vulgaris			5															
Scabiosa columbaria			60													23		6
Scabiosa ochroleuca			5													9		1
Senecio jacobaea			20													12		
Silene dioica			25													10		
Solidago virgaurea			20	225												1		7
Solidayo virgaurea			E	225														
Species x		20	5															
Spergula arvensis		30																
Spergularia rubra		50																
Stellaria graminea		5																
Succisa pratensis	6700	136	1250	700	210	570	330	325	480	77	305	30	275	32	68	125		22
Tanacetum vulgare					3					5		2						1
Trifolium dubium				25			80	70										
Trifolium pratense			10	350			60	65	410		305							5
Trifolium recens				3			15	13	9		7							
Verhena hastata			5	5												2		
Veronica chamacdam			5													6		
Veronica chamaeorys			5													0		
viola arvensis												-	-	-	-	47		
Total # plant taxa	2	10	32	16	11	3	18	18	15	6	15	8	2	6	2	11		

Appendix IV Insect Taxa per Population

		_					S	ess	ion	1							# transect	# indi-	On #
Nr Insect taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	observations	viduals	taxa
1 Helophilus trivittatus	16	8	75	49	1	20	1	4	21		17	-					10	212	11
2 Eristalis horticola	13	1	38	14			8	24	42	1	3		1			4	11	149	9
3 Heloohilus pendulus	43	3	33	18	3	2		6	5	2	6	1	10		4	13	14	149	9
4 Eristalis tenax/pertinax	39	5	19	34	2			4	14	2	17		3	1	1	7	13	148	9
5 Eristalis sp.	43		43	32	1	1	1	3	18								8	142	8
6 Other Diptera	7	21	4	4	5	1	25	19	17		10		1	2			12	116	12
7 Melanostoma sp.	4	8	5	31			1	2	3								7	54	9
8 Eristalis intracaria	1	1	11		1				4								5	5 18	2
9 Bombus pascuorum	1	1	1	1			3	4	2					1		2	9	16	3
10 Lycaena ohlaeas			1	6		6		1									4	14	6
11 Apidae							1	2			1			2			4	6	4
12 Sphaerophoria sp.	1						2	2									3	5 5	4
13 Sericomvia silentis				1									1	2			3	3 4	1
14 Episyrphus balteatus		1			1			1									3	3 3	3
15 Rhingia campestris	1			1					1								3	3 3	2
16 Lasiommata megera								1			1						2	2 2	1
17 Pieris napi	1					1											2	2 2	1
18 Pieris rapae	1							1									2	2 2	1
19 Symbid fly species x							1	1									2	2 2	1
20 Syrphus sp.	1						1										2	2 2	2
21 Vanessa cardui											2						1	2	1
22 Autographa gamma	1																1	1	1
23 Myathropa florea	1																1	1 1	1
24 Neoascia podagrica																1	1	1	1
25 Plusia festucae	1																1	1	1
26 Pyrophaena granditarsa		1															1	1	1
27 Vespidae				1													1	1	1
Total # individuals	175	50	230	192	14	31	44	75	127	5	57	1	16	8	5	27		1057	
Total # insect taxa	17	10	10	12	7	6	10	15	10	3	8	1	5	5	2	5			

							S	ess	sior	1							# transect	#	indivi-	On #
Nr Insect taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	observations	; (duals	plant taxa
1 Helophilus trivittatus	16	5	69	25	1	18	1	2	20		14						10	0	171	11
2 Eristalis horticola	13	1	33	10			6	19	39		3		1				ę	9	125	9
3 Helophilus pendulus	43	2	29	12	3	2		3	5	1	4	1	8		4	5	14	4	122	9
4 Eristalis tenax/pertinax	39	4	16	20	2			4	11	1	15		3	1	1	2	13	3	119	9
5 Eristalis sp.	43		41	19		1			13									5	117	8
6 Other Diptera	7		1	1	4	1	1	1	4				1	1			1(0	22	12
7 Eristalis intracaria	1	1	10		1				4									5	17	2
8 Bombus pascuorum	1	1	1				3	3	1					1			1	7	11	3
9 Melanostoma sp.	2	1	5	3														4	11	9
10 Lycaena phiaeas			1			3											:	2	4	6
11 Sericomvia silentis				1									1	2			:	3	4	1
12 Anidae								1			1						:	2	2	4
13 Lasiommata megera								1			1							2	2	1
14 Pieris napi	1					1												2	2	1
15 Pieris rapae	1							1										2	2	1
16 Rhingia campestris	1			1													:	2	2	2
17 Vanessa cardui											2							1	2	1
18 Autooranha gamma	1																	1	1	1
19 Myathropa florea	1																C 10 KE 10 KE 10	1	1	1
20 Plusia festucae	1																	1	1	1
21 Sphaerophoria sp.	1																1 II II II II II II II	1	1	4
22 Syrphus sp.	1																	1	1	2
Total # individuals	173	15	206	92	11	26	11	35	97	2	40	1	14	5	5	7			740	
Total # insect taxa	17	7	10	9	5	6	4	9	8	2	7	1	5	4	2	2				

Appendix V Insect Taxa on Succisa per Population

Appendix VI Plot Observation Data

Population	Date	Start	Tempera- ture (°C)	Cloudi- ness (?/8)	Wind- force (Bft)	# flower heads	Sex-ratio (M:F)	# visits	# insect taxa	Visitation rate
Wiiniewoude	3-9-02	16:57	22	-	2	32		85	2	2.66
Rotstergaast	4-9-02	11:22	20	0	-	30	14:16	72	5	2.40
Rotstergaast	4-9-02	11:50	20	0	-	32	12:20	52	9	1.63
Ekehaar	5-9-02	14:00	21	-	e	37	27:10	41	2	1.11
Ekehaar	5-9-02	14:18	21	-	ო	38	28:10	84	9	2.21
Eleveld	5-9-02	15:07	20	5	ო	38	32:6	104	80	2.74
Eleveld	5-9-02	15:39	20	5	ო	25	18:7	55	2 2	2.20
Wiiniewoude	6-9-02	12:05	18	œ	4	54	28:26	59	80	1.09
Wiiniewoude	6-9-02	12:18	18	œ	4	39	27:12	64	σ	1.64
Wiinjewoude	6-9-02	14:32	18	2	4	39	27:12	33	7	0.85
Annen	9-9-02	10:18	18	-	-	50	33:17	58	2	1.16
Annen	9-9-02	10:38	19	-	2	52	34:18	47	2	0.90
Annen	9-9-02	10:58	19	-	2	50	33:17	76	80	1.52
Gasteren	9-9-02	12:00	18	N	n	62	33:29	21	2	0.34
Gasteren	9-9-02	12:20	19	~	4	55	40:15	69	e	1.25
Assen	11-9-02	11:30	18	ω	2	47	41:6	61	2	1.30
Assen	11-9-02	11:53	18	ω	2	55	50:5	69	2	1.25
Assen	11-9-02	12:17	18	ω	2	47	41:6	113	2	2.40
De Haar	11-9-02	15:23	18	ω	e	38	25:13	46	4	1.21
De Haar	11-9-02	15:40	18	ω	3 C	40	22:18	26	Q	0.65
De Haar	11-9-02	17:04	18	ω	e S	38	25:13	22	00	2.03
Annen	13-9-02	13:03	22	0	1	51	36:15	195	9	3.82
Annen	13-9-02	13:40	22	0	1	45	34:11	103	Q	2.29
Rotstergaast	15-9-02	2 17:55	20	(4	0	30	16:14	29	m	0.97
Ekehaar	17-9-02	2 11:03	18	2	-	45	32:13	116	2	2.58
Ekehaar	17-9-02	2 12:38	18	w	3	45	32:13	40	2	0.89
Eleveld	17-9-02	2 14:55	18	w	3	45	33:12	56	2	1.24
Eleveld	17-9-02	2 15:14	18	w	3	24	15:9	62	~	2.58
Ekehaar	19-9-02	2 14:12	18	w	3 2	42	32:10	104	0	2.48
Annen	24-9-02	2 11:44	19		1	41	25:16	180	Q	4.39
Annen	24-9-02	2 11:47	19	0	1	34	15:19	102	G	3.00
Annen	24-9-02	2 15:00	18		. 1	34	15:19	45	LC)	1.32
Annen	24-9-02	2 15:00	18		1	41	25:16	34	4	0.83

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Appendix V1 Plot Observation Data (Continued)

(continued)										
Population	Date	Start	Tempera- ture (°C)	Cloudi- ness (?/8)	Wind- force (Bft)	# flower heads	Sex-ratio (M:F)	# visits	# insect taxa	Visitation rate
Gasteren	24-9-02	10.58	17	0	2	28	11:17	25	e	0.89
Fleveld	30-9-02	15:33	18	0	-	31	20:11	88	9	2.84
Fleveld	30-9-02	15:50	18	0	-	24	12:12	48	S	2.00
Fleveld	30-9-02	16:04	18	0	-	31	20:11	107	5	3.45
Ekehaar	1-10-02	15:26	16	4	~	14	8:0 8	15	n	1.07
De Haar	4-10-02	11:45	17	2	-	17	10:7	23	4	1.35
De Haar	4-10-02	12:00	17	2	-	17	10:7	18	n	1.06
Wiiniewoude	8-10-02	12:23	10	0	-	15	9:6	16	-	1.07
Wiiniewoude	8-10-02	12:42	12	0	-	15	2:13	25	0	1.67
Assen	9-10-02	13:05	14	F	4	10	4:6	48	4	4.80
Assen	9-10-02	14:20	14	L	4	10	4:6	26	n	2.60
Assen	9-10-02	14:47	14	1	4	17	5:12	4	1	0.24

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Population	Date	Start	Finish	Ter tu	npe re ('	era °C)	-	Te f bl	em) acl	ber ber	atu	re (°(C) I	Cloudi- ness (?/8)	Win force (d- Bft)
Ekehaar Wijnjewoude Wijnjewoude Wijnjewoude Annen	1-10-02 25-9-02 25-9-02 3-10-02 24-9-02	11:05 12:00 15:30 13:10 12:35	15:10 13:30 16:05 14:00 14:40			10 10 10 10 10	5 5 8 5					17 17	31 .5 .5 22 30	1 8 8 8 4		2 3 3 2 2
			1													

Appendix VII Following Insects – Weather Conditions

Appendix VIII Pollen Loads on Insect Bodies

						1		Plant	taxa					Total #	Total #
Population	Date	Insect taxor	Z	Achillea/ Tanacetum	Calluna/ Erica	Centaurea	Hleraclum/ Hypochaeris/ Leontodon	Persicarla	Plantago	Potentilla	Succisa	Trlfolium	Other	pollen grains p (mean <u>+</u> S.E.)	ollen species (mean <u>+</u> S.E.)
Gasteren	20-9-0	2 H. pendulus	0	5%	31%	%0	31%	%0	%0	%0	23%	%0	10%	399 ± 110	8 ± 0.38
Ekehaar	19-9-0	2 H. pendulus	10	5%	3%	%0	51%	%0	%0	%0	20%	%0	21%	287 ± 42	8 ± 0.61
Ekehaar	19-9-0	2 E. horticola	10	5%	2%	%0	40%	%0	%0	%0	37%	4%	11%	1847 ± 713	8 ± 0.31
Wijnjewoude	12-9-0	2 H. pendulus	10	1%	2%	1%	3%	%0	1%	%0	78%	1%	12%	78 ± 20	6 ± 0.40
Wijnjewoude	12-9-0	2 H. trivittatus	10	1%	2%	1%	19%	2%	1%	4%	38%	%0	32%	325 ± 171	8 ± 0.96
Wijnjewoude	12-9-0	2 E. horticola	10	1%	16%	1%	30%	%0	%0	1%	45%	1%	5%	193 ± 49	8 ± 0.63
Wijnjewoude	12-9-0	2 E. tenax	10	7%	1%	1%	53%	2%	%0	1%	25%	%0	10%	380 ± 113	99.0 + 6
Annen	18-9-0	2 H. pendulus	10	2%	4%	%0	. 26%	%0	1%	1%	54%	%0	%6	257 ± 90	7 ± 0.67
Annen	18-9-0	2 H. trivittatus	1	17%	%0	%0	35%	1%	4%	%0	41%	%0	2%	497 ± 249	6 ± 0.37
Annen	18-9-0	12 E. horticola	11	23%	%9	%0	3%	%0	%0	%0	67%	%0	1%	853 ± 141	6 ± 0.34
							- - -								

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Appendix IX Pollen Deposition

Population	Date	N	Fraction Succisa pollen	# pollen species	# Succisa pollen grains per stigma (mean <u>+</u> S.E.)	Potential seed set (mean <u>+</u> S.E.)
Gasteren	24-9-02	7	0.17 <u>+</u> 0.03	7 + 0.65	1.27 <u>+</u> 0.39	0.11 <u>+</u> 0.06
Ekehaar	1-10-02	7	0.18 + 0.04	10 + 0.42	2.04 <u>+</u> 0.54	0.21 <u>+</u> 0.05
Wijnjewoude	8-10-02	7	0.64 <u>+</u> 0.10	6 <u>+</u> 0.40	0.50 <u>+</u> 0.40	0.05 <u>+</u> 0.05
Annen	24-9-02	7	0.45 + 0.06	10 <u>+</u> 0.37	1.81 <u>+</u> 0.57	0.21 <u>+</u> 0.08

Population	Date	N	# seeds per flower head (mean <u>+</u> S.E.)	Fraction germinated seeds (mean <u>+</u> S.E.)
Gasteren	13-9-02	14	48 <u>+</u> 3.20	0.40 <u>+</u> 0.08
Gasteren	24-9-02	13	39 <u>+</u> 2.81	0.12 <u>+</u> 0.04
Wiiniewoude	25-9-02	22	44 <u>+</u> 2.41	0.49 <u>+</u> 0.05
Wijnjewoude	3-10-02	21	40 <u>+</u> 1.91	0.20 <u>+</u> 0.05
Annen	13-9-02	20	51 <u>+</u> 2.96	
Annen	24-9-02	9	53 <u>+</u> 3.80	
Assen	9-10-02	16	56 <u>+</u> 1.97	0.00 <u>+</u> 0.00

Appendix X Seed Set and Germination