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Impact of pollination and resource limitation on seed production in a border population of *Spiranthes spiralis* (Orchidaceae)

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SUMMARY

Experiments on pollination were carried out in a border population of the terrestrial orchid *Spiranthes spiralis* (L.) Chevall. in the southern part of The Netherlands, one of the two remaining sites in the country. Experiments revealed that *S. spiralis* is a non-autogamous species. Visiting insects, mainly bumblebees, are needed for seed production, either by geitonogamy or cross-pollination. Insect-pollinated plants had 35% fruit set compared to 75% for flowers geitonogamously pollinated by hand. Removal of flowers of other species surrounding clumped flowering stalks of *S. spiralis* increased fruit set, indicating interspecific competition for pollinators. Fruit set was independent of the number of flowers per inflorescence. Since the number of seeds per fruit increased with the number of fruits per inflorescence, available resources do not limit the seed production. This is probably a result of the phenology of this species. Insect pollination does not appear to be a bottle-neck in the sustainability of this border population of the rare and endangered *S. spiralis*. Appropriate vegetation management of the site is probably essential if the population is to persist.

Key-words: insect pollination, interspecific pollinator competition, pollination experiments, population fitness, resource allocation.

INTRODUCTION

Seed production is essential for the sustainability of plant populations and therefore is essential in the consideration of population fitness (Eriksson & Ehrlen 1992; Silvertown *et al.* 1993). Flowering and seed production are determined by both internal and external factors (Nilsson 1992), e.g. performance of individual plants (Wells & Cox 1989) and pollinator activity (Corbet *et al.* 1993). Although these groups of factors can be distinguished from each other, they are highly interactive (Firmage & Cole 1988; Campbell 1989; Rodríguez-Robles *et al.* 1992).

Many plant species reach the edge of their distribution in western Europe where they occur almost exclusively in man-made, semi-natural habitats (Meusel *et al.* 1978;

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Willems & Ellers 1996). The orchid *Spiranthes spiralis* (L.) Chevall., a species with a more southern origin (Meusel *et al.* 1978), reaches the north-western edge of its Continental European distribution in the south of The Netherlands (Tutin *et al.* 1964–1980), where it is both a rare (Mennema *et al.* 1980) and endangered species and has been placed in the most vulnerable category of the Dutch Red Species List (Van der Meijden 1990). Since it is known that pollinator limitations may affect species near the edge of their distribution area (Lloyd 1980), limits to seed production might be part of an explanation of species rarity in western Europe.

Although terrestrial orchids have attracted considerable attention in temperate areas, there have been relatively few quantitative assessments of pollinator activity and of costs of sexual reproduction in this group (cf. Nilsson 1992; Van der Cingel 1995). In this paper we present results of studies of *S. spiralis* designed to: (i) quantify impact of pollinator activities on fruit set, (ii) determine eventually impact of neighbouring other flowering species on pollination, and (iii) establish whether available resources limit seed production. The species has a very high conservation value (Margules & Usher 1981) in The Netherlands (Van der Meijden 1990). Therefore, the overall goal of the project is to gain insight in population fitness and demographic processes in *S. spiralis* in a border area of its distribution (Willems 1989; 1994). The aspect of seed production is the subject of this paper.

MATERIALS AND METHODS

The species

S. spiralis is widely distributed in southern Europe, in the Mediterranean region (e.g Aegean area, Asia Minor) and in coastal areas of North Africa (e.g. Morocco, Algeria, Tunisia) (Ziegenspeck 1936; Tutin *et al.* 1964–1980; Baumann & Künkele 1982). The species reaches the limits of its north-western distribution in the southern part of the United Kingdom and The Netherlands (Landwehr 1977; Meusel *et al.* 1978). In The Netherlands, only two populations have survived out *c.* 35 which were present in the first half of this century. Both extant populations occur in the southern part of the country, namely one in the south-western coastal dune area of the former Island of Goeree and the other one in the chalk district in the south-eastern part of the country, South-Limburg (Mennema *et al.* 1980). This population is the subject of this study and it is the only remaining one of at least 16 that were present in South-Limburg before 1950 (Kreutz 1992). Part of the population has been monitored since 1981 by following the fate of individual plants in a permanently marked plot (Willems 1989; 1994).

S. spiralis is a long-lived orchid species of which individual plants may live for some decades, with up to five wintergreen rosettes and one or more small below-ground tubers per plant (genet). During the winter and spring new tubers are formed. Both rosette leaves and inflorescence emerge in late summer, the latter some days earlier compared to the small leaves (1–5 cm length). Rosette leaves die off in late spring or early summer. The flowering period of *S. spiralis* is August–September (Ziegenspeck 1936; Wells 1981). The flowers are less than half a centimetre long, white and green with nectar and a honey scent that attracts indigenous bumblebee species, mainly *Bombus lapidarius* L. and *B. pascuorum* Scop., at our study site (personal observation). The flowers form a single row that spirals up the stalk. Individual plants produce one or more flowering stalks that bear up to 25 flowers. The flowers open sequentially from the bottom to the top and the total number of flowers open simultaneously varies from 3–5. The height of the flowering stalk is very variable (5–25 cm). In October–November the capsules are ripe and seeds are wind-dispersed. Vegetative reproduction is of minor importance in this species (Ziegenspeck 1936; Willems 1989).

Study site

The research site was the nature reserve of Berghofweide, some 12 km east of Maastricht, province of Limburg (50°50′N; 5°53′E), The Netherlands. *S. spiralis* occurs in a species-rich (*c.* 25 phanerogamic species/m^2) unfertilized grassland on a south-facing slope (inclination 20°, altitude 170 m) beneath the tall fruit trees of a traditional orchard of which the sward was mown or grazed by livestock. The subsoil consists of a mixture of gravel deposits and weathered chalk products (pH 6·0–7·5).

In 1980 the site was set aside as a nature reserve and as a consequence the focus of management changed from agricultural production goals to nature management with the aim of maintaining high species diversity. Since 1980, vegetation has been mown yearly in summer (July) and the hay has been removed. Sheep, at low stocking rate, grazed the site for several weeks in late autumn or early winter after seed dispersal. For more details on site management, see Willems (1989) and Willems *et al.* (1997).

Experimental design and data collection

The field work on the pollination biology of *S. spiralis* was carried out in September and October 1994. Seventy-nine inflorescences which had no or few open flowers were selected. These inflorescences were covered by cages of iron frame covered by cotton net (sieve size <0·1 cm) in order to prevent pollinator access.

The first experiment aimed at investigating self-pollination. Fifteen randomly chosen inflorescences were covered by the cages, five of these inflorescences were used to evaluate seed production without pollinators (autogamy). These inflorescences were covered before any flowers were open and the cages remained in place until the end of October. Flowers on the remaining 10 inflorescences were pollinated by hand to evaluate geitonogamy and pollinator limitation. Pollinia were removed with a needle and then placed on stigma surface of the next highest flower of the same inflorescence. Hand pollinations were carried out in the open flowers from bottom to top to imitate observed pollinator movements at the research site. As a control treatment, fruit production was recorded for 11 randomly chosen inflorescenses occurring under natural conditions, namely free pollinator access.

A second experiment was conducted to determine the effect of inflorescence clumping on fruit set in *S. spiralis*. Sixty-four microsites were chosen in which other flowering herbs surrounded *S. spiralis* inflorescences. An inflorescence was classified as being clumped (C) when there were at least two more flowering stalks of *S. spiralis* nearer than 50 cm. Inflorescences were considered isolated (I) when no other flowering stalk of this species was within 1·5 m. In half of each group, flowers of other species were removed from a circular area of *c.* 7·5 m^2 (radius of 1·5 m) around *S. spiralis* inflorescence (F-). To minimize disturbance of the vegetation structure, which might influence insect behaviour, we removed only the flower heads of the surrounding species. The mean density of inflorescences of other species (e.g. *Lotus corniculatus* L., *Trifolium pratense* L., *Leontodon hispidus* L., *Centaurea jacea* L., *Scabiosa columbaria* L., *Succissa pratensis* Moench., *Origanum vulgare* L.) around *S. spiralis* flowering stalks (F+) averaged 6/m^2.

Table 1. Fruit production in *S. spiralis* among different treatments in 1994: number of inflorescences (*n*), mean fruit set percentage (*x*) and standard deviation (SD). There was a significant ($P<0{\cdot}01$, *t*-test) difference between artificial geitonogamous pollination by hand and natural pollination by insects in the field

Treatment	(*n*)	Number of flowers	Fruits (%)	
			x	(SD)
No pollinator access	5	55	0	(–)
Artificial pollination	10	81	75	(18)
Natural pollination	11	128	35	(27)

In all four treatments (I/F –, I/F+; *n*=11, and C/F –, C/F+; *n*=21) cages were placed over *S. spiralis* inflorescences. Prior to the start of the experiment on either 13 September or 17 September, cages were removed after flowers had been picked from neighbouring forbs the previous day. The cages were removed for 6 h per day to record possible effect of the surrounding vegetation on pollinator activity. During the 6 h, the positions of open flowers were recorded on each inflorescence. This enabled us to establish the effectiveness of any pollinator visits by recording the position of swollen capsules afterwards.

In a third investigation we evaluated seed set per capsule in relation to the number of capsules per inflorescence. We hypothesized that a negative correlation between the number of capsules per flowering stalk and the number of seeds per capsule, especially in the upper part of the inflorescences, would indicate resource limitation in seed production. At the beginning of November, we randomly collected 124 ripe seed capsules in the population. If there were more than three capsules per inflorescence half of the number of capsules were collected. Capsules were stored in plastic vials at 4°C prior to counting seeds by using a binocular microscope (20 ×). Capsules were covered with glycerol before opening to prevent seeds from becoming airborne. Seeds were removed from the capsules, suspended in glycerol and placed into Petri dishes over a grid of 1 × 1 cm.

Data were analysed using ANOVA (general factorial) in the SPSS statistical package release 6·0. We tested for effects of isolated versus clumped inflorescence and for the effect of surrounding flowering species on capsule production by two-way analysis of variance. Data were tested after arcsin transformation, which is necessary for percentage data. We tested for correlations between number of flowers per inflorescence and number of capsules and between number of flowers per inflorescence and percentage of capsules using Pearson's correlation coefficients. Pearson's correlation was also used to test for the correlation between number of seeds per capsule and total number of capsules per inflorescence.

RESULTS

In the first experiment, inflorescences which has been covered during the entire flowering season did not produce any fruits (Table 1), demonstrating that *S. spiralis* must be considered a non-autogamous orchid species. Hand pollination of flowers within the same flowering stalk resulted in seed production, indicating that *S. spiralis* is a

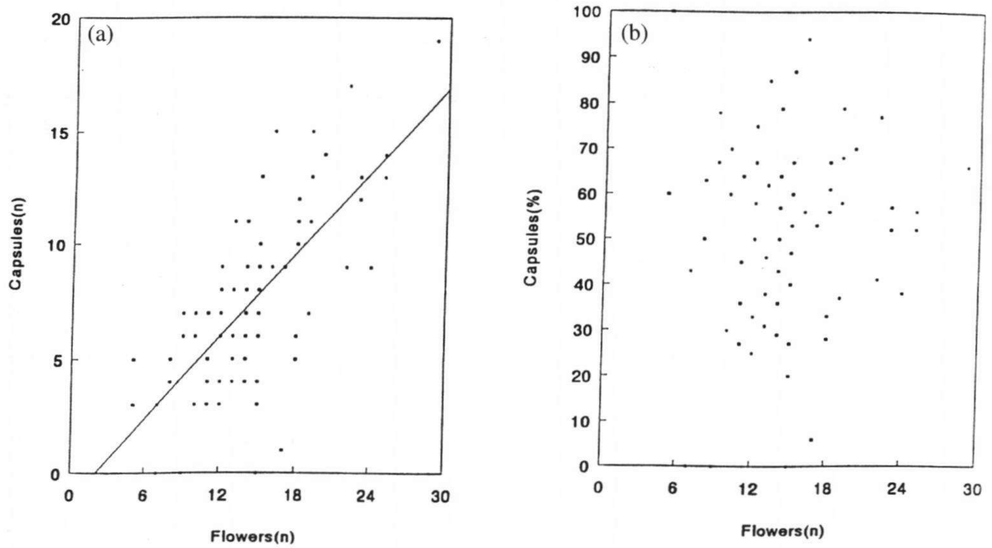

Fig. 1. Effect of inflorescence size on fruit production in *S. spiralis*; (a) number and (b) percentage of produced seed capsules ($n=62$).

self-compatible geitonogamous species that totally depends on insect pollinators for seed production. Insect pollination resulted in 35% fruit set compared to 75% by geitonogamously hand pollination. Fruit set by natural pollinators ranged from 0–78% for individual *S. spiralis* inflorescences, compared to 50–100% for inflorescences that were hand pollinated. This indicates that fruit production was pollen limited during the 1994 flowering season (Table 1).

The number of fruits is positively correlated with the total number of flowers per inflorescence ($P<0{\cdot}001$, $R^2=0{\cdot}567$), whereas the number of flowers had no effect on the percentage of flowers producing capsules ($P=0{\cdot}95$, $R^2=0{\cdot}07\times10^{-3}$) in *S. spiralis* ($n=62$) (Fig. 1a,b).

There was a significant difference in capsule production between isolated and clumped inflorescences of *S. spiralis*, irrespective of the presence or absence of other flowering plants in the adjacent vegetation. *S. spiralis* plants growing in clumps had higher fruit set than isolated plants (Table 2, Fig. 2). This indicates that isolated inflorescences receive fewer successful visits from pollinators compared to those that are clumped.

In the second experiment differences in fruit production were found in relation to the surrounding vegetation. Clumped plants of *S. spiralis* (three inflorescences or more) produced significantly more capsules in microsites where flowers of other species were removed compared to areas with other flowering species present. There was no effect of the difference in the surrounding vegetation if flowering stalks of *S. spiralis* were isolated (Table 2, Fig. 2).

In case of resource limitation, a negative correlation between the number of seeds per fruit and the number of fruits per flowering stalk was supposed. We found a significant positive relationship between the number of capsules per inflorescence and number of seeds per capsule ($P<0{\cdot}01$, $R^2=0{\cdot}067$), indicating that seed production was not limited by available resources. There was, however, a large variation in the seed number per capsule (Fig. 3).

Table 2. Analysis of variance. Tests of significance for arcsin transformed data using unique sums of squares. Effects of isolated and clumped position of inflorescence and effect of the surrounding vegetation on fruit production

Source of variation	SS	DF	MS	*F*	Sig. of *F*
Within + residual	16·26	47	0·35		
Isolated	1·63	1	1·63	4·70	0·035
Flower	0·35	1	0·35	1·01	0·319
Isolated by flower	1·45	1	1·45	4·20	0·046
(Model)	3·94	3	1·31	3·79	0·016
(Total)	20·20	50	0·40		

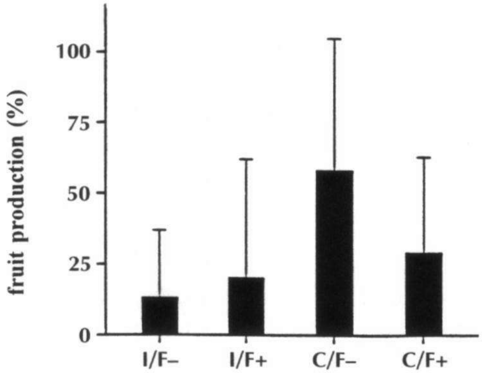

Fig. 2. Fruit production in *S. spiralis* (± standard deviation) as a result of different treatments. Isolated (I) and clumped (C) inflorescences in plots with (F+) and without (F−) other flowering species. Pollinator access was possible for two different days.

DISCUSSION

S. spiralis at Berghofweide is a non-autogamous species. This implies that seed production, and hence fitness of this population, totally depends on insect pollinators. Thus infrequent or ineffective pollinator visits may result in low seed production. Pollination by hand has often produced a significant increase in fruit production compared to pollination in a natural way, indicating that pollen limitation affects sexual reproduction of orchids (Snow & Whigham 1989; Zimmerman & Aide 1989; Ackerman & Montalvo 1990; Calvo 1990; 1993; Whigham & O'Neill 1991). Although insect pollination in the studied *S. spiralis* population resulted in a high percentage of pollinated flowers (35%), results of hand pollination (75%) clearly demonstrate that seed production was pollinator-limited in 1994. August and September were rainy and cold in 1994. This was also the reason that the pollination experiment with clumped and solitary flowering stalks was carried out on only a few days. Yearly variation in weather conditions may thus limit fruit set in *S. spiralis* at this site; e.g. in two previous years, one with unfavourable weather conditions (1987) and one with reasonable conditions during that period (1990), capsule production in this population was 24% and 49%, respectively (Willems 1994).

In the present population the total number of flowers per inflorescence contributes to the number of successfully pollinated flowers. Orchid inflorescences containing different

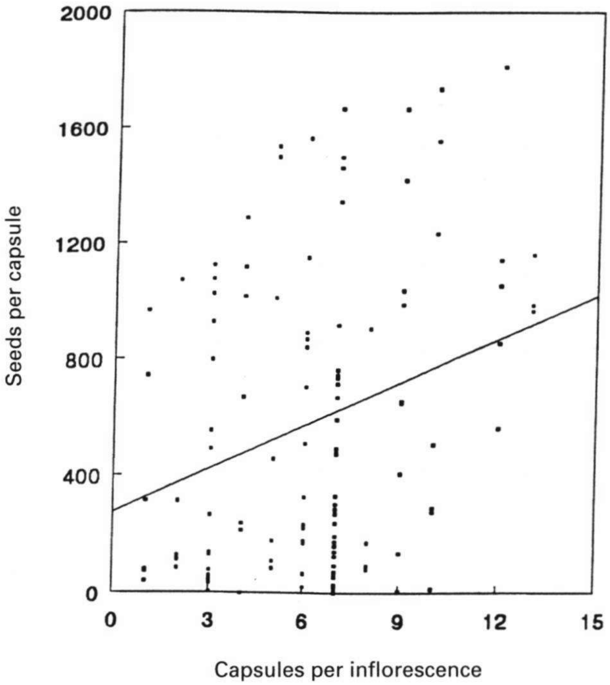

Fig. 3. The correlation between the number of capsules per flowering stalk and the seed number per capsule in *S. spiralis*.

numbers of flowers have been shown to be differentially successful as pollen recipients and donors (Firmage & Cole 1988; Rodríguez-Robles *et al.* 1992). Reproductive success has been observed to increase with increasing size of the inflorescence on orchids (Calvo 1990), as well as in other plant families, e.g. Solanaceae (Lawrence 1993). In the present study, however, larger flower numbers did not result in an increase in the percentage of fruit set, but were positively related to the number of fruits produced. That might be due to the sequential flowering in *S. spiralis*, which increased the probability of a pollinator visit to the inflorescence over the total blooming period. In *S. spiralis* large inflorescences do not provide a greater pollinator attraction than small ones, because all inflorescences only present a few open flowers (3–4) simultaneously, which means that these inflorescences show an equal floral display to possible pollinators.

The number of pollinator visits to *S. spiralis* also depended on the density of flowering stalks within the microhabitat. In dense floral patches bumblebees have been observed to visit more plants than in sparse patches (Rathcke 1983; Real 1983 and references therein). In dense patches individual plants may be able to attract more pollinators than each could independently, but in such patches individuals also compete and the number of flowers per plant visited after arrival of the pollinator may be low (Klinkhamer & De Jong 1990). In our study the higher fruit set in clumped inflorescences of *S. spiralis* indicate that clumping of inflorescences is advantageous in this species for seed production.

Other flowering species may increase the attractiveness of the site for pollinators. This might enhance the chance of pollination of the orchid flowers. However, flowering

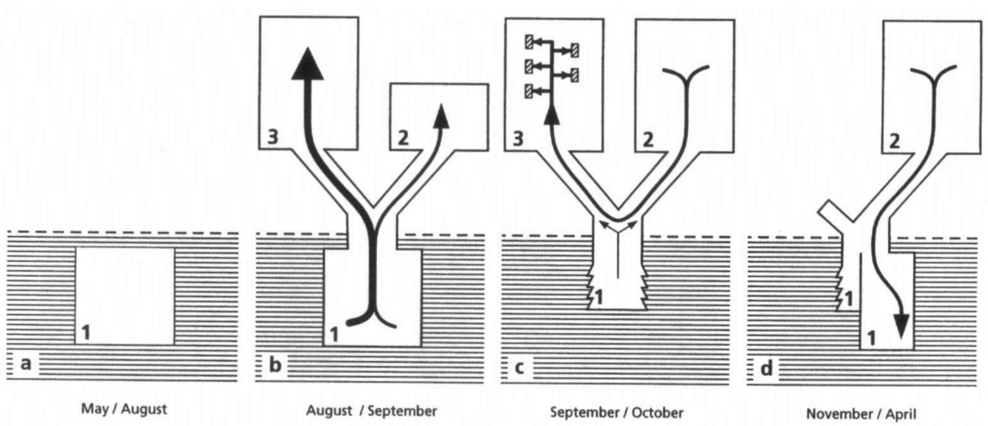

Fig. 4. Hypothetical generalized allocation pattern in *S. spiralis* illustrating the absence of resource limitation in seed production. Direction of the arrows indicate flow of photosynthates (and water), whereas the thickness of the lines roughly indicates the proportion. Plants are absent above ground during the period May–August. At the end of August or beginning of September an inflorescence may appear aboveground, preceding the rosette development. The rosette is fully developed at the time of fruit set and may allocate photosynthates to the seed formation depending on its demand. After seed shed in October photosynthates are allocated to new tuber formation. Remains of the shrivelled old tuber are present for still a long time (D). 1=belowground tuber, 2=aboveground rosette and 3=inflorescence (with capsules).

plants of different species may also compete for pollinator visits. Such competition occurs when plants of one species suffer reduction in fruit set as a result of sharing pollinators with other species (Campbell 1985). Competition for pollinators if those are infrequent is common for several orchid species (Dressler 1968; Waser 1983; Nilsson 1992). In *S. spiralis* the capsule production, and hence seed production, was lowered by the nearby presence of other flowering species, indicating that different species were competing for pollinators at Berghofweide during that time. The bumblebees *Bombus lapidarius* and *B. pasquorum* were also frequent visitors of the other flowering plant species surrounding *S. spiralis.*

Effects of resource limitation or provision limitation (Lawrence 1993) may be expressed either by declined fruit production or decreased number of seeds per fruit or by both (Zimmerman & Aide 1989; Ackerman & Montalvo 1990; Calvo 1990; 1993; Nilsson 1992). Due to the shape of the inflorescence and the flowering pattern of *S. spiralis* in combination with the pollinator's activity, following the subsequently open flowers, a lower fruit production and/or lower seed number per capsule could be a result of resource limitation in this species. However, the present study shows a significant positive correlation between fruit number per inflorescence and the seed number per fruit. This indicates that investment in sexual reproduction in this *S. spiralis* is not within-year restricted by resource limitation.

An explanation for this may be the phenology of the species, which has been described in detail by Wells (1981: 284). At the time of flowering and fruit set one or more underground tubers, formed as storage organs for carbohydrates in the previous growing season, are still present. Also, new rosette leaves appear aboveground shortly after emergence of the flowering stalk and these leaves are fully developed during the time of fruit and seed production. We hypothesize that the majority of the assimilates resulting from the young leaves are translocated to the reproductive organs after successful flower pollination. This may explain the absence of resource limitation in the sexual reproduction in *S. spiralis*. After seed release, the wintergreen rosettes stay aboveground for another 6–7-month period, during which a shift in allocation of carbohydrates in the newly formed tubers can take place as a result of photosynthesis. It can be concluded that photosynthesis during the period September to May is of crucial importance for the survival of *S. spiralis* populations (Fig. 4).

Conservation management introduced in 1981 and aiming to create a short sward during the period November to May, to avoid overtopping of the tiny rosettes by the surrounding vegetation, has resulted in a dramatic increase of the number of plants at Berghofweide over the last 10 years, as resulted from the yearly sensus in a permanent plot: from 25 plants (38 rosettes) in 1984 to 44 plants (56 rosettes) in 1993 (J. H. Willems, unpublished data). The number of plants of the total population at this site is approximately 10 times the number present in the permanent plot. There was no positive effect of the newly introduced management on the population size during the period 1981–88 (Willems 1989), since the time between seed germination and first emergence aboveground as an autotrophic plant last probably *c.* 10–15 years in *S. spiralis* (Wells 1981).

For the survival of one of the two remaining populations of *S. spiralis* in The Netherlands, situated at the border of its distribution area, it is important to know that this long-lived species is not limited severely by natural pollination, as demonstrated to be the main reason for low reproductive success of another rare plant species in The Netherlands, *Gentiana cruciata* L. (Petanidou *et al.* 1995). Due to the isolated position the sustainable survival of the *S. spiralis* population depends totally on local seed production. By knowing the factors affecting the seed production, e.g. pollination by indigenous insects and surrounding vegetation, population fitness can be enhanced by appropriate management of the site. At present *S. spiralis* produces enough seeds to increase population size. The present population increase indicates that the actual management regime also creates suitable germination conditions for seeds produced at the site, and that this population is not threatened with extinction in the immediate future.

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