ON THE RELATION BETWEEN VEGETATIVE AND GENERATIVE REPRODUCTION IN *SPIRANTHES SPIRALIS*

J.H. WILLEMS
Department of Plant Sciences, Section of Plant Ecology, Utrecht University

Abstract

This paper deals with the phenomenon of decreasing vegetative reproduction in the perennial orchid *Spiranthes spiralis* (L.) Chevall. at Berghofweide Nature Reserve in the southernmost part of The Netherlands (province of South-Limburg). The observation of the significant decline in average rosette number per plant ("genet") was a result of the research on two sub-populations during a period of 12 and 20 years, respectively. The results have been based on the census of individual plants several times in the growing season.
The number of rosettes per plant is positively correlated to the number of flowering stalks per plant. Clustered inflorescences have a significant higher pollination rate of the flowers compared to those in isolated ones, even if the flowers from surrounding herb species are removed artificially. Generative reproduction, and hence the population fitness, is evidently related to the vegetative multiplication in *Spiranthes spiralis* at the Berghofweide N.R. The number of rosettes per plant may be considered an indirect parameter for population fitness. The site management remained the same during this study: yearly mowing in July and aftermath sheep grazing after seed release in October. No evidence of senescence of plants in both sub-populations has been found. Therefore, no explanation for the decline in rosette number per plant can be offered so far. Continued monitoring of the population will probably contribute to a more profound insight in population processes of *Spiranthes spiralis*.

Samenvatting

In dit artikel wordt aandacht geschonken aan de relatie tussen vegetatieve en generatieve voortplanting bij *Spiranthes spiralis* in twee deelpopulaties in het Natuurreservaat Berghofweide in Zuid-Limburg. In beide deelpopulaties, waarvan individuele planten in permanente proefvakken zijn gevolgd gedurende respectievelijk 12 en 20 jaar, is een onmiskenbare afname te zien van het aantal rozetten per plant.
Het bestuivingsucces bij *Spiranthes spiralis* is groter als enkele bloeiwijzen dicht bij elkaar staan dan wanneer deze geïsoleerd zijn. Dit al dan niet geclusterd voorkomen van bloeiwijzen hangt samen met het aantal rozetten per plant. Hoe meer rozetten per plant aanwezig zijn hoe groter de kans op meer dan één bloeiwijze is. Op deze manier bepaalt de vegetatieve reproductie mede het succes van de zaadvorming en daarmee van het welzijn van de populatie van *Spiranthes spiralis*.
Het beheer op beide delen van de Berghofweide, eigendom van respectievelijk Staatsbosbeheer en Vereniging Natuurmonumenten, bestaat uit maaien in juli en schapenbegrazing na

de verspreiding van de zaden in de herfst. Dit beheer is sedert het begin van deze studie hetzelfde gebleven en kan derhalve niet de oorzaak zijn van de achteruitgang van het aantal rozetten per plant. Ook het eventueel verouderen van individuele planten, met als gevolg een geringere vegetatieve reproductie, is niet aan de orde omdat hiervoor elke aanwijzing ontbreekt. Zo is er bij individuele planten die al 15 jaar bovengronds aanwezig zijn, geen lagere bloeifrequentie of minder bloemen per bloeiwijze gevonden, twee criteria voor veroudering.
Tot nu toe ontbreekt elke aanwijzing voor de oorzaak van de afname van het aantal rozetten per plant in beide deelpopulaties van *Spiranthes spiralis* van de Berghofweide. Wellicht dat voortzetting van het jaarlijks monitoren uiteindelijk meer inzicht zal verschaffen in de populatieprocessen van deze alleszins bedreigde plantensoort en daarmee wellicht ook in de eventuele oorzaak van het hier geschetste verschijnsel.

Zusammenfassung

In diesem Artikel wird das Verhältnis zwischen vegetativer und generativer Reproduktion bei *Spiranthes spiralis* in zwei Teilpopulationen im Naturreservat Berghofweide in der Provinz Zuid-Limburg behandelt. In beiden Teilpopulationen, in denen die individuellen Pflanzen in Dauerquadraten seit 12 bzw. 20 Jahren studiert wurden, zeigt sich eine deutliche Abnahme der Zahl der Rosetten pro Pflanze.
Der Bestäubungserfolg bei *Spiranthes spiralis* ist größer wenn einige Blütenstände nebeneinander stehen als wenn sie isoliert vorkommen. Ob die Blütenstände gruppenweise vorkommen oder nicht, hängt mit der Zahl der Rosetten pro Pflanze zusammen. Je mehr Rosetten pro Pflanze es gibt, um so größer wird die Chance dass es mehrere Blütenstände gibt. In dieser Weise bestimmt die vegetative Reproduktion auch den Erfolg der Samenbildung und damit das Wohl der Population von *Spiranthes spiralis*.
Die Pflege beider Teile der Berghofweide, im Besitz von 'Staasbosbeheer' bzw. 'Vereniging Natuurmonumenten', besteht aus Mahd im Juli und Schafbeweidung nach der Samenverbreitung im Herbst. Diese Pflege hat sich seit Anfang der Forschungsarbeit nicht geändert und kann deshalb den Rückgang der Rosettenzahl pro Pflanze nicht verursacht haben. Auch eine etwaige Alterung der Pflanzen mit als Folge eine geringere vegetative Reproduktion kommt nicht in Frage, weil dafür jegliche Indiz fehlt. So gibt es bei individuellen Pflanzen, die schon 15 Jahre überirdisch existieren, keine niedrigere Blühfrequenz oder weniger Blüten pro Blütenstand - zwei Kriterien für Alterung.
Bis jetzt gibt es keine Erklärung für die Abnahme der Rosettenzahl pro Pflanze in beiden Teilpopulationen von *Spiranthes spiralis* auf der Berghofweide. Vielleicht kann die Fortsetzung der jährlichen Forschungsarbeit eine bessere Einsicht in die Populationsprozesse dieser in allen Hinsichten bedrohten Pflanzenart vermitteln und damit vielleicht auch in die Ursache des hier beschriebenen Phänomens.

Introduction

Within the relatively few number of indigenous orchids in The Netherlands, viz. about 30 species, a wide variety of life-history strategies can be distinguished. There are a number of long-lived (e.g. *Aceras anthropophorum*) and short-lived species (e.g. *Ophrys apifera*), species with a substantial vegetative reproduction (e.g. *Goodyera repens*) and species almost completely depending on generative reproduction (e.g. *Coeloglossum viride*), whereas the duration and period of above-ground presence may be short, up to 3 months a year (e.g. *Listera ovata*), or more than nine months (*Spiranthes spiralis*) (Wells 1981, Willems and Melser 1998, Kreutz and Dekker 2000, Willems and Dorland 2000). Therefore, generalizations with respect to orchid population biology and ecology are difficult to make. The variety of life-history characteristics can even been encountered in a small country without a large variety in geomorphology, like The Netherlands where the highest altitude is only about 300 m above sea level. However, life-history strategies can not always be distinguished very clearly and are sometimes related to each other.
In this paper attention will be given to the interaction between the two possible reproduction strategies in plants, viz. the generative and vegetative multiplication. The results are based on long-term demographic studies of two sub-populations of the long-lived *Spiranthes spiralis* (L.) Chevall.

Material and Methods

a. The species

Spiranthes spiralis is a long-lived orchid species with a wintergreen rosette and flowering in late summer to early autumn (August – September). It is a Sub-Mediterranean species with a number of border populations in Western Europe. The species consists of one or more underground tubers which are renewed yearly. Above-ground plants have one or more tiny rosettes with up to six leaves per rosette. If a plant consists of more than one rosette this clearly is the result of vegetative multiplication and the rosettes can be considered genetically identical ("genets") (Willems 1989).
The flowering stalks, up to 25 cm height, appear above ground some time before the green leaves. The small white-greenish flowers award the pollinators, mainly bumble bees and solitary bees, with nectar. Seed release mainly takes place in October. The number of seeds per fruit is rather high in *Spiranthes spiralis*, viz. up to 850 seeds per capsule (Wells 1981, Willems and Melser 1998, Willems and Dorland 2000).
Spiranthes spiralis is both a rare and endangered species in The Netherlands, of which the number of 5 x 5 km grid squares decreased from 35 to 3 in the 20th century (Mennema *et al.* 1981). The decrease in South-Limburg was even more spectacular over the period during which the number of 1 x 1 km grid squares declined from 16 before 1981 towards only one remaining population at present (Kreutz 1994). This population is the subject of this study. In total there are only two viable populations left in The Netherlands, one in the chalk district of South-Limburg and one in the dune area in the south-western part of the country, on the island of Goeree (Kreutz and Dekker 2000).

b. The study site

The present research is carried out in the Berghofweide Nature Reserve in South-Limburg, The Netherlands. The site is a south-facing slope of 4 ha covered by a species-rich grassland. The semi-natural grassland consists of a mixture of both calcareous and acid soil conditions. The slope is partly owned by the National Forestry Commission (Staatsbosbeheer), whereas the other part is the property of the Dutch Society for Preservation of Nature (Vereniging Natuurmonumenten). Site management consists of yearly mowing in the second half of July and aftermath grazing by sheep after seed release of *Spiranthes spiralis*, in November – December. For more detailed site information see Willems (1989) and Willems and Dorland (2000).

c. Methods applied

The population has been monitored since 1981 on the part of Vereniging Natuurmonumenten and from 1989 onwards on the Staatsbosbeheer section in permanently marked plots of 8 x 8 m and 3 x 5 m size, respectively. In these plots all individual plants have a set of unique coordinates from fixed underground markers, which enables to return to the same individual plant in successive years (Wells 1967, Willems 1982).

From each plant the number of rosettes and leaves, phenological status, height of the flowering stalk and the number of flowers has been measured. When the distance between the midst of two rosettes was more than 5 cm, they have been considered to belong to two different plants (Willems 1989).

Results

In 1981 the average number of rosettes per plant in the sub-population at the Vereniging Natuurmonumenten section was 1.83, whereas the largest number of rosettes per plant equalled 2. During the following 20 years the number of rosettes declined gradually, however statistically significantly to 1.34 per plant (Fig. 1a). The number of rosettes per plant never exceeded the number of 6 during the period of observation during which a total 170 different plants encountered within the plot. Only one plant (No. 4) consisted of 6 rosettes during three consecutive years (1983-1985). Three years later this specimen was dead, following 2 years of only 2 rosettes present.

The same general trend has been observed at the Staatsbosbeheer part of the site where monitoring started in 1989 because of the increasing number of plants as a positive reaction on the application of the above-mentioned management scheme beginning in 1986 (Fig. 1b). During the initial years of monitoring the average number of rosettes increased from 1.86 in 1989 to 2.21 two years later. The highest number of rosettes per plant was 4 during that period.

In the year 2000 the average number of rosettes per plant was declined towards 1.15 (Fig. 1b), whereas the highest rosette number per plant was only 2. The number of plants ever recorded in the plot from 1989 to 2000 was 98 in total.

In conclusion: the number of rosettes per plant decreased significantly in two sub-populations of *Spiranthes spiralis* at the nature reserve Berghofweide during a period in which the management of the site remained the same.

Discussion

In spite of the wide-spread attention for native orchids in western Europe by both amateur and professional botanists, very general mechanisms responsible for population structure or flowering frequency are often unknown. Therefore, it is outstanding that similar data on life history and demography of *Spiranthes spiralis* as mentioned for The Netherlands are also available from another part of the distribution area, viz. England (Wells 1967). Wells arranged in a population at Knocking Hoe (Bedfordshire) groups of more than two rosettes per plant in classes during three consecutive years, viz. 1963-1965. During this period the average number of rosettes per plant was 1.19, 1.27 and 1.29 respectively. These numbers are lower than the values recorded at Berghofweide. The average rosette numbers only of the last few years of observation are similar to the Knocking Hoe values. The highest number of rosettes per plant ever found was 7 in the Knocking Hoe population. This number was only recorded twice, whereas the total number of plants exceeded 300 yearly (Wells 1967), indicating the high degree of rarity of plants with such a high number of rosettes per plant.
With this respect it is interesting that the number of rosettes per plant was very high in a population of *Spiranthes spiralis* in the central part of Italy, viz. Morro near Camarino (province Marche), where plants of *Spiranthes spiralis* were found with up to 14 rosettes (personal observation, 1998). Whether these high numbers in Morro are related to the central position in the species' distribution area, is an interesting, but still unanswered question.
It is a well-known feature in orchid populations that the number of flowering plants may change dramatically from year to year (Sterk 1976, Wells 1981, Willems and Bik 1991). However, also the number of rosettes per plant may differ yearly in *Spiranthes spiralis* and this also holds for the number of flowering stalks per plant. A higher rosette number is positively related with the number of inflorescences per plant. It has been experimentally proven that clustered inflorescences are more attractive for insect pollinators compared to isolated flowering stalks and thus resulting in a higher fruit set and hence a higher seed production. This effect was observed when interspecific competition was either present or artificially excluded by removal of the flowers of accompanying herb species (Willems and Lahtinen 1997). This implies that the vegetative reproduction in *Spiranthes spiralis* has an impact on the generative reproduction capacity. Therefore, the number of rosettes per plant can be considered an indirect parameter for population fitness. A decreasing average rosette number per plant as monitored at Berghofweide, causes concern about the fate of this population of *Spiranthes spiralis*. The reason for the declining number of rosettes and hence the decrease in population fitness of both sub-populations is still obscure. The main external factor, the site management, did not change during the period of this research. The decreasing number of rosettes per plant can be seen as an indication that the population of *Spiranthes spiralis* can be considered as endangered in their future existence in the Berghofweide N.R.

Acknowledgements

I am pleased to acknowledge the help of Luciana Caretenuto (Camerino, Italy) both in the field and in working up the data and of members of the Natuurwacht Zuid-Oost Limburg, especially Wim Bult and Ed van Campen, for technical and logistic support during the field-work. I am grateful to the Dutch Society for Preservation of Nature (Vereniging Natuur-monumenten) at 's-Graveland and the National Forestry Commission (Staatsbosbeheer) at Roermond for permission to carry out long-term research in the Berghofweide Nature Reserve.

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J.H. Willems
Department of Plant Sciences, Section of Plant Ecology, Utrecht University
Sorbonnelaan 16
NL-3508 TB Utrecht

Figure 1.
The average number of rosettes per plant in *Spiranthes spiralis* in the course of time at the Berghofweide N.R., The Netherlands.

Figure 1a shows the number of rosettes in the part of the reserve owned by the Dutch Society for Preservation of Nature (NM) during the period 1981-2000.

Figure 1b shows the same trend in decreasing number of rosettes per plant in the sub-population at the section of the site owned by the National Forestry Commission (SBB) during the period 1989-2000.

Fig. 1a

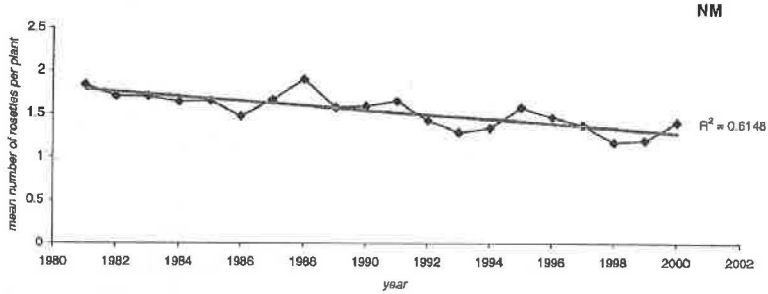
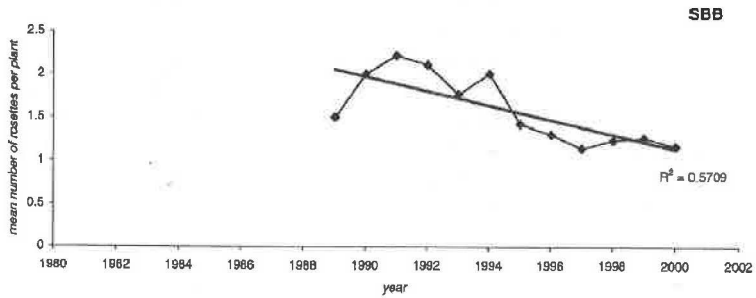


Fig. 1b



VERKLARING VAN ENIGE TERMEN

In het voorafgaande artikel van J.H. Willems en in het hierna volgende van M. Light komt de term "genet" voor. Ook de definitie van het begrip "plant" verdient nadere toelichting.
De heer Willems schreef op verzoek van de redactie naar aanleiding van zijn artikel over *Spiranthes spiralis*:

"Wat de kwestie "plant" en "rozet" betreft: een plant kan uit een rozet of verscheidene rozetten bestaan, dat kan van jaar tot jaar wisselen of ook hetzelfde blijven. Stel je hebt een plant bestaande uit een enkel rozet en op diezelfde plaats staan het volgend jaar twee rozetten. Deze zijn door vegetatieve vermenigvuldiging ontstaan en derhalve genetisch identiek. Ik beschouw een dergelijke verzameling van rozetten als één plant. In het Engels wordt daarvoor de term "genet" gebruikt, en de individuele rozetten heten "ramets". Als je een plant van *Goodyera repens* hebt met uitlopers met (bloeiende) rozetten, dan is dat toch ook één enkele plant. Hier zie je duidelijk de verbinding met elkaar. Dit laatste is wat moeilijk bij *Spiranthes*, maar de verbindingen zijn er ondergronds wel degelijk. We hebben dit op kleine schaal in Italië ondergronds nagegaan, op een plaats waar duizenden planten stonden. Ondergrondse tubers of knollen kunnen met de rozetten in alle mogelijke combinaties voorkomen: 1 rozet-1 knol, 1 rozet-2 knollen, 1 rozet-3 knollen, 2 rozetten-1 knol, enz. enz. Als je elk rozet als plant beschouwt kom je in de problemen met de interpretatie van de afwezigheid ervan gedurende een of meer jaren. Stel in jaar x heeft een plant 3 rozetten, in jaar x+1 slechts een enkel rozet en in jaar x+2 weer drie rozetten, dan kun je niet zeggen dat twee planten dormant, ondergronds aanwezig waren. Zo is Terry Wells aan dormantie bij *Spiranthes* gekomen in zijn artikel van 1967, terwijl dit geen echte dormantie is, zoals bijv. bij *Orchis simia* die gedurende een of twee jaar bovengronds als groene plant afwezig kan zijn. Omdat *Spiranthes* elk jaar zijn ondergrondse knollen geheel vernieuwt, kan zo'n plant niet dormant zijn, hij is er in een gegeven jaar of anders is hij dood, bv. door beschaduwing. Je moet een plant van *Spiranthes* zien als een individu met de mogelijkheid van jaarlijks wisselende aantallen spruiten, de rozetten. Wat voor factor die wisseling bepaalt, willen we graag te weten zien te komen. Maar het zal nog wel wat voeten in de aarde hebben voordat we dit weten.
Tenslotte: De termen "genets" en "ramets" zijn o.a. uitvoerig beschreven in het klassieke boek: POPULATION BIOLOGY OF PLANTS, door J.L. Harper, 1977. Academic Press, London. 892 pp. Een aanrader om te raadplegen!!"