Katholieke Universiteit Leuven
Faculteit Bio-ingenieurswetenschappen
Departement Aard- en Omgevingswetenschappen
Afdeling Bos, Natuur en Landschap

DISSERTATIONES DE AGRICULTURA

Gecombineerd doctoraatsproefschrift nr. 859 aan de faculteit Bio-ingenieurswetenschappen van de K.U.Leuven met Universiteit Gent

# Distribution, demography and metapopulation dynamics of *Cuscuta epithymum* in managed heathlands

Proefschrift voorgedragen tot het
Behalen van de graad van
Doctor in de
Bio-ingenieurswetenschappen

door

**Klaar MEULEBROUCK**

**APRIL 2009**

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Ik draag veel verhalen in me.

Ieder mens draagt veel verhalen in zich,

Als zaden die langzaam ontkiemen en

Op een dag zullen bloeien.

Het juiste moment is aangekomen om de geschiedenis van

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Enkele sfeerbeelden:

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# Samenvatting

Droge heide gedomineerd door struikhei was gedurende lange periode één van de meest kenmerkende en wijdverspreide halfnatuurlijke landschappen in Noordwest Europa. De grote meerderheid van deze vegetaties zijn ontstaan door degradatie van bossen naar een actief bewerkt agrarisch landschap. De combinatie van menselijk activiteit en een natuurlijke successie van de vegetatie naar bos creëerde een uniek dynamisch landschap met diverse gemeenschappen van dieren en planten kenmerkend voor verschillende successiestadia van heide. In de loop van de 20ste eeuw verdween in Europa een groot aandeel van het heide-areaal door een doorgedreven intensivering van landbouw, bebossing en verstedelijking. De resterende heidegebieden zijn sterk versnipperd. Momenteel wordt heide beschouwd als een internationaal bedreigd biotoop met een hoge beschermingswaarde. Ondanks deze bescherming bevinden een groot deel van de heidegebieden zich in een slechte toestand. Gezien hun halfnatuurlijk karakter hangen heiden volledig af van menselijk ingrijpen. Het ontbreken van een geschikt beheer vormt vandaag de dag dan ook een van de grootste bedreigingen voor het voortbestaan van heiden en van de soorten gebonden aan de specifieke heidecondities. Een van deze typerende heidesoorten is *Cuscuta epithymum* of klein warkruid, ooit een algemene plant die sterk achteruitgaat in heel West-Europa en momenteel op de Rode Lijst van Vlaanderen (België) en andere landen voorkomt. Deze holoparasiet wordt vooral teruggevonden op beheerde heidevlekken, welke zich in een vroeg stadium van de heidesuccessie bevinden. Klein warkruid onttrekt water en voedingsstoffen aan zijn gastheer nodig zijn voor het voltooien van zijn levenscyclus.

Dit doctoraatsonderzoek wil onderzoeken welke factoren de verspreiding van *C. epithymum* beïnvloeden en de habitatvereisten en verschillende demografische eigenschappen van klein warkruid ontrafelen. Verder heeft dit onderzoek tot doel een dieper inzicht te verkrijgen in de overlevingskansen van *C. epithymum*-metapopulaties onder verschillende beheersregimes, om uiteindelijk duurzame instandhouding- en gerichte beheermaatregelen aan te reiken die de verdere achteruitgang van deze interessante soort moeten verhinderen.

Op landschapsschaal wordt het voorkomen en de populatiedynamiek van klein warkruid zowel in tijd als ruimte voornamelijk gestuurd door de vegetatiestructuur en -dynamiek, die op zijn beurt sterk beïnvloed wordt door beheersmaatregelen. Zowel de grootte als de isolatiegraad van heidevlekken lijken daarentegen geen merkbare invloed te hebben op het verspreidingspatroon van de soort. De kolonisatie van groeiplaatsen hangt in sterke mate af van de regelmaat waarop het beheer plaatsvindt, maar wordt waarschijnlijk vooral beïnvloed door de aan- of afwezigheid van een persistente zaadbank. Op locale schaal presteert klein warkruid het best op heidevlekken van het jonge successiestadium (niet ouder dan 7 jaar na beheer). Deze heidevlekken worden gekenmerkt door een sterke aanwezigheid

van jonge, 10 à 30cm hoge struikhei (*Calluna vulgaris*), de belangrijkste gastheer van klein warkruid. De omvang van populaties neemt af met het verouderen van de vegetatie, om uiteindelijk locaal te verdwijnen 10 jaar nadat de laatste beheermaatregel plaatsvond. Verder blijkt dat de populatiedynamiek van klein warkruid niet enkel gedreven wordt door gerichte omgevingsveranderingen, maar eveneens door onvoorspelbare gebeurtenissen, zoals uitzonderlijke weerscondities.

Onderzoek van de kiemingsecologie van *C. epithymum* leert ons dat de zaden van klein warkruid een gecombineerde dormantie vertonen, waarbij zowel de ondoorlaatbare zaadhuid (i.e. fysische dormantie) als het embryo (i.e. fysiologische dormantie) het kiemingsmoment bepalen. Deze eigenschap beschouwen we als een dubbel veiligheidsmechanisme dat de kieming op ongunstige perioden belemmert. Verder vertonen ingegraven zaden een seizoenale cyclus van fysiologische dormantie, waardoor hun kiemingsactiviteit verandert doorheen het jaar. Deze dormantiecyclus zorgt dat de kieming nauwkeurig is afgesteld op seizoenale temperatuursveranderingen. Een periode van koude wintertemperaturen (< 5°C) haalt het embryo van klein warkruid uit een dormante toestand. Vanaf het moment dat de lentetemperatuur voldoende stijgt, kiemen de zaden en verschijnen de kiemplanten. Later in de lente lokken hogere temperaturen (> 10°C) een dormante toestand van het embryo uit, waardoor de kieming terug verhinderd wordt. Uit ons onderzoek blijkt verder dat niet-dormante zaden van *C. epithymum* geen licht of fluctuerende temperaturen vereisen voor hun kieming. Elk jaar kiemt slechts een gedeelte van de kiemkrachtige zaden, terwijl de overige zaden dormant in de zaadbank blijven. De zaden van klein warkruid kunnen meerdere jaren overleven in de bodem en vormen dus een persistente zaadbanken, minstens korte termijn. Toch neemt de kiemkracht van zaden in de bodem opvallend snel af met de tijd. Slechts 11% van de zaden overleefde een periode van 31 maanden.

*Cuscuta*-soorten worden doorgaans als eenjarig beschreven. In tegenstelling hiermee blijkt klein warkruid in staat de winter vegetatief te overbruggen aan de hand van haustoria en dit vooral op hun meest algemene meerderjarige gastheer, struikhei. De haustoria overleven de winter in het weefsel van de gastheer, terwijl het overige weefsel van klein warkruid afgesterft tijdens de herfst. Hoewel het aantal overwinterende haustoria sterk varieerde van populatie tot populatie, was gemiddeld 85% van de planten uit een *C. epithymum*-populatie afkomstig van uitgelopen overwinterde haustoria. Het pseudo-eenjarige karakter van klein warkruid is dus een belangrijke, maar vaak over het hoofd geziene levensstrategie van deze soort. Vooral de leeftijd van struikheiplanten bepaalt de slaagkans op vegetatieve overwintering en beïnvloedt ook de jaarlijkse populatiegroei. De meeste haustoria die de winter overleefden werden teruggevonden op 1 à 2 jaar oude takken van struikhei, gevolgd door een graduele afname in verdere successiestadia van de vegetatie.

Ook de vestiging van klein warkruid, gaande van het moment dat de zaden op de bodem vallen tot de succesvolle vestiging en ontwikkeling van volwassen planten, werd grondig bestudeerd. Uit dit onderzoek blijkt dat vooral de leeftijd van de gastheer *C. vulgaris* een hinderende factor is voor een succesvolle vestiging. Over het algemeen was de vestiging van de 6600 geïntroduceerde *C. epithymum*-zaden na twee groeiseizoenen laag. Slechts 5% van alle zaden verscheen als kiemplant en uiteindelijk kwamen slechts 89 planten (< 2%) tot bloei. Kiemplanten verschenen onder alle condities, zowel in gemaaide en geplagde, jonge heide als in onbeheerde volgroeide heide. Tijdens de groei van *C. vulgaris*-stengels wordt in de buitenste weefsellaag een mechanische barrière ontwikkeld die de infectie van *C. epithymum* belemmert. Vestiging was dan ook het meest succesvol in geplagde pionier-heide, gekenmerkt door een relatief open en lage vegetatie gedomineerd door jonge *C. vulgaris* planten. Deze resultaten benadrukken het belang van een goede gastheer- en habitatkwaliteit voor een geslaagde vestiging. Beide worden rechtstreeks beïnvloed door het gevoerde beheer van de vegetatie. Het volledig ontbreken van heidebeheer is dus nefast voor een geslaagde vestiging van deze bedreigde soort.

Om de overlevingskans van *C. epithymum*-metapopulaties in dynamische heidegebieden op lange termijn in te schatten, maakten we gebruik van een realistisch metapopulatiemodel geparameteriseerd met observationele en experimentele data. Er kwam duidelijk naar voor dat zowel occasionele, langeafstandsverbreiding van zaden als persistente zaden kritische eigenschappen zijn voor een duurzame overleving van *C. epithymum* in heidegebieden. Als de verbreiding van zaden sterk gelimiteerd is en/of de soort er niet in slaagt om genoeg zaden te produceren - wat een zeer realistisch scenario is voor *C. epithymum* in Vlaamse heidegebieden - dan is een relatief hoge beheersfrequentie vereist, met minder dan 15 jaren tussen twee opeenvolgende beheersmaatregelen, om het voortbestaan van *C. epithymum* metapopulaties te verzekeren. Extensieve begrazing kan de negatieve effecten van heidesuccessie deels compenseren.

Kort samengevat toont ons onderzoek aan dat *C. epithymum* sterk is aangepast aan een leven in een steeds veranderend heidelandschap, maar toch uiterst gevoelig is voor locale extinctie. Gezien de bijzondere habitatvereisten van klein warkruid, is een aangepast beheer noodzakelijk voor een duurzaam behoud van deze soort. Wij adviseren een kleinschalig cyclisch beheer van verschillende beheersvormen, zoals maaien en plaggen, eventueel gecombineerd met extensieve begrazing om steeds voldoende vroege successiestadia te verzekeren. Het gecreëerde mozaïek aan heidevlekken van verschillende ouderdom zal niet enkel *C. epithymum* vooruit helpen, maar zal ook een geschikte leefomgeving creëren voor een groot aantal andere kwetsbare heidesoorten. Dit onderzoek illustreert duidelijk hoe soort-specieke kennis aangewend kan worden om beheer- en inrichtingsplannen van heidegebieden op te stellen respectievelijk te verbeteren met als doel de kwaliteit en/of kwantiteit van droge heide te verhogen.

# Summary

*Calluna*-dominated dry heathlands were one of the most distinctive and widespread semi-natural landscapes of north-western Europe. They were derived from woodland by human agricultural activity, particularly by grazing and vegetation harvesting. The combination of the agricultural activity and natural vegetation succession created a unique dynamic system favourable for both early and later successional communities of plants and animals. During the 20th century, a large proportion of heathland was lost across Europe due to intensive agriculture, reforestation and urban development. Heathlands are now internationally endangered habitats of high conservation value. Heathlands entirely depend on anthropogenic intervention to prevent loss through natural succession and therefore, the lack of appropriate management is one of the main threats to the remaining heathlands.

One of the characteristic, dry heathland species is *Cuscuta epithymum* (Common dodder), once a common plant which has declined severely throughout western Europe and is currently red listed in Flanders (Belgium) and in other countries. This holoparasitic plant is mainly found on recently-managed heathland patches which are still in an early stage of succession. The parasite entirely depends on its host's resources to grow and complete its life cycle. This thesis aims to identify the factors determining *C. epithymum*'s distribution pattern, its habitat requirements and various demographic characteristics, and to assess the long-term survival probabilities of its metapopulations under various management regimes. It will provide guidelines for a more accurate conservation management which will prevent further decline of this interesting, dry heathland species.

At a landscape scale, the occurrence and population dynamics of *C. epithymum* both in time and space proved to be mainly controlled by the structure and dynamics of the vegetation, which in turn is heavily influenced by management events. Patch area and isolation had no noticeable influence on *C. epithymum* distribution patterns. Colonisation events primarily depended on the rate at which management events occurred and were believed to be strongly driven by the presence of a persistent seed bank. At a local scale, the plant performed best in early successional heathland patches in which *Calluna*-stems, *C. epithymum*'s main host, were not older than seven years and have a canopy height between 10 and 30cm. Population size decreased with ageing of the vegetation and local extinction occurred when heathland vegetation became too old, approximately 10 years after management. Next to these deterministic extinctions, the dynamics of *C. epithymum* are also influenced by unpredictable environmental stochasticity, such as aberrant weather conditions.

When exploring the germination ecology of *C. epithymum* more thoroughly, we found that its seeds have a combination of physical and physiological dormancy, where both the seed coat and embryo rule the timing of germination. This characteristic is interpreted as a

double safety mechanism preventing germination at unfavourable times. Furthermore, buried seeds exhibited a seasonal cycle of physiological dormancy, by which germination rates change during the year. This dormancy cycle fine tunes germination of *C. epithymum* to seasonal temperature changes. The nondormant state of the embryo was induced by a period of cold winter temperatures (< 5°C). Once spring temperatures rise sufficiently, *C. epithymum* seeds germinate and its seedlings emerge. Higher temperatures in late spring (> 10°C) provoked a dormant state of the embryos, preventing germination of the seeds. Non-dormant seeds did not require light nor fluctuating temperatures for germination. Each year only a proportion of the viable seeds germinated, while the remainder stayed dormant in the seed bank. Although *C. epithymum* seeds are demonstrated to be capable of surviving for several years in the soil and thus to form, at least short-term, persistent seed banks, seed viability decreased quickly in the field, with only 11% survival after 31 months of burial.

Although *Cuscuta* species were formerly often believed to be annual plants, *C. epithymum* overwintered vegetatively, especially on its most common perennial host, *C. vulgaris*. Haustoria remained alive within host plant tissue through the winter, although all other *C. epithymum* tissue dried and died during autumn. The number of overwintering haustoria varied between different populations, but on average 85% of each *C. epithymum* population was the result of resprouting haustoria. The pseudoannual growth is an important, but previously overlooked life-strategy. The age of host heather plants significantly determined the overwintering ability of *C. epithymum* and strongly influenced its annual growth. Most sprouted haustoria were found in 1- or 2-year old vegetation followed by a gradual decline at older successional phases.

The different establishment stages of *C. epithymum*, all steps from the time of seed rain on the soil surface to the adult plant, are predominantly limited by the age of its host *C. vulgaris*. In general, recruitment of 6600 introduced *C. epithymum* seeds was rather low. After two growing seasons, only 4.8% of the sown seeds germinated and just 89 flowering adults (< 2%) established. Seedling emerged under all conditions ranging from mown or turf-cut pioneer heath to unmanaged, dense mature heathland. Seedling attachment to the host, haustorial penetration, growth to full-grown size and vegetative overwintering were hampered in mature vegetation with older heather individuals. As *C. vulgaris* stems grow old, they develop a mechanical barrier in their outer tissue preventing *C. epithymum* infection. Establishment was most successful in turf-cut pioneer heathland, characterised by relatively open and low vegetation dominated by young *C. vulgaris* plants. These results emphasise the importance of both habitat and host quality for the recruitment of *C. epithymum*, which is directly affected by the management applied to the vegetation. Lack of appropriate heathland management will seriously restrict the establishment of this endangered parasite.

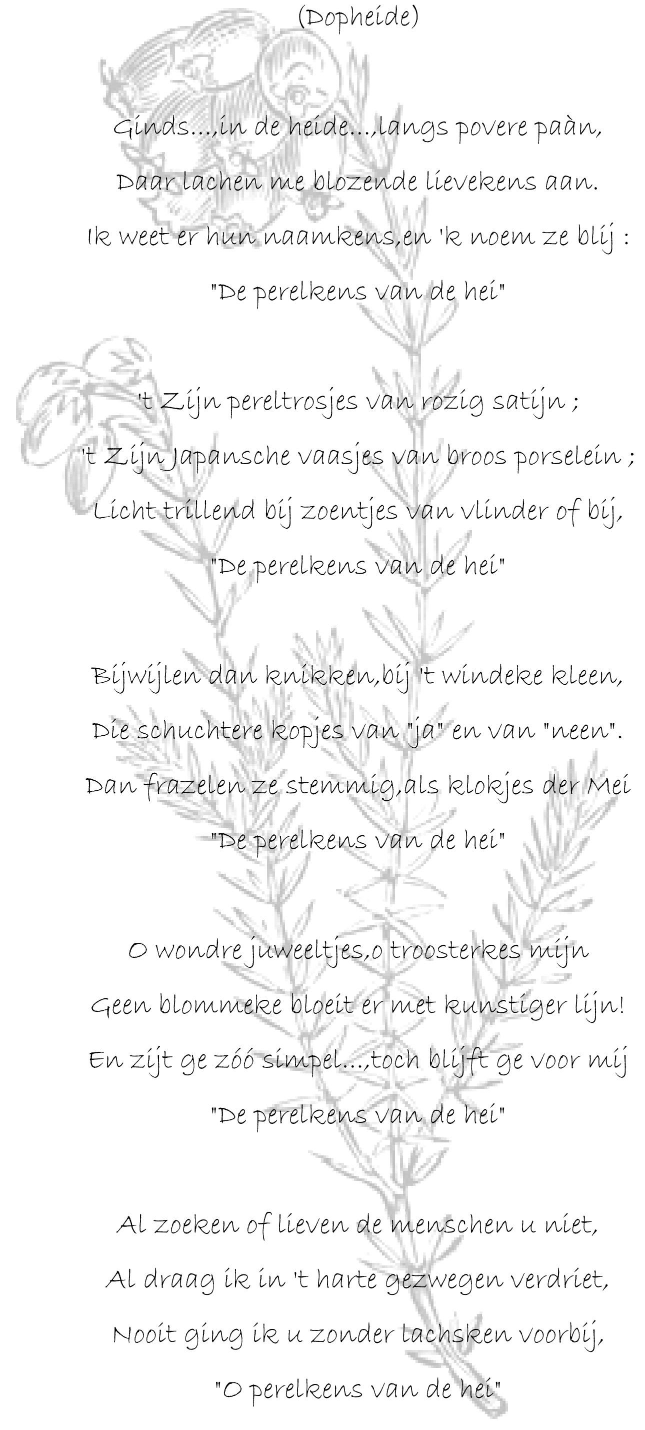
To understand the long-term survival of *C. epithymum* metapopulations in dynamic heathlands, a realistic metapopulation model was parameterised with observed and experimental data. Both occasional long-distance dispersal and persistent seeds were shown to be critical for the long-term survival of this species in heathlands. If seed dispersal rates are low, and/or the species fails to produce enough persistent seeds -a very realistic scenario for *C. epithymum* populations in Flemish heathland reserves- a relatively high management frequency with less than 15 years between two consecutive management events appears necessary to sustain viable *C. epithymum* metapopulations over a longer period. Grazing can, through creating a patchy environment with young, resprouting heather, however, partly reduce the negative effects of vegetation succession.

This thesis demonstrates that *C. epithymum* is strongly adapted to survive in dynamic heathlands, and is still highly sensitive to local extinction. Considering its highly specific habitat requirements, a particular management is required for the conservation of this species. A cylcic management of various management measures, such as mowing and turf-cutting, is recommended on a small scale to provide sufficient early successional vegetation. If possible, management should also include extensive grazing. The resultant mosaics of heathland patches of different successional phases will promote *C. epithymum*, and will also create a suitable habitat for a wide range of other heathland species. This study clearly illustrated how the incorporation of species-specific knowledge can be used to draw up and/or improve management and design plans for heathlands that aim to improve the quality and/or quantity of dry *Calluna* heath.

# 1

# Introduction

Photo: Klaar Meulebrouck (Ziepbeekvallei, 2008)



### *Erica tetralix*

(Dopheide)

Ginds…,in de heide…,langs povere paàn,
Daar lachen me blozende lievekens aan.
Ik weet er hun naamkens,en 'k noem ze blij :
"De perelkens van de hei"

't Zijn pereltrosjes van rozig satijn ;
't Zijn Japansche vaasjes van broos porselein ;
Licht trillend bij zoentjes van vlinder of bij,
"De perelkens van de hei"

Bijwijlen dan knikken,bij 't windeke kleen,
Die schuchtere kopjes van "ja" en van "neen".
Dan frazelen ze stemmig,als klokjes der Mei
"De perelkens van de hei"

O wondre juweeltjes,o troosterkes mijn
Geen blommeke bloeit er met kunstiger lijn!
En zijt ge zóó simpel…,toch blijft ge voor mij
"De perelkens van de hei"

Al zoeken of lieven de menschen u niet,
Al draag ik in 't harte gezwegen verdriet,
Nooit ging ik u zonder lachsken voorbij,
"O perelkens van de hei"

Alice Nahon from 'Vondelingsken, 1920'

# Problem setting

## *The decline of European heathlands*

In Europe many biotopes of high conservation value have been shaped by a long history of traditional management, such as calcareous grasslands, heathlands and woodlands (e.g. Gormsen, 1991; Tack et al., 1993; Webb, 1998; Burny, 1999; Odé et al., 2001; Piessens, 2006; Adriaens et al., 2008). Heathland is one of the widespread semi-natural landscapes on the poorer soils of the Atlantic region of western Europe (Allemeersch et al., 1988; Webb, 1998; Piessens et al., 2004; De Blust, 2007). The vegetation is characterised by the dominance of evergreen dwarf shrubs such as *Calluna vulgaris*, *Erica tetralix* and/or other ericoid shrubs, usually with a high diversity of plant and invertebrate species (Schaminée et al., 1996; De Blust, 2004; van Landuyt et al., 2006). Under natural conditions, heathland occurs spontaneously at high altitudes above the natural tree-line, on exposed Atlantic coasts where tree-growth is excluded, and in temporary gaps in woodland (Goodall and Specht, 1981).

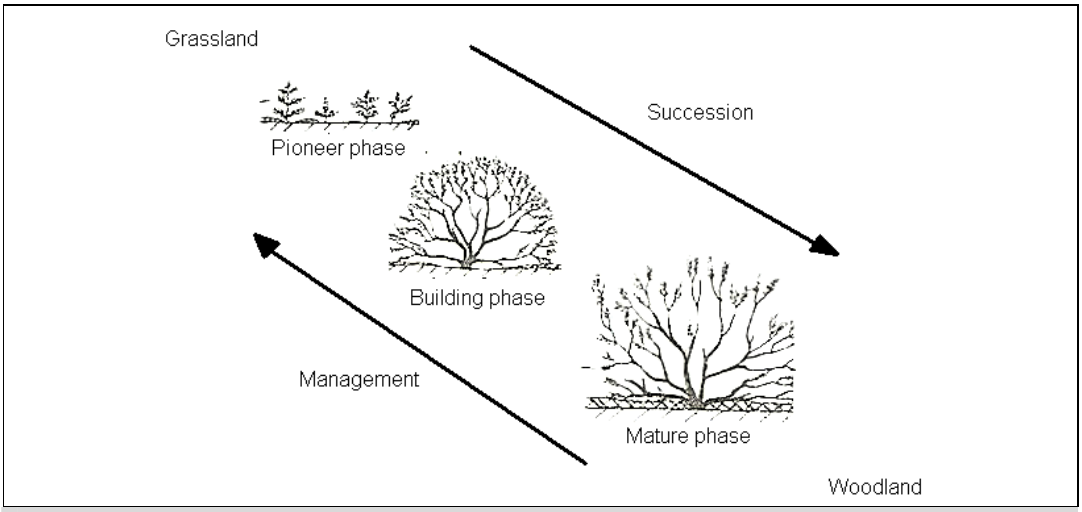


**Figure 1.1.** Heathland succession with three different phases in the succession. Heath vegetation can be considered as a transitional stage in the succession from grassland to woodland. While successional forces tend to drive this change in one direction towards woodland, management such as grazing and mowing slows or reverses the trend. Excessive management may change heath vegetation into grassland. The in Flanders less common 'degradation phase' has not been included.

The bulk of European heathland is, however, semi-natural and would have developed into woodland if no regular human intervention occurred (Fig. 1.1). The majority of heathland developed since the second half of the Holocene (ca. 7000-5000 years ago) as a result of agricultural practices (Bastiaens and Deforce, 2005). After woodland clearance, the resultant vegetation was used for livestock grazing, cut as turf, cut for fodder and fuel, and burned to produce fresh young heather for improved grazing. Turf was used as fuel or for the construction of buildings, and in some countries it was mixed with animal dung and spread over arable fields as fertiliser (Gimingham, 1972; Werger et al., 1985; Sibbett, 1993; Webb, 1998; Burny, 1999; De Blust, 2004). As these agricultural activities prevented succession to forest, heathland was a stable system for centuries (i.e. plagioclimax), with its maximum extent in Flanders around the year 1000 AD (Tack et al., 1993; Piessens et al., 2004). This continuous management of heathland also created dynamic landscapes which were characterised by intermittent disturbance activities and subsequent secondary heathland succession, resulting in typical vegetation cycles. Each time that human tilled heathland dominated by *C. vulgaris*, a succession of *C. vulgaris* growth phases occurred, often known as heathland succession (see Gimingham, 1972; Fig. 1.1). Species that were already adapted to the conditions found in specific phases of the heathland succession were able to take advantage of the anthropogenic increase in those phases.

As a result of changes in land use and agricultural intensification from the end of the 18th century European heathlands increasingly lost their economic value, resulting in a dramatically decline of heathland area throughout much of western Europe (Gimingham, 1979; Tack et al., 1993; Webb, 1998; Odé et al., 2001; De Blust, 2004; Piessens et al., 2004). In some regions, such as Flanders and the Netherlands, less than 5% of the mid-19th century heathland area is left (Odé et al., 2001; Piessens et al., 2004; Fig. 1.2). Remaining heathlands are often strongly fragmented and are less diverse, in terms of both species composition and vegetation structure (Aerts and Berendse, 1988; Piessens, 2006). Consequently, many species found in heathlands are threatened and have ended up on Red Lists. At present, in Flanders, no less than 30% of all characteristic heathland species belongs to the Red List (De Bruyn, 2003). One of those Red List species is *Cuscuta epithymum* (chapter 2). For biodiversity reasons and as remnants of formerly widespread cultural landscapes, heathlands are nowadays regarded as an internationally endangered habitat of high conservation value (cf. EU Habitats Directive 92/43/EEC; Webb, 1998).

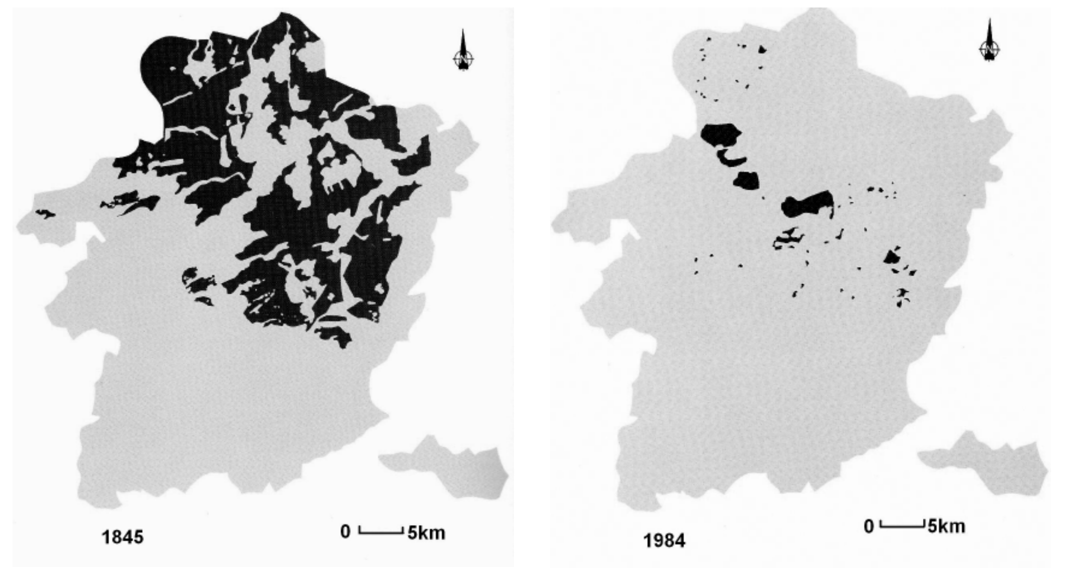


**Figure 1.2.** Maps showing the decline in heathland extent (black shading) in the province of Limburg, north-eastern Belgium. (From Allemeersch et al., 1988). The figure illustrates habitat destruction, decreasing fragment area and increasing isolation between heathland relicts.

The abandonment of management is still one of the problems for the remaining heathlands, and the species that have specialised and adapted to it. Modern management cannot just replicate the historic land management, because heathlands are now smaller, more isolated and their management is not financially profitable anymore (Webb, 1998). Current heathland management is primarily directed towards nature and landscape conservation, and focused on decreasing modern impacts, for example caused by atmospheric nutrient deposition (e.g. Webb, 1998; Barker et al., 2004; Maes and Van Dyck, 2005; Piessens et al., 2006). For the management of semi-natural traditionally managed biotopes, such as European heathlands, there is an increased need for understanding species-specific requirements, which are not necessarily achieved by copying traditional management techniques (Schemske et al., 1994; Maes, 2004). In particular, species adapted to disturbed habitats do not always survive during modern nature conservation management (e.g. Simberloff, 1998; Maes et al., 2004; Verheyen et al., 2005). To improve the management, detailed information on the habitat requirements, distribution, dispersal and colonisation capacities of particular species is required. Consequently, detailed studies on the demography, distribution and abundance of threatened species and of the interactions with the surrounding dynamic landscape are needed.

## A metapopulation approach for conservation objectives

Understanding the reasons for species decline, and the consequences for viability and long-term persistence, are major goals of conservation research (Schemske et al., 1994; Brook et al., 2000). Over the years several useful approaches have been developed to address the challenge of species loss. At local scale most organisms are grouped as populations of interacting individuals living in a certain area. Assessing the demographic structure of these populations provides valuable insights into their viability for conservation purposes. In practice, population viability analyses (PVA) are increasingly used for this goal (Akçakaya and Sjögren-Gulve, 2000). This collection of methods uses species-specific demographic models incorporating detailed information on species ecology and behaviour, in order to evaluate the threats faced by the populations, their risks of local extinction or decline, and their chances of recovery (Akçakaya and Sjögren-Gulve, 2000). Most PVA's are applied for single populations at local scale (Akçakaya and Sjörgen-Sulve, 2000; Münzbergová and Ehrlén, 2005). Populations of most species do, however, not necessarily function as spatially isolated units, but instead interact with other populations in the surrounding landscape within a larger spatial scale. Such spatially structured assemblages of sexually reproducing populations which are connected to each other by dispersal from one local population to at least one other population are known as metapopulations (Levins, 1969; Fig. 1.3). The long-term persistence of these metapopulations depends on a balance between the formation of new populations by dispersal and (re)colonisation, and local population extinctions (Hanski, 1999; Fig. 1.3). Metapopulation ecology deals with the regional dynamics of single species in fragmented landscapes. Therefore, it may offer a fruitful approach to anticipate on species' requirements at landscape scale and to test how various management designs influences target species. Additionally, metapopulation approaches may improve management planning to influence the abundance and distribution of the species.

Since the introduction of the spatial implicit metapopulation model by Levins (1969), metapopulation theory has developed as a popular framework for analysing the dynamics of species in patchy landscapes and for understanding the factors of species decline (e.g. Akçakaya and Raphael, 1998; Hanski and Gilpin, 1997; Matlack and Leu, 2007; O'Brien et al., 2008). Yet, because of the simplicity of Levins' model, it cannot be applied to real landscapes. Several later studies have aimed to include a greater biological realism in spatial explicit metapopulation models. First most attention was given to static landscapes, where patches are neither created nor destroyed (Fig. 1.3a). In natural ecosystems the quality and spatial distribution of habitat patches are rarely constant in time (Matlack and Monde, 2004; Kleyer et al., 2007). Particularly in ecosystems such as heathlands, where management actions and natural regeneration cause a constantly changing mosaic of patches of changing successional phase, the metapopulation survival of species is strongly determined by landscape dynamics (e.g. Webb, 1988; Fisher and Stöcklin, 1997; Verheyen et al., 2004; Bossuyt and Honnay, 2006). Species inhabiting such dynamic landscapes are faced with varying qualities (or suitabilities), numbers and spatial arrangement of patches (Biedermann, 2004; Bossuyt and

Honnay, 2006; Kleyer et al., 2007) and the viability of their metapopulations depends on the balance between the rate of appearance, disappearance and the spatial arrangement of patches. Therefore, both habitat dynamics and population dynamics should be considered simultaneously (Akçakaya et al., 2004; Bossuyt and Honnay, 2006; Ranius, 2007). This can be achieved by spatial realistic metapopulation models that apply PVAs to metapopulation level by combining spatial population structure, patch-specific habitat quality and changes in habitat quality both in space and time (Fig. 1.3b).

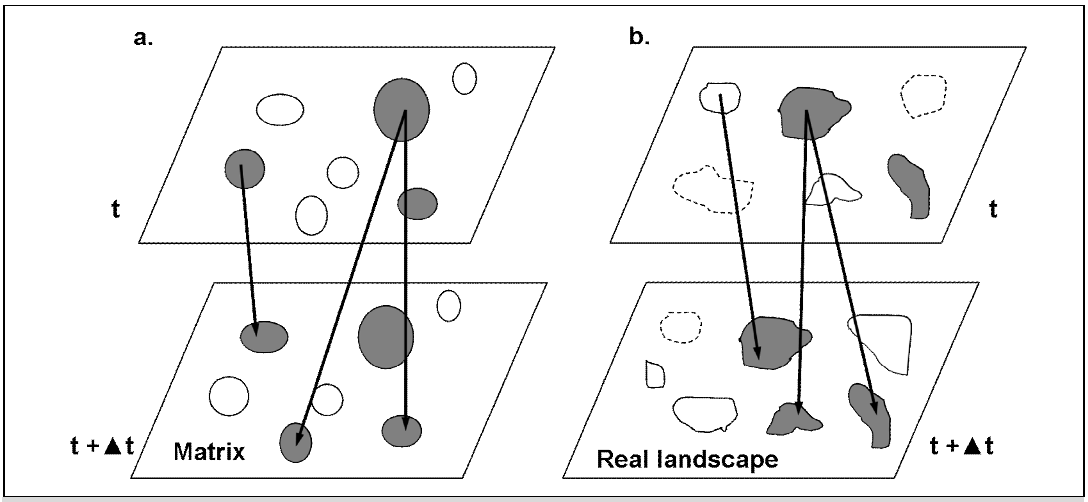


**Figure 1.3.** Schematic representation of **a)** a spatial implicit metapopulation approach and **b)** a spatial realistic approach of a metapopulation at two points in time, namely t and t + ▲t. Filled shapes: suitable habitat patches occupied by a population; empty shapes: vacant suitable habitat patches; dotted lines: vacant and unsuitable (inadequate quality) habitat patches; arrow: dispersal of individuals from one patch to another. In contrast with the static landscape in the spatial implicit metapopulation approach, patches in the spatial realistic metapopulation may be dynamic, including appearance, disappearance or change in habitat quality.

The most important challenge by using a spatial realistic metapopulation approach comes from obtaining sufficiently precise estimates of model parameters (Moilanen and Hanski, 1998), particularly for plant metapopulations. The majority of metapopulation models have been used for studying regional distribution patterns of highly mobile species, such as birds and insects, in which turnover dynamics are relative easily to observe (e.g. Hanski, 1994a; Biedermann, 2000; Akçakaya et al., 2004; Ranius, 2007). In contrast, studies of metapopulation dynamics in plants are limited (for review see: Eriksson, 1996; Husband and Barrett, 1996; Freckleton and Watkinson, 2002; Ouburg and Eriksson, 2004). Many researchers believe that plants possess a number of life-cycle features that make them difficult to incorporate in metapopulation models, typically envisaging short lived, highly mobile species (see Ouburg and Eriksson, 2004). Plants are sedentary and usually disperse via seeds. Many plant species have long life-spans, often exceeding the life-span of the observer and making it difficult to monitor turnover dynamics in the field (Freckleton and Watkinson, 2003; Verheyen et al., 2004). In addition, many species possess persistent seed banks (Thompson et al., 1997; Piessens et al., 2004 for heathlands), which makes it complicated to estimate population dynamics. Some authors therefore doubt the applicability of metapopulation theory for describing the regional dynamics of plants (Husband and Barrett, 1996; Bullock et al., 2002; Freckleton and Watkinson, 2002; Ehrlén and Eriksson, 2003). However, some researchers proved that a metapopulation framework can be useful to study patchily distributed plant species under certain conditions (e.g. Valverde and Silvertown, 1997; Ehrlén and Eriksson, 2003; Verheyen et al., 2004; Snäll et al., 2005; Bossuyt and Honnay, 2006). But there is only a handful of empirical studies dealing with plant metapopulations in dynamic landscapes (e.g. Dupré and Ehrlén, 2002; Verheyen et al., 2004; Jäkäläniemi et al., 2005; Snäll et al., 2005; Bossuyt and Honnay, 2006; Dostál, 2007) and empirical evidence of the direct impact of seed banks in maintaining plant metapopulations is still missing. Detailed field studies of plants coping with dynamic landscapes are urgently needed to better understand how real plant populations cope with transient habitats and how they react to changes in the management.

This thesis investigates successional heathland communities containing *Cuscuta epithymum* (Common dodder) populations. If heathlands are managed, they have a dynamic arrangement of heathland patches which are created by management and then disappear again due to natural heathland succession. *Cuscuta epithymum* is faced by continuously changing habitat quality both in time and space. The thesis aims to identify the factors determining the distribution of *C. epithymum* and to discover various components of its demography, to incorporate the information in a spatial realistic metapopulation model, simulating the long-term persistence of *C. epithymum*. The data collected during this research period will provide pragmatic recommendations for heathland management and conservation. The combination of a relatively short life-cycle, fast regeneration in suitable habitat patches, and the simplicity of the creation of suitable habitat patches makes this system suitable to study using a metapopulation framework.

## Aims and outline of this thesis

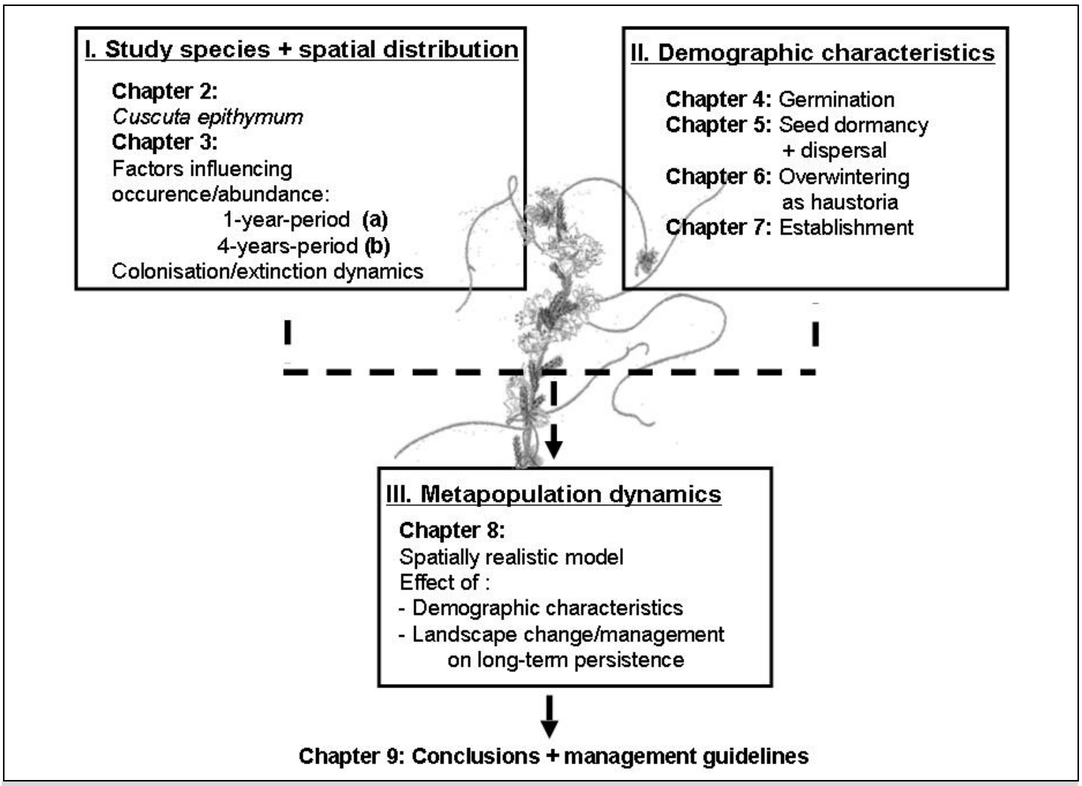


**Figure 1.4.** Schematic outline of this thesis referring to the different research topics covered by chapters 2 to 9. The first 8 chapters are positioned in boxes representing the three study parts of this thesis. Chapter 9 translates the findings from the previous chapters to management recommendations. The arrows point to the relation between the different parts.

The significant decline of *C. epithymum* in Belgium and other parts of western Europe suggests a requirement for targeted management plans for its long-term conservation. Accordingly, the main objective of this thesis is to increase our knowledge about the ecological requirements and life history characteristics of the endangered holoparasite *C. epithymum* in dynamic semi-natural heathlands. The ecology of populations is embedded in a landscape context, so we have worked on individual plant, population and metapopulation level. The thesis consists of three parts (Fig. 1.4). In this thesis, we consider the dynamics of *C. epithymum* at landscape scale in terms of the changes in distribution and abundance among and/or within patches in one particular nature reserve. The **first part** introduces several aspects of plant parasitism and presents the study species. This part further describes the factors explaining the spatial distribution of the study species in a landscape scale. Several aspects of the life-cycle of *C. epithymum* are investigated in the **second part** of this thesis. The **last part** uses the knowledge obtained from the first two parts to build up a spatial realistic metapopulation model to explore the long-term survival of this species under different management regimes. This will be the basis for habitat management guidelines. Boxes are used to present the results of smaller experiments executed during the PhD research which are not extensive enough to fill a whole chapter.

## Part 1: Study species and spatial distribution patterns

**Chapter 2** introduces the study species *C. epithymum*, with a review of all aspects of the biology of *C. epithymum* relevant to its ecology and behaviour. This chapter starts with a description of plant parasitism.

Disentangling the key habitat characteristics and identifying the driving forces behind regional distribution patterns and population dynamics remains a central issue in conservation ecology (e.g. Hanski and Gilpin, 1997; Brys et al., 2005; Ameloot et al., 2006; Ramsay and Fotherby, 2007). **Chapter 3** therefore investigates how regional and local environmental factors affect the distribution and abundance of *C. epithymum* populations within four Flemish heathland nature reserves. In a first part of this chapter the impact of the direct environment and surrounding landscape on its distribution observed in 2004 is assessed by relating population presence and abundance to six local environmental factors and three regional variables. The short-term census used in the first part of **Chapter 3** can only give an indication of temporal and spatial population dynamics. Data on regional-scale spatial and temporal dynamics of both *C. epithymum* populations and suitable heathland patches (including patch creation and disappearance) were therefore obtained from a longer-term study over four years (**Chapter 3b**). The effects of landscape dynamics on *C. epithymum* population dynamics are discussed.

## Part 2: Demographic characteristics

Subsequent chapters focused on the demographic characteristics of *C. epithymum* and their relationship with successional vegetation changes. This is needed to understand how *C. epithymum* establishes populations at a certain stage of succession and disappear again in a later stage.

Germination is one of the most critical steps in a plant life-cycle, depending on seeds' dormancy degree and the presence of suitable conditions allowing germination to occur. A broadening of the limited knowledge about *C. epithymum* seed ecology proved necessary to have a better idea of how seed germination functions and of how germination in nature is timed to coincide with the presence of suitable host plants. In **Chapter 4** the results of a detailed germination experiment are presented.

In ever-changing landscapes such as heathlands, seed dormancy which spreads seeds across multiple years, and seed dispersal which spreads offspring across multiple locations are ways in which plants cope with habitat uncertainty. **Chapter 5** deals with both of these seed features of *C. epithymum*. Fresh seeds were buried in the field under natural conditions. The viability, depletion and dormancy-break of the buried seeds were studied at regular intervals over 31 months. In addition, the results of a small dispersal experiment are presented in a supplementary box (**Box 5.1**).

Although *C. epithymum* is often considered to be an annual, several reports have referred to the possible perennial character of the parasite. In **Chapter 6** the capability of *C. epithymum* to overwinter vegetatively, and the impact of successional stages on this capability, were examined. The ecological importance of perennation for this species in transient habitats is discussed.

Introduction experiments offer a direct test of recruitment limitation, but generally only the seed germination and seedling phase are monitored. The relative importance of different establishment stages in the process of recruitment is often not considered. **Chapter 7** reports on an experiment designed to find out how establishment of *C. epithymum* precisely happened during two consecutive growing seasons and how heathland management applications, the degree of vegetation succession and seed-density exactly influenced the different steps in parasite's recruitment and establishment.

## Part 3: Metapopulation dynamics

By creating dynamic patch mosaics, heathland management influences the dynamics of interacting populations. Little empirical evidence is, however, available on the impact of current management applications on metapopulations of single species occurring in such dynamic landscapes. **Chapter 8** evaluates metapopulation viability of *C. epithymum* by the use of the existing software RAMAS Metapop (Akçakaya, 2002). Using the information obtained from Chapters 3-7, a spatial realistic metapopulation model was parameterised. The model was designed to explore the impact of demographic characteristics and of spatiotemporal landscape patterns created by management interventions on *C. epithymum*'s long-term metapopulation viability within a particular heathland site (here nature reserves).

## Conclusions and management guidelines

In **Chapter 9**, the results of the previous chapters are briefly summarised, paying special attention to the advantages and disadvantages of various *C. epithymum* characteristics for coping in dynamic heathland. Based on the information gathered in the previous chapters, several practical guidelines for the management of heathlands obtaining *C. epithymum* populations are proposed. Finally, suggestions for future research are offered.

# 2

# Spotlight *Cuscuta epithymum* (L.) L.

Photo: Klaar Meulebrouck (Heiderbos, 2006)

In this chapter, we first introduce several aspects of plant parasitism and afterwards we throw more light on the taxonomy, morphology, distribution, conservation status and ecology of our study species, *C. epithymum*, paying special attention to its parasitic characteristics. During the four years of research we have gathered a lot of additional data concerning *C. epithymum* in Flanders, some of which is mentioned in this chapter. The results of two smaller research projects are presented in boxes to distinguish them from the descriptive information on *C. epithymum* retrieved from the scientific literature.

## Plant parasitism

The vast majority of all flowering plants are autotrophic organisms that obtain necessary resources, namely water, minerals and carbon, from their environment and via photosynthesis. In contrast, approximately 4200 plant species or ~ 1 % of all flowering plants exhibit a parasitic mode of life whereby they partially or completely rely on host plants for their nutritional requirements (Kuijt, 1969; Nickrent, 2002). These parasitic plants represent a wide variety of life forms from trees and shrubs (e.g. sandlewoods) to vines (e.g. *Cuscuta* spp. and *Cassytha* spp.) and herbs (e.g. *Rhinanthus* spp.) occurring in most plant communities throughout the world from tropical rain forests to the high Arctic (Press and Graves, 1995). Recent phylogenetic studies indicate that parasitism has arisen independently in at least 11 lineages during the evolution of flowering plants and occurs in approximately 265 genera within 22 different families (Nickrent, 2002; Nickrent et al., 1998; APG II, 2003). The invasion of the host tissues and extraction of resources is achieved through haustoria, which are specialised multifunctional organs that uniquely characterise all the parasitic plants (Estabrook and Yoder, 1998; Fig. 2.1). Haustoria are physiological bridges that join the host and the parasite by transferring nutrients from the former to the latter (Kuijt, 1969). The position of haustorial attachment to the host is used to classify the parasites as either a root or shoot parasites, whereas the presence or absence of functional chloroplasts further defines the parasitic plants as being either hemiparasitic or holoparasitic, respectively (Kuijt, 1969). Approximately 20% of all parasites are holoparasitic, lack functional chloroplasts and thus obtain all their water, nutrients and carbohydrates via haustorial connections to the host xylem and phloem. This classification is, however, not always that clear, as some holoparasites contain small amounts of chlorophyll (Kuijt, 1969; Nickrent, 2002; Heide-Jorgensen, 2008).

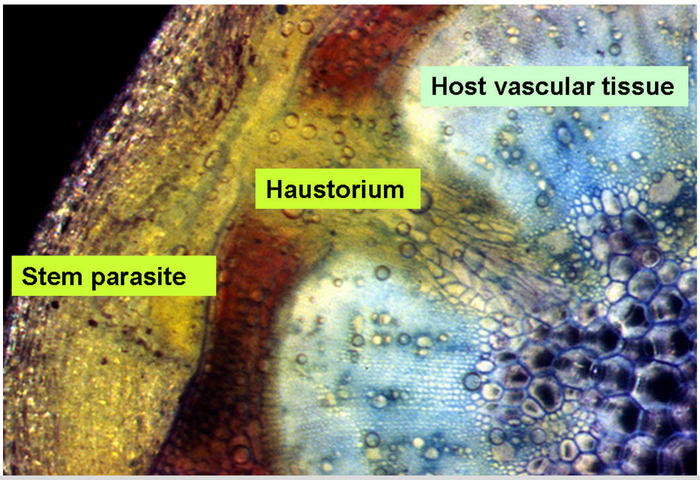


**Figure 2.1.** Cross-section of the stem of the holoparasite *Cuscuta salina* parasitising its host. The haustorium connects to the host phloem and xylem (Photo: Collin Purrington).

Parasitic plants have direct consequences on their hosts. The acquisition of host resources can exert strong effects on host growth, allometry, reproduction and physiology (e.g. Press et al., 1999; Gómez, 1994; Ameloot, 2007). The effect of the parasite will depend on the state of the host, including its stage of development and the availability of resources (Press and Graves, 1995; Nickrent, 2002). Because of this reduction in host performance, the presence of parasitic plants may lead to changes in the competition interactions between host and non-host plants which in turn will affect the community structure and dynamics (e.g. Pennings and Callaway, 2002; Ameloot et al., 2005; 2008; Grewell, 2008). The latter has, for instance, been proved for the holoparasite *C. salina* in Californian salt marshes (Pennings and Callaway, 1996). Here, the parasite prefers the competitively dominant species, *Salicornia virginica*. By selectively suppressing this species, other less competitive plants are temporarily allowed to expand in the patches infected with *C. salina*. As *S. virginica* becomes less abundant, *C. salina* will evanesce from that place and the other less competitive plants may reinvade again. By promoting competitively inferior species, parasitic plants can thus play a significant role in increasing the community diversity (e.g. Pennings and Callaway, 2002; Niemelä et al., 2007). Such cycling interactions may also explain why some parasites, such as *Rhinanthus minor*, appear to 'move through' vegetation (Gibson and Watkinson, 1992). Within communities, parasites not only affect other plants, but their presence can also directly or indirectly influence other species, including herbivores and pollinators (Press and Phoenix, 2005). Goméz (1994), for example, observed that parasitism by *C. epithymum*, reduced feeding of herbivores on *Hermathophylla spinosa* hosts. The combination of these top-down (e.g. as a natural enemy of the host) and bottom-up (e.g. as a resource) effects within communities lead to the conclusion that parasites can be considered as 'ecosystem engineers' or 'keystone species', whose presence is crucial for the organisation and diversity of their community (see

Press and Phoenix, 2005 for a review). Nevertheless, not only parasites have strong impacts on their hosts and on the community structure and dynamics. Hosts can also affect their parasites through both direct and indirect pathways (Adler, 2002). Because parasitic plants acquire both nutrients (Press and Graves, 1995) and defensive compounds from their hosts, many attributes of parasitic plants are dependent on the qualities of their hosts (Adler, 2002). *Castilleja miniata*, for example, showed a reduced herbivory by alkaloid uptake from lupine hosts (Adler, 2002). Thus, parasite-host plant interactions may alter community composition via several mechanisms. Gómez (1994) even concluded that the relationship between an individual dodder plant and an individual host plant is neutral, due to the array of direct and indirect, positive and negative effects.

Despite the widespread prevalence of parasitic plants, they are largely ignored in plant community science. Our current knowledge of the biology of parasitic plants is dominated by laboratory studies, and by studies on crop pests. In order to place the knowledge gained from these studies into a broader context, there is need for more field studies of parasitic plants in the communities in which they naturally occur.

**It is *Kushut:***

***No root, no leaves***

***No fruit, no breeze***

***No shade***

*[Poem written by*
*unknown Arab poet]*

# *Cuscuta epithymum* (L.) L.

## *Taxonomy*

*Cuscuta epithymum* (L.) L. belongs to the genus *Cuscuta* (subgenus *Cuscuta*), the only parasitic group evolved within the Convolvulaceae which comprises more than 200 species (Stefanovic et al., 2003; 2007; Mihai Costea, pers. comm.). The subgenus *Cuscuta* is distinguished from the two other subgenera by distinctly linear free styles and elongated stigmas (Yuncker, 1932; García and Martín, 2007). *Cuscuta epithymum* exhibits considerable variation (Henrard et al., 1942; Feinbrun, 1970; García and Martín, 2007). Based on morphological differences, the species was subdivided into two subspecies and three varieties. In addition, the taxa are strongly characterised by distinctive eco-geographic features, but this variability is of low taxonomic value and is often influenced by the host species (Feinbrun, 1970). Consequently, many names have been proposed to describe this morphological variability (van Ooststroom, 1951; García and Cafferty, 2005). The individuals that are present in Flanders belong to the most common form of *C. epithymum*, namely *C. epithymum* ssp. *epithymum* var. *epithymum* (Feinbrun, 1970; identified by Mihai Costea).

### *Morphology*

*Cuscuta epithymum* is a holoparasitic vine with twining, filiform, reddish stems, reduced scale-like leaves and no roots (Fig. 2.2a). The stems have an average diameter of 0.25 to 0.40mm; the thickness varies within the same plant depending on the amount nutrients acquired from their hosts (Kelly, 1994). Some plants contain small amounts of chlorophyll which makes them capable of limited and localised photosynthesis. Nevertheless, the amount of assimilation is insufficient for completing its life cycle, which practically makes *C. epithymum* an obligate parasite (Verdcourt, 1948; Hibberd et al., 1998; van der Kooij et al., 2000; Birschwilks et al., 2006). The proportion of chlorophyll present is ten times less compared to autotrophic plants, and 99% of the carbon used is extracted from the host plant(s) (Smith et al., 2001; Jeschke et al., 1994). If present, photosynthetic activity mainly occurs in the tip of the seedlings, and when insufficient nutrients are available, e.g. when growing on unsuitable hosts or in the shade (Verdcourt, 1948; Dawson et al., 1994; pers. obs.; Fig. 2.2c). The parasite coils around the stems or leaves of the host and penetrates to its vascular system with specialised absorptive organs, called haustoria, in order to absorb water, carbohydrates and minerals (Kuijt, 1969; Parker and Riches, 1993; Fig. 2.1). The infection process is facilitated by the secretion of enzymes, such as peroxidase, which is associated with the invasion and destruction of host tissue (López-Curto et al., 2006). Under favourable conditions, *C. epithymum* produces a clearly recognisable interlaced aggregation of reddish stems on the vegetation of host plants (Fig. 2.2b). The stems often branch and coil around almost every plant they come in contact with.

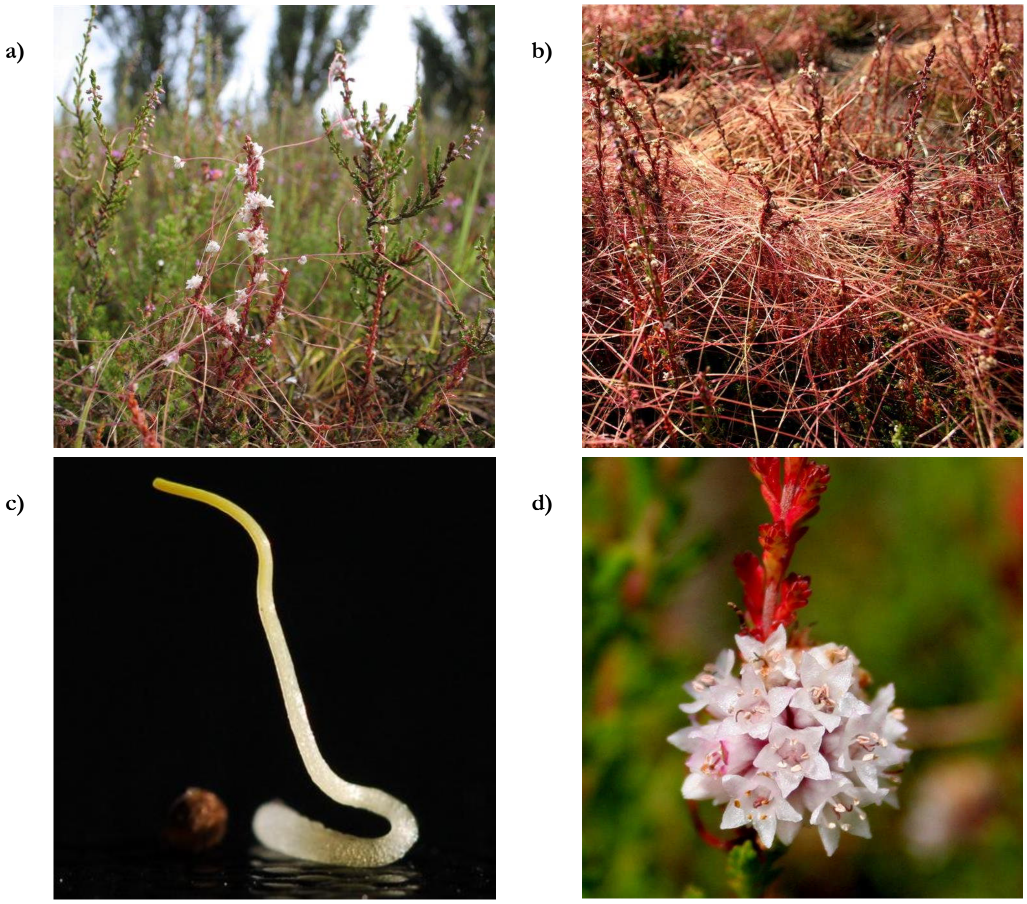


**Figure 2.2.** **a)** *Cuscuta epithymum* parasiting *C. vulgaris*, **b)** aggregation of red *C. epithymum* stems, **c)** *C. epithymum* seedling with green photosynthetic tip, **d)** inflorescence of *C. epithymum*. (Photo c: Frederik Lerouge)

Each reproductive plant can display several hundred tiny, bell-shaped flowers of on average 2.29 ± 0.26 mm in diameter (Gómez, 1994; Fig. 2.3a). The white to pink flowers are fleshy, sessile and grouped in lateral cymes with on average (± SE) 7 ± 0.13 flowers per inflorescence (n = 208; min = 1; max = 20; Fig. 2.2d). They are bisexual, regular and typically pentamerous. Calyx and corolla lobes are triangular and fused at the base (Fig. 2.3b, d). Like all the other species of the genus *Cuscuta*, *C. epithymum* is characterised by a unique feature, called infrastaminal scales (also called “hypostaminal scales” or “corolla appendages”; Kuijt 1969; Costea and Tardif, 2006). These distally fringed spatulate appendages are located at the interior of the corolla tube, at the base of the five stamens (Yuncker, 1921; Fig. 2.3c).

The somewhat spherical epigynoecium is composed of two carpels each containing two anatropous ovules (Kuijt, 1969). *Cuscuta epithymum* has two styles per flower, both with a linear stigma rising above the corolla (Fig. 2.3e, f). The latter is an important distinguishing feature of species belonging to the subgenus *Cuscuta*.

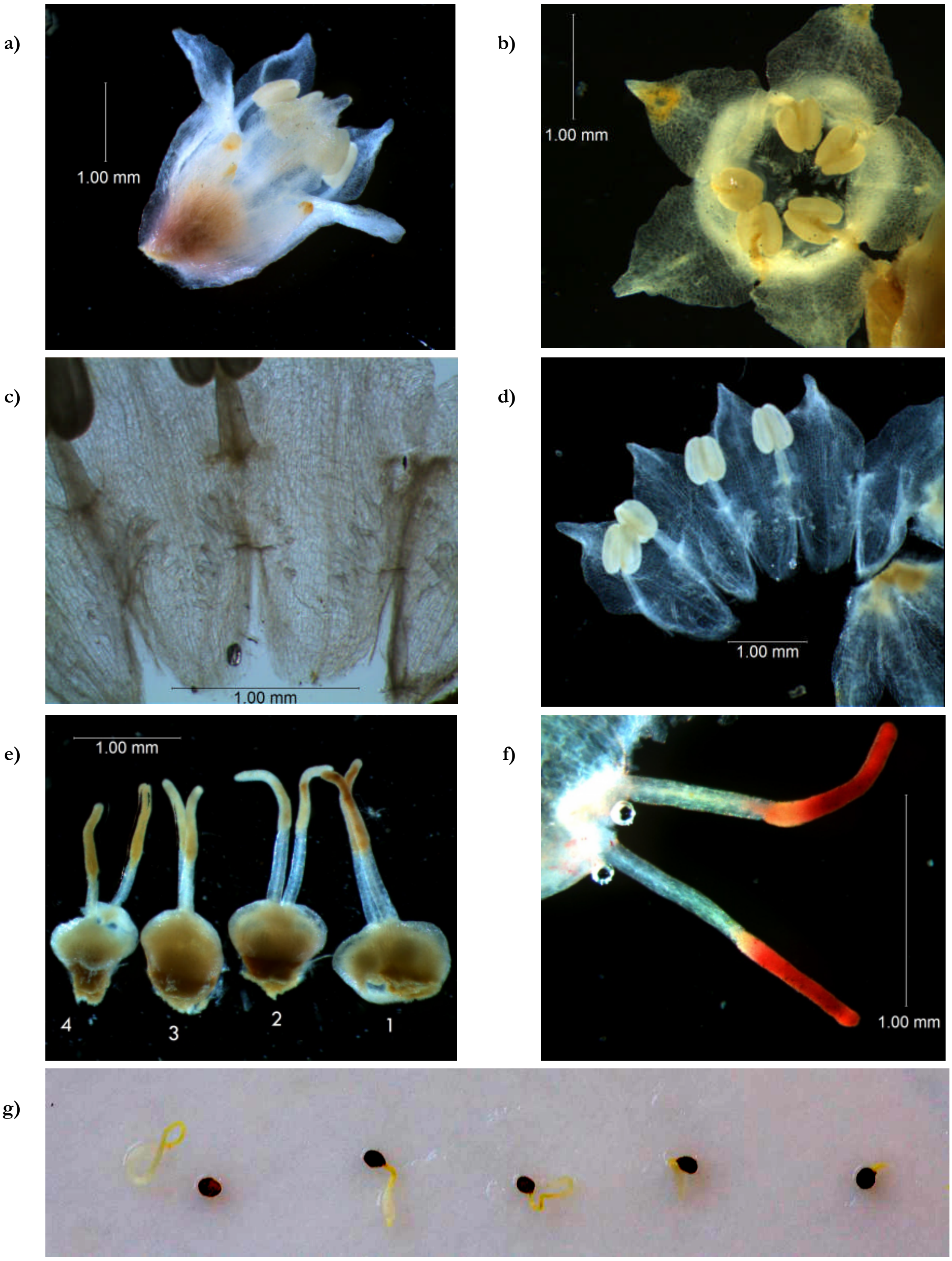


**Figure 2.3.** *Cuscuta epithymum* **a)** flower, **b)** corolla and infrastaminal scales in 3D, view from above, **c)** infrastaminal scales, **d)** corolla, stamens and infrastaminal scales, **e)** gynoecia, **f)** styles and stigma, stained with neutral red, **g)** successional steps in seed germination from germinating seed at the right to seedling at the left side. (Photos a-f: Mihai Costea; from herbarium voucher 'Belgium, Flanders, Limburg province, Maasmechelen, August 2004, K. Meulebrouck; see Costea, 2007-onwards)

The gynoecium develops into a circumscissile spherical capsule which is capped by the withered corolla and contains up to four seeds (Yuncker, 1921). Mature seeds have a multi-layered seed coat (Lyshede, 1992). For a more detailed description of seed morphology see Chapter 4.

The spirally-wrapped embryo has no radicle or cotyledons, reflecting its parasitic way of life (Sherman et al., 2008). Seeds usually germinate on or near the soil surface. The leaf- and rootless seedlings are arch-shaped and exhibit a pale green tip when they emerge (Fig. 2.2c). The swollen basal radicle of the seedling functions as a root in absorbing water and anchoring the plant, but it should be considered as a highly modified basal portion of the stem (Sherman et al., 2008). Soon after emergence, the seedling straightens itself, discards the empty seed coat and begins to circumnutate counterclockwise in search of a suitable host (Kuijt, 1969; Dawson et al., 1994; Fig. 2.3g). Seedlings usually reach 1.2 to 8.0cm in length. In developing seedlings, resources are allocated to the growing shoot, as well as to the process of attachment and penetration of the host. After attachment, the portion of the seedling that extended from the ground desiccates and dies. The remaining part of the stem acquires an intense red colour. The developing haustoria penetrate the epidermis and advance deeper into the cell layers of the host, connecting eventually to both the phloem and xylem vasculature tissues (Dawson et al., 1994; Hibberd and Jeschke, 2001). When the haustorial connection between the vascular bundles of the host and the parasite is completed, *Cuscuta* becomes a very strong sink, competing with the host sinks for assimilates (Jeschke et al., 1994).

## *Geographical distribution*

The genus *Cuscuta* is nearly cosmopolitan in distribution with its species found on every continent with the exception of Antarctica, ranging from 60”N in Europe and Asia, to the Cape region of South Africa, and in the south to 47”S in Argentina and Chile (Yuncker, 1932). In Belgium, two native species, *C. europaea* and *C. epithymum*, and two aliens, *C. lupuliformis* and *C. campestris* are present (Lambinon et al., 1998).

*Cuscuta epithymum* is native to Europe, western Asia and northern Africa, and has been introduced to North America, eastern Asia, Africa, South America, Australia and New Zealand by contamination of seeds of crops, mainly alfalfa and clover (García and Cafferty, 2005; Costea and Tardif, 2006). It can be found almost throughout Europe, except the extreme north (Tutin et al., 1972) and up to heights of 3000 m (e.g. Sierra Nevada, Gómez, 1994). The optimal distribution of *C. epithymum* is in the temperate and subtropical parts of Europe, western Asia and the Atlas region (Parker and Riches, 1993; Preston et al., 2002). Before the 20th century, the parasite was quite common in Belgium, but it is rare now (Duvigneaud, 1945; van Landuyt et al., 2006; Fig. 2.4a,b). A recent survey in Flanders (summer 2007) found that the species is confined to 28 sites, of which 93% are situated in the

Campine region, in the provinces of Antwerp and Limburg (Fig. 2.4c; Peeters, 2008; Rombouts, 2008). The species is found in only one site in the province 'Vlaams-Brabant' and one in the north-western province 'West-Vlaanderen' (Eikelberg and Warandeduinen, respectively on Fig. 2.4c). In Flanders, the distribution of *C. epithymum* is mainly associated with the distribution of dry heathland and particularly with *C. vulgaris* (Fig. 2.4a).

## *Habitat and communities*

In order to determine the phytosociological position of *C. epithymum*, an extensive survey of all sites in Flanders containing *C. epithymum* was carried out during the summer of 2007 (Peeters, 2008; Rombouts, 2008; Fig. 2.4c). Based on a hierarchical classification method (Twinspan, PCORD-software) and the phytosociological classification used in Schaminée et al. (1996), three vegetation types were distinguished. These vegetation types in Flanders are described below, together with other communities from all over Europe where *C. epithymum* has been observed. Finally, a picture is drawn from the management performed in the 28 Flemish sites containing *C. epithymum* populations.

### Heathlands

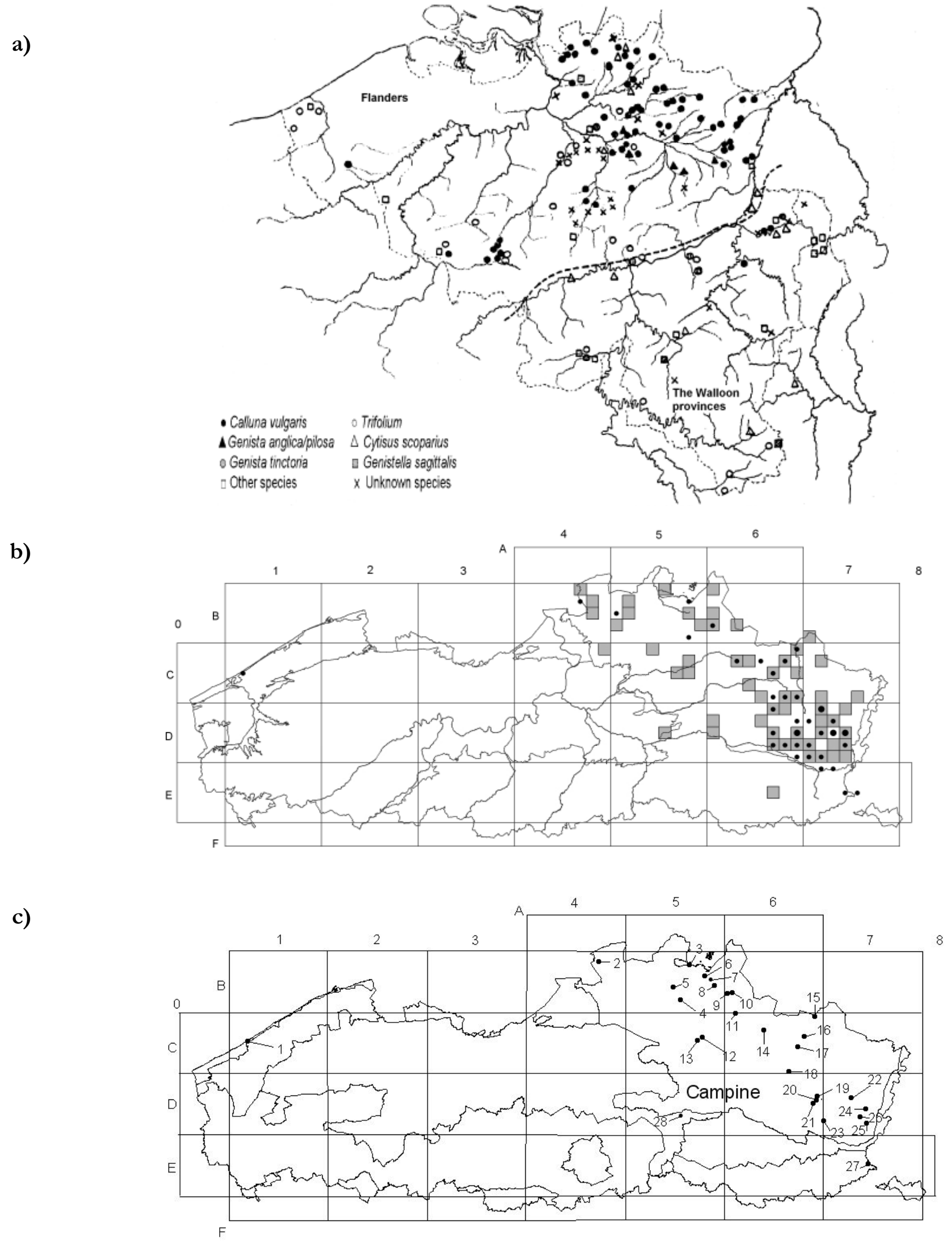
In its native range in Europe, *C. epithymum* is an indicator species of the *Calluno-Genistion pilosae* Duvigneaud 1944 association, which has it its main distribution in North-western lowland and mountain ridges of Central and South-western Europe and is included in the *Calluno-Ulicetea* Braun-Blanquet et Tüxen 1943 class (Laurent, 1901; Duvigneaud, 1945; Gimingham, 1972; Doyle, 1993; Schaminée et al., 1996). These lowland heathlands are usually found on acid oligotrophic sandy soils that are phosphorus deficient and are characterised by a podzolic profile (Gimingham, 1972). *Cuscuta epithymum* is found to be already a common part of dry heathland communities in Denmark during the Bronze Age, as the fragments of the species were found in 31% of some sod samples (14th century B.C.; Karg, 2008). In Flanders, 26 of the 28 sites containing *C. epithymum* belong to this vegetation association. In these sites, the vegetation is characterised by the dominance of the shrub *C. vulgaris* and the typical presence of *Carex pilulifera*, *Deschampsia flexuosa*, *Erica tetralix, Festuca ovina, Genista anglica* and/or *G. pilosa* (data not shown), which are all potential hosts of *C. epithymum* (Box 2.1). The soils of these 26 sites had an average pH of 3.4 ± 0.04 (min. = 2.9; max. = 4.0; Peeters, 2008; Rombouts, 2008). *Cuscuta epithymum* was found mainly on the early successional stages of this semi-natural vegetation association. In France, it was also observed in the heathland association *Daphno cneori-Callunetum*, which is characterised by both sub-atlantic and continental species (Muller, 1997). In Asturias (Spain), *C. epithymum* parasitises *Ulex europaea* in gorse-rich heathlands of the Atlantic coast (pers. obs.). These heathlands belong to the *Ulici europaei-Ericetum vagantis* Guinea 1949 association which is also included in the *Calluno-Ulicetea* class. They are typically found under wet, oceanic climatic conditions and are dominated by *Daboecia cantabrica, E. cinerea,* , *E. vagans* and *U. europaea* (Rivas-Martínez et al., 2001). In Spain, *C. epithymum* is further associated with the *Gentiano pneumonanthe - Ericetum mackaianae* association (*Calluno - Ulicetea*) containing *C. vulgaris*, *E. cinerea* and *Ulex gallii* as diagnostic species (Fagúndez, 2008). This habitat type occurs mostly at the North-western end of Spain and Portugal.

### Grass-heaths

In Europe, *C. epithymum* also occurs in vegetation belonging to the grass-heathland class *Nardetea* Rivas Goday and Borja Carbonell 1961 (e.g. Ireland: Doyle, 1993; The Netherlands: Schaminéé et al., 1996). In Flanders, it grows on this vegetation class at one location (Sint-Pietersberg, Fig. 2.4c). On that site, *C. epithymum* was found in *Betonico-Brachypodietum* Willems et Blanchenborg ex Schaminée nom. nov., an association combining acidophilous species such as *Danthonia decumbens*, *G. anglica* and *Potentilla erecta*, with species that are characteristic for mesophilous calcareous grasslands such as *Leontodon hispidus*, *Pimpinella saxifraga* and *Sanguisorba minor* (Sint-Pietersberg, Fig. 2.4c). Other distinctive species are *Achillea millefolium*, *Agrostis capillaris*, *Campanula rotundifolia*, *Centaurea jacea*, *Hieracium umbellatum*, *Lotus corniculatus* ssp. *corniculatus*, *Plantago lanceolata* and *Rumex acetosa.* This association mainly occurs on relatively dry and warm, west- to south-exposed hills. The limestone hills of the Sint-Pietersberg are covered by acid gravel deposits and therefore contain an unique vegetation having affinities with both *Nardo-Galion saxatilis* heathland grasslands, and *Mesobromion erecti* calcareous grasslands (Smits et al., 2006). The soil is acid with an average pH of 4.3 (Peeters, 2008; Rombouts, 2008).

### Dune grasslands

Flemish *C. epithymum* populations were found in a dry, calcareous dune grassland belonging to *Anthyllido-silenetum* De Leeuw in Braun-Blanquet et Moor 1938, an association assigned to the dry grasslands on sandy soils of the class *Koelerio-corynephoretea* (Schaminée et al., 1996; Warandeduinen, Fig. 2.4c). Characteristic and differential species of this association that were present in the vegetation of the Warandeduinen include *A. millefolium*, *Asparagus officinalis, Carex arenaria*, *Galium verum*, *Leontodon saxatilis*, *L. corniculatus*, *P. lanceolata* and *Rhinanthus minor* (data not shown). The mean pH of this location was 7.6, with a maximum of 7.7, indicating that *C. epithymum* also occurs in habitats with a strong calcareous substrate. In other European sites *C. epithymum* is found on sandy soils near the sea (e.g. The Netherlands, Henrard, 1942). In Ireland *C. epithymum* has been found growing in mature dune limestone grasslands belonging to the *Polygalo-Koelerion* association of the *Koelerio-Corynephoretea* class (Doyle, 1993).



1. Warandeduinen (Middelkerke, WV, D)
2. Kalmthoutse Heide (Kalmthout, A, H)
3. Wortel-Kolonie (Hoogstraten, A, H)
4. Duivelskuil (Beerse, A, H)
5. Bonte Klepper (Rijkevorsel, A, H)
6. Hoogmoerheide (Merksplas, A, H)
7. 't Zwart Water (Turnhout, A, H)
8. Dombergheide (Turnhout, A, H)
9. De Liereman (Oud-Turnhout, A, H)
10. Korhaan (Arendonk, A, H)
11. Looiendse Bossen (Retie, A, H)
12. Korte Heide (Lichtaart, A, H)
13. Het Olens Broek (Olen, A, H)
14. Buitengoor (Mol, A, H)
15. Hageven (Neerpelt, L, H)
16. 't Plat (Overpelt, L, H)
17. Veewei (Eksel, L, H)
18. Kamp van Beverlo (Leopoldsburg, L, H)
19. Schietveld (Houthalen-Helchteren, L, H)
20..Tenhaagdoornheide(Houthalen-Helch., L, H)
21. Teut (Zonhoven, L, H)
22. Heiderbos (As, L, H)
23. De Maten (Genk/Diepenbeek, L, H)
24. Mechelse Heide (Maasmechelen, L, H)
25. Kikbeekvallei (Maasmechelen, L, H)
26. Ziepbeekvallei (Lanaken, L, H)
27. Sint-Pietersberg (Kanne, L, G)
28. Eikelberg (Gelrode, VB, H)

**Figure 2.4.** Geographic distribution of *C. epithymum* **a)** in Belgium during 1945 with its main host species (adapted from Duvigneaud, 1945), **b)** in Flanders from 1939 to 1971 (grey 4 × 4km² grid squares) and from 1972-2004 (circles with the size representing the proportional frequency; from van Landuyt et al., 2006) and **c)** in Flanders during summer 2007 (Peeters, 2008; Rombouts, 2008). The numbers refer to the places where *C. epithymum* populations have been found. The name of the city, province and vegetation type wherein *C. epithymum* was growing are given in brackets. Provinces: A = Antwerpen; L = Limburg; VB = Vlaams-Brabant; WV = West-Vlaanderen. Vegetation types: D = dune grassland; G = grass-heath; H = dry heathland.

## Other vegetation types

*Cuscuta epithymum* also occurs on dry, calcareous grasslands belonging phytosociologically to the *Festuco-Brometea* class (Butaye et al., 2005; Doyle, 1993). There are records of *C. epithymum* growing in calcareous grasslands on limestone in south-western Belgium outside Flanders (Laurent, 1901; Butaye et al., 2005; Fig. 2.4a). In Ireland, *C. epithymum* has been found in areas which are typically stable landward dunes classified as a *Mesobromion erecti* (Br-Bl et Moor 1938) Oberdorfer 1957 association of the *Festuco-Brometea* class. The vegetation of these stable dunes is grass-dominated, with *Festuca rubra* forming the main cover. Other notable plants of this vegetation include *G. verum*, *L. corniculatus*, *P. lanceolata*; *Thymus praecox* and *Trifolium pratense*, all potential hosts of *C. epithymum* (see Box 2.1). *Festuco-Brometea* grasslands are mainly distributed in the Atlantic, Central Europe and sub-Mediterranean regions of Europe (Schaminée et al., 1996).

In the Mediterranean high mountains of South-eastern Spain (Sierra Nevada), *C. epithymum* grows on unspecified mountainous low shrub vegetation dominated by *Hormathophylla spinosa* (Gómez, 1994). In addition, *C. epithymum* has been recorded in avalanche tracks in the Swiss Alps in a *Festuco-Thymetum serpylli* Tüxen 1937 association belonging to the *Koelerio-Corynephoretea* class containing dry grasslands on sandy soils (Rixen et al., 2007), and in shrubby vegetation of the western Mediterranean mountains (García and Martín, 2007). *Cuscuta epithymum* is sometimes found on agricultural fields. In Flanders, there are no recent reports of this occurrence, but herbarium material (National Plant Garden; Box 2.1) and the distribution map of 1945 (Fig. 2.4a; see *Trifolium*) indicate that the parasite was found on alfalfa and clover in the past. Dijkstra (1968) mentioned that in the Netherlands *C. epithymum* is only found on clover fields situated on chalk rich soils.

In conclusion, in Europe the parasite is a strongly connected to oligotrophic soils, both acid and calcareous. In Flanders, the parasite was only found on vegetation characteristic to nitrogen-poor soils, with a $pH_{(KCl)}$ ranging from 2.9 to 7.7. Ammonium and functional nitrogen concentrations were found to be extremely low (Peeters, 2008; Rombouts, 2008). Furthermore, the parasite mainly occurs now on vegetation communities with a semi-natural character, with a long history of human intervention by traditional vegetation management. In Europe, these semi-natural vegetation types were once widespread in hilly calcareous and sandy areas, but the extent of these vegetation types has diminished considerably since 1850 due to the abandonment of the traditional management regime, afforestation and urbanisation (Webb, 1998; Butaye et al., 2005; Adriaens et al., 2006). The habitats with *C. epithymum* are also characterised by a lack of temperature extremes and by an abundant and well-distributed rainfall.

## Management

In summer 2007, all 28 Flemish sites containing *C. epithymum* populations (Fig. 2.4c) were either managed by the Agency Nature and Forest of the Ministry of Flemish Community or by the largest private non-governmental nature organisation Natuurpunt vzw, with exception of one where no management was applied (Peeters, 2008; Fig. 2.4c). Two of the 28 current Flemish *C. epithymum* populations are located in military areas and all present-day populations are situated in areas under protection of the European Habitat Directive and/or Bird Directive. Nevertheless, most sites are only recently managed as nature reserves, on average from 1985 (min: 1956; max: 2000). Compared to other European countries or regions, the sites containing *C. epithymum* populations are relatively small; the average ± SE area is 293 ± 117ha (median = 77ha), with only 10 larger than 100ha (min: 1ha; max: 3000ha), of which approximately 114.9 ± 52ha is heathland (both dry heathland and grass-heaths; min: 0ha; max: 1136ha). A range of management practices is carried out in the 28 sites on the patches containing *C. epithymum*. Mowing of the vegetation and turf-cutting are applied in the majority of the sites (57% and 29%, respectively), which is in accordance with the most important heathland management practices in the Netherlands and Great Britain (Williams, 2003). Cutting vegetation (e.g. removing of saplings; 18%), controlled burning (7%) and choppering (i.e. creating open ground by mowing into the ground with a brushcutter; 14%) are practiced on a lower proportion of the 28 sites. Grazing with small groups of cows and small sheep flocks used to be relatively common in Belgian heathlands until about 1950 (Burny, 1999). At present, grazing also seems an important heathland management practice. Grazing is applied as a management tool in 61% of the 28 surveyed sites, while grazing of the *C. epithymum* populations is allowed in only 12 sites (43% of all sites). In the 17 sites where grazing is applied, a range of domesticated animals is used for nature conservation benefits, namely cattle (47%, mainly Galloway), sheep (47%), horses (29%), donkeys (12%) and goats (6%).

Recommended stock densities are a maximum of one cow or horse per 5-6ha for year-round grazing and a maximum of five sheep per ha (Williams, 2003). For the cattle and horses similar stock densities were used in the 28 reserves (one cow/horse per 6ha), although the used stock density for sheep was considerable lower than the recommended density (1 sheep per 7ha) and mostly no year-round grazing was applied. On 13 sites, a combination of practices such as grazing and mowing is used on the patches with *C. epithymum* to achieve the desired results.

## Box 2.1: Parasitism

*Cuscuta epithymum* is an obligate holoparasite because, as mentioned earlier, it is largely dependent upon suitable plant hosts for carbohydrates, water, nutrients and physical support. Although its name suggests that it is a specialist mainly infecting host species from the genus *Thymus*, personal observations (n = 967, Appendix 2.1), specimens deposited in of the Belgian national herbarium (n = 305; Appendix 2.1), as well as literature (n = 13; Appendix 2.2) clearly revealed that *C. epithymum* is a generalist. During 2004-2008 *C. epithymum* was found attached with haustoria upon at least 67 different species from 31 families in the Flemish heathlands, dune grassland and calcareous grassland (Appendix 2.1). Another 12 and 8 additional host species were observed in herbarium samples from Flanders and the Walloon provinces, respectively (Appendix 2.1). *Cuscuta epithymum* is capable of using a wide range of hosts in Belgian habitats. Nevertheless, from our observations we can not conclude that all species on which haustoria were found are effective hosts. The parasite needs to develop fully differentiated and functional haustoria through which nutrient flow from host to parasite occurs (Christensen et al., 2003). Indeed, a successful contact and initial penetration of haustoria does not necessarily imply a successful subsequent parasitising process (Dawson et al., 1994; Christensen et al., 2003). Sometimes, *C. epithymum* twines around a species without penetrating the vascular system and only uses the plant as a support to a more suitable host (for grasses, pers. obs. and Costea Mihai, pers. comm.). Because we can not determine the functional viability of haustoria in the field, the number of recorded hosts may be exaggerated. During field observations we could not distinguish between primary hosts, i.e. plants on which the parasite establishes during the seedling stage, and secondary hosts, which are those species on which the parasite can infect only as an adult (Parker and Riches, 1993). In the field, *C. epithymum* is often observed to simultaneously infect different species (pers. obs.). According to Marvier (1998), a simultaneous attack of a mixture of host species may lead to an improved parasite performance, because different types and quantities of compounds are obtained from different hosts. However, results from Matthies (1996) and Svensson and Carlsson (2004) do not support this hypothesis. Our study was not designed to test the performance of *C. epithymum* while simultaneously parasitising multiple hosts.

Although the literature indicates that numerous parasitic plants can grow on a diverse range of hosts, many of them are known to show high levels of host preference as they typically develop well on only a subset of the available hosts (e.g. Pennings and Callaway, 1996). For instance, experiments with other *Cuscuta* spp. have proved the capacity of their seedlings to selectively choose the most nutritional hosts (Kelly 1990; 1992; Koch et al., 2004). Apparently, *Cuscuta* spp. can "forage" in plant communities by preferentially responding to the volatile cues produced by their favourite hosts (Runyon et al., 2006). Host specificity is believed to be related to both the abundance and diversity of potential hosts (Press and Phoenix, 2005; Thorogood and Hiscock, 2007). For *C. epithymum* there is, however, far less information on the degrees of preference of various hosts. Although we did not perform a research targeted to host selection, from our field observations we can, however, conclude that *C. epithymum* does not use all the potential hosts equally, and that it is locally host-specific. The five most frequently infected host species for Flemish heathlands, calcareous and dune grasslands are listed in Table B.2.1.1. While in heathland by far the most common host was *C. vulgaris*, the most infected plant species in calcareous and dune grasslands were *Centaurea jacea* and *Thalictrum minus* ssp. *dunense*, respectively (Table B.2.1.1). In heathland, *C. epithymum* consistently infected *C. vulgaris* (young individuals, see Chapter 3), *Erica cinerea* and *E. tetralix* where they occurred. However, *C. vulgaris* was by far the most abundant host species and was present in 98% of the investigated aggregations of *C. epithymum* stems. Other species, including *Molinia caerulea* and *Deschampsia flexuosa* were less infected in proportion to their abundance at the sites (since haustorial support in grasses is unknown, these species were probably only used as support). At the calcareous grassland, haustoria of *C. epithymum* were found on only 25 of the 45 plant species present in the vegetation. Furthermore, the mean levels of infection were lower on *Danthonia decumbens*, *Festuca filiformis* and *Plantago lanceolata* than on *Achillea millefolium*, *Centaura jacea* and *Lotus corniculatus*, even though these hosts were more abundant at this site (Fig. B.2.1.1).

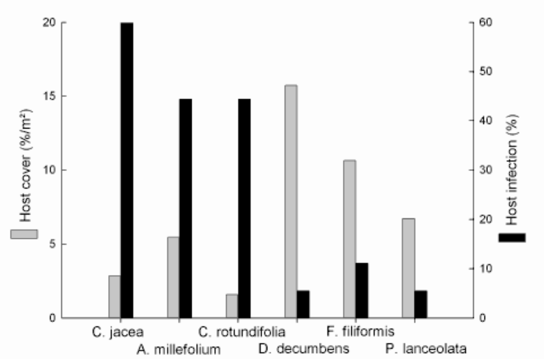


**Figure B.2.1.1.** Mean cover (%/m²) and infection level of the three most frequent infected (left) and the three most abundant plant species (right) in the investigated calcareous grassland (Sint-Pietersberg, Kanne). Infection level refers to the percentage of the C. epithymum aggregations in which this host was infected, relative to the total number of observed aggregations (18).

Thus, some hosts are used disproportionately to their abundance and host selection is not simply a reflection of host availability in the environment. In other words, host abundance does not always predict the prevalence of *C. epithymum*. A similar pattern has been observed with the parasite *C. costaricensis*, where 88% of the parasite cover grew on only 54% of available host cover (Kelly et al., 1988). Although it is difficult to determine the exact factors determining the observed host selection, several explanations may be proposed. Firstly, following the resource availability hypothesis, a particular host species may be ‘preferred’ simply because it is the most common component of the community (e.g. *C. vulgaris*). For these hosts, the probability of proximity is always high, which is an advantage for the parasitic plants. Second, hosts such as the legumes (Fabaceae), are less frequent, but are capable of fixing the atmospheric nitrogen and as a result they may be preferred because they are more nutritious (Svensson and Carlsson, 2004). In this case, the host quality is more important than host abundance. For *C. salina* it is shown that it is strongly affected by host nitrogen content (Pennings and Simpson, 2008). Furthermore, some annual plants may be more suitable hosts compared to perennial species due to the absence of secondary tissues (e.g. periderm) allowing an easier penetration of haustoria. Finally, some plant species are capable to oppose an active defense reaction against the invading haustoria and are thus systematically less infected. For example, *Gossypium hirsutum* and *Hibiscus rosa-sinensis* (Malvaceae) prevent the advance of haustoria by formation of wound tissue (Capderon et al., 1985; Sahm et al., 1995 in Albert et al., 2006).Young tomato (*Solanum lycopersicum*) plants are able to block and repel *Cuscuta* searching tissue by initiating a localised production of suberin barriers (Ihl et al., 1998 in Albert et al., 2006).

**Table B.2.1.1.** Five host species on which haustoria were most frequently found for heathland, calcareous grassland and dune grassland habitats.

| *Heathland (n = 944)* | | *Grassland (n = 18)* | | *Dunes (n = 15)* | |
|---|---|---|---|---|---|
| **Host (27)** | % | **Host (25)** | % | **Host (26)** | % |
| *Calluna vulgaris* | 97.0 | *Centaurea jacea* | 61.1 | *Thalictrum minus ssp. dunense* | 93.3 |
| *Molinia caerulea* | 10.2 | *Achillea millefolium* | 44.4 | *Galium verum* | 86.7 |
| *Carex pilulifera* | 4.3 | *Campanula rotundifolia* | 44.4 | *Achillea millefolium* | 80.0 |
| *Deschampsia flexuosa* | 4.2 | *Lotus corniculatus* | 27.8 | *Plantago lanceolata* | 80.0 |
| *Erica cinerea* | 3.6 | *Genista anglica* | 27.8 | *Lotus corniculatus* | 46.7 |

%: percentage of the *C. epithymum* aggregations in which this host was infected, relative to the total number of observed aggregations (= n). Per habitat type the total number of different host species is given between brackets.

The resistance of rice to *C. campestris* infestation was due to several layers of hard and thick walled sclerenchyma, which prevented the penetration of haustoria (Mishra et al., 2007). In stead of these mechanical protections, some host plants use chemical defense by producing phytoalexins or other inhibitors (e.g. Bringmann et al., 1999; Costea and Tardif, 2006).

We can conclude that *C. epithymum* is not specific in its host attachments because the parasite has been observed to form parasitic connections with a wide range of host species. It has, however, been demonstrated that the parasite has a degree of selectivity in the hosts it uses, so that some species are more frequently infected than others. As Pennings and Callaway (1996) proved for *C. salina*, host specifity has consequences for the structure and dynamics of natural vegetation assemblages (see before). However, more detailed research is needed to investigate host selection in *C. epithymum* and its potential impact on vegetation patterns.

## Phenology and life cycle

In Flanders, the germination of *C. epithymum* occurs in the spring, mainly during April and May, but sometimes as late as in July (pers. obs.; see Chapter 6). The first flower buds usually appear at the beginning of July and the first flowers start to open about two weeks later. Flowering prolongs then to the end of September. The first fruits are formed at the beginning of August and seed maturation occurs during the following two months. Flowering and fruiting extend throughout a large period of the growing season, due to the indeterminate growth pattern. The majority of the plant body dies by the end of October and usually falls from its host, although the parasite can overwinter as haustoria and regenerate in the following spring (see Chapter 6).

## Reproduction

*Cuscuta epithymum* is a hermaphrodite, having both male and female reproductive organs in the same flower. The species is able to reproduce sexually via cross- and self-pollination (Yuncker, 1921; Verdcourt, 1948). Even though *C. epithymum* flowers are visited by a large number of insects and are probably cross-pollinated (Müller (1883) in Yuncker, 1921; Verdcourt, 1948; Gómez, 1994; see Box 2.2), some authors believe that under natural conditions selfing is the rule (Verdcourt, 1948). *Cuscuta epithymum* growing in the Sierra Nevada shares pollinators with its host plant, *Hormathophylla spinosa* (Gómez, 1994).

Although each flower has four ovules, relatively high levels of seed abortion generally result in a limited number of seeds produced per fruit (Kuijt, 1969; pers. obs.). This was shown in our reproductive data by the relatively low numbers of seed set per flower (mean number of seeds produced per flower in the study area is 1.7 ± 0.07). Population size is found to substantially affect reproductive output, because individuals in small populations produced significantly fewer flowers per area unit than those in large populations. In contrast, population size did not significantly influence the number of seeds per fruit (Chapter 3b).

## Box 2.2: Pollination by insects

### Introduction

Many, if not all, plant species rely on interactions with other species for the completion of their life cycle. The most obvious example is the interaction that many flowering plant species have with specialist or generalist pollinating animal species (Kearns and Inouye, 1997; Brys et al., 2008; Forupt et al., 2008). For many flowering plants the contribution of insects to their pollination success is critical in ensuring a sufficient reproductive output. Without the presence of these pollinators, seed production is often shown to be low or even absent (e.g. Kearns and Inouye, 1997; Traveset and Sáez, 1997; Jacquemyn et al. 2005). Because pollinators tend to respond to the abundance of flowers, especially small and fragmented populations of insect-pollinated species are found to be sensitive to pollinator availability (e.g. Brys et al., 2004; 2008; Jacquemyn et al., 2005; Campell and Husband, 2007). For the endangered *C. epithymum*, little information is available concerning its pollinators and breeding system (Press and Graves, 1995; Costea and Tardif, 2006), and the impact of reduced population size on its reproductive output. This small study, therefore, focused on the insect-pollination biology of *C. epithymum* in Flemish heathlands.

### Methods

To investigate the composition and abundance of the pollinators visiting *C. epithymum* populations in Flemish dry heathland pollinator visits to its flowers were observed on three 1-m² plots in August 2008. Those plots were composed of young *Calluna vulgaris* individuals infected with *C. epithymum* and were situated in the nature reserve 'Mechelse heide' minimal 400 m apart from one another (Chapter 2). In March 2006 these plots were created by mowing late-successional heath as part of a dispersal-experiment (Box 5.1). Each plot was observed during 3 periods of 20 minutes on a sunny afternoon, when most pollinators are active (6th August, 30°C, no clouds). Within these three plots, the *C. epithymum* plants had a high flower density (> 450 flowers per 0.04m²) and thus provided the same signal to attract possible pollinators or offer the same rewards in terms of pollen and/or nectar. Because the main host, *C. vulgaris*, had not yet flowered at the time of these pollinator surveys, there was no competition between the species for potential pollinators. Every 20 minutes we recorded all the insects visiting *C. epithymum* flowers within the selected plots, and documented the type of insect (species), the visit frequency of each species (number of visits), the number of flowers probed per visit, and the visit time (duration of foraging). We observed the flowering plants for a total of 3 hours and only the insects that were obviously feeding in the *C. epithymum* flowers were recorded. Representative individuals of all flower visitors were collected for identification, at least to the level of family.

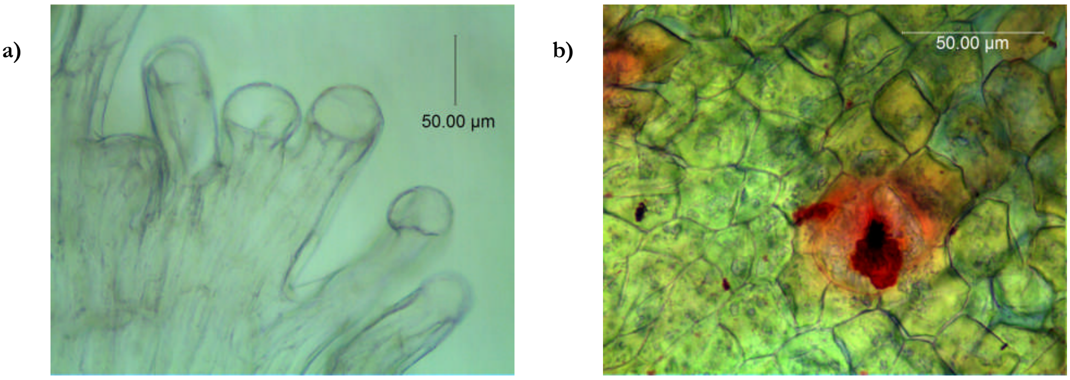


**Figure B.2.2.1.** Flower structures hypothesised to be involved in the attraction of pollinators by *C. epithymum*: **a)** Secretory cells in the *C. epithymum* infrastaminal scale **b)** Modified nectar-producing stomata at the base of *C. epithymum* ovary [Photos: Mihai Costea; from herbarium voucher 'Belgium, Flanders, Limburg province, Maasmechelen, August 2004, collected by K. Meulebrouck; see Costea, 2007-onwards].

## Results and discussion

Our observations demonstrate that *C. epithymum* flowers attract a large number of insects that may function as pollinators. Up to 121 insect visits were observed during the 3h of observation (Fig. B.2.2.2). Each of these floral visitors made visible contact with the anthers and/or stigmas during their visits. These observations may suggest that at least a part of the seed produced in these *C. epithymum* plots are the result of insect pollination. The functioning and dependence of the breeding system on insect pollinators is further suggested by the occurrence of nectar secreting floral structures in the flowers of *C. epithymum* (Fig. B.2.2.1; unpublished data by Costea M.). Similar observations of nectar secretion at the base of the *C. epithymum* ovary and of shrinking infrastaminal scales soon after fertilization for *C. reflexa* have also been made (Müller (1883) in Yuncker, 1921; Kuijt, 1969). With a diverse assemblage of potential pollinators, some species may be more effective pollinators than others.

Our results show that the insect types differ in the time that they spend per flower, number of visits, duration of foraging and the number of probed flowers per visit (Table B.2.2.1). Ohara and Higashi (1994) demonstrated for *Corydalis ambigua* that the visit time per flower rather than the visit frequency is a more critical component for the reproductive output of the visited flower. Pollinators staying longer at flowers are established to deposit more pollen grains, as a result of the increased chance of touching a stigma. Based on this information, our observations suggest that pollinators belonging to the Fanniidae might be good pollinators for *C. epithymum* (Tabel B.2.2.1). However, because the number of visits is generally accepted to provide an increased chance of reception of pollen from different individuals (Ohara and Higashi, 1994), Crabronidae should increase the chances for pollen export, promoting both self- and cross pollination (Table B.2.2.1).

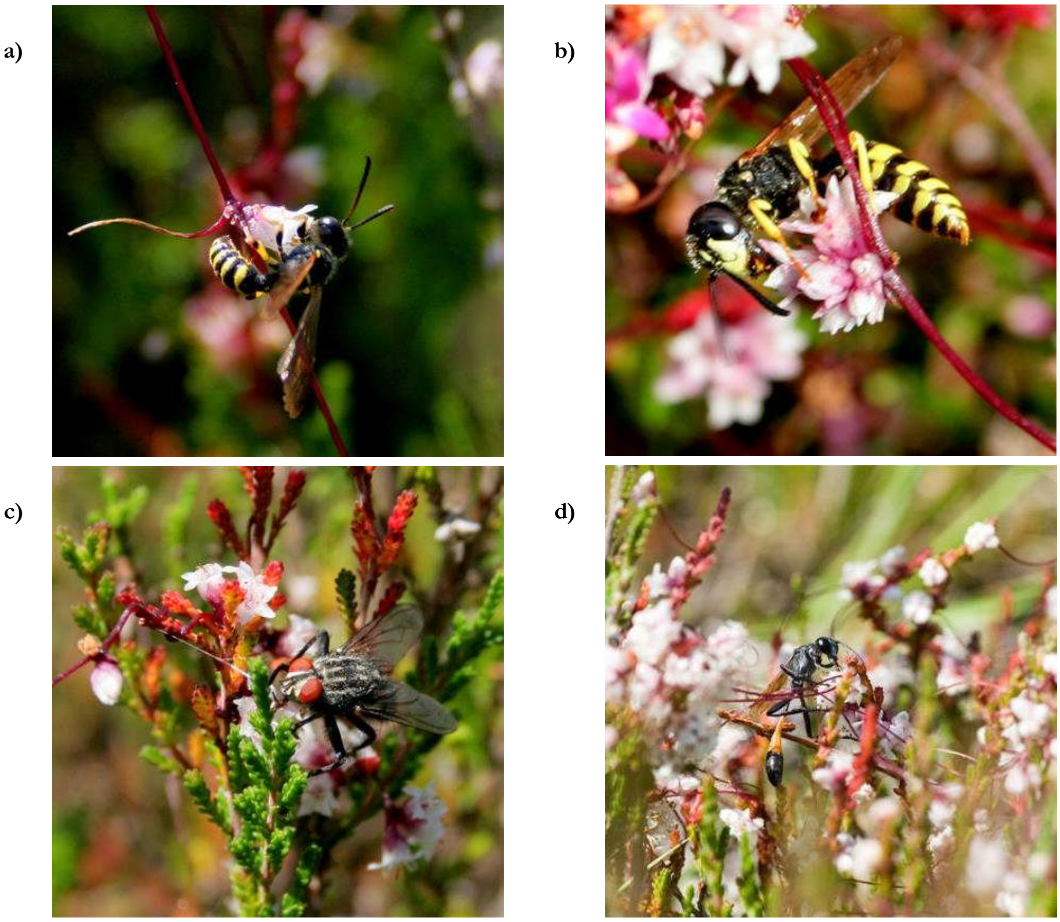


**Figure B.2.2.2.** Insect pollinators observed on the flowers of *C. epithymum*. **a)** + **b)** *Dolichovespula media*; **c)** *Sarcophaga carnaria*; **d)** *Ammophila sabulosa* [Pictures: **a)**, **b)** and **c)**: Meulebrouck K.; **d)**: Smits Jap].

**Table B.2.2.1.** Insect families which were observed feeding in *C. epithymum* flowers listed in decreasing order of importance determined by percentage of 121 visits made by each insect family. Further, differences in mean (± SE) time (seconds) spend per flower, number of visits per 20 min observation period, visit time and number of flowers probed per visit. Insects identified to species level and additional pollinators are represented below.

| *Family (Order)* | *Visits* | *Time/flower* | *Visits/20min* | *Visit time (s)* | *Flowers/visit* |
|---|---|---|---|---|---|
| Crabronidae (H)▪ | 35.5% | 6.7 ± 1.8 | 4.8 ± 1.5 | 23.0 ± 6.0 | 2.3 ± 0.7 |
| Sarcophagidae (D)* | 15.7% | 4.5 ± 1.7 | 2.1 ± 1.0 | 29.2 ± 8.6 | 2.4 ± 1.1 |
| Vespidae (H) ▪▪ | 15.7% | 3.5 ± 1.2 | 2.1 ± 1.0 | 93.3 ± 32.4 | 8.0 ± 3.3 |
| Tachinidae (D)** | 11.6% | 4.8 ± 1.6 | 1.6 ± 0.6 | 45.9 ± 17.3 | 1.6 ± 0.6 |
| Fanniidae (D) | 9.1% | 9.8 ± 3.2 | 1.2 ± 0.5 | 32.7 ± 17.8 | 1.7 ± 1.1 |
| Syrphidae (D)*** | 8.3% | 3.2 ± 1.5 | 1.1 ± 0.5 | 18.2 ± 8.7 | 1.2 ± 0.6 |
| Apidae (H) ‡ | 2.5% | 2.8 ± 1.5 | 0.3 ± 0.2 | 10.0 ± 0.0 | 0.4 ± 0.2 |
| Sphecidae (H) | 1.6% | 1.3 ± 0.9 | 0.2 ± 0.2 | 22.5 ± 7.5 | 1.0 ± 0.8 |

▪: *Philanthus triangulum*; ▪▪: *Dolichovespula media*; *: *Sarcophaga carnaria*; **: *Eriothrix rufomaculata*; ***: *Ammophila sabulosa*, *Ammophila probescens*; ‡: *Apis mellifera*; Additional observed pollinators during the period of research (2006-2008): *Episyron rufipes* (Pompilidae); *Polyommatus icarus* (Lycaenidae); *Formica pratensis* (Formicidae). H: Hymenoptera (here bees and wasps); D: Diptera (here only flies).

From the literature it is known that *Cuscuta* species can be self-pollinated (Verdcourt, 1948; for *C. obtusiflora* Rodrigurez-Pontes, 2008). Considering our findings and previous documented results, we may conclude that *C. epithymum* is neither an exclusive self-pollinator nor a pure outcrosser, but is probably a species that shows a mixed mating system, in which floral traits promote outcrossing, but allow self-pollinating.

More empirical evidence is needed in order to obtain a clearer view of the pollinator assemblage of *C. epithymum*, the functioning of its breeding system, the impact of population size, and the implications of self- and cross-pollination on seed production rates and fitness of the progeny.

## Seed germination and seed dispersal

For *C. epithymum* occurring in Flanders a thorough discussion of seed germination and dispersal is presented in the Chapters 4 and 5. In this section, a summary of seed dispersal is given for *Cuscuta* species, based on the review of Costea and Tardif (2006).

Seed dispersal in the genus *Cuscuta* is considered to be “unspecialised” (Kuijt, 1969). There is almost no information available concerning “natural” means of seed dispersal. Although *Cuscuta* seeds do not possess adaptations for wind dispersal, the pits in the seed coat may suggest possibilities for wind dispersal. The light weighted capsule, wrapped in papery withered flower parts is easily blown away at short distances (pers. obs.). Further, endozoochory could play a role in the dispersal of some species. Kuijt (1969) mentioned that seeds remain viable while passing through the digestive system of sheep. Nevertheless, Gómez (1994) reported that hosts infected by *C. epithymum* were avoided by the main herbivore in the area. Since the seed coat becomes gelatinous when wetted (pers. obs. for *C. epithymum*), seeds may also be dispersed by epizoochory or even anthropochory (e.g. by means of farm machinery). Finally, hydrochory is suggested for some *Cuscuta* species, as their seeds or the capsules containing the seeds are capable of floating (Verdcourt, 1948; Prather and Tyrl, 1993). These species frequently occur near water, e.g. *C. europaea* (Verdcourt, 1948). The dispersal methods of any *Cuscuta* species have not previously been studied in detail. The principal means of world-wide dispersal *Cuscuta* species has been through contamination of crop seed (see above).

## Conservation status of the species

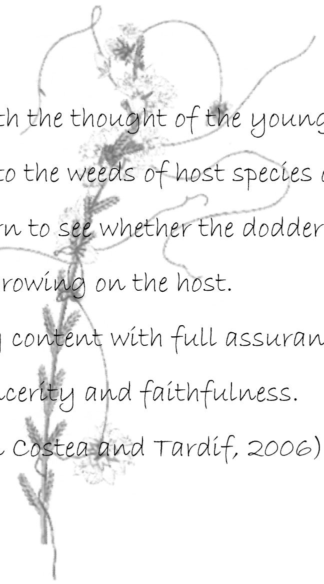
Although only relatively few species attack crops (Dawson et al., 1994; Parker and Riches, 1993), *Cuscuta* is mainly renowned as a group of detrimental parasitic weeds. Some *Cuscuta* species, mainly *C. campestris*, can indeed cause substantial damage by affecting crop production, particularly in the subtropics and Mediterranean countries, but also in North America and Central Europe (Takabayashi et al., 1981; Parker and Riches, 1993; Dawson et al., 1994). Although recent observations in Europe are lacking, *C. epithymum* was also mentioned in the past as a locally troublesome pest of alfalfa and clover crops (Engelmann, 1887; Laurent, 1901; Stewart and French, 1909; Anthon, 1913; Henrard et al., 1942; Verdcourt, 1948). Nevertheless, it is less known that numerous *Cuscuta* species are endangered or even threatened with extinction (Costea et al., 2006). From 1850 onwards, *C. epithymum* has become increasingly rare within its natural range (e.g. Berten, 1990). The loss of its habitat has caused a strong decline of *C. epithymum* populations since the beginning of the 20th century (Preston et al., 2002; see 'heathland'). Consequently, the plant is categorised as 'endangered' on the recent Red List of Flemish phanerogamic plants (van Landuyt et al., 2006). Likewise, in the rest of north-western Europe the parasite is strongly declining (e.g. The Netherlands: Weeda et al., 1988; Schaminée et al., 1996; Ireland: Doyle, 1993; the United Kingdom: Cheffings and Farrel, 2005; France: Guillaume Decocq, pers. comm.).

## Etymology

The etymology of *Cuscuta* is thought to be Aramaic and/or Hebrew deriving from K-S-W-T (in Hebrew) and K-S-W-T-A (in Aramaic) meaning "cover" or "clothing" (Costea and Tardif, 2004). The origin of the English name "dodder" is more obscure, but may be from an old German word "dotter" used to describe the yolk of an egg (Costea and Tardif, 2004).

The rapid development of *C. epithymum* and its stranglehold on hosts have earned it a place in vernacular names, such as the German 'teufelsswirn', the Dutch 'duivelsnaaigaren', the French 'cheveux de Venus' and the English 'strangle vine', 'devil's gut' and 'witches hair'. It was believed to have contraceptive effects and was also called "woman without children" by Paiute Indians (Costea and Tardif, 2004). A commonly used synonym of *C. epithymum* is *C.*

*trifolii* (e.g. Laurent, 1901; Yuncker, 1932; Henrard, 1942). This name is used for *C. epithymum* individuals infecting clover or alfalfa fields.



> A girl having plucked a vine, with the thought of the young man in mind
> tossed the vine over her shoulder, into the weeds of host species of this dodder [..].
> The second day after, she would return to see whether the dodder had attached itself
> and was growing on the host.
> If so, she went away content with full assurance
> of her lover's sincerity and faithfulness.
> (Gilmore, 1914 in Costea and Tardif, 2006)

# Appendix

**Appendix 2.1.** Species and family to which *C. epithymum* haustorial attachment has been observed during field observations (PO: personal observation, including the data of 2 thesisstudents (Peeters, 2008; Rombouts, 2008)) in populations belonging to heathland (H), calcareous grassland (G) and dune grassland (D) and in herbarium samples of *C. epithymum* individuals found in Flanders (F) and in the Walloon provinces (W) from the Belgian National Herbarium (HB) (n = number of investigated *C. epithymum* aggregations). Host species are classified in four groups: (1) forbs; (2) graminoids including all grasses and grasslike plants, like sedges and rushes; (3) legumes, which are herbaceous species of the Fabaceae and (4) woody species. Number of host species is given between brackets. Nomenclature follows Lambinon et al. (1998). X refers to the presence of *C. epithymum* infection on that particular species.

| **Groups** | **Species (87)** | **Family (31)** | **PO (n = 967) (67)** | **HB (n = 180/125)** | |
|---|---|---|---|---|---|
| | | | | **F (27)** | **W (28)** |
| **Forbs (43)** | | | | | |
| | *Achillea millefolium* | Asteraceae | X (D/G) | X | X |
| | *Agrimonia eupatoria* | Rosaceae | | X | |
| | *Asparagus officinalis* | Liliaceae | X (D) | | |
| | *Campanula rotundifolia* | Campanulaceae | X (G) | | |
| | *Centaurea jacea* | Asteraceae | X (G) | X | X |
| | *Cerastium fontanum ssp. vulgare* | Caryophyllaceae | X (D) | | |
| | *Convolvulus arvensis* | Convolvulaceae | | X | |
| | *Daucus carota* | Apiaceae | X (G) | | X |
| | *Diplotaxis spp.* | Brassicaceae | X (D) | | |
| | *Galium saxatile* | Rubiaceae | X (H) | X | X |
| | *G. pumilum* | Rubiaceae | | | X |
| | *G. verum* | Rubiaceae | X (D) | X | |
| | *Helianthemum nummularium* | Cistaceae | X (G) | | |
| | *Hieracium pilosella* | Asteraceae | X (G) | | |
| | *H. umbellatum* | Asteraceae | X (D/G/H) | | |
| | *Hypericum perforatum* | Hypericaceae | X (G/H) | | |
| | *H. pulchrum* | Hypericaceae | | | X |
| | *Hypochoeris radicata* | Asteraceae | X (D) | | |
| | *Jasione montana* | Campanulaceae | | X | |
| | *Knautia arvensis* | Dipsacaceae | | X | |
| | *Leontodon saxatilis* | Asteraceae | X (D) | | |
| | *Linum usitatissimum* | Linaceae | | | X |
| | *Lysimachia nummularia* | Primulaceae | | | X |
| | *Oenothera spp.* | Onagraceae | X (D) | | |
| | *Pimpinella saxifraga* | Apiaceae | X (G) | | |
| | *Plantago lanceolata* | Plantaginaceae | X (D/G) | X | X |
| | *Potentilla erecta* | Rosaceae | X (G) | | |
| | *Ranunculus repens* | Ranunculaceae | X (G) | | |
| | *Rhinanthus minor* | Scrophulariaceae | X (D) | | X |
| | *Rumex acetosella* | Polygonaceae | X (H) | X | |
| | *Sanguisorba minor* | Rosaceae | X (G) | | X |
| | *Scabiosa columbaria* | Dipsacaceae | | | X |
| | *Senecio jacobaea* | Asteraceae | X (D) | | |
| | *Sinapis arvensis* | Brassicaceae | | X | |
| | *Sisymbrium altissimum* | Brassicaceae | | X | |
| | *Stachys officinalis* | Lamiaceae | X (G) | | |
| | *Succisa pratensis* | Dipsacaceae | X (G) | | |
| | *Teucrium chamaedrys* | Lamiaceae | | X | |
| | *T. scorodonia* | Lamiaceae | X (H) | X | X |
| | *Thalictrum minus ssp. dunense* | Ranunculaceae | X (D) | | |
| | *Thymus pulegioides* | Lamiaceae | X (D) | | |
| | *T. serpyllum* | Lamiaceae | | | X |
| | *Tragopogon pratensis* | Asteraceae | X (D) | | |

| Groeps | Species | Family | PO | HB | |
|---|---|---|---|---|---|
| | | | | F | W |
| **Graminoids (16)** | | | | | |
| | *Agrostis capillaris* | Poaceae | X (G/H) | | |
| | *Ammophila arenaria* | Poaceae | X (D) | | |
| | *Brachypodium pinnatum* | Poaceae | | | X |
| | *Carex arenaria* | Cyperaceae | X (D/H) | | |
| | *C. demissa* | Cyperaceae | X (H) | | |
| | *C. panicea* | Cyperaceae | X (D) | | |
| | *C. pilulifera* | Cyperaceae | X (H) | | |
| | *Cynosurus cristatus* | Poaceae | X (G) | | |
| | *Deschampsia flexuosa* | Poaceae | X (H) | X | |
| | *Elymus repens* | Poaceae | X (D) | | |
| | *Festuca filiformis* | Poaceae | X (G/H) | | |
| | *Juncus squarrosus* | Juncaceae | X (H) | | |
| | *Luzula multiflora* | Juncaceae | | X | |
| | *Molinia caerulea* | Poaceae | X (H) | | |
| | *Phleum bertolonii* | Poaceae | X (D) | | |
| | *Sieglingia decumbens* | Poaceae | X (G) | | |
| **Legumes (7)** | | | | | |
| | *Lotus corniculatus ssp. corniculatus* | Fabaceae | X (D/G) | X | X |
| | *Medicago lupulina* | Fabaceae | | X | X |
| | *M. sativa* | Fabaceae | | X | X |
| | *Ononis repens ssp. repens* | Fabaceae | X (D) | | |
| | *Trifolium campestre* | Fabaceae | | | X |
| | *T. pratense* | Fabaceae | X (G) | X | X |
| | *T. repens* | Fabaceae | X (G) | | |
| **Shrubs + Tree species (21)** | | | | | |
| | *Acer pseudoplatanus* | Aceraceae | X (H) | | |
| | *Betula pendula* | Betulaceae | X (H) | | |
| | *Calluna vulgaris* | Ericaceae | X (H) | X | X |
| | *Crataegus monogyna* | Malaceae | X (G) | | |
| | *Erica cinerea* | Ericaceae | X (H) | X | |
| | *E. tetralix* | Ericaceae | X (H) | X | |
| | *Frangula alnus* | Rhamnaceae | X (H) | | |
| | *Genista anglica* | Fabaceae | X (G/H) | X | |
| | *G. pilosa* | Fabaceae | X (G/H) | X | X |
| | *G. tinctoria* | Fabaceae | | X | X |
| | *Genistella sagittalis* | Fabaceae | | X | X |
| | *Hippophae rhamnoides* | Elaeagnaceae | X (D) | | |
| | *Pinus sylvestris* | Pinaceae | X (H) | | |
| | *Prunus spinosa* | Amygdalaceae | X (G) | | |
| | *Quercus robur* | Fagaceae | X (H) | | |
| | *Q. rubra* | Fagaceae | X (H) | | |
| | *Rosa canina* | Rosaceae | X (D) | | |
| | *R. rugosa* | Rosaceae | X (D) | | |
| | *Rubus spp.* | Rosaceae | X (D/H) | | X |
| | *Sarothamnus scoparius* | Fabaceae | X (H) | X | X |
| | *Vaccinium myrtillus* | Ericaceae | X (H) | | |

**Appendix 2.2:** Species which were infected by *C. epithymum* mentioned in various references. The host species are categorised in four groups: (1) forbs; (2) graminoids including all grasses and grasslike plants, like sedges and rushes; (3) legumes representing herbaceous species of the Fabaceae and (4) woody species. Per reference, for each group, the number of different host species is given between brackets. If known, the country in which the observations are made is given under the reference. Nomenclature follows Lambinon et al. (1998).

| Author | Species |
|---|---|
| Laurent (1901)<br>Belgium | **Forbs (6)**: *Achillea millefolium*, *Euphorbia cyparissias*, *Galium verum*, *Origanum vulgare*, *Teucrium scorodonia*, *Thymus serpyllum*<br>**Legumes (4)**: *Medicago sativa*, *Trifolium hybridum*, *T. repens*, *T. pratense*<br>**Shrubs (2)**: *Sarothamnus scoparius*, *Genista tinctoria* |
| Stewart and French (1909)<br>United States | **Forbs (2)**: *Erigeron annuus*, *Taraxacum* spp.<br>**Legumes (3)**: *Medicago lupulina*, *M. sativa*, *Trifolium pratense* |
| Nevue (1913)<br>France | **Legumes (3)**: *Lotus corniculatus*, *Trifolium* spp., *Vicia* spp.<br>**Shrubs (2)**: *Sarothamnus scoparius*, *Ulex europaeus* |
| Johansson (1914)<br>Sweden | **Forbs (55)**: *Achillea millefolium*, *Alchemilla pubescens*, *Allium vineale*, *Arabis hirsuta*, *Arenaria serpyllifolia*, *Armeria maritima*, *Artemisia absinthium*, *Campanula rotundifolia*, *Carum carvi*, *Centaurea jacea*, *C. scabiosa*, *Cerastium tomentosum*, *Chrysanthemum leucanthemum*, *Cirsium acaule*, *Convolvulus arvensis*, *Daucus carota*, *Echium vulgare*, *Filipendula vulgaris*, *F. ulmaria*, *Fragaria viridis*, *Galium boreale*, *G. verum*, *Gnaphalium sylvaticum*, *Gypsophila fastigiata*, *Helianthemum nummularium*, *Hieracium pilosella*, *H. umbellatum*, *Hypericum perforatum*, *Inula salicina*, *Knautia arvensis*, *Leontodon autumnalis*, *Linaria vulgaris*, *Pimpinella saxifraga*, *Plantago lanceolata*, *Polygala vulgaris*, *Polygonum aviculare*, *P. convolvulus*, *Potentilla argentea*, *P. reptans*, *Ranunculus bulbosus*, *Rumex acetosa*, *R. acetosella*, *Scabiosa columbaria*, *Sedum acre*, *S. album*, *Selinum carvifolia*, *Silene nutans*, *S. vulgaris*, *Stellaria graminea*, *Succisa pratensis*, *Taraxacum officinale*, *Thymus serpyllum*, *Urtica dioica*, *Veronica chamaedrys*, *Viola canina*<br>**Graminoids (15)**: *Agrostis capillaris*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Avena pratensis*, *A. pubescens*, *Briza media*, *Carex flacca*, *Dactylis glomerata*, *Elymus repens*, *Festuca ovina*, *Luzula pilosa*, *Melica ciliata*, *Phleum pratense*, *Poa compressa*, *P. pratensis*<br>**Legumes (12)**: *Anthyllis vulneraria*, *Lathyrus pratensis*, *Lotus corniculatus*, *Medicago falcata*, *M. lupulina*, *Ononis repens*, *Tetragonolobus maritimus*, *Trifolium arvense*, *T. campestre*, *T. pratense*, *T. repens*, *Vicia cracca*<br>**Shrubs + Trees (7)**: *Calluna vulgaris*, *Juniperus communis*, *Malus sylvestris*, *Pinus sylvestris*, *Prunus spinosa*, *Rhamnus catharticus*, *Rosa rubiginosa* |
| Johansson (1918)<br>Sweden | **Forbs (10)**: *Agrimonia eupatoria*, *Campanula persicifolia*, *Geranium sanguineum*, *Hypochoeris maculata*, *Linum catharticum*, *Plantago maritima*, *Primula veris*, *Pulsatilla pratensis*, *Ranunculus acris*, *Rhinanthus major*<br>**Graminoids (3)**: *Festuca arundinacea*, *Sesleria caerulea*, *Sieglingia decumbens*<br>**Legumes (1)**: *Trifolium medium* |
| Henrard et al. (1942)<br>The Netherlands | **Forbs (25)**: *Achillea milllefolium*, *Cenaurea jacea*, *Chrysanthemum* spp., *Convolvulus arvensis*, *Daucus carota*, *Euphorbia seguieriana*, *Euphrasia* spp., *Galium mollugo*, *G. saxatile*, *G. verum*, *Hieracium umbellatum*, *Knautia arvensis*, *Origanum vulgare*, *Phleum arenarium*, *Plantago lanceolata*, *Polygala serpyllifolia*, *Potentilla erecta*, *Ranunculus flammula*, *Sanguisorba minor*, *Senecio jacobaea*, *Succisa pratensis*, *Teucrium scorodonia*, *Thymus serpyllum*, *Verbena officinalis*, *Veronica officinalis*<br>**Graminoids (3)**: *Agrostis capillaris*<br>**Legumes (9)**: *Anthyllis vulneraria*, *Lotus corniculatus*, *Medicago lupulina*, *M. sativa*, *Ononis* spp., *Ornithopus perpusillus*, *Trifolium arvense*, *T. pratense*, *T. repens*<br>**Shrubs + Trees (9)**: *Calluna vulgaris*, *Sarothamnus scoparius*, *Erica tetralix*, *Genista anglica*, *G. pilosa*, *Pinus sylvestris*, *Rosa* spp., *Salix repens*, *Vaccinium myrtillus* |

| Author | Species |
|---|---|
| Duvigneaud (1945)<br>Belgium | **Forbs (3)**: *Centaurea jacea*, *Meum athamanticum*, *Sanguisorba officinalis*<br>**Legumes (3)**: *Lathyrus linifolius*, *Lotus corniculatus*, *Trifolium* spp.<br>**Shrubs (7)**: *Calluna vulgaris*, *Sarothamnus scoparius*, *Genista anglica*, *G. pilosa*, *G. tinctoria*, *Genistella sagittalis*, *Ulex* spp. |
| Gaertner (1950) | **Forbs (83)**: *Achillea millefolium*, *Agrimonia eupatoria*, *A. parviflora*, *Amaranthus blitum*, *Allium vineale*, *Antirrhinum* spp., *Arabis hirsuta*, *Arenaria serpyllifolia*, *Artemisia absinthium*, *Bellis perennis*, *Calamintha clinopodium*, *Campanula rotundifolia*, *Carduus crispus*, *C. defloratus*, *Carum carvi*, *Centaurea jacea*, *C. scabiosa*, *Cerastium glomeratum*, *C. fontanum*, *Cirsium acaule*, *C. arvense*, *C. vulgaris*, *Coriandrum sativum*, *Convolvulus arvensis*, *C. cantabricus*, *Crepis capillaris*, *Daucus carota*, *Echium vulgare*, *Erigeron acer*, *E. annuus*, *Equisetum arvense*, *Euphrasia nemorosa*, *E. stricta*, *Foeniculum vulgare*, *Fragaria viridis*, *F. vesca*, *Galium boreale*, *G. mollugo*, *G. verum*, *Geranium dissectum*, *Gymnocarpium robertianum*, *Gypsophila fastigiata*, *Hieracium pilosella*, *H. umbellatum*, *Hypericum perforatum*, *Leontodon autumnalis*, *Linaria vulgaris*, *Linum catharticum*, *Matricaria recutita*, *Melandrium album*, *Origanum vulgare*, *Pimpinella anisum*, *P. saxifraga*, *Phlox* spp., *Polygala vulgaris*, *Polygonum aviculare*, *P. convolvulus*, *Potentilla argentea*, *P. reptans*, *Prunella vulgaris*, *Ranunculus arvensis*, *R. bulbosus*, *R. repens*, *Rumex acetosa*, *R. acetosella*, *Sedum acre*, *S. album*, *Selinum carvifolia*, *Senecio jacobaea*, *Sherardia arvensis*, *Silene nutans*, *Solanum tuberosum*, *Stellaria graminea*, *Succisa pratensis*, *Taraxacum officinale*, *Teucrium montanum*, *Thymus serpyllum*, *Thymus* spp., *Urtica dioica*, *U. urens*, *Veronica chamaedrys*, *Viola canina*, *Vitis vinifera*<br>**Graminoids (19)**: *Agrostis canina*, *A. alba*, *A. gigantea*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Avena pubescens*, *Avenula pratensis*, *Briza media*, *Carex flacca*, *Dactylis glomerata*, *Festuca pratensis*, *F. ovina*, *Lolium* spp., *Luzula pilosa*, *Phleum pratense*, *Poa compressa*, *P. pratensis*, *Triticum repens*, *Zea mays*<br>**Legumes (15)**: *Anthyllis vulneraria*, *Lathyrus pratensis*, *Lotus corniculatus*, *Medicago falcata*, *M. lupulina*, *Ononis repens*, *Ornithopus sativus*, *Phaseolus vulgaris*, *Tetragonolobus maritimus*, *Trifolium arvense*, *T. campestre*, *T. hybridum*, *T. pratense*, *T. repens*, *Vicia cracca*<br>**Shrubs (11)**: *Calluna vulgaris*, *Crataegus laevigata*, *Cucubalus baccifer*, *Erica* spp., *Genista* spp., *Juniperus communis*, *Malus sylvestris*, *Pinus sylvestris*, *Prunus spinosa*, *Rosa rubiginosa*, *Viburnum lantana* |
| Dean (1954) | **Forbs (2)**: *Erigeron strigosus*, *Taraxacum officinale*<br>**Legumes (2)**: *Medicago sativa*, *Trifolium dubium* |
| Dijkstra (1968)<br>The Netherlands | **Forbs (1)**: *Thymus serpyllum*<br>**Shrubs (4)**: *Calluna vulgaris*, *Sarothamnus scoparius*, *Erica tetralix*, *Genista pilosa* |
| Weeda et al. (1988)<br>The Netherlands | **Forbs (2)**: *Galium verum*, *Thymus pulegioides*<br>**Legumes (3)**: *Medicago sativa*, *Lotus corniculatus*, *Trifolium pratense*<br>**Shrubs (1)**: *Calluna vulgaris* |
| Doyle (1993)<br>Ireland | **Forbs (30)**: *Achillea millefolium*, *Antennaria dioica*, *Asperula cynanchica*, *Asplenium ruta-muraria*, *Bellis perennis*, *Blackstonia perfoliata*, *Campanula rotundifolia*, *Centaurea nigra*, *Cerastium fontanum*, *Daucus carota*, *Dryas octopetala*, *Eryngium maritimum*, *Euphrasia salisburgensis*, *Galium verum*, *Geranium sanguineum*, *Hieracium pilosella*, *Hypochoeris radicata*, *Leucanthemum vulgare*, *Minuartia verna*, *Plantago lanceolata*, *Polygala vulgaris*, *Potentilla erecta*, *Ranunculus bulbosus*, *Rhinanthus minor*, *Senecio jacobaea*, *Succisa pratensis*, *Taraxacum officinale*, *Teucrium scorodonia*, *Thymus praecox*, *Viola* spp.<br>**Graminoids (4)**: *Briza media*, *Festuca rubra*, *Koeleria macrantha*, *Luzula campestris*<br>**Legumes (7)**: *Anthyllis vulneraria*, *Lathyrus pratensis*, *Lotus corniculatus*, *Medicago lupulina*, *Ononis repens*, *Trifolium pratense*, *Trifolium* spp.<br>**Shrubs (6)**: *Calluna vulgaris*, *Erica mackaiana*, *E. tetralix*, *Rosa pimpinellifolia*, *Prunus spinosa*, *Ulex gallii* |
| Gómez (1994)<br>Spain | **Forbs (6)**: *Alyssum purpureum*, *Arenaria tetraquetra*, *Campanula hermenii*, *Carduus carlinoides*, *Eryngium glaciale*, *Thymus serpyllum*<br>**Shrubs (2)**: *Genista versicolor*, *Hormathophylla spinosa* |

# 3

# Distribution and dynamics of *Cuscuta epithymum*

Photo: Klaar Meulebrouck (Ziepbeekvallei, 2008)

This chapter is divided into two parts. The first part throws more light on the factors affecting the distribution patterns of *C. epithymum* in a one-year study, paying special attention to the role of persistent seed banks. In the second part of this chapter, the effects of landscape dynamics on *C. epithymum* population dynamics are investigated in a longer-term study of 4 years. Both parts deal with the same populations, but focus on different time scales (1 vs. 4 years). The subjects discussed in both parts are therefore inextricably bound up with eachother.

**Photo: Klaar Meulebrouck (Mechelse Heide, 2008)**

# 3a. Local and regional factors affecting the distribution of *Cuscuta epithymum*

Adapted from: Meulebrouck K., Ameloot E., Verheyen K. and Hermy M. 2007 – Biological Conservation 140: 8-18.

## Introduction

Research has stressed the importance of processes operating at a regional scale in explaining species' distribution, including the concept of metapopulations (Hanski and Gilpin, 1997). Metapopulations are seen as systems of local populations persisting through a balance between local (within-patch) extinctions and (re)colonizations of patches by migrants (dispersing individuals) from occupied patches (Hanski and Gilpin, 1997). Metapopulation theory predicts that the survival of these populations largely depends on the amount (area-related extinction rate) and spatial arrangement of suitable patches (isolation-related colonisation rate) (Hanski and Gilpin, 1997; but see Pellet et al., 2007). Only a fraction of suitable habitat patches is expected to be occupied, because species continually become extinct locally and the dispersal ability of most plant species is expected to be limited (Turnbull et al., 2000). Finally, temporal landscape dynamics, characteristic of many human-dominated landscapes, have an important influence on the viability of certain metapopulations (e.g. Verheyen et al., 2004; Snäll et al., 2005).

As well as these regional processes, local environmental features such as soil characteristics are often stated as the predominant drivers for species distribution (Whittaker, 1967; Roem and Berendse, 2000). Parasitic plants in particular may show a response to changing environmental conditions. Frost et al. (2003), for example, showed for *Cuscuta salina*, that abiotic factors, such as different salinity regimes, can influence parasitic plant's performance, whereas biotic factors, including host availability, host quality (Koch et al., 2004) and host preference (Kelly, 1992), have also been shown to affect the parasite's distribution.

In this study we investigate the effect of local and regional conditions on *C. epithymum*'s distribution. In most of western Europe, this holoparasitic plant is mainly limited to recently-managed heath patches, which are still in an early stage of succession (Schaminée et al., 1996). Not only in Belgium, but also in other countries such as the United Kingdom (Cheffings and Farrell, 2005) and the Netherlands (Weeda et al., 1988), there has been a rapid decline of this habitat and in this species. A better understanding of factors determining the distribution pattern of this species may also provide some general insights into pioneer species, both plants and animals, frequently overlooked in heathland studies. Indeed, most studies until now have focused on the dominant species of dry heath vegetation (e.g. *Calluna vulgaris* in Chapman, 1967; Calvo et al., 2002; Nilsen et al., 2005; but see Oostermeijer et al. for *Gentiana pneumonanthe* in wet heathland, 1998 and Nordbakken et al., 2004 for *Drosera* spp. in peatland). Furthermore, the fact that parasitic plants may play an important role in determining the structure and dynamics of communities, as demonstrated by the study of *C. salina* in a southern California salt marsh (Pennings and Callaway, 1996) and *Rhinanthus angustifolius* in a Dutch semi-natural grassland (Ameloot et al., 2006), makes it an interesting study species.

If *C. epithymum* functions as a metapopulation inhabiting a dynamic heathland, we expect isolation, patch size and patch age to be the overriding factors determining its distribution. However, if mainly local environmental features explain the observed patchy distribution, there should be detectable differences in local conditions between occupied and unoccupied patches. In this paper we report how regional and local environmental factors affect the distribution and abundance of *C. epithymum*. Its distribution in heathland was analysed in more than 160 locations within four heathland reserves. To our knowledge, the current study is the first empirical study simultaneously testing effects of local and regional factors on the distribution patterns of a parasitic plant. From the viewpoint of conservation and management, the results of this study may allow predictions of the effects of management on the parasite and other species with similar life cycles.

## Materials and methods

### *Study sites*

During August 2004, we investigated *C. epithymum* populations in four nature reserves (Heiderbos, De Maten, Mechelse Heide and Ziepbeekvallei) located in the north-east of Flanders (Belgium) in the province of Limburg at ca. 91 m above sea level (Fig. 3.1, Table 3.1a). Before 1850, these reserves used to be part of a huge, continuous heath occupying two thirds of Limburg (Allemeersch et al., 1988). During the past few decades heathland has become severely fragmented (see Chapter 1), and the study reserves are now isolated heathland fragments (Fig. 3.1). All reserves are predominantly covered by dry heathland vegetation typically dominated by *C. vulgaris* with *Erica tetralix*, *Molinia caerulea*, *Deschampsia flexuosa* and/or *Erica cinerea* making up a smaller part of the vegetation. The soils are sandy and strongly podsolised. In each reserve, management aims at restoring and conserving the heath vegetation; it includes mowing, and in some cases, burning, turf-cutting and grazing. So far, no specific management actions have been taken for the conservation of *C. epithymum*.

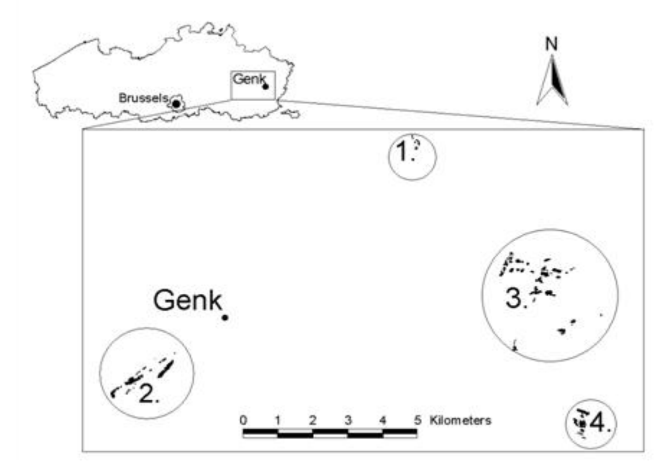


**Figure 3.1.** Location of the 166 management patches in the four study sites in Flanders (Belgium). 1 = 'Heiderbos', 2 = 'De Maten', 3 = 'Mechelse Heide', 4 = 'Ziepbeekvallei'. Distance between the study sites ranges between 2.4 km (between Mechelse Heide and Ziepbeekvallei) and 12. 4 km (between Ziepbeekvallei and De Maten).

### *Recording Cuscuta epithymum and vegetation*

Within the four heathland study sites (reserves), 166 management patches, assumed to be suitable for *C. epithymum*, were surveyed in August 2004. Management patches are recently-managed patches with a very open to closed young dwarf shrub vegetation and a canopy height ranging from 5 to 40cm. Because of the often abrupt transitions between these management patches and the surrounding unsuitable, older heathland matrix, these patches were generally easily recognisable in the field.

In each management patch, the presence/absence of all phanerogamic plant species and *C. epithymum* were recorded. Additionally, in each patch a variable number (ranging from 2 to 10 in proportion to the patch area) of randomly located 1m × 1m plots was established in which (1) the cover of the phanerogamic plants species, bryophytes, lichens and bare ground (i.e. area not covered by live vegetation) was estimated visually using a percentage scale and (2) the mean canopy height was assessed as the average of 5 randomly chosen points per 1m² plot. If *C. epithymum* occurred in the patch, an equal number of plots within the management patch with and without the parasite were investigated using a stratified random method. In total 461 plots were surveyed. The number of *C. epithymum* aggregations (aggregations of red threads the parasite forms in the vegetation), the total area (m²) covered by these aggregations (*s*) and the cover (%) of the *C. epithymum* threads in a 1m² plot within these aggregations were recorded as a measure for population size per management patch. The latter variable was measured because the *C. epithymum* thread density may drastically vary on a small scale. The

absolute abundance of *C. epithymum* per management patch was then determined as:
$$\sum\left(\frac{s_i \times \%\text{cover}_i}{100}\right),$$

where *i* represents an aggregation within the management patch. In each *C. epithymum* aggregation we scored the flower density, defined as the number of flowers per (20 x 20) cm² (3 categories: few = 0-125 flowers, moderate = 125-450 flowers, many = > 450 flowers). Finally, in each of the 692 aggregations we listed all species to which *C. epithymum* was attached with haustoria and these are further referred to as 'hosts'. Functionality of haustoria, i.e. if there was a nutrient flow from host to parasite, was, however, not assessed.

### *Regional and local patch characteristics*

For all management patches, three regional characteristics were calculated, namely patch size, patch age and isolation. First, the patches were digitised using ArcView 3.2 (ESRI 2000), enabling the calculation of patch area (14 m² - 195 000 m², median 564 m²). The management patches in Heiderbos were rather small compared to the relatively large patches observed in the other study sites (Table 3.1).

Second, for each of the 166 patches, connectivity was calculated as:

$$\text{IFM}_i = \sum A_j \exp(-\alpha d_{ij}) \qquad \text{Hanski (1994b)} \qquad \text{(eq. 1)}$$

Connectivity refers to the degree to which the landscape facilitates or impedes movement among patches. In equation 1, the sum is taken over all patches $j \neq i$, where *i* represents the focal patch and *j* each of the other patches in which the species is present. The edge-to-edge distance from patches *i* to *j* is $d_{ij}$ and $A_j$ is the absolute abundance of *C. epithymum* in patch *j*. Finally, the parameter *a* represents a constant, expressing the survival rate of the species over distance. Because we had no indication of what the value for *a* would be, we initially used $a$ = 0.1, 0.35, 0.5, 1 and 5 (cf. Moilanen and Nieminen, 2002). The obtained IFM-values were strongly correlated (all $p < 0.001$ with minimal $r_s = 0.93$) and gave similar results in the analyses described below. Since $a = 1$ gave the most significant result, this value was used in the further data analysis. This outcome was not surprising because of the expected limited dispersal rate and the sedentary character of the parasite.

Third, patch age, i.e. the interval since last practice expressed as the number of years since the last management intervention (continuous variable), was identified through interviews with local nature managers. The patches were later classified in three age classes, namely 0-3, 4-7 and 8-14 years old.

The six local patch characteristics consisted of the type of last management application (turf-cutting, burning, mowing and none (i.e. longer than 14 year without management), canopy height, % cover of bare ground, mean Ellenberg N and F and the pH-KCl (1:2.5 ratio of soil to KCl (1 M)). After litter removal, ten topsoil samples (10cm deep, diameter 3.5cm) were taken from each management patch and thoroughly mixed before direct pH measurements. The mean Ellenberg indicator values (Ellenberg et al., 1992) for soil moisture (F) and productivity (N) status were determined based on the total species list (*C. epithymum* was excluded) per management patch (presence/absence).

### Data analysis

The data were analysed using a nested approach. First all 166 patches were included in the analyses, secondly only the 69 occupied patches were considered and finally, only the paired m$^2$-plots within the occupied patches were taken into account.

In a first step, the effect of regional and local characteristics on *C. epithymum*'s presence/absence was investigated. Therefore, an indirect gradient analysis was applied to retrieve possible vegetation differences between management patches with and without the parasite. Given the relative low length of the gradient (2.78) in the Detrended Correspondence Analysis, a Principal Component Analysis (PCA, CANOCO 4.5; ter Braak and Smilauer, 1998) was applied on species abundances (*C. epithymum* was removed from this dataset). This ordination was applied both on all 166 patches together and on the patches grouped per study site. In order to minimize the impact of abundant species (especially *Calluna vulgaris*), log(x + 1)-transformed cover data were used. The scores of the patches on the first and second PCA-axes were then correlated with the 8 continuous environmental variables using Pearson product-moment correlations. Additionally, using independent samples t-tests, the significance was tested of differences in first and second axis scores between patches with and without the parasite. Next, using binary logistic regression analysis (Hosmer and Lemeshow, 1989), the relative importance of the three regional and the six local variables on parasite's presence was assessed. In this analysis, the study site was included as a control variable as well. Backward stepwise selection, with $p = 0.05$ as criterion to omit variables from the model, was used.

In the second step, only the 69 occupied management patches were included. In an exploratory phase Pearson product-moment correlations were used to assess the relationships between *C. epithymum*'s abundance and the 8 continuous environmental variables. One-way ANOVA was performed to test whether the abundance was significantly different between the four study sites, four management types, patch age (3 classes), canopy height (4 classes) and the parasite's reproduction success (3 classes). Finally, a General Linear Model (GLM: McCullagh and Nelder, 1989) was applied to synthesize the importance of these variables on the abundance of the parasite. A backward stepwise procedure was used to end up with the model with the highest adjusted R$^2$.

In the last step, paired m$^2$-plots within the occupied management patches were examined. A paired-samples t-test was used to evaluate differences in canopy height and *Calluna vulgaris*' abundance between plots with and without the parasite.

All statistical analyses were performed with SPSS for Windows 12.0 (SPSS 2003).

# Results

## *Cuscuta epithymum in the four study sites*

We found 91 phanerogamic plant species, with mean species richness (± SE) 10.04 ± 0.38 species per patch (Table 3.1b).

**Table 3.1.** **a)** Characterisation of the four studied Flemish nature reserves **b)** Means (± SE) of management patch area, number of all phanerogamic plant species per patch (S), pH-KCl and mean Ellenberg indicator values for soil nitrogen (N) and soil moisture (F) per study site.

| | *Study sites* | *Heiderbos (n = 24)* | *De Maten (n = 57)* | *Mechelse Heide (n = 66)* | *Ziepbeek (n = 19)* |
|---|---|---|---|---|---|
| **a)** | Geographical position | 51° 00’ 51”N,<br>5° 27’ 02’’E | 50° 57’ 29”N,<br>5° 27’ 55”E | 50° 58’ 17”N,<br>5° 37’ 38”E | 50° 56’ 22”N,<br>5° 38’ 49”E |
| | Site area (ha) | 42 | 310 | 700 | 167 |
| | Area heath veg. (ha) | 14 | 65 | 452 | 88 |
| | Soil type | Dry sand + gravel deposits | Inland dunes | Dry to moist sand | Moist to wet sand |
| | No. of o.p. | 19 | 28 | 9 | 13 |
| | % of o.p. per study site | 79 | 48 | 13.5 | 68.5 |
| **b)** | Patch area (m$^2$) | 260 ± 39$^{a}$ | 1500 ± 463$^{a}$ | 3285 ± 467$^{b}$ | 3908 ± 685$^{b}$ |
| | S | 13.25 ± 0.59$^{a}$ | 11.98 ± 0.78$^{a}$ | 8.11 ± 0.42$^{b}$ | 7 ± 0.65$^{b}$ |
| | pH (KCl) | 3.15 ± 0.04$^{a}$ | 3.17 ± 0.04$^{a}$ | 3.01 ± 0.03$^{b}$ | 2.90 ± 0.02$^{b}$ |
| | N | 2.06 ± 0.07$^{a}$ | 2.33 ± 0.07$^{ab}$ | 2.35 ± 0.06$^{b}$ | 1.99 ± 0.08$^{a}$ |
| | F | 6.00 ± 0.08$^{a}$ | 5.94 ± 0.15$^{a}$ | 5.87 ± 0.12$^{a}$ | 6.97 ± 0.19$^{b}$ |

Different letters correspond to significant differences (Tukey’s pairwise means comparison test, $p < 0.05$); n = number of surveyed management patches per study site (166 in total); veg. = vegetation; o.p. = occupied patches.

*Cuscuta epithymum* was found in all study sites, but only occurred in 69 of the 166 (42%) surveyed management patches, unequally divided over the reserves (Table 3.1). There was a significant difference in mean absolute abundance per occupied patch between the study sites, with the highest percentage in Ziepbeekvallei (ANOVA $F = 3.37$, $p < 0.05$; Fig. 3.2a). In all management patches, *C. epithymum* occupied only a fraction of the total patch area (maximum: 2.8%, min: 0.002% and mean (± SE): 1.4 ± 0.41%).

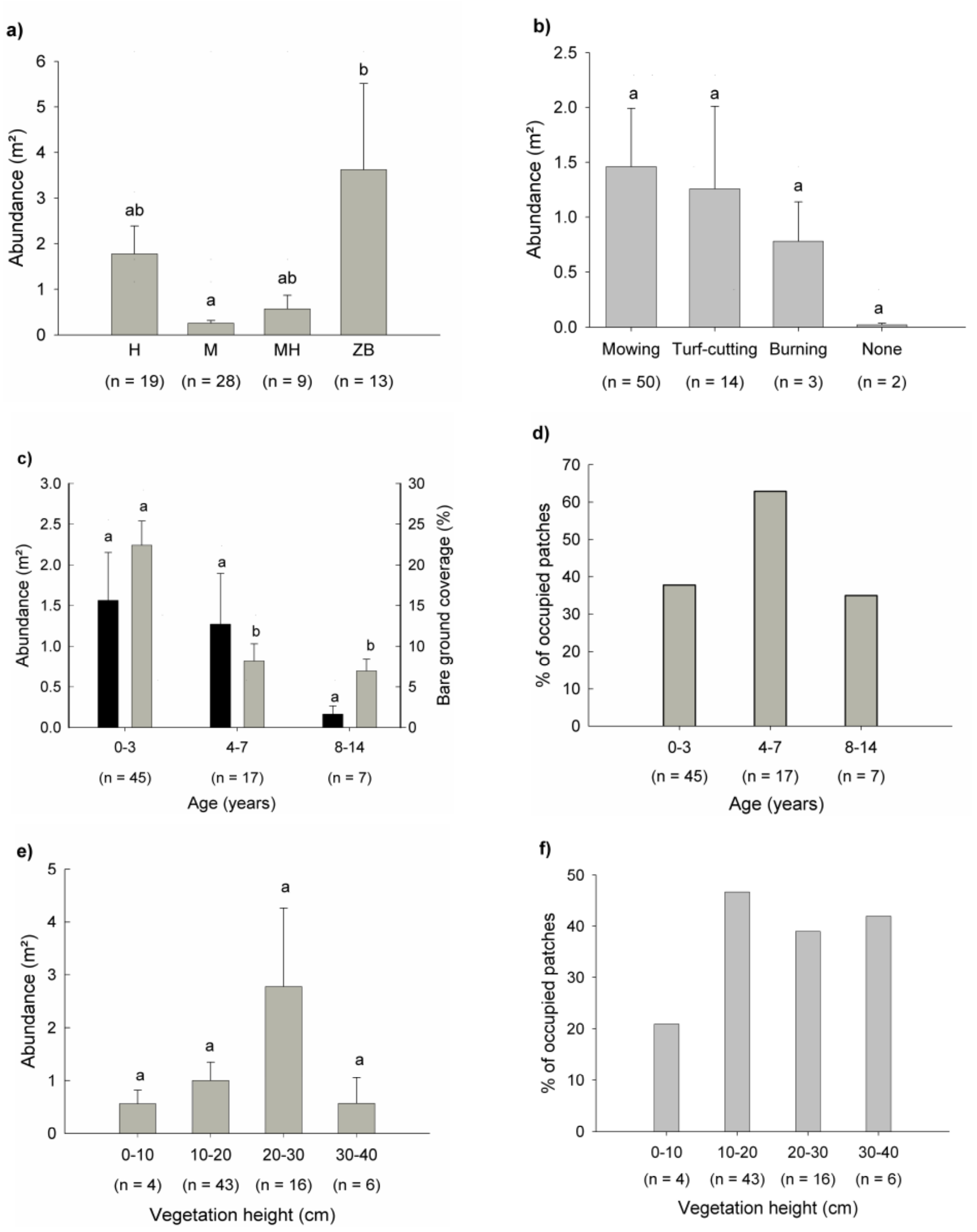


**Figure 3.2.** Mean (± SE) ***C. epithymum*** abundance in the occupied patches (69 in total) for **a)** each study site (H = Heiderbos, M = De Maten, MH = Mechelse Heide, ZB = Ziepbeekvallei) and **b)** each management type. **c)** Mean (± SE) parasite abundance (black bars) and percentage bare ground cover (grey bars, right vertical axis) in occupied patches per age class. **d)** Percentage of occupied patches per age class relative to the total number of patches belonging to that class. **e)** Mean (± SE) abundance of the parasite per patch in the four classes of canopy height and **f)** percentage of occupied patches per canopy height class, relative to the total number of patches belonging to that class. n = number of patches belonging to a particular class. Different letters correspond to significant differences (Tukey's pairwise means comparison test, $p < 0.05$).

All management applications had a positive but not significant effect on parasites abundance (Fig. 3.2b). Furthermore, for younger patches situated in the early successional stages, there was a (non-significant) tendency for higher *C. epithymum* abundance (ANOVA $F = 1.25$, $p = 0.30$, Fig. 3.2c). Patches belonging to earlier successional stages showed a significantly greater percentage of bare ground (ANOVA $F = 6.03$, $p < 0.05$, Fig. 3.2c). Moreover, most occupied patches were situated in young heath succession stages of 4 to 7 years old (Fig. 3.2d), containing relatively open vegetation (Fig. 3.2c). During heathland succession, canopy height increases. If patches are ordered by canopy height class, parasite's abundance per patch increased until a threshold of 25cm (Fig. 3.2e). Hereafter, *C. epithymum* abundance decreased not significantly with increasing height and the parasite completely disappeared in vegetation taller than 40cm. Nevertheless, most occupied patches were found in vegetation of 10 to 40cm high (Fig. 3.2f). Flower density was positively correlated with absolute abundance, with large populations having a higher flower density than small populations (ANOVA $F = 4.89$, $p = 0.01$, Fig. 3.3).

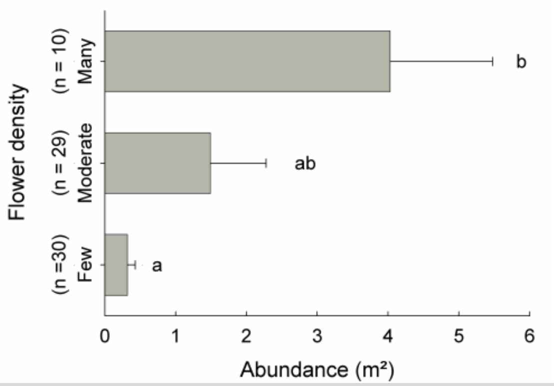


**Figure 3.3.** Parasite's flower density in function of their population size, expressed as mean (± SE) absolute abundance per flowering class. Different letters correspond to significant differences ($p < 0.05$), as determined with a Tukey a posteriori test. n = number of management patches per flowering class. Three flowering classes: few = 0-125, moderate = 125-450, many = more than 450 flowers per 0.04m².

## *Occurrence of C. epithymum*

Scores on the first two PCA-axes were significantly higher for the occupied patches than for the unoccupied patches ($t = -4.27$ (axis 1), $t = -5.04$ (axis 2), both $p < 0.001$, Fig. 3.4). This can be attributed to the clustering of patches of the Heiderbos reserve, of which a high percentage was occupied (Fig. 3.4). Correlating the first two axes with environmental variables shows a significant influence of canopy height, % bare ground, patch area and patch age on vegetation composition (Fig 3.4). Within the study sites, no significant differences in axis scores between occupied and unoccupied patches were found, except for Mechelse Heide (occupied patches significantly higher scores on second PCA-axis: $t = -3.38$, $p < 0.01$). Hence, there was hardly any difference in vegetation composition between occupied and unoccupied management patches.

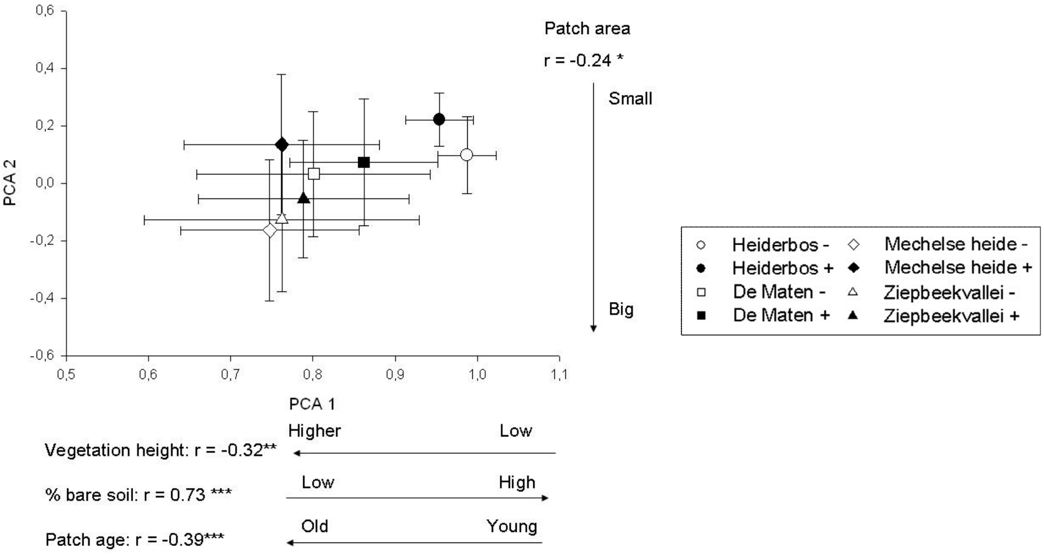


**Figure 3.4.** A two-dimensional PCA-ordination plot of the 166 management patches using vegetation data. Mean ± SD values for occupied and unoccupied patches per reserve are presented. Different symbols refer to the four heathland reserves. Filled symbols: management patches with *C. epithymum*, empty symbols: unoccupied patches. Arrows along the axes indicate significant correlations between the patches scores on the axes and environmental variables (Pearson product-moment correlations, *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$).

Soil properties only varied significantly between study sites (Table 3.1) and not between occupied and unoccupied patches within the study sites, with the exception of soil nitrogen (N) and soil moisture (F) in Ziepbeekvallei ($p < 0.01$). Furthermore, results of the logistic regression analysis revealed that presence/absence of *C. epithymum* was not related to one of the 10 variables, except for 'study site' (Nagelkerke $R^2 = 0.37$, Wald = 38.02, $p < 0.001$). Yet, although only marginally significantly, management type also had a positive impact on the presence of the parasite (Wald = 6.85, $p = 0.08$): after mowing (exp($B$) = 6.88) and burning (exp($B$) = 10.92) the chance of finding the parasite increased.

## *Differences in abundance in occupied patches*

No significant correlation between *C. epithymum*'s absolute abundance per patch and patch size (patch area) was found ($r_p = 0.30, -0.20, -0.17$ and $0.17$; $p > 0.05$; for Heiderbos, De Maten, Mechelse Heide and Ziepbeekvallei, respectively). Consequently, there was also no correlation between abundance and patch size when considering all the occupied patches together ($r_p$ = 0.13, *p* = 0.30).

**Table 3.2.** *F*-values and significance levels of the GLM analysis on the parasite's abundance within the 69 management patches occupied by *C. epithymum*, and on the parasite's abundance within the same patches, but without two outliers for IFM (n = 67). Three regional and six local variables (as listed in the text) were tested and study site was included as a control variable. The model with the highest adjusted R² was retained.

| *Variables* | *df(n,d)* | *F* | *F without outliers* |
|---|---|---|---|
| IFM | 1,61 | $59.55^{***}$ | $1.52^{ns}$ |
| Study site | 3,61 | $2.26^{ns}$ | $3.71^{*}$ |
| % bare ground | 1,61 | $2.52^{ns}$ | / |
| N | 1,61 | $1.70^{ns}$ | / |
| F | 1,61 | $2.63^{ns}$ | / |
| Canopy height | 1,61 | / | $1.39^{ns}$ |
| Adjusted R² | | 0.53 | 0.11 |

IFM: connectivity measure, N and F: Ellenberg indicator values based on presence/absence for soil nitrogen (N) and soil moisture (F), df (n,d) = degrees of freedom (nominator, denominator); ***: p < 0.001; * : 0.01 < p ≤ 0.05; ns: p > 0.05

Soil nitrogen (N), soil moisture (F) and % bare ground were retained in the final GLM-model, despite being non-significant (Table 3.2) and only connectivity (IFM), a regional variable, explained a significant part of the observed variation in the parasite's abundance per management patch (Table 3.2). Indeed, *C. epithymum* abundance and connectivity are positively correlated ($r_p$ = 0.71, *p* < 0.01). However, when visualizing this correlation, two outliers were detected, with a very large population size and extremely high IFM values. The correlation without these outliers is not significant ($r_p$ = -0.08, *p* > 0.05). Moreover, a GLM without these outliers removed the significant impact of connectivity on the parasites' abundance (Table 3.2). Only connectivity, study site and canopy height were maintained in this final GLM-model, where only the effect of study site was significant.

### Distribution in the local vegetation and host range

In the four reserves, we identified 21 species to which *C. epithymum* was attached with haustoria (Table 3.3). *Calluna vulgaris* (Ericaceae) was by far the most frequent host. Haustoria were found on heather in 98% of the 692 *C. epithymum*-aggregations, while the next most common host (*M. caerulea*) was infected in only 4% of the observed aggregations. Only *C. vulgaris*, *M. caerulea*, *E. tetralix* and *Genista pilosa* were found as hosts in all the study sites where these species were present, while the parasite was attached to *D. flexuosa* and the seedlings of

*Pinus sylvestris* and *Betula pendula* at three study sites (Table 3.3). It was observed that when *C. epithymum* parasitised tree species, only seedlings and saplings were affected.

**Table 3.3.** Species to which *C. epithymum* was attached with haustoria in the four study sites listed in decreasing order of importance.

| *Species* | *H* | *M* | *MH* | *ZB* | *%* |
|---|---|---|---|---|---|
| *Calluna vulgaris* | + | + | + | + | 98.1 |
| *Molinia caerulea* | + | + | + | + | 3.90 |
| *Deschampsia flexuosa* | + | - | + | + | 3.18 |
| *Carex pilulifera* | - | - | + | + | 3.18 |
| *Genista pilosa* | + | + | + | 0 | 2.02 |
| *Teucrium scorodonia* | 0 | - | + | 0 | 1.88 |
| *Pinus sylvestris* sdl | - | + | + | + | 1.73 |
| *Galium saxatile* | - | - | + | 0 | 1.73 |
| *Erica tetralix* | + | + | + | + | 1.45 |
| *Erica cinerea* | + | - | + | 0 | 1.30 |
| *Rubus fruticosus* | - | + | + | - | 1.16 |

The following species were infected in only one study site: *Genista anglica*, *Betula pendula* sdl, *Rumex acetosella*, *Carex arenaria*, *Juncus squarrosus*, *Vaccinium myrtillus*, *Quercus rubra* sdl, *Quercus robur* sdl, *Carex demissa*, *Festuca filiformis*., all with infection level < 1%.

+ or - refers to the presence or absence of haustorial connection; 0: absence of the host species in the surveyed management patches; %: percentage of the *C. epithymum* aggregations in which this 'host' was infected, relative to the total number of observed aggregations (692), sdl = seedlings, H = Heiderbos, M = De Maten, MH = Mechelse Heide, ZB = Ziepbeekvallei.

**Table 3.4.** Differences in mean (± SE) canopy height (cm) and mean (± SE) *Calluna vulgaris* abundance (%) in all 1 m² plots with (+) and without (-) *C. epithymum* in the four study sites together and per study site (n = number of 1m² plots).

| | + C. epithymum | - C. epithymum | *t-Value* | *Trend* |
|---|---|---|---|---|
| **All paired plots** | (n = 131) | (n = 131) | | |
| Height (cm) | 13.46 ± 0.67 | 15.95 ± 0,67 | -2.83** | ↓ |
| *Calluna vulgaris* (% cover) | 58.03 ± 2.47 | 49.56 ± 2.70 | 3.25*** | ↑ |
| **Heiderbos** | (n = 38) | (n = 38) | | |
| Height (cm) | 10.52 ± 1.18 | 13.20 ± 1.49 | -1.70* | ↓ |
| *Calluna vulgaris* (% cover) | 43.21 ± 3.85 | 52.55 ± 4.13 | -2.68** | ↓ |
| **De Maten** | (n = 46) | (n = 46) | | |
| Height (cm) | 13.89 ± 1.10 | 18.37 ± 1.66 | -2.66** | ↓ |
| *Calluna vulgaris* (% cover) | 83.40 ± 12.14 | 61.43 ± 4.52 | 4.93*** | ↑ |
| **Mechelse Heide** | (n = 18) | (n = 18) | | |
| Height (cm) | 16.71 ± 2.12 | 17.04 ± 2.16 | -0.14$^{ns}$ | |
| *Calluna vulgaris* (% cover) | 34.33 ± 6.42 | 33.22 ± 6.05 | 0.23$^{ns}$ | |
| **Ziepbeekvallei** | (n = 29) | (n = 29) | | |
| Height (cm) | 14.62 ± 1.28 | 15.04 ± 1.28 | -0.28$^{ns}$ | |
| *Calluna vulgaris* (% cover) | 51.90 ± 4.44 | 36.93 ± 6.10 | -2.55** | ↑ |

T-values from paired samples t-tests are given with significance level. ↑: higher value or ↓: lower value in presence of *C. epithymum*. *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns: not significant (2-tailed).

Canopy height was significantly lower in occupied plots than in unoccupied ones (Table 3.4) but in only two of the four study sites this difference was significant. Overall, the cover of *C. vulgaris* was significantly higher in occupied plots than in unoccupied ones (Table 3.4).

# Discussion

## Vegetation succession influences population size at the local scale

*Cuscuta epithymum* can be described as a species that appears after the vegetation has been set back to its initial successional state by some form of management. Our observations confirm the association of the parasite with young (Fig. 2c), recently-managed heathland, dominated by *C. vulgaris* (Table 3.3) (see also Weeda et al., 1988). On the local scale the variation of population size in the occupied patches was mainly the result of changes in vegetation structure during the heathland succession cycle (Fig. 3.2c-f). Furthermore, an interview with 29 managers of Flemish reserves containing *C. epithymum* revealed that in 97% of these reserves the parasite only appears after management. In fact, vegetation patches mainly composed of young individuals of *C. vulgaris* of 10-30cm height, corresponding to maximal seven years after the last management application, offer an optimal habitat for the parasite's growth (Fig. 3.2c-f). Similar results were found by Mitchell et al. (1997) who did not find *C. epithymum* in older successional stages. As larger populations have a higher flower density (Fig. 3.3), management practices also indirectly affect the reproductive capacity of the parasite. Additionally, within occupied patches, *C. vulgaris* had a larger cover in proximity to the parasite (Table 3.4). This observation may suggest that larger available host cover supports parasite growth as long as host morphology sustains infection. Nevertheless, this hypothesis requires further research.

In addition, the vegetation composition also influences the parasite's abundance on the local scale. In Flanders, *C. epithymum* occurs in several vegetation types and in our study the parasite was attached to many different plant species (Table 3.3, see also Kuijt, 1969), which confirms a broad host range (Csiky et al., 2004). Nevertheless, by far the most haustoria were found on *C. vulgaris*. This may merely result from the dominance of *C. vulgaris* in these systems (see Box 2.1). Moreover, the parasite may mainly attack young heather shrubs because of a preference for higher quality hosts (cf. Kelly, 1992; Koch et al., 2004).

## Is a persistent seed bank the main driver?

*Cuscuta epithymum* only occurred in 42% of the surveyed management patches and clearly showed a patchy distribution. Some authors emphasised the importance of patch size and isolation on patch occupancy (e.g. Hanski and Gilpin, 1997; Bruun, 2001), while other studies demonstrated that mainly ecological interactions between plants and their environment influenced species incidence (Brose, 2001; Fleishman et al., 2002). The results of our study in heathland, however, show that there was no clear difference in vegetation composition and that none of the local or regional variables really explained the occurrence of *C. epithymum*. In contrast with the results of Frost et al. (2003), soil factors did not influence the parasite's presence, as they only varied between study site and not between the different patches within the sites (Table 3.1). Hence, these variables vary along environmental gradients on a wider geographical scale and cannot be used to define suitable patches. The expectations derived from metapopulation theory failed also to help unraveling the distribution pattern of the parasite. We did not find a significant influence of patch size ($r_P = 0.08$, $p = 0.52$) and isolation ($r_P = 0.14$) on population size. These results are consistent with the study of Piessens et al. (2005) in which almost none of the investigated heathland species were affected by patch area and species differed strongly in isolation sensitivity. The low importance of area and isolation, however, indicates that in a dynamic heath system the patchy distribution of *C. epithymum* results from other, yet undescribed, variables, such as the existence of a persistent seed bank in combination with very limited seed dispersal. The presence of suitable, but unoccupied habitats may be attributed to restricted dispersal, as in other connected plant populations (Verheyen et al., 2004; Snäll et al., 2005). Indeed, *C. epithymum* does not show any dispersal specialisation and is expected to be severely dispersal limited (Chapter 2, Box 5.1). Further, the parasite may be well-adapted to survive under the changing conditions of a frequently disturbed heathland ecosystem (Gimingham, 1972). Since *C. epithymum*, as an early successional species, occurs only in recently-managed heathland patches embedded in an inhospitable matrix of old heathland, it has to "track" the dynamic patch network in space or in time for long-term survival at the landscape level (see Snäll et al., 2005 for a patch tracking model). Here, seed dormancy or dispersal in time, next to long-distance dispersal, is often considered as a major strategy allowing organisms to avoid harsh environmental conditions (Amarasekare and Possingham, 2001; Adams et al., 2005). Moreover, the ability to build up a long-term persistent seed bank is one of the most important traits ensuring survival of heathland species (e.g. Pywell et al., 2002; Bossuyt and Hermy, 2003; Piessens and Hermy, 2006). This hypothesis is confirmed by results of an interview with 29 Flemish heath reserve managers (data not presented). In 40% of these reserves *C. epithymum* could survive an unfavourable period of several decades in the seed bank, e.g. if a forest replaced heathland. When forest allotments were cut down, or old heath was rejuvenated, the parasite reappeared, assuring its survival for another few decades, at least after seed set. Dormancy of *Cuscuta* seeds is also observed in other species (e.g. *C. campestris*: Benvenuti et al., 2005). Therefore, we can conclude that *C. epithymum* populations not really behave as strict metapopulations, but are better described as a special form of 'regional ensembles', namely remnant populations, as defined by Freckleton and Watkinson (2002). Such system of populations contains a low migration rate and includes populations that persist as seed banks. Hence, current distribution of the parasite may in fact reflect past land-use and management history (see Eriksson et al., (1995) looking at *Thymus serpyllum*). It is therefore expected that a positive relationship exists between the period a site has been suitable for *C. epithymum* in the past and the chance of finding the species in a suitable patch today. Unfortunately, as such detailed management records for our study sites do not exist, we were not able to test this hypothesis.

This raises the question of managers mainly have to concentrate on occupied patches for the maintenance of established populations by a long, continuous management or to vary management applications over the whole heath reserve to create a dynamic mosaic of management patches in the hope the parasite will disperse.

### *Conclusion for management and future research*

Having investigated the effect of both small- and large-scale factors on the distribution and abundance of *C. epithymum*, results have clearly highlighted the importance of heathland management to ensure the persistence and survival of *C. epithymum* in early-successional heath communities. The tight relationship of the parasite with the structure and dynamics of the vegetation has important implications for conservation management in general, and management of early-successional lowland heath communities in particular.

In general, all management strategies leading to a more open vegetation structure including younger phases of *Calluna*, but without removing the topsoil (seed bank), will be favourable for the growth of *C. epithymum*. This can be achieved by a combination of cyclical management by mowing, burning and shallow turf cutting. The created pioneer phases are not only important for *C. epithymum*, but also for species that are adapted to conditions of bare ground. Since there is a decline in seed density and viability with time, heathland managers should promote seven- or ten year management cycles on patches including *C. epithymum*. Furthermore, since the parasite does not seem to be negatively affected by a reduced patch area, it can be concluded that even small-scaled management is sufficient for its survival. Finally, the finding of a persistent seed bank would give some good possibilities for re-establishment of heathland communities with *C. epithymum* (see also Chapter 9).

Despite the fact that none of the analysed variables showed strong effects on the distribution of the parasite, the conceptual framework of the study is an important step forward, since our study revealed several new aspects of the ecology of this parasite and certainly offers new possibilities for further research on this and other early successional species.

# 3b.Population responses to landscape changes during a four-year period

## Introduction

Despite an increasing interest in the interaction between population and landscape dynamics and in the factors explaining the spatial structure of populations (see Hanski, 1999; Hanski and Gaggioti, 2004), the spatial and temporal effects of landscape dynamics on population dynamics remain largely underexplored (Bullock et al., 2002; Freckleton and Watkinson, 2003; Ouburg and Eriksson, 2004). There is a consensus amongst ecologists that the persistence of many annual and short-living plants depends upon regular disturbance such as management interventions in grasslands and heathland (Eriksson, 1998; Webb, 1998; Lennartsson and Oostermeijer, 2001; Brys et al., 2004; Sletvold and Rydgren, 2007). Because of these disturbances and subsequent vegetation development, habitats may have a fluctuating quality, with continuous variation in suitability to support a population in time as well as in space (Kleyer et al., 2007). The first part of this Chapter studied the impact of local and/or regional factors on *C. epithymum*'s distribution and abundance, and clearly demonstrated that this was largely influenced by fluctuating habitat quality. The key factor determining *C. epithymum*'s population size was canopy structure, which in turn is influenced by management interventions followed by vegetation succession. However, the census in the first part of this chapter gave only a snapshot of its regional distribution in one year, while floristic responses to changing vegetation are timescale dependent. The point at which a snapshot of occupancy and abundance is taken can affect results, and can miss potentially important patterns (Bullock et al. 2002b; Fleishman et al., 2002). There is indeed some evidence that the dynamics of suitable patches may influence *C. epithymum* population dynamics and viability in the long-term. Furthermore, to conserve it successfully we need to understand how this species responds to natural and/or management-induced habitat changes. In this part of Chapter 3 we want to explore the longer-term effects of landscape change on *C. epithymum* populations, and we mainly want to investigate the interaction between its population dynamics and management driven landscape and vegetation dynamics. Patch and population dynamics and the response of *C. epithymum* populations on landscape changes are reported for four consecutive summers. Changes in reproductive related characteristics were also studied.

# Methods

## Data collection

During 2004-2007 we investigated 108 *C. epithymum* populations distributed over 289 management patches in four Flemish nature reserves (Heiderbos, De Maten, Mechelse Heide and Ziepbeekvallei; Fig. 3.5; see Chapter 3a for details). During each summer we investigated all potential *C. epithymum* habitats (i.e. management patches, see Chapter 3a), allowing us to understand patch dynamics, including changes in suitability, establishment and disappearance of appropriate habitat patches, and population dynamics of the *C. epithymum* populations present.

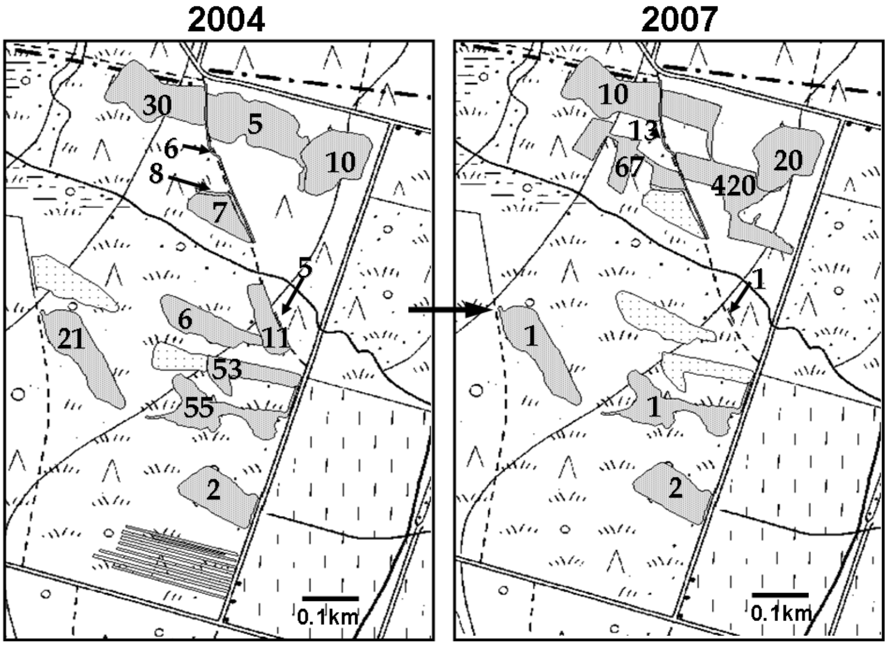


**Figure 3.5.** Spatiotemporal changes in all management patches and *C. epithymum* populations from 2004 till 2007 in the reserve 'Ziepbeekvallei'. Numbers refer to the number of *C. epithymum* aggregations per management patch, giving an indication of population size. Management patches containing *C. epithymum* populations are grey tinted, while unoccupied ones are white dotted. The arrows on the maps indicates locations of small management patches.

In each patch, annual measurements of vegetation height and *Calluna* density were made in order to assess changes in vegetation structure, and for each *C. epithymum* population the size (expressed as absolute abundance per m$^2$) was surveyed annually (see Chapter 3a for detailed explanation). For all management patches three regional characteristics were calculated, namely patch size, patch age (i.e. number of years since last management intervention, no more then 14 years), connectivity and the type of last management application within the last 14 years was determined (turf-cutting, burning, mowing or none).

The patches were later classified in three age classes, namely 0-3, 4-7 and 8-14 years since last management (see Chapter 3a). In addition, the use of stock grazing was recorded.

To determine the effect of vegetation succession on reproduction-related characteristics, a subsample of 10 *C. epithymum* populations was selected at each reserve, in which reproduction was investigated during 3 subsequent summers (2006-2008). Within each population, 10 *C. epithymum* aggregations (see Chapter 3a) were randomly selected. If there were <10 aggregations, they were all investigated. For each of these aggregations, the number of flowers per 0.04 $m^2$ was recorded, from which the mean flower-density per population was calculated. For each population the mean number of seeds per fruit was assessed by counting the seeds in each fruit for a variable number of inflorescences (ranging from 1 to 10 in proportion to the population size).

### *Data analysis*

The data were analysed on three different levels. First, all occupied patches were considered, secondly only the patches in Heiderbos and De Maten which were occupied during the whole study period were included in the analyses (n = 15 and 18, respectively) and finally, only the 10 patches (populations) per reserve in which reproduction was studied were analysed.
One-way ANOVA was used to test whether the population size differed significantly between the four study sites, the four management types and between the three age classes per year. Pearson product-moment correlations were used to assess the relationships between *C. epithymum* abundance and the regional characteristics of isolation and patch size. Finally, an independent t-test was applied to discover potential differences in connectivity and patch size among patches which were colonised by *C. epithymum* during the four-year period and non-colonised patches.

For the subsample of patches in Heiderbos and De Maten, one-way ANOVA was performed to test whether the population size and vegetation height changed significantly during the four studied years. In other words, the population dynamics and vegetation changes were investigated. Pearson product-moment correlation was applied to retrieve possible relationships between canopy heights and patch age for those patches.

To investigate time-related changes in reproduction capacity and the relative importance of vegetation structure and population size ($m^2$) for reproduction related characteristics, a two step analysis was carried out using data from the ten patches in each nature reserve. Since the variables of vegetation height and *Calluna* density were partly interdependent, we performed a principal component analysis (PCA). Sample scores for the rotated principal component were used as the 'vegetation structure' variable for further analysis (first PCA axis explained 85 % of the variance, correlation with variables: $r_S = 0.92$; $p < 0.001$ each). In the first step, the influence of vegetation structure and time on flower density and population size was studied. To see whether larger populations produced significantly more flowers a non-parametric Spearman's rho-test was used. Because these two variables are related, only flower density was examined in the following analyses. To investigate if flower-density was related to vegetation structure, year and their interaction a General Linear Model (GLM; McCullagh and Nelder, 1989) was applied. Significant difference in flower-density between the three studied years was determined using Kruskal-Wallis test, followed by a post-hoc analysis ($p < 0.05$) and Spearman's rho correlation was used to test whether or not there was a relation between flower-density and vegetation structure. In the second step, the mean seed production per fruit was investigated. To investigate the hypothesis that larger populations produce more seeds per flower than smaller ones, a GLM was applied with population size, year and their interaction as independent variables. Vegetation structure was not added in the GLM-analysis, since this variable is negatively correlated with population size ($r_S = -0.37$, $p < 0.001$). A Kruskal-Wallis test, followed by post-hoc analysis ($p < 0.05$) was also used to determine differences in seed production between the three studied years. Both flower-density and seed production were log-transformed prior to analysis. Statistical tests were carried out using SPSS 15.0 (SPSS, 2008).

# Results

During the whole period of research, *C. epithymum* was present in the four reserves (e.g. Fig. 3.5). However, population size differed significantly from year to year and annual changes in population size strongly varied from site to site (Table 3.5a, b; Fig. 3.6a). In all sites, except for De Maten, a strong fall in metapopulation abundance (i.e. sum of all population sizes per reserve) was observed in 2006 (Fig. 3.6a). Mowing was by far the most frequent management type. Rejuvenation of the heath vegetation was achieved by mowing for 70% of all 289 studied patches. There was a (non-significant) tendency for higher *C. epithymum* abundance on mown patches (Fig. 3.6c). Consequently, most *C. epithymum* populations were found on mown patches (Fig. 3.6c). Every year, patches situated in the early successional stage contained significantly larger *C. epithymum* population than older patches. Most populations were found in young heath succession stages of 0-3 years old (Fig. 3.6d).

**Table 3.5.** Patch and population dynamics. **a)** Number of management patches and patches occupied with *C. epithymum*, **b)** mean ± SE population size expressed as abundance ($m^2$; see chapter 3a) and **c)** dynamic of the management patches (appearance and disappearance) and the *C. epithymum* populations (colonisation and extinction) for each year and each nature reserve.

| | | *Year* | *H* | *M* | *MH* | *ZB* |
|---|---|---|---|---|---|---|
| a) | No. of patches (% occupied patches) | 2004 | 24 (79) | 57 (49) | 66 (14) | 19 (68) |
| | | 2005 | 25 (88) | 59 (37) | 66 (11) | 18 (44) |
| | | 2006 | 42 (62) | 42 (57) | 99 (11) | 16 (69) |
| | | 2007 | 49 (76) | 38 (66) | 106 (16) | 13 (77) |
| b) | Mean ± SE abundance ($m^2$) | 2004 | 1.77 ± 0.63$^{ab}$ | 0.26 ± 0.06$^{a}$ | 0.57 ± 0.30$^{ab}$ | 3.62 ± 1.89$^{b}$ |
| | | 2005 | 1.31 ± 0.60$^{a}$ | 0.03 ± 0.02$^{a}$ | 13.69 ± 9.75$^{b}$ | 5.57 ± 4.53$^{ab}$ |
| | | 2006 | 0.72 ± 0.24$^{ab}$ | 0.12 ± 0.08$^{a}$ | 1.51 ± 0.57$^{b}$ | 0.16 ± 0.15$^{a}$ |
| | | 2007 | 1.96 ± 0.92$^{a}$ | 0.15 ± 0.03$^{a}$ | 1.06 ± 0.43$^{a}$ | 1.20 ± 0.90$^{a}$ |
| c) | No. of new patches | 2004-7 | 21 | 6 | 83 | 2 |
| | No. of disappeared patches | 2004-7 | 6 | 26 | 43 | 8 |
| | No. of col + recol | 2004-7 | 23 + 2 | 3 + 4 | 10 + 0 | 2 + 3 |
| | No. of extinctions | 2004-7 | 5 | 9 | 4 | 8 |

Different letters correspond to significant differences (Tukey pairwise means comparison test, $p < 0.05$). col = colonisations; recol = recolonisations.

Population size was not significantly correlated with patch size or connectivity ($r_p$ = 0.20 and -0.03, respectively, each $p > 0.05$). In addition, no significant difference in connectivity was found between colonised patches and uncolonised ones ($t = 1.52$, $p > 0.05$). Nonetheless, uncolonised patches were significantly larger than colonised ones ($t = -2.69$, $p < 0.01$; patch size = 270 ± 38.5 and 200 ± 31.4$m^2$, respectively). The latter difference is, however, quite small and besides, colonisation events might be expected to be more frequent on larger patches than on smaller ones. It is believed that the difference is probably not meaningful or that factors are involved that are at present completely unknown.

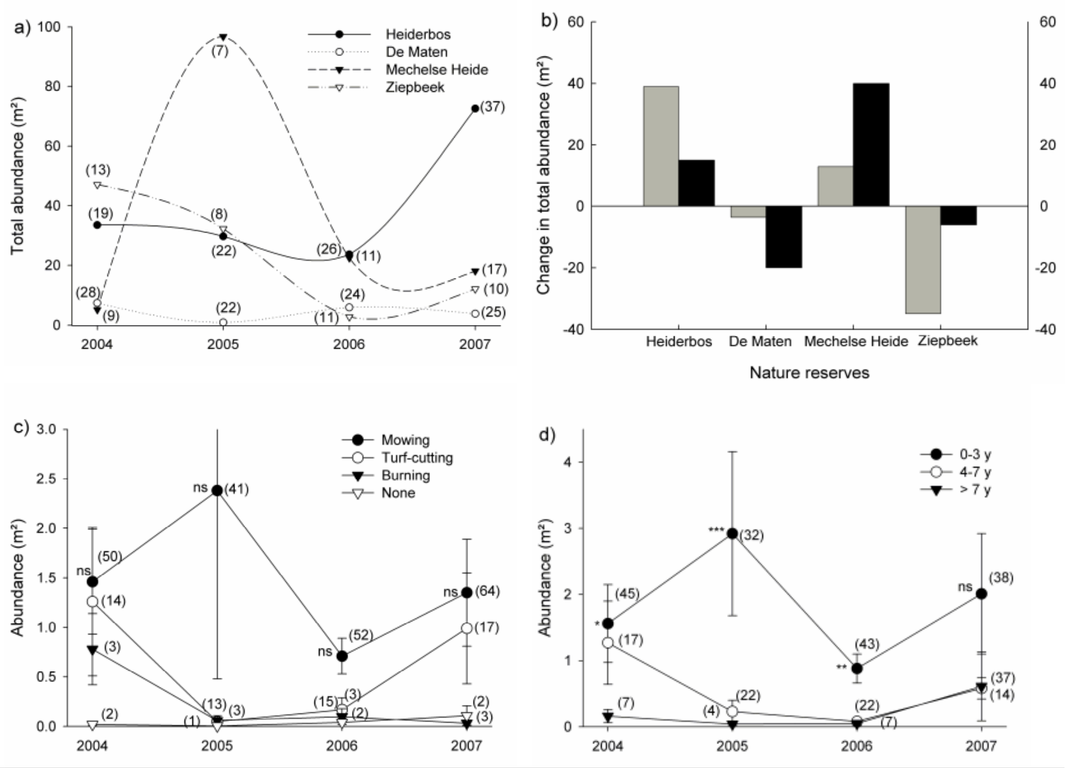


**Figure 3.6.** Changes in population size expressed as abundance (m²) over time. **a)** Change in total *C. epithymum* abundance (i.e. sum of all population sizes) over the four studied years for each study site, **b)** total population growth or decline (grey) and increase or decrease in number of management patches (black) between 2004-2008 per study site, and change in mean (± SE) *C. epithymum* abundance **c)** per type of management intervention and **d)** per vegetation age class over a period of four consecutive years.

One-way ANOVA (*: $0.01 < p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant) showed that young vegetation (0-3 y: managed 0-3 years ago) contains significant larger *C. epithymum* populations compared to the older vegetation. Numbers in parentheses represent the number of patches occupied with *C. epithymum* belonging to a particular reserve, management type or vegetation age class for each year.

Higher patch dynamics had a positive effect on both *C. epithymum*'s population dynamic and the total metapopulation size. The chance of new *C. epithymum* colonisations increased with increasing patch creation (Table 3.5c). Consequently, a positive growth in metapopulation size was only observed in the two reserves where patch creation events outnumbered patch disappearances (i.e. when during the four years more new patches were created than patches were lost by advanced heathland succession; Fig. 3.6b).

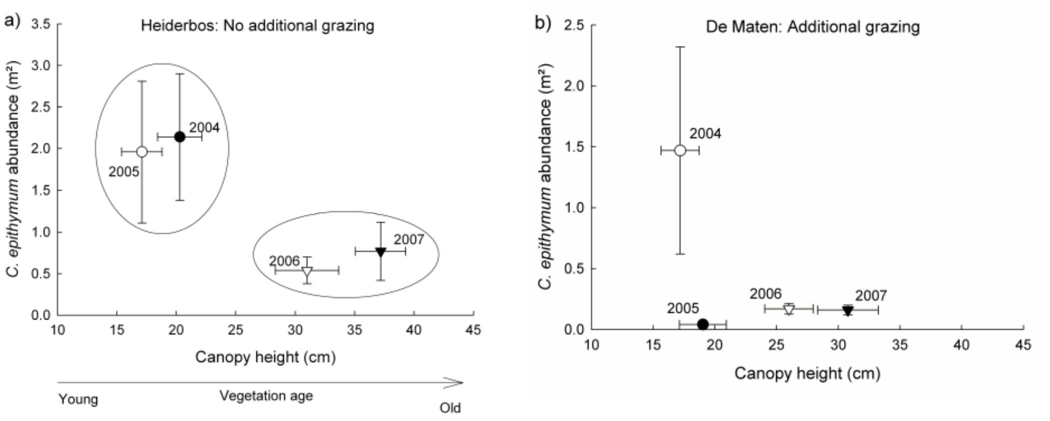


**Figure 3.7.** Change in mean ± SE *C. epithymum* population size (i.e. abundance ($m^2$)) in relation with mean ± SE canopy height in patches occupied in each of the four studied years for **a)** Heiderbos (n = 15) and **b)** De Maten (n = 18). The data points encircled by different lines correspond to significant differences in vegetation height and population size (Tukey's pairwise means comparison test, $p < 0.05$). The arrow along the axis of the graph on the left side indicates the significant positive correlations between canopy height and patch age, for the graph on the right side no significant relationship was found between these variables (Spearman rank correlations, $r_s = 0.54$; 0.002, with $p \leq 0.001$; $p > 0.05$, respectively).

For the subsample of 15 patches situated in Heiderbos, *C. epithymum* abundance decreased significantly with increasing vegetation height, which is significantly correlated with patch ageing (Fig. 3.7a). The older the vegetation was in a particular patch, the smaller was the population size. When patches had not been managed for more than 10 years, no *C. epithymum* individuals were found. On the contrary, for the subsample of patches studied in De Maten, no relationship was found between population size and vegetation height or patch age. Some patches at De Maten where management interventions (mowing, burning, or turf-cutting) had not occurred for over 10 years contained *C. epithymum* individuals (n = 18; Fig. 3.7b). The patches of De Maten differ from those in Heiderbos by the presence of extensive grazing by Galloway cattle from May to September at a density of about 0.1 cow $ha^{-1}$.

Flower density decreased significantly with reduced population size ($r_P$= 0.65, $p < 0.001$). The highest numbers of flowers were found in the largest populations (ANOVA $F$ = 25.16, df = 2, $p < 0.001$; Fig. 3.8). In addition, flower density was significantly influenced by year and vegetation structure, with a decreasing flower density with increasing vegetation height and *Calluna* cover (Table 3.6a). Finally, only year explained a significant part of the observed variation in the number of seeds per fruit, while population size did not significantly influence seed production (Table 3.6b).

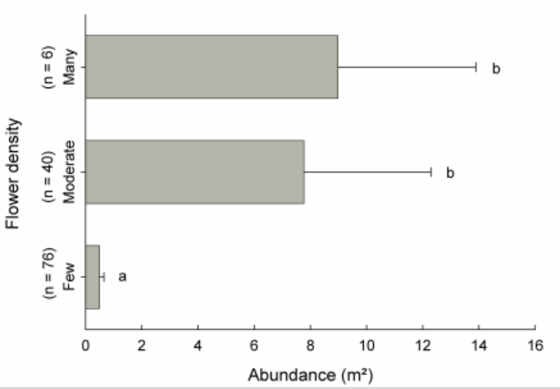


**Figure 3.8.** *Cuscuta epithymum* flower density as a function of their population size, expressed as mean (± SE) absolute abundance per flowering class. Different letters correspond to significant differences ($p < 0.05$), as determined with a Tukey a posteriori test. n = number of management patches per flowering class. Three flowering classes in accordance with Chapter 3a: few = 0-125, moderate = 125-450, many = more than 450 flowers per $0.04 m^2$.

**Table 3.6.** Effects of **a)** vegetation structure, year (2006, 2007, 2008) and the interaction of both on flower density (i.e. mean numbers of flowers per $0.04\ m^2$) and of **b)** population size ($m^2$), year and the interaction of both on seed production (i.e. mean number of seeds per fruit).

| a) | *Flower density* | | b) | *Seed production* | |
|---|---|---|---|---|---|
| | *df (n,d)* | *F* | | *df (n,d)* | *F* |
| Vegetation structure | 1,115 | 9.5*** | Vegetation structure | 1.96 | $0.5^{ns}$ |
| Year | 2,115 | 15.1** | Year | 2.96 | 0.5** |
| Veg × Year | 2,115 | 3.3* | Veg × Year | 2.96 | $0.2^{ns}$ |
| Adjusted R² | 0,34 | | Adjusted R² | 0.11 | |

*F*-values and significant levels from General Linear Models (GLM) are given. ns: not significant; *: $p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$; df(n,d) = degrees of freedom (numerator, denominator).

# Discussion

## *Patch size and isolation*

Consistent with the patterns observed in 2004, landscape isolation and patch size did affect neither patch occupancy nor *C. epithymum* abundance. Further, in contrast with observations for several other species (e.g. Hanski, 1994a; Quintana-Ascencio and Menges, 1996), the regional colonisation dynamics of this parasite were not dependent on isolation of suitable patches and the number of colonisations did not increase with increasing habitat patch size. For the latter a reverse pattern was observed. Other factors than patch geometry may drive *C. epithymum* population distribution and dynamics. Similar results were, however, found by Brose (2001) and Dupré and Ehrlén (2002) for other vascular plants. As *C. epithymum* is believed to disperse its seeds only over relatively short distances (see Box 5.1), these results suggest that seed exchange between populations is very limited. The studied populations may be seen as remnant populations (Freckleton and Watkinson, 2002), for which the existence of a persistent seed bank allows *C. epithymum* to "track" dynamic patches in time (Snäll et al., 2005). Seed banks have been shown to enable persistence during long periods of unfavourable conditions of unmanaged late-successional heathland (Bossuyt and Hermy, 2003; Piessens et al., 2004). This hypothesis is confirmed by the observation that new *C. epithymum* populations mostly appeared on patches the same year when the vegetation in these patches was set back to an early successional state by management interventions, independent of the presence of other *C. epithymum* populations in the vicinity.

### Patch dynamics

The dynamics of *C. epithymum* populations and the final changes in metapopulation abundance were positively influenced by patch dynamics. The more new patches were created during the study period, the more *C. epithymum* colonisations were observed (Table 3.5). Only when the creation of suitable patches by management outnumbered the patches which became unsuitable for the growth of the parasite due to advanced heathland succession, a positive metapopulation growth was observed over the four studied years (Fig. 3.6b). These results led us to conclude that colonisation mainly depends on the availability of suitable habitats and the rate at which new suitable habitats are created. This agrees with Johst et al. (2002), who underlined the importance of the rate at which disturbances occur for metapopulation persistence in dynamic landscapes. Second only to management frequency, the management method had an influence on *C. epithymum* populations. In particular, the prevailing management method 'mowing' resulted in considerably higher population abundances in comparison with turf-cutting or burning for all studied years. Nevertheless, this result is distorted by the predominance of mowing applications in the studied reserves in comparison with other management types (Fig. 3.6c). From our personal observations we can, however, conclude that all management interventions setting back the vegetation to early successional stages benefits *C. epithymum*, as observed for 2004 (Chapter 3a).

### Changes in vegetation structure

The spatial distribution of *C. epithymum* is showed to be very sensitive to vegetation age (or patch age i.e. time since last management) and height, which are governed by management activities. Our longer-term results confirm the association of *C. epithymum* with young, recently managed heathland, with largest population sizes found in early successional stages of 0-3 years old (Fig. 3.6d). The population size significantly decreased with increasing vegetation height. As soon as heathland vegetation became too old, *C. epithymum* disappeared from the vegetation. Nevertheless, these relationships were found in only three of the four studied nature reserves. In the reserve 'De Maten' population size did not significantly decrease with vegetation ageing (Fig. 3.7b). This observation may be attributed to the presence of extensive cattle grazing in addition to the other heathland management applications. As demonstrated by Bullock and Pakeman (1997), the presence of grazers in heathlands generally increases the cover of forbs and bare ground, while it reduces the cover of dwarf shrubs. By affecting small-scale vegetation heterogeneity, the presence of grazing in addition to other management applications extends the period of *C. epithymum* presence in successional heath patches. It must be noted, however, that the mean population size observed in De Maten is relatively low in comparison with those in the other reserves, which may be caused by the low number of new patches created by management in this reserve (Fig. 3.6a; Table 3.5c). Extensive grazing should therefore be combined with sufficient other management interventions to increase the densities and number of *C. epithymum* populations.

Although local extinctions were mostly found to occur when vegetation succession caused the loss of suitable habitat, our results show that, at least in some cases, extinctions can be caused by aberrant weather patterns. The strong fall in population size observed in 2006 is probably attributed to the extreme summer drought in July 2006, with a precipitation of only 34mm instead of the average of 89mm (recorded at weather station 'Maasmechelen'). In this unusually dry summer, withering and even death of *C. vulgaris* individuals was observed, which caused in its turn the death of *C. epithymum* individuals infecting this heather (pers. obs.). These results suggest that *C. epithymum* is sensitive to climatic changes which influence precipitation, although more research is needed to confirm this hypothesis. Nevertheless, we may conclude that changes in *C. epithymum* population size are mostly deterministic, but stochastic changes can occur.

### *Reproduction*

Our data clearly showed that the number of flowers per area reduce with decreasing population size. This might suggest that small populations suffer from increased inbreeding and consequently from loss of genetic variation (Richards, 2000; Hermy et al., 2007; Honnay and Jacquemyn, 2007), both of which are expected to reduce sexual reproduction. Consequently, flower density was higher in the larger populations occurring in patches with a low vegetation height and *Calluna*-cover. The data suggests a significant decrease in sexual reproduction during heathland succession, as well as a decline in population size. Management practices therefore indirectly affect the reproductive capacity of the parasite, as mentioned in Chapter 3a. Just as flower density was highly variable, the mean number of seeds per fruit was highly variable during the study period, implicating demographic stochasticity. Surprisingly, the per-flower seed production was not influenced by population size. This is in contrast with other studies which observed smaller populations to show lower seed set because of reduced pollinator attention (e.g. Oostermeijer et al., 1994; Morgan, 1999; Jacquemyn et al., 2002; Brys et al., 2004). For *C. epithymum* no information is available regarding to the influence of population size or flower density on the attraction on potential pollinators. Because of the parasitic nature of *C. epithymum*, one could hypothesise that the reproduction output of the holoparasite is rather related to its host quality than to its population size. Frost et al. (2003), for instance, demonstrated for *C. salina* that flower and fruit production was lower when it infected hosts which experience salt stress. The applicability of this hypothesis for *C. epithymum* needs, however, further research.

## Conclusion

We found evidence that *C. epithymum*'s occupancy and turnover patterns are not best explained in terms of patch size or isolation, but that population dynamics interact with spatiotemporal dynamics in habitat quality generated by management applications. The change in *C. vulgaris* canopy structure, characterised by management and subsequent succession, was the key factor controlling *C. epithymum* population dynamics and fecundity over several years. The frequency by which management activities are conducted seems to affect colonisation events. This agrees with Dupré and Ehrlén (2002) who stated that habitat configuration (i.e. patch area and isolation) may be less important for vascular plant distribution than habitat quality or the effects of land use history. Local colonisations, extinctions and population size are shown to be partly influenced by environmental and demographic stochasticity. As noted in several other studies (e.g. Fleishman et al., 2002; Guo et al., 2002; Ameloot et al., 2006) our findings highlight the need for, and importance of, multiple years of population and demographic monitoring to give an insight to factors influencing plant population dynamics. The results of one-year observations will depend on the point at which a snapshot is taken because of the influence of certain occasional events. Data collected over multiple years may reduce the effect of environmental and demographic stochasticity and therefore yield more accurate conclusions.

# 4

# Germination ecology of the holoparasite *Cuscuta epithymum*

Adapted from: Meulebrouck K., Ameloot E., Van Assche J.A., Verheyen K., Hermy M and Baskin C.C. 2008 – Seed Science Research 18: 25-34.

**Photos: Frederik Lerouge (2006)**

# Introduction

Seed germination and seedling establishment are critical stages in the life cycle of a plant, and their success depends on seeds being nondormant and in a suitable location when conditions permit germination. It is well established that seed dormancy and germination of higher plants are complex adaptive traits influenced by a large number of environmental factors (e.g. Mayer and Poljakoff-Mayber, 1975). Furthermore, the environmental conditions required to break dormancy may, or may not, be the same as those required for germination (Baskin and Baskin, 1998). Vleeshouwers et al. (1995) considered seed dormancy to be important in the life cycle of plants because it prevents germination at a time when environmental conditions are not (or will not remain) favourable for seedling establishment. On the other hand, nondormant seeds may not germinate because environmental conditions do not correspond to those required for germination (Vleeshouwers et al., 1995). That is, correlations between germination requirements of nondormant seeds and the ecological state of a plant's habitat offer a mechanism to avoid unfavourable conditions (e.g. cold winter) for subsequent plant establishment and reproductive growth (Finch-Savage and Leubner-Metzger, 2006).

The long-term persistence of any species at a site requires that at least occasionally seeds germinate and plants grow to maturity and produce seeds. Thus, timing of seed germination in relation to environmental conditions in the habitat can be an important part of the adaptation of a species to its habitat. Annual species, in particular, have received considerable research attention with regard to timing of germination in nature (Baskin and Baskin, 1998); however, little is known about timing of germination in annual holoparasites such as *Cuscuta*. In addition to favourable environmental conditions in the habitat, host plants must be available when seeds of holoparasites germinate, or seedling establishment can not occur (Kuijt, 1969). Seedlings of *Cuscuta*, in particular *C. epithymum*, only have 14-21 days to locate a host and establish a haustorial connection or they will die (Benvenuti et al., 2005; Chapter 2).

In *C. epithymum*, another intriguing aspect of the germination ecology is the presence of water-impermeable seed coats, i.e. physical dormancy (Gaertner, 1950). Seeds of many species of *Cuscuta* have a water impermeable seed coat surrounding fully developed coiled embryos (Baskin and Baskin, 1998; Benvenuti et al., 2005); consequently, scarification promotes germination of seeds of many *Cuscuta* species, including *C. epithymum* (Gaertner, 1950; Hutchinson and Ashton, 1979; Flynn et al, 2004). Furthermore, because *C. epithymum* seeds do not germinate to high percentage following acid scarification (maximal 5% germination: Gaertner, 1950), they also may have physiological dormancy. As far as we know, this should be the first report of combined dormancy in seeds belonging to the Convolvulaceae and the genus *Cuscuta*.

The holoparasitic *C. epithymum* grows in the temperate zone of the Northern Hemisphere, with its main distribution areas in western and central Europe and the Atlas Mountains in northwestern Africa (Verdcourt, 1948; Schaminée et al., 1996; Chapter 2). In northwestern Europe, *C. epithymum* is mainly associated with dry heathland vegetation (Doyle, 1993), and is mostly restricted to the pioneer phase of the heather succession (Chapter 3). Unlike some other holoparasites, for example, in *Orobanche* and *Striga*, the germination of *C. epithymum*, does not depend on host-exudates to stimulate germination (Baskin and Baskin 1998, Matusova et al., 2004). However, relatively little information is available about other factors responsible for seed dormancy break, germination requirements and on the relevance of these characteristics for *C. epithymum* in dynamic heath systems. For example, it is generally known that seeds of many species with physical dormancy can become nondormant during dry storage at room temperature (Baskin and Baskin, 1998). Little is known about this phenomenon in *C. epithymum* seeds, although Ewart (1908) reported only 6% germination after 4 years of dry storage.

In spite of this knowledge, several aspects of the germination of *C. epithymum* are still unclear. The patchy occurrence of *C. epithymum* in managed heath communities and the ongoing decline of this species raise several fundamental questions which were the objective of our research. (1) What kind of dormancy (physical and/or physiological) do freshly-collected and dry-stored seeds have? (2) What are the temperature requirements for the loss of dormancy and for optimal germination? (3) Do nondormant seeds require light for germination? (4) If physiological dormancy is present, how much cold stratification is required to break it?

## Materials and methods

### *Species*

*Cuscuta epithymum* (L.) L. is generally described as a summer annual, although it also has been reported to be perennial in certain conditions (Dean, 1954; Costea and Tardif, 2006; Chapter 6). Each individual plant may produce hundreds to thousands of spherical to somewhat 3-angled seeds with an average diameter and mass of 0.9mm and 0.3mg, respectively (Gaertner, 1950; Gómez, 1994; Costea and Tardif, 2006). Mature *Cuscuta* seeds have a four layered seed coat consisting of an epidermis, outer and inner palisade layers, and compressed parenchyma cells (Lyshede, 1992, Costea and Tardif, 2006). The testa epidermis is peculiar in that the outer walls of dry seeds are invaginated, while they bulge outwards in wetted seeds. Together with the epidermis the specialised two-layered cell palisade tissue is likely responsible for impermeability (Lyshede, 1992) and the entrance of dormancy is correlated with anatomical changes in the seed coat (Hutchinson and Ashton, 1979). Seeds show no morphological adaptations for dispersal (Chapter 2).

### *Seed collection*

Seeds were collected from ripe fruits during the last 2 weeks of September 2004, 2005 and 2006 from different *C. epithymum* populations located in heath vegetation of three nature reserves. These reserves, namely 'Heiderbos' (2004, 2005, 2006), 'Kikbeekbron' (2005, 2006) and 'Ziepbeekvallei' (2005), are situated in the province of Limburg (see Chapter 3) and separated by less than 9km. The climate is temperate, with an annual mean temperature of 10-11°C and an annual total rainfall of 750-800mm. In 2004 and 2005, seeds were stored dry at room temperature after collection until the start of imbibition treatment, germination experiment or the stratification treatment. In contrast, seeds collected in 2006 were used within 1 week for different experiments. While collecting seeds in autumn 2005, we marked some locations where plants occurred, and *C. epithymum* reappeared (as seedlings) in these exact places the next spring, confirming the summer annual character of this species. Seeds undergo three distinct developmental stages on the mother plant. First, immature seeds are large and green, then they change from green to light brown and finally become darker brown and shrink in size as they dry. For the germination tests, only the last category was used. The diameter of *C. epithymum* seeds was 0.9 ± 0.03mm (mean ± SE; n = 20). Freshly collected seeds weighed on average 0.31 ± 0.01mg (n = 100), while seeds stored dry for 3 months weighted only 0.26 ± 0.01mg (n = 160).

### *Seed viability*

To determine the maximum germination potential of fresh seeds, seed viability of seeds collected in 2006 was assessed with tetrazolium tests (Moore, 1973). Five replicates of 40 seeds each were placed on moist filter paper (Schleicher and Schuell N° 2282) at 23°C for 16 hours and then cut in half with a scalpel. Seed sections were incubated in a 0.1% aqueous solution of tetrazolium chloride for 24h at 30°C in darkness. Only seeds showing a strong red-stained embryo were considered viable.

### *Imbibition experiment*

To test if *C. epithymum* seeds are water impermeable, i.e. possess physical dormancy, the rate of water uptake was monitored for scarified and for nonscarified dry-stored (December 2005) and fresh (2006) seeds. Four replicas of 40 seeds individually scarified with a razor blade and of 40 nonscarified seeds were placed on filter paper moistened with distilled water in 9cm diameter Petri dishes and kept in the laboratory at room temperature (*ca.* 21°C). Following this procedure, seed mass was determined at 1h intervals for the first 8h, then after 13h and later at intervals of 24h for the next 120h. Following Hidayati et al. (2001) percentage water uptake was calculated as actual increase in seed mass based on seed mass at $t_0$:

$$\%W_s = [(W_i - W_d)/W_d] \times 100$$

where $W_s$ = increase in mass of seeds, $W_i$ = mass of seeds after a given interval of imbibition, and $W_d$ = seed mass at $t_0$. Initial ( at $t_0$) seed mass was determined for air dried seeds that had been wetted for *ca.* 2min, blotted dry (standard treatment), and weighted to the nearest 0.1mg (mean of four seeds).

Increase in mass of the seed may not necessarily indicate that germination will occur. Thus, to determine if the scarified and nonscarified seeds would germinate, seeds were incubated under a 12h photoperiod at 23°C for 3 weeks.

## *Germination experiments*

Different germination experiments were carried out to study the effect of temperature and light on the loss of dormancy and on germination. In a first experiment, a preliminary treatment of cold stratification and three temperature regimes (15/6, 23 and 30/20°C) for testing after cold stratification were chosen based on results from a pilot study in 2005. The 15/6°C regime simulates spring conditions in a temperate climate, 23°C represents a constant temperature and 30/20°C was chosen because a previous study had found optimum germination of *C. epithymum* seeds at relatively high temperatures, and because we expected a positive influence of fluctuating temperatures. Lados (1999) obtained high germination percentages at 26°C.

To determine if cold stratification is a prerequisite for germination, 18 dishes of 50 nonscarified seeds each were placed on moist filter paper at 5°C for 12 weeks in December 2005. To obtain dark conditions, nine of the 18 dishes were placed in light-tight boxes. Filter paper was replaced every 2 weeks to avoid fungal infection. The controls consisted of 18 dishes of 50 nonscarified seeds, each on dry filter paper stored at room temperature (21°C) for 12 weeks. Dry stored and cold stratified seeds were tested for germination under a 12h daily photoperiod or in continuous darkness (Petri plates inside wooden light tight boxes and only opened under green light) at alternating (12/12 hour) temperature regimes of 15/6 and 30/20°C or at a constant temperature of 23°C. Light (PAR = *ca.* 36 $\mu$mol m$^{-2}$s$^{-1}$, 400-700nm photon irradiance) was provided by fluorescent tubes (Philips TLD 80) during the 12h higher temperature portion of the cycle.

In 2006, during an extensive experiment we determined if *C. epithymum* seeds had both physical and physiological dormancy. Three replicas of 50 scarified and of 50 non-scarified fresh seeds were cold stratified at 5°C for 0, 4, 8 and 12 weeks and then tested for germination at 15/6, 23 and 30/20°C in a 12h daily photoperiod for minimum 9 and maximum 15 weeks. Several periods of cold stratification were used to determine how much cold stratification was required to break physiological dormancy. During stratification a low concentration (3ppm) of merthiolate (Na-ethylmercury-thiosalicylate) was added to the Petri dishes to prevent microbial infections. No inhibition of germination was observed. Following Baskin and Baskin (1998) nongerminated seeds were checked for viability at the end of the experiment. Seeds that collapsed when pinched gently with forceps were considered nonviable, while those that did not collapse following pinching and had a firm, white embryo, were regarded as viable.

In all experiments, distilled water was added as needed. Germinated seeds were counted three times a week until no germination was observed for 10d. Protrusion of the radicle was the criterion for germination.

### Effect of dry storage

Seeds collected in September 2004 and stored for 16 months at room temperature were used to determine the effect of dry storage on dormancy level and germination. Nine replicates of 50 seeds were placed in 9cm diameter Petri dishes on moistened filter paper. Seeds in three replicas were individually scarified with a razor blade, while those in six replicates were not scarified. The scarified seeds were incubated in a 12h daily photoperiod at 23°C, and the nonscarified seeds were incubated under 12h daily photoperiod 23°C and at an alternating temperature of 15/6°C. All seeds were incubated until no germination was observed for 10d (after 4 weeks). Furthermore, the effect of dry storage was tested by comparing germination of fresh seeds (2006) with that of seeds stored dry for 3 months (2005).

### Data analysis

Following Meyer and Monsen (1991) germination rate was expressed as the number of days until 50% of the final germination was reached. The maximal potential germination percentage was calculated as the sum of percentage germinated seeds and percentage non-germinated, but viable seeds. Means and standard errors were calculated for germination percentages and percentage increase in seed mass. To achieve normality and homogeneity of variances, data were arcsine transformed before analysis. Effects of cold stratification, scarification, temperature regimes and/or light regimes on the (maximum) germination of *C. epithymum* seeds were analysed with a two- or three-way ANOVA, followed by a Tukey pair-wise means comparison test ($p < 0.05$). Potential differences between treated and untreated seeds (scarification or dry storage), differences between the temperature regimes and differences between various stratification cold periods were analysed using an independent sampled *t*-test and one-way ANOVA test, respectively. All statistical analyses were performed with SPSS for Windows 14.0 (SPSS 2006).

# Results

## Seed viability

Tetrazolium tests revealed that 26 ± 0.77 of 40 freshly-collected seeds were viable, indicating a maximum potential germination percentage of 65%. Of the nonviable seeds, 9 ± 0.81% coloured only light red and 26 ± 1.17% showed no colouring. Therefore, 35% of freshly-collected seeds were nonviable rather than dormant.

## Imbibition experiment

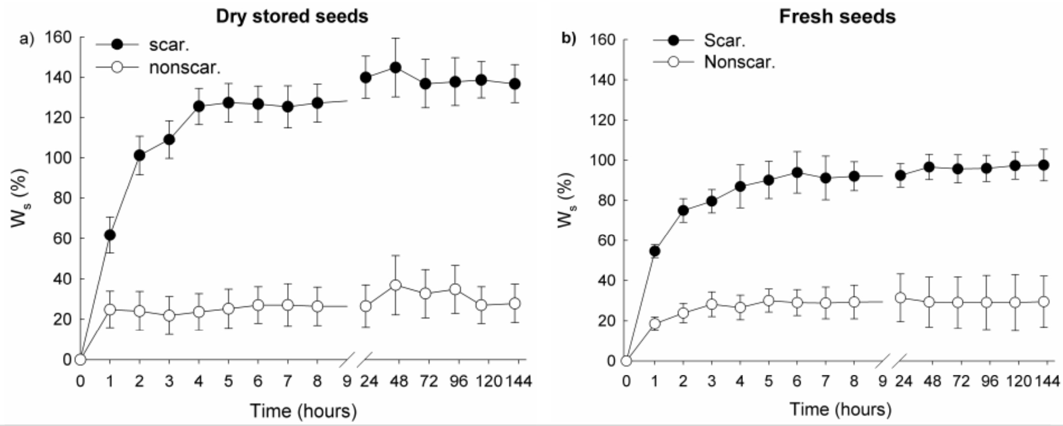


**Figure 4.1.** Mean (± 1 SE) water uptake by intact and by scarified *C. epithymum* seeds **a)** stored dry for 3 months or **b)** freshly collected. Seeds were incubated at room temperature (23°C) on moist filter papers for *ca*. 6d. Filled symbols: scarified seeds; open symbols: non-scarified seeds. $W_S$ = percentage of increase in seed mass.

After 2h (dry-stored seeds) or 1 hour (fresh seeds), scarified seeds had imbibed significantly more water than nonscarified ones (Fig. 4.1a, at time 2h for dry stored: $t = 2.99$, $p < 0.05$; Fig. 4.1b, at time 1h for fresh seeds: $t = -14.81$, $p < 0.001$). After 4-5h, the increase in seed mass of scarified seeds reached a plateau value of 126 ± 9% for dry-stored seeds and 90.19 ± 9.27% for fresh seeds. Furthermore, only 0.6 ± 0.6% dry-stored and 0% fresh, nonscarified seeds germinated within 20d at 23°C, while 27 ± 4% dry stored and 21 ± 3% fresh scarified seeds germinated in the same germination period (Germination % after 21d: $t = 4.46$, $p < 0.05$ and $t$ =-14.56 respectively, $p < 0.001$). However, after 21h of incubation, all scarified seeds (fresh and dry-stored) were totally swollen and 0 % of the dry-stored and 21 ± 2% of the fresh nonscarified seeds were partly swollen.

## Germination experiments

After 4 weeks of incubation in either light or darkness at the three temperature regimes, none of the nonstratified seeds had germinated (Table 4.1).

**Table 4.1.** Germination of nonscarified *C. epithymum* seeds at constant (23°C) and alternating temperatures (15/6 and 30/20°C) at a 12h daily photoperiod (L/D) or continuous darkness (D) for 30d. Stratified (nonscarified) seeds were kept at 5°C for 8 weeks. Germination percentages (%) are means ± SE after three weeks of incubation. Germination rate was scored as the time (d) to 50% of the final germination percentage.

| Light | *Temperature (C°)* | *Stratification (%)* | *Rate (d)* | *No stratification (%)* |
|---|---|---|---|---|
| L/D | 15/6a | 4.6 ± 1.8 | 9 | 0 |
| | 23a,b | 4.6 ± 2.1 | 8 | 0 |
| | 30/20b | 2.1 ± 1.1 | 5 | 0 |
| D | 15/6a | 10.4 ± 1.7 | 9 | 0 |
| | 23a,b | 7.1 ± 1.5 | 6 | 0 |
| | 30/20b | 2.8 ± 1.1 | 6 | 0 |

Different letters correspond to significant differences (Tukey's pair wise means comparison test, $p < 0.05$).

Stratification significantly increased germination both in light and darkness ($t$-test after 21d: $t = 8.49$, $p < 0.001$, Table 4.1), but at all test temperatures germination percentages were relatively low (Table 4.1). Furthermore a significant temperature effect was observed, with significantly less germination at 30/20°C compared to the other temperature regimes (Tukey test, Table 4.1 and 4.2). Daily alternation of temperature did not enhance germination (Table 4.1). Light was also not required for germination of the stratified seeds and seeds germinated as fast in dark as in light conditions (Table 4.1). When only considering the seeds incubated at 16/5°C and 23°C, more seeds germinated in dark than in light, although this result was not significant (Table 4.2).

**Table 4.2.** *F* and *p*-values of the two-way ANOVAs testing the effects of germination temperature, light regime and interaction on germination percentage of 3-month-old dry stored *C. epithymum* seeds after 30d of incubation.

| *Variables* | *df(n,d)* | *F* | *p-value* |
|---|---|---|---|
| Temperature | 2,12 | 5.54 | 0.02 |
| Light | 1,12 | 2.51 | 0.139 |
| Temperature × Light | 2,12 | 0.84 | 0.457 |
| $R^2_{adj}$ | | 0.38 | |

Df (n, d) = degrees of freedom (nominator, denominator).

For scarified fresh seeds at all temperatures, germination percentages increased with increasing length of the cold stratification period (Fig. 4.2, Tukey pair wise means comparison test, $p < 0.05$).

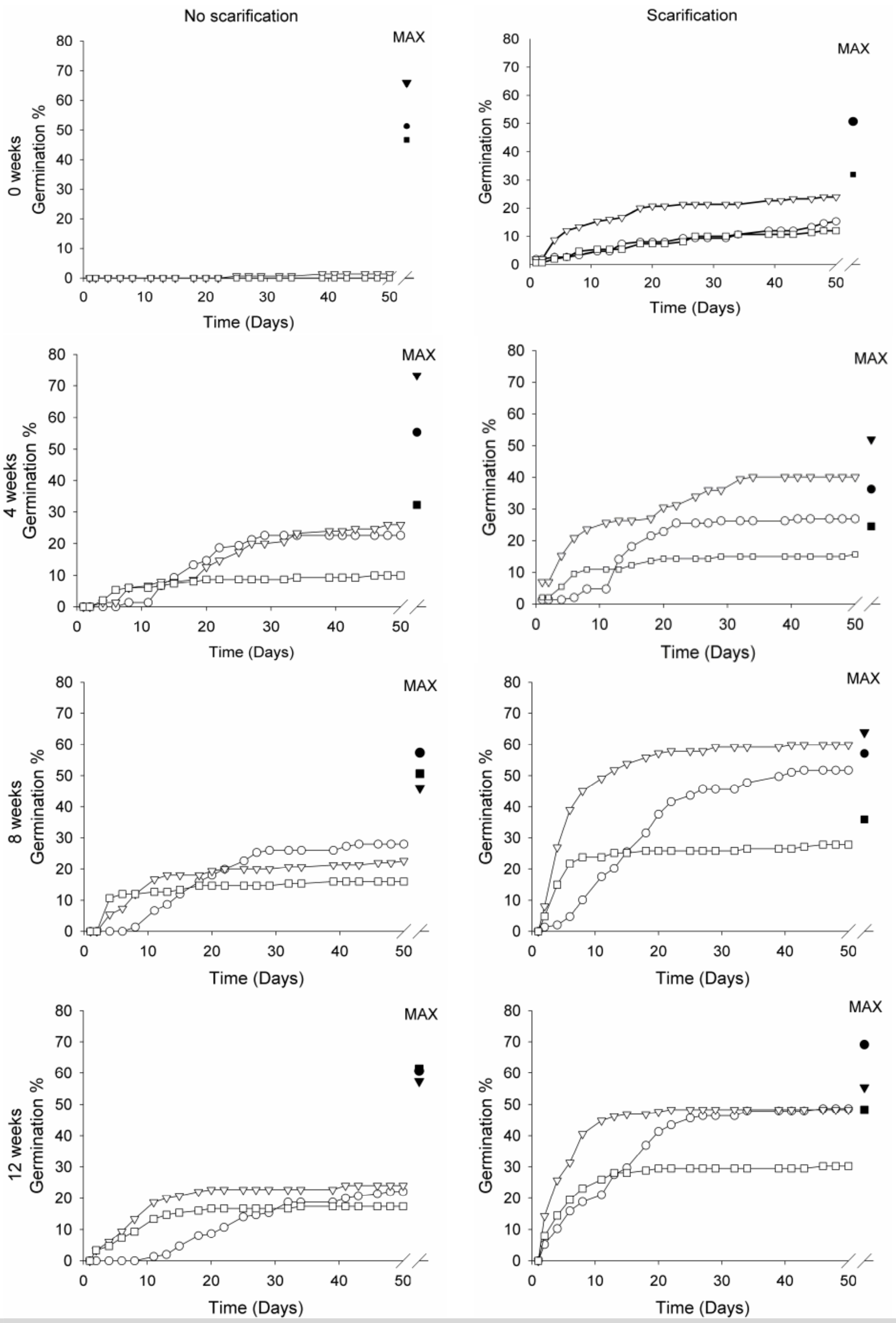


**Figure 4.2.** Cumulative germination percentages of fresh *C. epithymum* scarified (right) or nonscarified (left) seeds in 12h photoperiod, under temperature regimes of 15/6°C (●), 23°C (▼) or 30/20°C (■) for 50d, after a stratification period of 0, 4, 8 or 12 weeks at 5°C (open symbols). Maximal potential germination percentages for the three temperature regimes, i.e. the sum of the percentage germinated seeds and the percentage viable ungerminated seeds, are shown by closed symbols.

Increasing germination was most significant for seeds incubated at 15/6°C (Fig. 4.2, effect of stratification on germination: one-way ANOVA $F = 32.49$, $p < 0.001$). Increasing germination with increasing length of cold period was not significant for the temperature regime of 30/20°C (one-way ANOVA: nonscarified seeds: $F = 6.94$, scarified seeds: $F = 1.69$, $p > 0.05$). An 8-week period of cold stratification gave optimal results; 12 weeks of this treatment showed no further increase in germination of seeds at 15/6, 23 or 30/20°C. After 8 weeks of cold stratification, the percentage of germinated seeds came close to the maximal potential (Fig. 4.2). None of the seeds germinated at 5°C. Germination only occurred after temperature elevation. Temperature and scarification, as well as stratification, significantly affected seed germination (Table 4.3).

The effect of scarification was dependent on temperature regime (Fig. 4.2, Table 4.3), and scarified seeds incubated at 15/6°C or 23°C germinated to significantly higher percentages than those at 30/20°C (*t*-test: $t = -3.98$, $p = 0.001$, $t = -3.06$, $p = 0.006$, $t = -2.23$, $p = 0.04$, respectively for 23, 15/6 and 30/20°C). In all treatments, the slowest germination was for seeds incubated at 15/6°C (Fig. 4.2); it generally took 1 week before these seeds started germinating. Furthermore, after 3 weeks of observation some seeds, especially those not exposed to a cold period, continued germinating even after more than 50d of incubation (last observed germination after 110d of incubation, 15/6°C, scarified seeds). At the end of this experiment, 49 ± 3% of the seeds were dead (i.e. collapsed when pinched gently), 25 ± 3% had germinated and 27 ± 3% were viable, but not germinated (Fig. 4.2). The maximum potential germination percentage was influenced by temperature and scarification as well as by stratification (Table 4.3). At 30/20°C or after scarification viability was significant lower than at the other temperature regimes or without scarification (Fig. 4.2, Tukey pair wise means comparison test, $p < 0.001$).

**Table 4.3.** *F* and *p*-values of the three-way ANOVAs testing the effects of germination temperature, scarification, stratification and interactions on germination percentage of fresh *C. epithymum* seeds after 50d of incubation and on maximum potential germination percentage.

| | | *Germination %* | | *Max germination %* | |
|---|---|---|---|---|---|
| *Variables* | *Df(n,d)* | *F* | *p-value* | *F* | *p-value* |
| Temperature | 2,54 | 15.86 | <0.001 | 19.34 | <0.001 |
| Scarification | 1,54 | 63.56 | <0.001 | 8.91 | 0.004 |
| Temperature × Scarification | 3,54 | 27.38 | <0.001 | 5.43 | 0.002 |
| Scarification × Stratification | 2,54 | 3.36 | 0.042 | 1.62 | 0.21 |
| Temperature × Stratification | 3,54 | 2.61 | 0.061 | 2.87 | 0.05 |
| $R^2_{adj}$ | 6,54 | 1.13 | 0.360 | 3.45 | 0.006 |
| | | 0.72 | | 0.53 | |

Df (n, d) = degrees of freedom (nominator, denominator); Max. germination = sum of percentage germinated seeds and percentage non germinated, but viable seeds.

### *Effect of dry storage*

No germination of nonscarified seeds was observed after 16 months of dry storage at room temperature followed by 4 weeks of incubation at 15/6 or 23°C. In contrast, after 4 weeks of incubation at 23°C, 32 ± 5% of the scarified seeds had germinated. Although not significantly, the germination percentage of scarified seeds at 23°C increased with the length of dry storage (Fig. 4.3; one-way ANOVA after 21d: $F = 2.13$, $p = 0.19$). However, it should be mentioned that the seeds were collected in different years and sites (see 'seed collection').

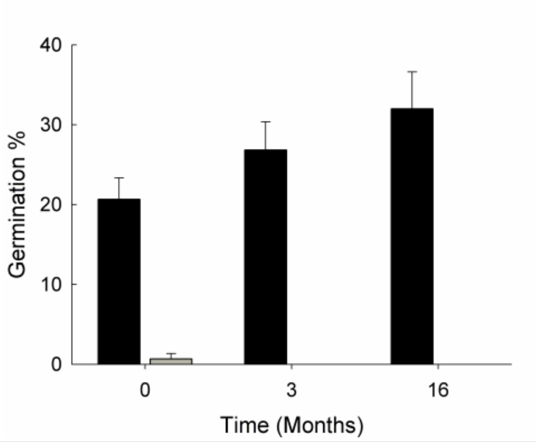


**Figure 4.3.** Germination percentages (mean + SE) after 21d of incubation at 23°C of scarified (black) and nonscarified (grey) *C. epithymum* seeds stored dry for 0, 3 and 16 months at room temperature. The seeds are collected in different years, namely in 2006 (0), 2005 (3) and 2004 (16).

## Discussion

### *Environmental control of dormancy breaking*

Various mechanisms regulate seed germination of *C. epithymum* in its natural habitat, some of which are internal, whereas others are external, environmental factors. These experiments clearly revealed that both naturally matured and dry-stored seeds were physically dormant, and that physical dormancy was not broken during long periods (3 and 16 months) of dry storage (Fig. 4.2 and Fig. 4.3). In accordance with other *Cuscuta* spp. data (e.g. *C. campestris*: Hutchison and Ashton, 1979; Benvenuti et al., 2005), the exclusion of water by the seed coat was the main factor preventing germination. Indeed, physical dormancy had to be broken before germination was possible. Consistent with Hutchison and Ashton (1979) for *C. campestris*, impermeability of *C. epithymum* seeds probably develops during maturation on the mother plant and remains after shedding under dry conditions (but Gaertner, 1950 reported seed germination when still in the capsule). Furthermore, in contrast to seeds of some other species from different families that become water-permeable (nondormant) during dry storage - e.g. *Sida spinosa* (Malvaceae) (Egley, 1976) and *Stylosanthes macrocephala* (Fabaceae) (Silva and

Felippe, 1986) - our imbibition experiments suggest that dry storage strengthened physical dormancy of *C. epithymum* seeds. While 20% fresh, nonscarified seeds imbibed fully, none of the dry-stored nonscarified seeds did so. While water absorbed by the epidermis of dry-stored *Cuscuta* seeds causes them to bulge outwards, water could pass through the two palisade cell layers lying just underneath the epidermis (Lyshede, 1992); thus, the seeds do not imbibe. The ecological significance of physical dormancy can be seen as an adaptive seed trait allowing *C. epithymum* seeds to germinate over time and thus spread germination risks across generations. Physical dormancy may allow species to quickly colonise burned areas, be dispersed over long distances and/or survive for long periods in the seed bank (Fenner and Thompson, 2005). Indeed, *C. epithymum* grows on recently burned heath (Chapter 3); it appears after management of the vegetation (e.g. mowing and turf cutting, Chapter 3); and may therefore possess a persistent seed bank, as suggested for *Cuscuta* spp. by Costea and Tardif (2006). Many other heathland species have a persistent soil seed bank (Grime, 1981; Bossuyt and Hermy, 2003), and 15% of *C. campestris* seeds were viable after 12 years of storage, suggesting that this species may be able to form a persistent seed bank (Benvenuti et al., 2005). However, survival of seeds in dry storage is not necessarily an indication of viability in the soil.

Although Hutchison and Ashton (1979) found that mechanical scarification of *C. campestris* seeds increased germination to over 90% at a temperature of 30°C, scarified seeds of *C. epithymum* without cold stratification germinate to a maximum of only 24% (Fig. 4.2). Thus, physiological dormancy occurs in seeds of *C. epithymum*. Germination only occurred in scarified and/or stratified seeds, with the highest germination observed in scarified seeds that were cold stratified (Fig. 4.2). Although seed coats are made permeable and can imbibe water, only some of *C. epithymum* seeds are capable of germinating (Fig. 4.3 and Fig. 4.2), while other seeds possess dormant embryos. Germination after dry storage increased slightly for scarified seeds and decreased for nonscarified seeds, suggesting after-ripening or a progressive loss of physiological dormancy as a function of time (Fenner, 2000). Another possible explanation is the presence of annual cycles of physiological dormancy, as found for various species, including annuals, e.g. Baskin and Baskin (1985), Bouwmeester and Karssen (1993), Van Assche and Vanlerberghe (1989) and Van Assche et al. (2003), but both hypotheses needs further research for seeds of *Cuscuta* spp. (e.g. burial experiments). In addition, seeds should orgininate from one collection in the same year, since the yearly reproductivity of *C. epithymum* is proved to be highly variable (see Chapter 3b). We conclude that *C. epithymum* seeds possess both physical and physiological dormancy (combinational dormancy), and that seed dormancy is a crucial safety mechanism in its parasitic life cycle. Previously, embryo dormancy combined with coat impermeability has also been observed in both leguminous and non-leguminous species and is interpreted as a double safety mechanism preventing accidental premature germination of seeds (Kigel, 1995), although, as far as we know, this is the first report of combinational dormancy in the genus *Cuscuta* and in the Convolvulaceae.

As reported for seeds of many other plant species germinating in spring under field conditions (Baskin and Baskin, 1998), our experiments showed that spring germination would be strongly influenced by temperature. A treatment of 8 weeks at 5°C (stratification) was most effective in releasing both the physical and physiological dormancy state (Fig. 4.2). This safety mechanism ensures that intact, as well as slightly damaged, seeds germinate in early spring and not before the winter, or when they are exposed to the high temperature fluctuations occurring in summer. The advantage of this low temperature requirement is obvious for *C. epithymum*, which sheds its seeds in autumn, but its seedlings require young host plants to parasitise, which are mostly available in spring. Furthermore, if the parasite would germinate in autumn, it would not have enough time to complete its life cycle before winter. As mentioned before, after seed shed some *C. epithymum* seeds may still be water permeable, but at that time physiologically dormant embryos can not germinate. Soon, after drying, all seeds become impermeable until a cold winter period leads to changes in the dormancy status of the seeds. A cold stratification likely inactivates the physical barrier in the seed coat by means of structural and biochemical alterations in the cell walls so that water can reach the developing embryo (pers. com., M. Costea). More importantly, while 65% of the fresh *C. epithymum* seeds were viable, only 22% of the intact stratified seeds germinated after a cold period of 8 weeks. Our research thus emphasises that a cold winter period of 8 weeks followed by a temperature elevation totally inactivates the physiological dormancy, but that this cold period only partially (30%) eliminates physical dormancy since more seeds germinated after scarification (Fig. 4.2). As a result, only a part of the seed crop will germinate in the following spring, while the other still physically dormant fraction will remain in the seed bank until some subsequent growing season. In other words, physiological dormancy ensures germination in spring, while physical dormancy provides a spread of germination in time, illustrating the presence of a double safety mechanism. In managed heathland landscapes, where the vegetation undergoes successional dynamics due to regular human disturbance activities, a stock of viable seeds is an advantageous regenerative strategy. In this way, the parasite may remain in the seed bank when heather is too old and quickly reappear after management, such as mowing, turf-cutting or burning. The chief advantage of such a risk-spreading strategy is the avoidance of total reproductive failure of any given generation. Also *C. campestris* possesses a mechanism that allows germination of a few seeds at a time for several years (Dawson in Kuijt 1969). Confirmation of this hypothesis needs further experimentation, including burial and sowing experiments (see Chapter 5 and 7).

## *Germination requirements*

In western European heathlands, *C. epithymum* mostly occurs in early successional stages and disappears after *ca.* 7 years, but reappears in young vegetation when heathland is managed by mowing, turf-cutting or burning (Chapter 3). Therefore, an absolute light requirement or the need for fluctuating soil temperatures, seen as indicators of the surrounding vegetation and the depth of seeds in the soil, are expected to occur in *C. epithymum* seeds. These mechanisms frequently occur in small-seeded pioneer species (Putz and Appanah, 1987; Van Assche and Vanlerberghe, 1989; Van Assche et al., 2003) and in buried seeds of many annual weeds (Pons 1991). Nevertheless, our laboratory data showed no evidence of requirements for light or fluctuating temperature for germination (Table 4.1 and Fig. 4.2). This is in accordance with Benvenuti et al. (2005) who demonstrated the photo-insensitive germination ecology of *C. campestris*. In general, seeds with physical dormancy germinate over a wide range of temperature and light conditions after they become permeable (Baskin et al. 2004). How *C. epithymum* seeds avoid germination in older heath vegetations still remains unclear, knowing that germination does not depend on chemicals produced by hosts (Baskin and Baskin 1998). Maybe the seeds cannot germinate because of other factors, e.g. inhibition through allelopathy by other plants (Costea and Tardif, 2006; Baskin and Baskin, 1998).

At least in some species, temperature can have a dual effect on seeds since it not only affects dormancy, but it also may have a direct effect on germination (Bouwmeester and Karssen, 1993). As mentioned above, low autumn and winter temperatures tend to partially break physical dormancy in the summer annual *C. epithymum*. Our results showed that seeds never germinated at 5°C, and germinated best after being exposed to moderate temperatures (15/6°C or 23°C, see fig. 4.2), suggesting that chilling followed by rising spring temperatures is needed to remove physical dormancy. After physical dormancy is broken, seeds with non-dormant embryos can germinate at prevailing spring temperatures. For some legumes with physical dormancy, germination also occurred as a respons to sudden shift to higher, alternating temperatures after a cold winter period (Van Assche et al., 2003), and such a sudden shift to higher temperatures may be a widespread season-sensing mechanism among species with persistent seed banks. When seeds are near the soil surface, seeds undergo a cold period followed by higher temperatures in spring resulting in germination. Indeed, fresh *C. epithymum* seeds germinated best at a constant temperature of 23°C, while 15/6°C yielded almost comparable results. The latter exhibited no germination in the first week, followed by a slow germination during several weeks, but the final germination percentages were as high as at 23°C (Fig. 4.2). This may be a mechanism for preventing germination on one or a few relatively warm days during the cold winter period. Furthermore, the seeds may be nondormant, but they may not germinate because it is too cold. Once temperature becomes suitable in spring, a large number of seeds of this parasite may germinate within a few days followed by a period with intermittent germination. High temperatures (e.g. 30/20°C) would not stimulate cold stratified seeds to germinate, causing *C. epithymum* seeds to remain ungerminated in the summer period. Here, we hypothesise again the annual cycling of physiological dormancy, although confirmation is needed.

We do not know if the processes observed under laboratory conditions occur in field, and more studies are certainly needed, but this study provides evidence that double seed dormancy may be an important survival strategy of *C. epithymum*, allowing for its persistence in dynamic heathland vegetation from northwestern Europe. The combined dormancy creates a double safety strategy: because of physiological dormancy, germination can occurs in spring, and due to the physical dormancy only a portion of the seeds will germinate, which will potentially lead to the formation of a persistent seed bank. In this way, the parasite may be adapted to the specific conditions that now occur in managed heathlands in a temperate climate.

# 5

# Will the sleeping beauties wake up? Seasonal dormancy cycles in the seeds of the holoparasite *Cuscuta epithymum*

Photo: Klaar Meulebrouck (Ziepbeekvallei, 2007)

# Introduction

It is well recognised that in many ecosystems seed banks are crucial for ensuring persistence of a taxon at a population site (e.g. Fisher and Mattheis, 1998; Piessens et al., 2005), especially for many short-lived species that reproduce mainly by seeds (Venable and Lawlor, 1980; Valleriani, 2006). Although theory emphasises the importance of seed banks in plant species population dynamics (e.g. Grime, 1981; Russi et al., 1992; Meyer et al., 2006), empirical estimates of this important life stage are scarce, particularly in parasitic plants (but see López-Granados and Garcia-Torres, 1999; Benvenuti et al., 2005). Often we are much better informed about the laboratory responses of seeds than about their behaviour under natural conditions (Fenner, 2000). Baskin and Baskin (1998) pointed out, however, that seeds stored under natural conditions may not respond in the same way as those studied under controlled laboratory conditions. To understand the role of seeds in the population dynamics of a specific species, it would thus be very helpful to track the fate of buried seeds exposed to natural temperature and moisture cycles. For many species seed death and/or death of seedlings that fail to emerge from the soil accounts for a substantial seed loss and strongly influences the establishment response and ecology of the species (Klinkhamer et al., 1998; Grundy et al., 2003). Some seeds may be attacked by pathogens, others lose viability in the course of time and die of old age, and still others may be lost from the seed bank via early germination (Baskin and Baskin, 1998). During the burial period, seeds are not necessarily in a constant state of being able to germinate if placed under appropriate temperature, light and moisture conditions. In fact, annual cycles of germinability, in which germination changes during the course of a year, occur in seeds of many species (e.g. Baskin and Baskin, 1985; 1998; Van Assche and Vanlerberghe, 1989; Van Assche et al., 2003) and it is probably very common among plants with persistent seed banks (Van Assche and Vanlerberghe, 1989; Jayasuriya et al., 2009). Germination cycling is indeed regularly found in seeds having physiological dormancy (PD) during which the embryos cycle back and forth between dormancy and nondormancy many times before seeds eventually germinate or die (e.g. Baskin and Baskin, 1985; Bouwmeester and Karssen, 1993). The same phenomenon has been documented in a few species producing physical dormant (PY) seeds. Nonetheless, because PY seeds can not return to a dormant state once dormancy is broken (Jayasuriya, 2009), cycling in these seeds is due to alternations between being sensitive and insensitive to dormancy breaking treatments (i.e. sensitivity cycling; some species from Fabaceae in Van Assche et al., 2003; *Ipomoea lacunosa* in Jayasuriya et al., 2008a; *Cuscuta australis* in Jayasuriya et al., in 2008b). Essentially nothing is, however, known about the germination cycling in seeds with combinational dormancy (PY + PD), i.e. seeds that possess both water impermeable seed coats and dormant embryos.

In the present study we focus on the seed bank of *C. epithymum*, an early successional holoparasite. In north-western Europe the species mostly occurs in recently managed dry heathland vegetation (see Chapter 2 and 3). The high variability in frequency and spatial configuration of management activities in successional heathlands (Gimingham et al., 1979; Kleyer et al., 2007) causes spatiotemporal cycles in habitat quality, thus making germination and establishment of the short lived parasite highly unpredictable. Chapters 3 and 4 suggest that dormancy is one of the most important traits ensuring long-term survival in dynamic heath landscapes, but empirical data are absent. Seeds of this species have a combination of PY and PD, and after cold stratification only 15-30% of the seed crop becomes water permeable and germinates under optimal temperatures. The remaining seeds have PY and potentially form a persistent soil seed bank (Chapter 4). Furthermore, the results obtained in Chapter 4 may indicate an annual germination cycle, just as other species living under seasonally predictable conditions which are typical for the temperate zone (Van Assche and Vanlerberghe, 1989; Van Assche et al., 2003), but this hypothesis has not been tested. Also for *C. epithymum* the response of its seeds during burial under natural conditions has not been investigated. Therefore, we studied the viability, depletion and dormancy-break of buried seeds exposed to the normal seasonal changes for 31 months. More specifically the following questions are posed. (1) Do the *C. epithymum* seeds exhibit germination cycles, and if cycles are present what mechanism is involved? (2) What is the loss of viability in nature compared to that of seeds stored dry at room temperature? (3) Do seeds of the parasite remain viable for extended periods of time in the soil? The results of this study are interpreted in an ecological context to provide insight into the fate of buried seeds subjected to natural conditions.

## Materials and methods

On 16 September 2006, during the period of natural seed shed, mature seeds were collected by hand from different *C. epithymum* populations growing in 'Hoge Kempen' National Park (50° 58' 17"N; 5° 37' 38"E), Limburg, Belgium. This national park includes remnants of formerly extensive heathland landscapes, once a characteristic landscape feature in much of northwestern Europe (Webb, 1998). The climate in the region of the park is temperate, with an annual mean temperature of 10-11°C and an annual total rainfall of 750-800mm. Mean daily maximum and minimum monthly air temperatures and precipitation were calculated from records made by the weather station at Maasmechelen (~ 3 km from study site; Fig. 5.1 a,b).

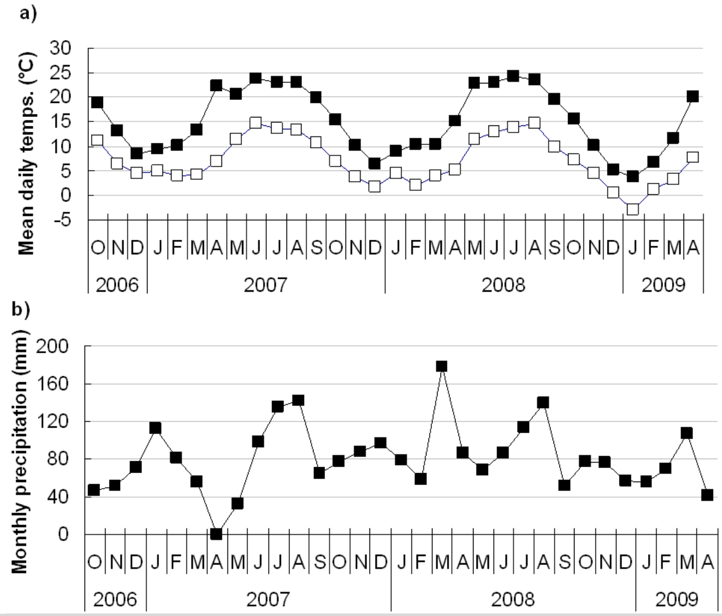


**Figure 5.1. a)** Mean daily maximum (black) and minimum (white) air temperatures per month and **b)** monthly precipitation in the study area for the duration of research (weather station 'Maasmechelen').

To determine the fate of *C. epithymum* seeds under natural conditions, fresh mature seeds were placed in 10 fine mesh (0.13mm² average mesh) nylon bags within a few days after harvest. Each bag contained 200 seeds mixed with 20g white acidophilous sand, and it was buried at a depth of 7cm in a 15cm diameter clay pot with a drainage hole in the bottom filled with local soil. The pot was buried so that the rim was even with the soil surface in early successional heathland vegetation which was mown in 2005. This experimental set up allows seeds to be exposed to natural temperature and soil moisture conditions. Another part of the harvested seeds was stored dry at room temperature (about 20°C) until the start of germination experiments (hereafter called control seeds). Furthermore, to determine the viability of the freshly collected seeds, we performed tetrazolium viability tests on 200 of the harvested seeds (see Chapter 4 for details).

The first day of every 3 months, one randomly selected bag was exhumed, allowing 10 exhumation dates starting from the first of October 2006 (fresh seeds) and ending the first of April 2009. After exhumation, soil was washed off the bag, and its contents were immersed in distilled water to separate seeds from sand. Three replicates of a maximum of 50 exhumed seeds and of 40 control seeds each were tested for germination on filter paper (Machery & Nagel 440B) moistened with distilled water in 9cm diameter Petri dishes at a constant temperature of 23°C for 3 weeks in light- and temperature controlled incubators. Light (PAR = *ca.* 36 µmol $m^{-2}$ $s^{-1}$, 400-700nm photon irradiance) was provided by fluorescent tubes during 12h a day. This germination condition was chosen because previous research had shown that the optimum germination of *C. epithymum* seeds was 23°C (Chapter 4). Following Baskin and Baskin (1998), non-germinated seeds were checked for viability at the end of the first part of this experiment. Seeds that collapsed when pinched gently with a forceps were considered non-viable, while those that did not collapse following pinching and had a firm, white embryo, were regarded as viable. To determine if intact, viable non-germinated seeds possessed PD embryos, the seeds were individually scarified with a razor blade and then tested for germination at 23°C for another 3 weeks (results of January and April 2007 are not available). For all germination tests, distilled water was added as needed. Germinated seeds were counted three times a week, for 6 weeks. Protrusion of the radicle was the criterion for germination. Percentages of germinated seeds, dead seeds, viable non-germinated and decomposed (i.e. disappeared during burial) seeds were calculated. The method by which exhumed seeds were classified is summarised by a dichotomous key (Fig. 5.2).

To achieve normality homogeneity of variances, data were arcsine transformed before analysis. Potential differences between buried and control seeds were analysed using a t-test for independent samples. This analysis was performed with SPSS for Windows 15.0 (SPSS, 2008).

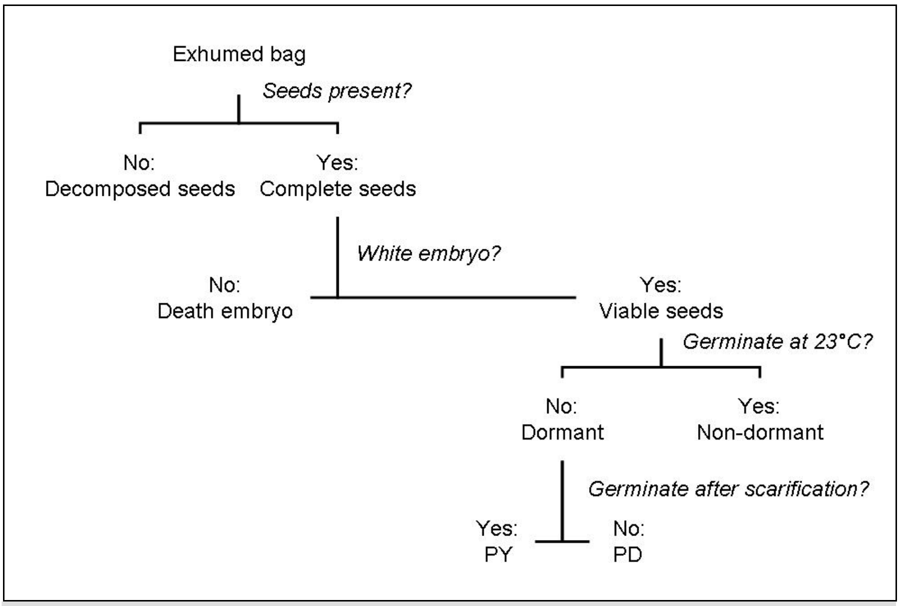


**Figure 5.2.** Schematic overview of the dichotomous decision key used in this study to classify exhumed seeds.

# Results

The tetrazolium test revealed that on average (± SE ) 65 ± 2% of the freshly-collected seeds was viable (see Chapter 4). Figure 5.3 shows the results of the germination experiments on freshly matured seeds and seeds buried or stored dry for different periods up to 31 months.

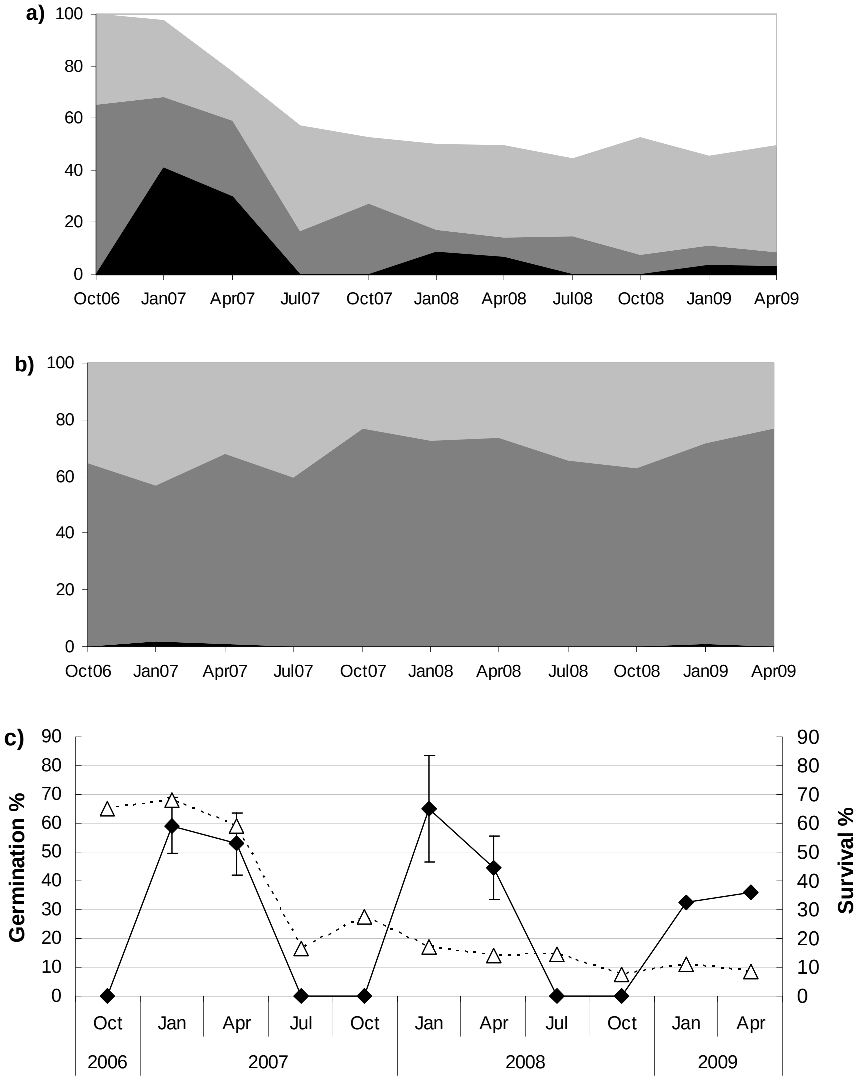


**Figure 5.3.** Percentage germinated seeds (black), dormant viable seeds (dark gray), seeds with dead embryo (pale gray), decomposed seeds (white) following 0 to 31 months of **a)** burial in the soil or **b)** storage at room temperature (control). **c)** Mean (± 1 SE) germination percentages calculated on the basis of surviving seeds (black diamonds) and survival percentages of seeds buried for 0 to 31 months in natural conditions (white triangles). The first point in the three graphs represents the result of fresh seeds.

Freshly collected mature *C. epithymum* seeds failed to germinate after 3 weeks of incubation at 23°C in light and were dormant (Fig. 5.3a). After burial, *C. epithymum* seeds clearly exhibited a seasonal cycle in germination, with a peak of germination in winter and spring and no germination in the other seasons (Fig. 5.3a, c). These germination peaks were observed for three successive years. After the 3-week period of incubation at 23°C, seeds exhumed in summer and autumn were not imbibed.

The sum of the germination percentages of the scarified viable non-germinated seeds and the nonscarified germinated seeds revealed seasonal cycling in PD (Fig. 5.4a). These seasonal changes in germination were clearly synchronised with seasonal changes in temperature, whereas the limited changes in precipitation did not promote germination (Fig. 5.1a, b; Fig. 5.3a, c). Exposure of seeds to winter temperatures (min. temperature ≤ 5°C, Fig. 5.1) partially promote germination of seeds subsequently exhumed and tested at 23°C, while exposure to late spring and summer temperatures (min. temperature > 10°C, Fig. 5.1) inhibited germination of seeds exhumed and tested at 23°C. Nevertheless, the germination percentage never reached the maximal germination potential (i. e. when all viable seeds should germinate; Fig. 5.3a); on average (± SE) only 39.5 ± 7% of all viable seeds germinated and so were released of both PY and PD. After burial and incubation at 23°C, only a low portion of the non-germinated viable seeds germinated, with an average (± SE) germination over the 31 months of only 17.2 ± 4%. The other ungerminated part of these viable seeds contained physiologically dormant embryos (Fig. 5.4b).

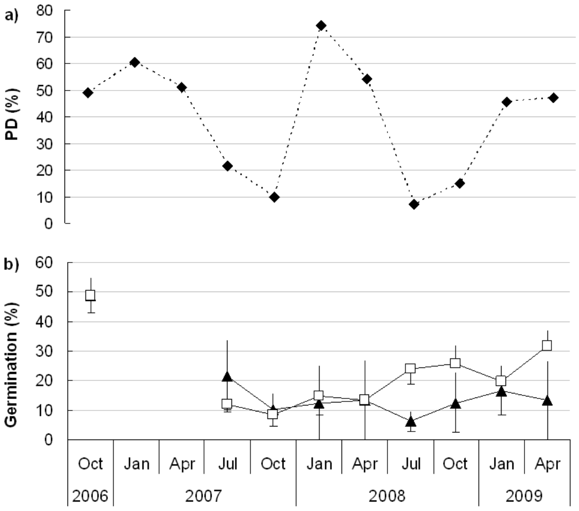


**Figure 5.4. a)** Percentage of the viable seeds that are physiological nondormant (PnD; i.e. sum of all germinated seeds (scarified and nonscarified). **b)** Mean (± SE) germination percentage of the scarified dormant seeds (see Fig. 5.3) following three weeks of incubation at 23°C. Before scarification the seeds were incubated three weeks at 23°C following 0-31 months of burial in the soil (black triangle) or storage at room temperature (white square). For January and April 2007 there are no results available of nongerminated scarified seeds.

During burial under natural conditions, seeds lost viability quite rapidly, especially in the first year (Fig. 5.3a; exponential decline $y = 78.8\ e^{-0.22x}$). At the end of the study only 8.5%

of the seeds were viable; 41% of the seeds contained a dead embryo and 50.5% of the seeds had disappeared, i.e. had decomposed. During the 31 months, the portion of nonviable buried seeds containing dead embryos stayed almost constant (on average (± SE) 33 ± 2%), while the portion of decomposed seeds increased strongly the first year and then reached a plateau of an average (± SE) 50 ± 1.4% (Fig. 5.3a).

Under constant room temperature conditions, almost all viable seeds were PY for the whole research period (Fig. 5.3b). Germination was only observed in a few exceptional cases and annual changes in germination were not observed (Fig. 5.3b). Further, there was no loss of seed viability during the 31 months, with an almost constant ratio viable on nonviable seeds. Consequently, the portion of viable seeds was significantly higher for seeds stored at room temperature than for those buried in soil ($t = -6.6$; $p < 0.001$). In contrast to the results of the buried seeds, PD did not show any seasonal changes. The percentage of non-dormant embryos was relatively low (on average (± SE) 22 ± 4%) and increased slightly during 31 months (Fig. 5.4b).

## Discussion

Not only did seeds of *C. epithymum* exhibit a marked seasonal germination periodicity (Fig. 5.3a), but scarification of exhumed seeds that failed to germinate at 23°C revealed that embryos cycle back and forth between physiological dormancy and nondormancy (Fig. 5.4a). That is, the changes in physiological state of the embryo are comparable to the annual changes in the dormancy state observed in many species whose seeds have physiological dormancy (PD) (Baskin and Baskin, 1998). To the best of our knowledge, this is the first report of PD cycling in seeds with combinational dormancy.

Benvenuti et al. (2005) and Van Assche et al. (2003) reported cycling of germinability in hard seeded *C. campestris* and some Fabaceae, respectively. Nevertheless, seeds of these species have only impermeable seed coats, while the embryo is nondormant. Thus, as explained by Jayasuriya et al. (2009) these observed germination periodicities are due to cycling in sensitivity to dormancy breaking-treatments during burial in the soil and not to dormancy cycling. Sensitivity cycling is also observed in *C. australis* (Jayasuriya et al., 2008b). From our results it is, however, not completely clear if *C. epithymum* 's physical dormant seed coat undergoes such cycles in sensitivity or is subjected to a gradually loss of PY during the winter periods. This aspect of *C. epithymum*'s seed biology needs, however, some additional experiments, as described in Jayasuriya et al. (2009). Nevertheless, the germination mechanism present in *C. epithymum* seeds is clearly fine tuned with predictable seasonal temperature shifts in such way that germination only occurs in spring when conditions are favourable for both germination and completion of the life cycle. Seedling emergence in the field showed similar strong seasonal periodicity with appearance from April until July (pers. obs.). In addition, dry storage at constant room temperatures did not break PY or PD at all, which reinforces the importance of changing in temperatures for dormancy loss. So, in addition to *Orobanche crenata* (López-Granados and Garcia-Toures, 1999), *C. campestris* (Benvenuti et al., 2005) and *C. australis* (Jayasuriya et al., 2008b), we present another example of a parasitic species in which germination is controlled by temperature changes instead of chemical cues of the host plant (e.g. *Striga* and *Orobanche* in Matusova et al., 2005).

In accordance with results obtained under controlled laboratory conditions (Chapter 4), each spring only a portion of the viable *C. epithymum* seeds (~ 60%) germinated (Fig. 5.3a). In contrast to laboratory results, where PD was broken in 100% of viable seeds after a cold period followed by temperature elevation, results from this study demonstrate that under natural field conditions PD was broken in only 74% of the viable seeds (Fig. 5.4a, b). So both PY and PD are responsible for delayed germination of a portion of the seed crop by which total reproductive failure is avoided. In this way, only the portion of the seeds containing both nondormant embryos and water permeable seed coats can respond to temperature elevation occurring during spring. The remaining dormant fraction with PY and PD remains ungerminated in the soil seed bank until some subsequent growing season. These data thus confirm the risk-spreading strategy of seeds hypothesised in Chapter 4.

In contrast with our expectations, there was substantial, exponential loss of buried seeds due to seed decomposition (Fig. 5.3a). Obviously, to ensure re-establishment of a few individuals at the time vegetation is set-back to an early-successional stage by management, large amounts of seeds must be produced before the vegetation becames too old to allow *C. epithymum* infection. Although the observed strong seed decline, this study provides unequivocal evidence that a small portion of the seeds of *C. epithymum* can survive during longer periods (min. 31 months; Fig. 5.3a) and thus have the potential to form persistent seed banks (cf. Grime, 1981). Since our results only give a view on seed germination three springs after the seed input, the maximum period of viability of *C. epithymum* seeds under natural conditions is still unknown. Thus, from these results we can not conclude that *C. epithymum* forms a long-term persistent seed bank (Bakker et al., 1996). However, seeds of this species re-established on several sites in Flemish heathland which were unsuitable for growth (forest/old heath) for several decades, e.g. when early-successional vegetation was created by cutting down forest allotments or by rejuvenating old heath vegetation (mentioned in Chapter 3). In addition, *C. epithymum* can reappear from the soil seed bank after heath vegetation that not been managed for > 15 years was mowed (Box 5.1). These observations and the gradually decline of seed viability the last 2 years observed in this research (Fig. 5.3c) confirmed the hypothesis that *C. epithymum* forms persistent seed banks. As for many other species living in landscapes with spatiotemporal cycles in habitat quality, these persistent seeds may be crucial for population survival (e.g. Venable and Brown, 1988; Pons, 1991; Piessens et al., 2005).

In contrast, seeds stored in the laboratory did not lose seed viability (Fig. 5.3b). This is in accordance with results found for seeds of *Orobanche crenata* (López-Granados and Garcia-Torres, 1999). Therefore, caution should be used in interpreting viability data as longevity estimates under laboratorial conditions have a tendency to overestimate natural longevity.

## Conclusion

Given the predictability of temperature dynamics in temperate climates and the unpredictable spatiotemporal cycles in habitat quality in managed heathlands, the combination of seasonal dormancy cycling, delayed germination and persistent seeds make *C. epithymum* well adapted for living in dynamic temperate zone heathlands. Nevertheless, the rapid decrease in viability of seeds buried in the field alerts us to the sensitivity of this parasite to local extinction if seed reserves at the site are not large enough. This may partly clarify the endangered character of this species.

### Box 5.1: Seed dispersal in space and time

**Introduction**

In heathlands management activities alternate and subsequently also secondary vegetation succession is scattered both in space and time (Webb, 1998). Species inhabiting such dynamic landscapes will experience those changes as spatiotemporal cycles in habitat quality (Kleyer et al., 2007). The ability of plant species to establish populations at suitable newly created patches is critical to deal with those mosaic cycles (Olff and Ritchie, 1998). In this context, seed dispersal, both in time and space is considered as essential (Fisher and Mattheis, 1998; Amarasekare and Possingham, 2001; Adams et al., 2005). Nonetheless, a trade-off between these features is expected (Venable and Brown, 1988; Rees and Long, 1993; Bullock et al., 2002). In addition, it is expected that the structure of the landscape surrounding habitat patches may influence dispersal among patches, e.g. by ‘corridors’ or by ‘stepping-stone dispersal’. Dispersal between populations should for instance be increased by building or protecting habitat corridors, which are linear strips of habitat that connect larger patches of habitat. Little is known, however, about the processes controlling colonisation of *C. epithymum*, or how management may anticipate on these processes.

In order to have a better view on the colonisation possibilities of *C. epithymum*, we created experimental “landscapes “ in which dispersal processes are observed. The objectives were to examine the distance *C. epithymum* can disperse, the potential of *C. epithymum* to cross unsuitable vegetation of old heath, the influence of the structure of surrounding habitat on dispersal activities and the capacity to recruit from seed banks.

**Method**

In March 2006, in the nature reserve ‘Mechelse heide’, experimental landscapes including corridors, isolated patches and possibilities for directed dispersal via stepping stones were created on three dry heath locations in which *C. epithymum* was absent before the experiment (Fig. B.5.1.1). Those locations had not been subjected to management for more than 10 years, were dominated by *Calluna vulgaris* (~ 100 % cover) of the late successional stage and were a minimum of 400m apart from one another. At each of these locations, three circles of ca. 380m$^2$ containing different types of “micro-landscape” were created by mowing and removing all aboveground vegetation (9 circles in total, two replicas of each landscape type; Fig. B.5.1.1). Each landscape is composed of the same amount of mown patches of the early-successional stage (~ 83m$^2$) surrounded by heath matrix of the late-successional stage unsuitable for the growth of *C. epithymum*.

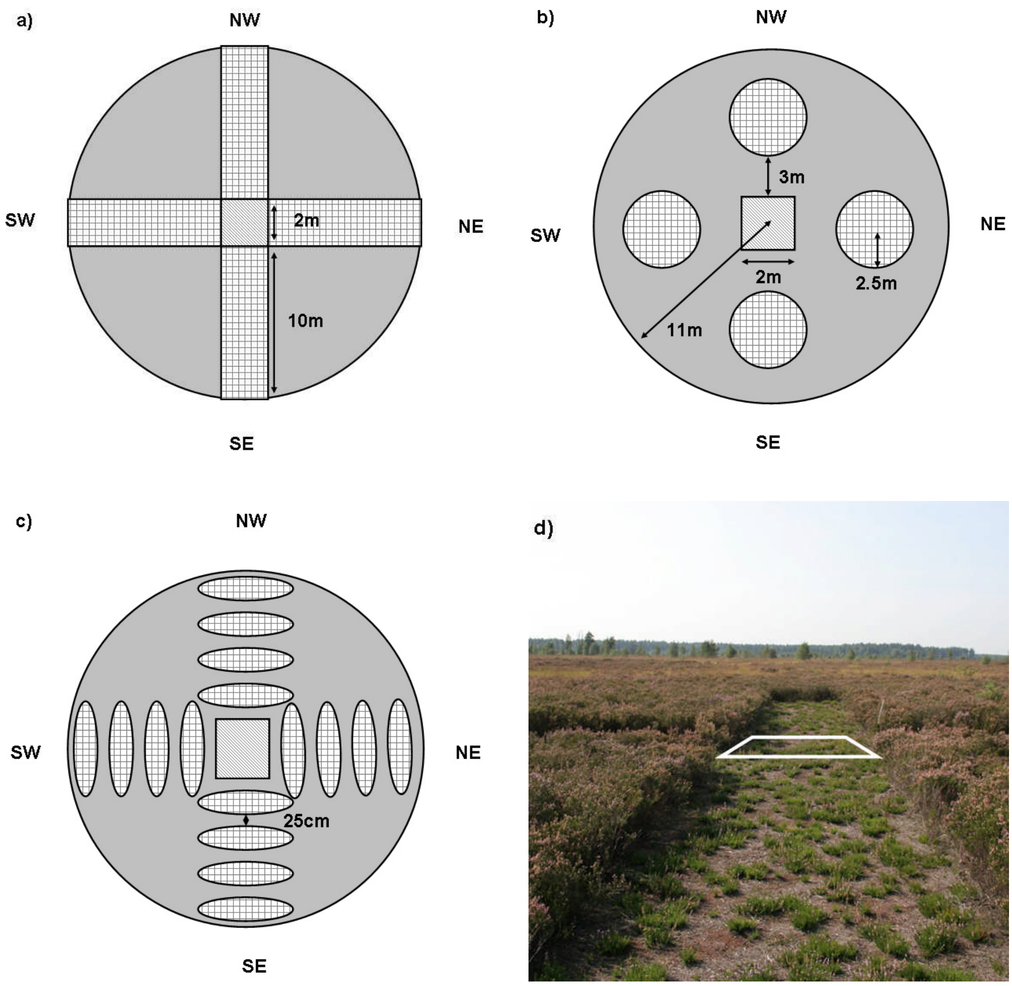


**Fig. B.5.1.1.** Layout of the experimental design of the three types of experimental "micro-landscapes". Each landscapes, created in circles with a radius of 11m, consist of a same amount of early-successional mown heath vegetation (light grey; ~83m²) surrounded by unsuitable late-successional heather (dark grey). Each landscape contains a central suitable patch (4m²), which is **a)** connected to four 2 × 10m corridors, i.e. 'corridor'-landscape, **b)** surrounded by four isolated circles (radius = 2.5m), i.e. 'isolation'-landscape or **c)** surrounded by four ellipsoids in each of the four wind directions representing stepping stones which are 25cm isolated from each other, i.e. 'stepping stone'-landscape. **d)** Photo of a 'corridor'-landscape'. The central suitable patch is accentuated by white lines. Each central square contains a *C. epithymum* source population which may migrate to the other suitable patches.

Each circle contains a central suitable patch of 2 x 2m² from which *C. epithymum* movement was monitored (Fig. B.5.1.1). In the 'corridor-landscape' the central patch was connected with four suitable corridors of 2m x 10m facing to four different wind directions (Fig. B.5.1.1a). The central patch of the 'isolation-landscape' was surrounded by four suitable circles (radius = 2.5m) separated from the central patch by 3m unsuitable late-successional heath (Fig. B.5.1.1b). Finally, the 'stepping stones-landscape' is composed of the central patch and in each of the four wind directions four ellipsoids ($r_1$ = 1m; $r_2$ = 1.6m) which are separated from each other by 25cm unsuitable heather (Fig. B.5.1.1c). On 15 September 2006 a source population of *C. epithymum* was created by adding 500 seeds per m². All experimental landscapes into which *C. epithymum* was introduced were surveyed in detail from April 2007 until August 2008 in order to compare movement rates of *C. epithymum* from the central patch to surrounding suitable patches. The 1st week of August 2007 only a few mature individuals were settled in the central patches and flowering of these individuals was absent or very limited. Therefore strongly parasitised *C. vulgaris* branches were collected from large and viable nearby *C. epithymum* populations and introduced in each central suitable patch in order to strengthen the source populations. From this moment onwards viable source populations containing flowering individuals established. From April until August 2008 establishment in the surrounding suitable patches was checked. At the last visit, in August 2008 migration distance into the suitable patches was measured. In addition the total area (m²) covered by *C. epithymum* and the cover (%) of *C. epithymum* threads within the central and surrounding patches were recorded in order to quantify the performance of the populations in the suitable patches. Further we scored the flower density, defined as the number of flowers per (20 x 20) cm² (few = 0-125 flowers, moderate = 125-450 flowers, many = > 450 flowers). Finally, in each surrounding patch the number of *C. epithymum* aggregations of red threads the parasite forms in the vegetation was recorded. Per "landscape type", for each wind direction means and standard errors of the different variables were calculated.

In order to examine the capacity of *C. epithymum* to disperse in time, in February 2006 four circles (radius = 2.5m) of early-successional heath vegetation were created by mowing and removing litter in a dry heathland site of 130m × 260m in 'Mechelse heide' reserve. The circles were separated from each other by at least 2m unsuitable old heathland. Before the onset of the experiment, the vegetation was not managed for more than 15 years. Nevertheless, on this location a *C. epithymum* population was present when the vegetation was still suitable (pers. comm. J. Gorissen). We tested the hypothesis that *C. epithymum* can bridge unfavourable periods in the seed bank and will reappear when old heath is rejuvenated. Therefore, from April 2007, when sufficient young Calluna individuals were present, until August 2008 the presence of *C. epithymum* was recorded. If *C. epithymum* occurred in the circle, the same variables as mentioned above were recorded.

## Results and discussion

Migration of *C. epithymum* from the central source population to surrounding suitable patches only succeeded when the central patch was connected by a suitable corridor, without any difference in migration direction (Table B.5.1.1). *Cuscuta epithymum* did not succeed in crossing physical barriers of old heath vegetation, even not small ones. In addition, movement into the corridors was just observed by means of growth of *C. epithymum* threads from one host to another during summer periods and from regrowing overwintering haustoria in spring 2008. In none of the surrounding suitable patches where seeds or seedlings were found.

**Table B.5.1.1.** Means (± SE) of *C. epithymum* cover (%/m²), mean number of *C. epithymum* aggregations, maximal movement distance of *C. epithymum* from the central source population into suitable patches (cm) and flower density for each central patch and wind direction per landscape type (corridor-, isolation- and stepping stone-landscape.

| | *Wind* | *Cover (%/m²)* | *No. of aggregations* | *Distance (cm)* | *Flower density* |
|---|---|---|---|---|---|
| **Corridor** | Centre | 4.7 ± 1.5 | | | Many |
| | NE | 0.1 ± 0.0 | 1.3 | 45 | Moderate |
| | SE | 0.1 ± 0.0 | 1.3 | 45 | Few |
| | SW | 0.1 ± 0.0 | 1.7 | 60 | Few |
| | NW | 0.4 ± 0.3 | 2.3 | 110 | Few |
| **Isolation** | Centre | 10.7 ± 5.2 | 0 | 0 | Many |
| | NE | 0.0 ± 0.0 | 0 | 0 | / |
| | SE | 0.0 ± 0.0 | 0 | 0 | / |
| | SW | 0.0 ± 0.0 | 0 | 0 | / |
| | NW | 0.0 ± 0.0 | 0 | 0 | / |
| **Stepping stone** | Centre | 4.0 ± 2.0 | 0 | 0 | Many |
| | NE | 0.0 ± 0.0 | 0 | 0 | / |
| | SE | 0.0 ± 0.0 | 0 | 0 | / |
| | SW | 0.0 ± 0.0 | 0 | 0 | / |
| | NW | 0.0 ± 0.0 | 0 | 0 | / |

Centre = central patch; four wind directions: NE = northeast, SE = southeast, SW = southwest, NW = northwest. Three flowering classes: few = 0-125, moderate = 125-450, many = more than 450 flowers per 0.04 m².

These results suggest that *C. epithymum* is strongly dispersal limited, as found for many other plants (e.g. Eriksson, 1998; Jacquemyn et al., 2002). In concordance with Tewksbury et al. (2002), studying both plant and animal species, we may assume that corridors function as conduits for migration of *C. epithymum* individuals. Nevertheless, migration rates were very low, i.e. maximum 1.1m per year (Table B.5.1.1). Given the successional character of heathland vegetation, it may be concluded that corridors are not effective in increasing *C. epithymum*'s colonisation chances to newly created suitable patches. Van Dorp et al. (1997) came to a similar conclusion for plants with short-range seed dispersal.

In August 2007, seven *C. epithymum* aggregations were found in one of the four circles mown in late-successional heath where the parasite was present in the past (extend 0.3m²; cover < 0.1 %, only two of the seven aggregations had some flowers). In August 2008, these *C. epithymum* aggregations had expanded to a viable flowering population of 2.5m² (cover: 5% and many flowers). Our results thus prove that *C. epithymum* is able to recruit from persistent soil seed bank and thus disperse in time, as demonstrated for other heathland species (Bossuyt and Hermy, 2003). No *C. epithymum* individuals were observed in the other three circles, suggesting a clustered occurrence of *C. epithymum* seeds in the soil. Similar clustering of seeds has been reported by Thompson (1986) and by Plue et al. (in press) for some grassland and woodland species, respectively.

These results thus demonstrate that *C. epithymum*'s colonisation is controlled by seed dispersal in time rather than in space. Nevertheless, more thorough research is needed to examine seed dispersal in space, including experiments with seed traps. Accidental dispersal of one seed to a suitable patch may indeed be sufficient to establish a whole new population. Finally, this study indicates that management planning of heathland to enhance the (re)colonisation of *C. epithymum* might be best based on the current and/or past spatial distribution of *C. epithymum* populations. Creation of new early-successional patches must preferably take place on locations where previously *C. epithymum* is known to have occurred or on locations adjacent to current occupied patches.

# 6

# Hidden in the host – Unexpected vegetative hibernation of the holoparasite *Cuscuta epithymum*

Adapted from: Meulebrouck K., Ameloot E., Brys R., Tanghe L., Verheyen K., Hermy M. 2009 – Flora 204: 306-315.

**Photo: Frederik Vanden Abeele (Ziepbeekvallei, 2007)**

# Introduction

Organisms, including parasitic plants, exploit their environment in a manner that maximises energy intake (e.g. Levins and MacArthur, 1969; Kelly, 1990; Marvier, 1998). Besides host choice, life history is an important life trait affecting a parasite's performance in a particular ecosystem (Schneeweiss, 2007). Schneeweis stated that parasites which specialise in predictable resources, i.e. long-lived hosts, are potentially perennial themselves. In absence of any constraint, individuals can increase their fitness by developing traits that maximise their longevity (Ehrlén and Lehtilä, 2002). Following this line of thought, especially for obligate parasitic plants, which entirely depend on their hosts and for which successful establishment is crucial, it would be a great advantage to be perennial, as it reduces the costs and risks to find and infect an appropriate host every year anew. Perennial parasites are further expected to have earlier access to nutrients than annual individuals originating from seeds and so the former may have a head start on growth and reproduction. An additional advantage of being perennial is that it is less dependent on stochastic events affecting germination and recruitment. As Bossuyt and Honnay (2006) stated, plant species investing in a longer life span require less energy for offspring to avoid extinction in unfavourable years, even in landscapes with deterministic changes in habitat quality, such as heathland. The question thus arises as to how the life span of a holoparasitic plant can affect their population viability in the long-run?

One of the better known groups of parasitic plants is the genus *Cuscuta* (Kuijt, 1969), which represents a holoparasitic group within the Convolvulaceae (Yuncker, 1932; Stefanovic et al., 2003). *Cuscuta* species occurring in the temperate zones are generally described as annuals, of which the whole population dies at the end of the growing season (e.g. Verdcourt, 1948, Costea and Tardif, 2006). However, in some species such as in *C. reflexa* (Rao, 1938) and *C. gronovii* (Dean, 1954; Truscott, 1958), there are reports that individual haustoria survive an unfavourable season in tissues of a perennial plant host. Nevertheless, these reports are highly descriptive, and as far as we know the effect of overwintering on the ecology of the parasite living under natural conditions has not been studied. Therefore, we investigated the possibility of *C. epithymum* overwintering via haustorial tissue in its hosts. For this study, *Calluna*-dominated vegetation in various stages of succession was examined for presence of overwintering haustoria. Since *C. epithymum* is a rare and threatened species occurring in dry heaths of north-western Europe (van Landuyt et al., 2006; Chapter 2), knowledge about this aspect of the species life-cycle and its impact on population vigour is essential to design optimal management strategies for the long-term survival of this declining species.

We addressed the following questions: (1) How common are overwintering haustoria in *C. epithymum* populations living in *Calluna*-dominated heath? (2) Under what conditions, on which hosts and in which location on its host are overwintering haustoria found, and is there a link with heath successional stage? (3) Does haustorial overwintering affect *C. epithymum* population performance in the following growing season and is there a relation between vegetative perennation and sexual recruitment?

## Materials and methods

### Study site

We investigated 10 *C. epithymum* populations in Heiderbos Nature Reserve (see Chapter 3), where a relative large number of *C. epithymum* populations is present. This nature reserve is situated in As (north-eastern Flanders, Belgium) at ca. 91 m above sea level and is 42 ha in area (see Chapter 3a). The study area is characterised by dry heathland vegetation typically dominated by *C. vulgaris* with *Erica cinerea*, *E. tetralix*, *Genista anglica*, *G. pilosa* and *Juniperus communis* making up considerable parts of the vegetation. Since 1970, management strategies have focused on the removal of colonising trees and shrubs and rotational mowing or turf-cutting for rejuvenation of *C. vulgaris* (cf. Verheyen et al., 2005). The rotational management has created a dynamic heathland landscape consisting of patches of different successional stages, each with a characteristic structure and plant composition.

### Data collection

In the summer of 2006, we selected 10 *C. epithymum* populations occurring in various successional stages of *Calluna* vegetation. For each selected population, we determined population size (i.e. population size 2006), mean cover per m² (i. e. parasite cover 2006) and the exact period of time since the last management intervention, namely heather mowing, took place (i. e. vegetation age). Three populations each occurred in sites that were mowed 2 or 4 years ago, whereas only one population was sampled in a *Calluna*-dominated vegetation that was mowed 1, 3, 6 and 7 years ago. Within each population we selected three permanent plots of 1m × 1m, at places where the parasite grew extensively during the 2006 growing season. Using a wooden 1m × 1m frame, divided in 100 squares of 10cm × 10cm, these permanent plots were monitored in 2007 at the end of March (1th visit), mid May (2nd) and mid July (3th visit). During the first visit, the extent of the old *Cuscuta* aggregation, i.e. the total number of 10cm × 10cm squares where dead *C. epithymum* segments of the growing season of 2006 were present, was recorded in each plot.

To investigate the extent of overwintering, we counted the number of overwintering haustoria (recognised as small, green stem pieces wrapped around the host's stem) per plot during our first visit in March and marked each of these haustoria with blue string to ensure relocation. For each haustorium found, we noted the host to which it was attached. During the following visit (May), all marked haustoria were surveyed again to determine the number of sprouted haustoria per m$^2$.

The impact of different vegetation characteristics and the capacity of *C. epithymum* to resproute (or not) via vegetative haustoria was evaluated. Therefore, we determined cover of *C. vulgaris* and bare ground (i.e. area not covered by vegetation) and canopy height (cm), which was assessed as the mean of five randomly chosen points in the 1m$^2$ plots. In each plot, a maximum of 10 haustoria (n = 270) was randomly selected, and the height above ground of the haustoria on the host plant and the total height of the infected host plant were determined. Also, we determined if the haustoria were located on lignous or unlignous parts of its host. To evaluate the degree of *C. vulgaris* lignification in vegetation of different ages and to assess the impact of infection of *C. epithymum* on above-ground biomass production of *C. vulgaris*, we determined above-ground biomass of infected and uninfected *C. vulgaris* individuals in *Calluna*-dominated vegetation of different age. We therefore randomly placed eight 0.0625m$^2$ plots, comprising four plots with infected *C. vulgaris* plants and four plots with uninfected ones, in each of two *C. epithymum* populations occurring in a 2 year old *Calluna*-dominated vegetation and two populations in a 4 year old vegetation in the same study area. After cutting the above-ground vegetation, in each plot, *Calluna* plants were separated into unlignous and lignous tissues. These were oven-dried at 80°C for 50h, weighted to the nearest 0.1g and converted to g m$^{-2}$.

To determine the impact of overwintering haustoria on population performance of *C. epithymum* the next growing season we determined the cover of *C. epithymum* in mid July for each 1m$^2$ plot, counted the number of 10cm × 10cm squares where *C. epithymum* flowers were present (i.e. extent of flowering) and quantified the mean number of flowers in a 10cm × 10cm square (i.e. flower density divided in three categories: few = 0 – 50 flowers, moderate = 51 – 200 flowers, many = > 200 flowers). To quantify sexual recruitment, we counted the number of seedlings per m$^2$ during the first two visits.

## *Data analysis*

As a first preliminary step, the number of variables was reduced by means of three PCAs (Principal Component Analysis; Kent and Cooker, 1992). The variables 'vegetation age', 'bare ground cover' and 'canopy height' were synthesised as one factor 'successional stage' (first PCA axis explained 70% of the variance, correlation with variables: $r_P = 0.79$, - 0.87 and 0.84, respectively; $p < 0.001$ each). The variables 'populations size 2006', 'parasite cover 2006' and 'extent of old *Cuscuta* aggregation' were reduced to one variable 'population performance 2006', expressing the population extent in summer 2006 (first PCA axis explained 59% of the variance, correlation: $r_P = 0.78$, 0.81 and 0.71, respectively; $p < 0.001$ each). The variables 'extent of flowering' and 'flower density' were compressed to the variable 'reproduction performance', expressing the number of flowers in a m² (first PCA axis explained 97% of the variance, correlation: $r_p = 0.99$; $p < 0.001$ for both). In a second preliminary step, dependent variables were arcsine-transformed if they represented percentages or log transformed if they represented count data to comply with the assumptions of statistical analyses.

Pearson product-moment correlation was used to determine the relation between height at which overwintering haustoria were located and total height of the host plant and canopy height. Pearson product-moment correlations were also applied to investigate the association between vegetation characteristics, such as dominance of *C. vulgaris*, openness of the vegetation and canopy height, and the number of sprouted haustoria. We used an ANOVA followed by a Tukey post-hoc analysis ($p < 0.05$) to analyse the effect of vegetation age on the number of sprouted haustoria, the bare ground cover and vegetation height. To test whether sprouting success of overwintering haustoria is related to the above-ground lignous biomass, we applied three repeated measures General Linear Models (GLM; O'Brien and Kaiser, 1985). Finally, a GLM (McCullagh and Nelder, 1989) was applied to assess the impact of successional stage, population performance 2006 and the cover of *C. vulgaris* on the number of sprouted haustoria. Non-significant factors were excluded by stepwise backward elimination at $p = 0.05$ to obtain a final model.

Pearson product-moment correlation was used to test whether there was a relation between perennation and sexual reproduction and between the number of seedlings and the number of sprouted haustoria per plot or not. To evaluate the impact of overwintering haustoria on population performance in the following growing season, the proportion of all established individuals originating from overwintering haustoria in relation to establishment from seedlings was expressed as $\frac{h}{(h+s)} \times 100$ %, where *h* represents the number of sprouted haustoria and *s* the number of seedlings. Again Pearson product-moment correlation was used to test for a possible association between the number of seedlings, 'reproduction performance' and successional stage.

All statistical analyses were performed with SPSS for windows 14.0 (SPSS, 2006).

# Results

## *General observations*

Over all the surveyed plots, a total of 4998 overwintering haustoria was found during the beginning of the growing season (the end of March), ranging from a minimum of 1 to a maximum of 1168 (mean 167) overwintering haustoria per $m^2$. Although 22 different species were present in the vegetation, only four species were found to function as appropriate host species on which *C. epithymum* developed overwintering haustoria, with the majority being found on *C. vulgaris* (Table 6.1).

**Table 6.1.** Host species on which overwintered haustoria were found listed in decreasing order of importance.

| *Host species* | *% plots (n = 30)* | *% overwintered haustoria (n = 4998)* |
|---|---|---|
| *Calluna vulgaris* | 100.0 | 97.40 |
| *Erica cinerea* | 36.7 | 1.42 |
| *Erica tetralix* | 16.7 | 0.94 |
| *Hypnum jutlandicum*[a] | 10.0 | 0.12 |
| *Genista pilosa* | 3.3 | 0.12 |

Percentage of the 30 investigated $1m^2$ plots and percentage of the 4998 recovered overwintered haustoria in which this host was infected. [a] This bryophyte is not regarded as real host.

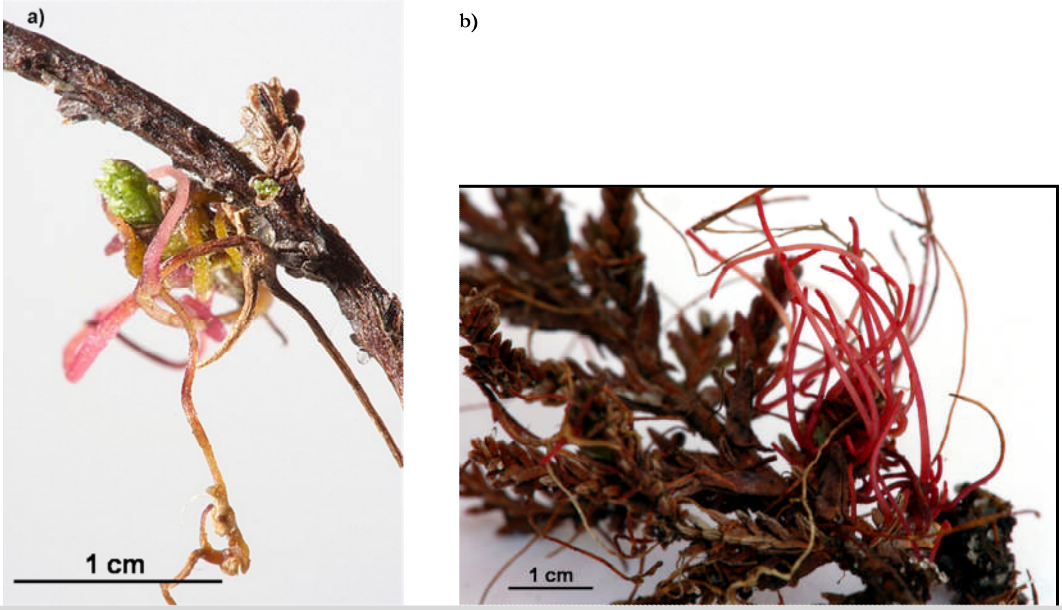


**Figure 6.1. a)** Detail of sprouted haustorium attached to its host *C. vulgaris*, **b)** several *C. epithymum* branches sprouting from one overwintering haustorium. Photos are taken by Adriaens D. (a) and Lerouge F. (b).

In mid-May, only 76.5% of all overwintering haustoria had sprouted (mean per m²: 65%; Fig. 6.1a). The stems of sprouted haustoria were reddish and about twice as wide as the white, about 2cm long *C. epithymum* seedlings present at the same time. In some cases, several *C. epithymum stems* were sprouting from one overwintered haustorium (Fig. 6.1b).

### *Location of overwintering haustoria*

Most haustoria were located near ground level at the base of the plants at a mean (± SE) height of 1.6 ± 0.07cm (Fig. 6.2). The host on which the overwintering haustoria were attached had a mean (± SE) height of 8.92 ± 0.32cm (Fig. 6.2).

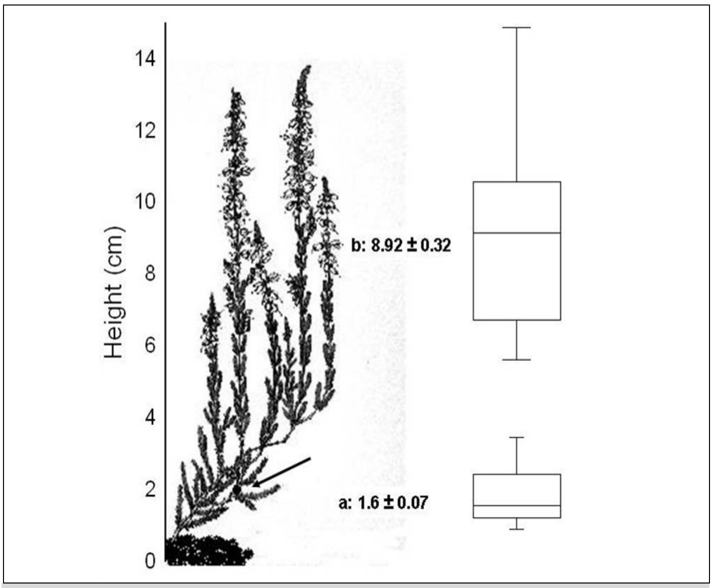


**Figure 6.2.** Location of overwintering haustoria on its host (here *C. vulgaris*) measured at the end of March. Mean (± SE) height and boxplots of **a)** haustorial growth location **b)** total height of the host. The arrow indicates location of an overwintering haustorium.

As *Calluna* canopy height increases during heath succession ($r_P = 0.84$; $p < 0.001$; n = 30), the height of *C. vulgaris* above the overwintering haustoria and the location of the haustoria on its host increased significantly with successional stage ($r_P$ = 0.66, 0.49, respectively; $p < 0.001$; n = 30). Consequently, the taller the heather plants, the higher the haustoria were attached ($r_P = 0.55$; $p < 0.01$, n = 30). Of the 270 examined haustoria, all haustoria were attached to unlignous parts of the host; not even one haustorium was attached to lignous host tissue.

## *Biomass of the most important host*

Young *C. vulgaris* plants (2 years old) had a significant lower proportion of lignous parts and a significant higher unlignous contribution to total above-ground biomass than older (4 year-old) plants (Table 6.2, Fig. 6.3). Furthermore, the unlignous contribution to total biomass was also significantly higher for parasitised individuals than for uninfected ones, showing that infected host plants have relatively more unlignous parts than uninfected individuals (Table 6.2, Fig. 6.3). Parasitised *C. vulgaris* lignous and unlignous above-ground biomass was significantly lower than unparasitised biomass (Table 6.2).

**Table 6.2.** Effects of vegetation age (2 versus 4 years), the infection of the parasite *C. epithymum* (pres-abs. = presence or absence of *C. epithymum* in the 0.0625 m² plots) and their interaction on the unlignous, lignous and total above-ground biomass and the ratio of unlignous to total biomass of the host *C. vulgaris*.

| *Variables* | *df (n,d)* | *Biomass (g/m²)* | | | |
|---|---|---|---|---|---|
| | | *Unlignous F* | *Lignous F* | *Total F* | *Unlignous/total F* |
| **Between subjects** | | | | | |
| Vegetation age | 1,14 | $0.85^{ns}$ | 33.11*** | $3.84^{ns}$ | 113.07*** |
| **Within subjects** | | | | | |
| Pres-abs. | 1,14 | 18.22*** | 12.40** | 21.13*** | 10.75** |
| Pres-abs. × veg. age | 1,14 | $3.64^{ns}$ | $0.88^{ns}$ | $1.02^{ns}$ | $0.01^{ns}$ |

*F*-values and significant levels from repeated measures General Linear Models (GLM) are given. **: $0.001 < p < 0.01$; ***: $p \leq 0.001$; ns: not significant; df (n, d) = degrees of freedom (nominator, denominator).

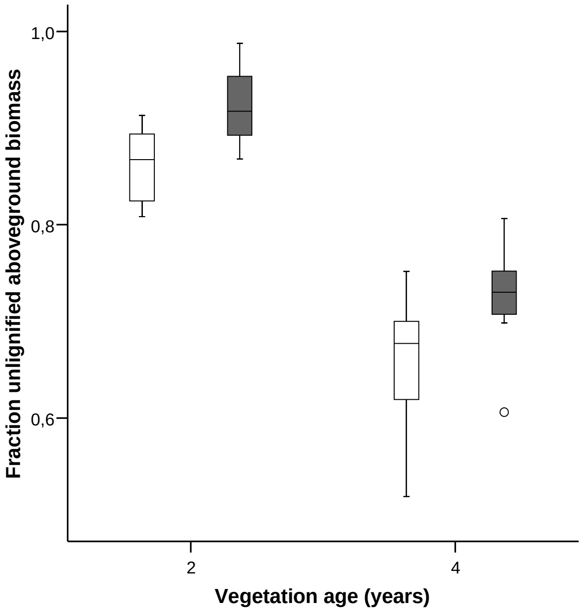


**Figure 6.3.** Boxplots of the fraction of unlignous above-ground biomass of infected (gray boxes) and uninfected (white boxes) *C. vulgaris* individuals in patches of 2- and 4-years old. Independent samples *t*-test: uninfected individuals: $t = 7.0$, infected individuals: $t = 8.0$ and all individuals together: $t = 9.2$, $p < 0.001$.

## *Effect of vegetation characteristics on sprouting haustoria*

The number of sprouted haustoria was significantly related to successional stage of the *Calluna*-dominated vegetation characterised by height of the canopy, percentage bare ground and period of time since the vegetation had been mowed (Fig. 6.4, Table 6.3). The number of sprouted haustoria decreased significantly with reduced percentage bare ground and increased canopy height ($r_P$ = 0.67 and -0.74, respectively; $p$ < 0.001; n = 30). Indeed, the highest number of sprouted haustoria was found in the youngest successional stages of the vegetation (1 or 2 years since the vegetation had been mown) (ANOVA $F$ = 4.04, df = 5,29, $p$ < 0.01, Table 6.3), since these early successional stages also showed the highest percentage bare ground and lowest canopy heights (ANOVA $F$ = 13.45 and 11.81, respectively, df = 5.29; $p$ < 0.001; Table 6.3). Finally, only successional stage explained a significant part of the observed variation in the number of sprouted haustoria per 1m² plot (df (n,d) = 1,28; $F$ = 40.16; $p$ < 0.001).

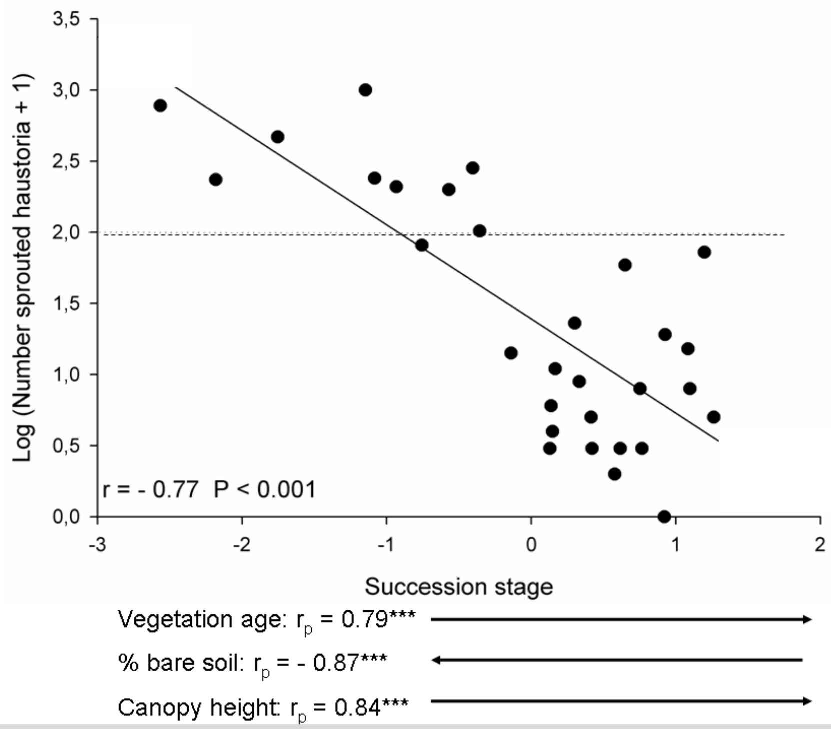


**Figure 6.4.** Number of sprouted haustoria per $m^2$ (log(x + 1) transformed) against 'successional stage', explaining the highest variation of vegetation height, percentage bare ground and vegetation age (Y = 1.39 + (-6.62 $e^{-1}$)X). Arrows along the x-axis indicate significant correlations between 'successional stage' scores (first PCA axis) and three vegetation variables; r values refer to Pearson product-moment correlations, ***: $p$ < 0.001. The dotted line at 2.0 indicates a borderline of about 100 sprouted haustoria per m² above which considerable changes are visible.

### Contribution to the next generation

Seedlings were found in only 20 of the 30 plots, with a total of 860 seedlings. The first seedlings were observed at end March with 21 seedlings being distributed over 10 plots. The other 839 seedlings, found in 15 plots, had germinated by mid-May. However, one plot in the 2 years old vegetation had more than 600 seedlings per m². Both with and without data from this plot in the analysis, no significant correlation was found between the total number of seedlings and the number of sprouted haustoria ($r_P = 0.37$ and 0.32, respectively, $p > 0.05$; n = 30). There was also no correlation between the number of seedlings and population performance 2006 ($r_P = 0.12$ and 0.02, respectively; $p > 0.05$; n = 30). Nevertheless, the number of seedlings decreased significantly with increased successional stage ($r_P = -0.40$ and -0.38, respectively; $p < 0.05$; n = 30, Table 6.3). In all studied *C. epithymum* populations, overwintering haustoria were found to contribute relatively more to the population than seedlings did (Table 6.3), and this independently of vegetation age and/or successional stage. On average (± ES) 85.4 ± 4.6% of the present individuals originated from sprouted haustoria.

**Table 6.3.** Characterisation of heath vegetation at six different ages of *Calluna*-dominated vegetation. Mean (± SE) percentage bare ground, canopy height, number of overwintered haustoria, number of sprouted haustoria and number of seedlings per m² plot and the proportion of all established individuals originating from overwintering haustoria in relation to establishment from seedlings (Rel. imp. haustoria).

| *Variables* | *Vegetation age in years (n = number of plots)* | | | | | |
|---|---|---|---|---|---|---|
| | *1 (3)* | *2 (9)* | *3 (3)* | *4 (9)* | *6 (3)* | *7 (3)* |
| Bare ground (%) | 40.0 ± 5.8$^{a}$ | 13.3 ± 3.4$^{b}$ | 2.7 ± 1.2$^{b}$ | 4.0 ± 0.9$^{b}$ | 2.0 ± 0.6$^{b}$ | 8.3 ± 4.9$^{b}$ |
| Canopy height (cm) | 7.0 ± 0.7$^{a}$ | 15.1 ± 1.3$^{ab}$ | 28.6 ± 1.9$^{c}$ | 19.5 ± 1.0$^{b}$ | 18.8 ± 4.3$^{b}$ | 21.9 ± 0.9$^{b}$ |
| # overwintered haustoria | 663 ± 237$^{a}$ | 293 ± 118$^{ab}$ | 18.7 ± 14.8$^{b}$ | 9.8 ± 2.5$^{b}$ | 16.0 ± 3.8$^{b}$ | 60.7 ± 29.9$^{ab}$ |
| # sprouted haustoria | 489 ± 157$^{a}$ | 235 ± 100$^{ab}$ | 6.7 ± 5.7$^{c}$ | 6.2 ± 2.2$^{c}$ | 9.7 ± 2.2$^{bc}$ | 44.7 ± 20.7$^{abc}$ |
| # seedlings | 27.3 ± 25.8$^{a}$ | 79.6 ± 67.1$^{a}$ | 0.0 ± 0.0$^{a}$ | 4.2 ± 2.7$^{a}$ | 0.7 ± 0.3$^{a}$ | 7.3 ± 3.8$^{a}$ |
| Rel. imp. Haustoria (%) | 96.8 ± 3.0$^{a}$ | 79.1 ± 10.1$^{a}$ | 100 ± 0.0$^{a}$ | 79.8 ± 10.6$^{a}$ | 94.1 ± 3.2$^{a}$ | 91.3 ± 5.3$^{a}$ |

Numbers between brackets refer to the number of surveyed 1 m² plots in vegetation of a specific age. Different letters correspond to significant differences (Tukey's pairwise means comparison test, $p < 0.05$).

Furthermore, the area that *C. epithymum* individuals occupied (m²) in summer, increased exponentially with increasing number of sprouted haustoria observed in spring ($r_P = 0.56$, $p < 0.001$; n = 30, Fig. 6.5a). Consequently 'reproduction performance' was also significantly positively influenced by the number of sprouted haustoria in spring ($r_P = 0.84$; $p < 0.001$, Fig. 6.5b), whereas it did not increase with a higher number of seedlings ($r_P = 0.24$, $p < 0.05$).

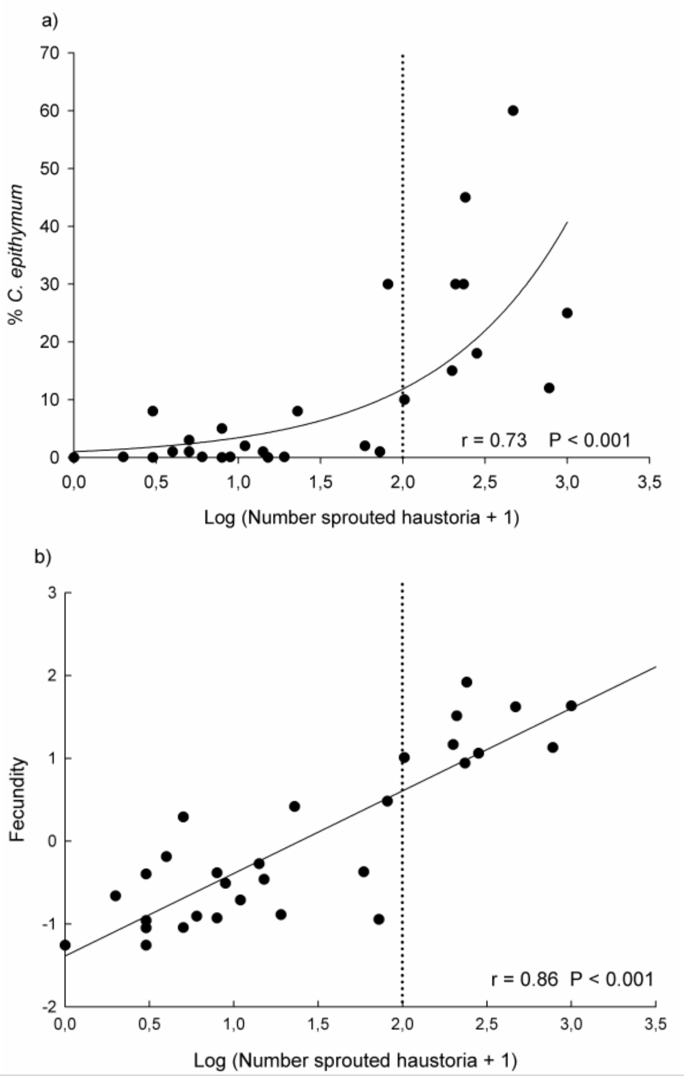


**Figure 6.5.** Relationship between number of sprouted haustoria (Log (x + 1) transformed) and **a)** cover of *C. epithymum* in summer 2007 ($Y = e^{(1.24 \times X)}$), **b)** 'reproduction performance', a variable expressing the amount of *C. epithymum* flowers in 1m² in summer 2007 ($Y = (-6.41\ e^{-1}) + (4.49\ e^{-1})X$). Pearson product-moment correlation (r) is added. The dotted line at 2.0 indicates a borderline of about 100 sprouted haustoria per m² above which considerable changes are visible.

## Discussion

This study clearly shows that overwintering through haustorial tissue occurs frequently, and is an important contributor to the local population dynamics of *C. epithymum* in *Calluna*-dominated heathland and consequently offers new insights on this parasite's life cycle.

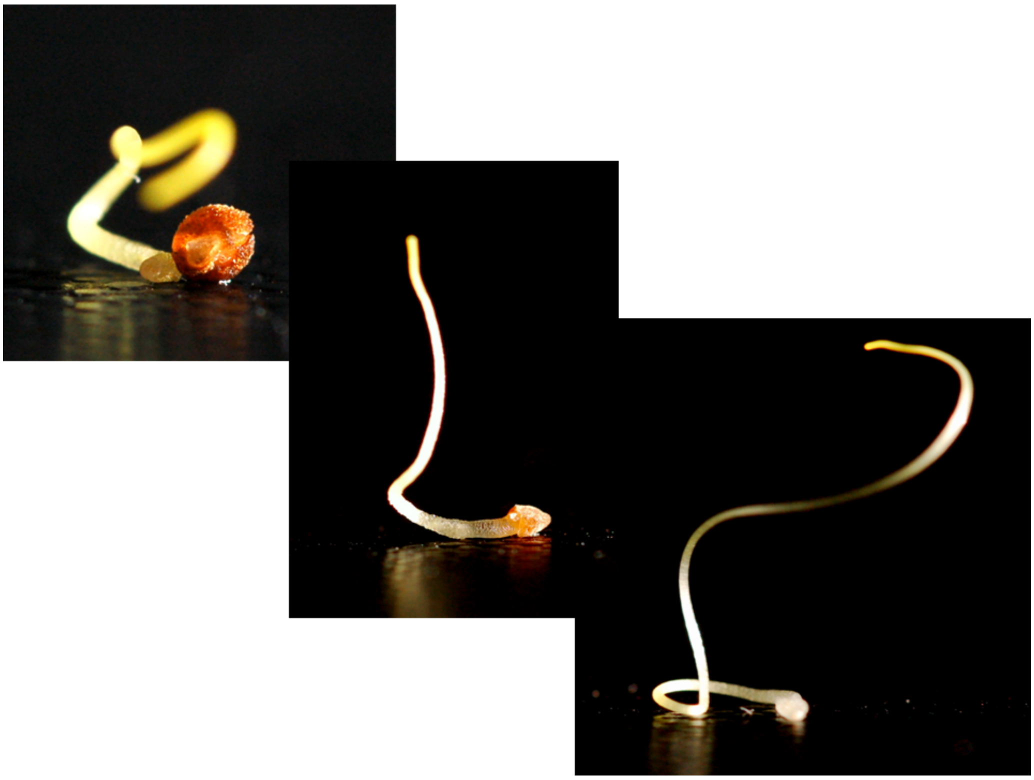
Overwintering haustoria were found in all studied populations located in heath vegetation of different ages (Table 6.3). More than 75% of these overwintering haustoria resulted effectively in new *C. epithymum* stems, growing out of the host tissue and foraging for an adjacent host. From one overwintering haustorium different shoots could arise and increased the density of the red threads and subsequent flowering. These observations led us to conclude that this holoparasite behaves more like a pseudoannual than an annual. A pseudo-annual can be described as a perennial plant that behaves as a vegetatively propagating annual, of which the majority dies off each autumn and the connections between vegetative propagules or hibernacles, are severed. In the next growing season, new plants emerge either from these hibernating organs or from seeds, resulting in a new, independent cohort (Verburg et al., 1996). Similarly, the haustoria of *C. epithymum* serve as vegetative propagules hidden in the host tissue during winter and resproute the next growing season. The number of overwintering haustoria and resprouting cohorts were strongly, negatively correlated with the successional stage of dominant Ericaceous shrubs, with most overwintering haustoria in recently mown *Calluna*-dominated vegetation (Table 6.3; Fig. 6.4). *Cuscuta epithymum* was only found to penetrate its host successfully via unlignous stems. Since *C. vulgaris* stems gradually lignify with age, this means *C. epithymum* must penetrate older hosts higher up in the canopy. Indeed, our data revealed that the older the *Calluna* vegetation, the higher the position of established *C. epithymum* haustoria and the fewer the number of haustoria. Once *C. vulgaris* individuals were too old to have sufficient non-woody stems, no *C. epithymum* individuals could be found. Apart of the absence of a mechanical barrier, these non-woody and younger host plant stems contain more nutrients and carbohydrates than older ones (Gimingham 1972; 1985), which make them much more profitable for the parasite. As *C. epithymum* is a stem holoparasite, one would expect that overwintering is only possible on evergreen, long-lived hosts. Although *C. epithymum* is considered as a host generalist (Box 2.1), our results proved that on our site only four perennial evergreen shrub species served as overwintering hosts (Table 6.1). Predictability of resources (i. e. long-lived hosts) is an important factor for successful establishment of a parasite on a host (Schneeweiss, 2007). This led us to conclude that *C. epithymum* selectively infects the most rewarding place on the most rewarding host (see also Kelly et al., 1988; Koch et al., 2004).

Our observations thus suggest that vegetative overwintering via haustoria may primarily promote *C. epithymum* populations to persist in the vegetation. Indeed the larger the number of haustoria in early spring, the more rapidly the species may re-establish. Further, early establishment increases the number of *C. epithymum* individuals that may flower and consequently set seed. The fact that *C. epithymum* individuals originating from overwintering haustoria are able to grow and infect host plants early in the growing season (several weeks before most seeds germinate), gives the opportunity to infect host stems when available nutrients and carbohydrates are abundant (Weih, 1998). Indeed *C. epithymum* stems of sprouted haustoria were twice as large and flowered much more abundantly compared to individuals originating from seeds that germinated the same growing season. Likewise, Kelly (1990) reported that *C. subinclusa* individuals starting from overwintering tissue are significantly larger at flowering than those starting from seed and that the biomass accumulation within a season is correlated with seed set. Furthermore, in analogy with observations in *C. reflexa* (Rao, 1938), the overwintering haustorial tissue of *C. epithymum* gave rise to a number of young shoots attacking different available hosts, resulting in much denser *Cuscuta* aggregations at locations where many overwintering haustoria sprouted (Fig. 6.5a). To survive, seedlings of parasitic flowering plants need to find a suitable host as quickly as possible. This consequently results in high mortality rates at the seedling stage and explains why this parasite invests in the development of overwintering haustoria as a strategy to perennate. The pattern of yearly clonal growth means *C. epithymum* populations can remain growing for several years in successional heath vegetation. Hence, vegetative reproduction via overwintering haustoria may play an important, but overlooked role in the life-cycle of this pseudoannual parasite. In other words, this strategy gives this species the opportunity to survive via vegetative propagation under unsuitable conditions, especially in less suitable older *Calluna* vegetation in which seedling recruitment is inhibited due to the dense vegetation and increased lignification of the host stem at the base of the plants. Perennation via overwintering haustoria makes the parasite thus much less sensitive to environmental influences that may largely affect the process of pollination, seed set, germination and recruitment (Honnay and Bossuyt, 2005). On the other hand, the occurrence of a persistent seed bank gives the opportunity to re-establish a *Cuscuta* population after disturbance activities, such as heath management including mowing, burning or turf cutting, providing appropriate growing conditions (Box 5.1). Nonetheless, if the *Calluna*-dominated vegetation becomes too old, due to a lack of a natural disturbance events or management, established *C. epithymum* populations will, however, gradually decline in size and finally disappear from the vegetation. Nevertheless, the species survives at a site via its persistent seed bank until a disturbance may cause appropriate conditions for germination and establishment. These insights thus strongly emphasise the importance of disturbances, like traditional management of semi-natural heathlands where *C. epithymum* occurs.

# 7

# Limited by the host: Host age hampers establishment of the holoparasite *Cuscuta epithymum*

Adapted from: Meulebrouck K., Verheyen K., Brys R., Hermy M. – Acta Oecologica 35: 533-540.



Photos: Frederik Lerouge (2006)

## Introduction

A good understanding of the relationship between plant establishment and the ecosystem of which they are part of is needed to provide adequate conservation and management strategies for rare declining plant species (e.g. Bullock et al., 1994; Oostermeijer et al., 2003; Endels et al. 2007). A point of particular interest for conservators is to find out the reasons why plant species not recruit as seedlings and/or establish at a particular site. The absence of a species may indicate either the lack of suitable microsites for regeneration (microsite limitation), or it may reflect insufficient seed production to saturate all suitable gaps (seed limitation; e.g. Clark et al., 1999; Turnbull et al., 2000; Isselstein et al., 2002). Apart from the question whether recruitment is seed limited, microsite limited or both, the relative importance of the different life-stage transitions during plant establishment is also often unknown (see Clark et al., 1999). In fact, practically any life-history stage following seed rain can act as strong bottlenecks for final adult establishment (Schupp, 1995; Gómez-Aparicio, 2008; Baeten et al., 2009). The great majority of studies provide only a snapshot of the establishment process at a single stage and single point in time, while successful establishment is a multiphase process including all steps from the time of seed arrival on the soil surface until the time adult plants have successfully established (Clark et al., 1999; Turnbull et al., 2000; Fenner and Thompson, 2005). This is especially true for parasitic plants, as an additional step must be overcome. Since these species depend on host plants for water, nutrient and/or carbon compounds, successful attachment to the vascular system of an appropriate host is ultimately required before reproductive adults may establish (e.g. Kuijt, 1969; Press and Graves, 1995). This chapter attempts to unravel the complete process of establishment of *C. epithymum* under variable natural growing conditions.

*Cuscuta epithymum* is a holoparasitic indicator species of dry European heathland (Schaminée et al., 1996). It appears in young, recently managed heath vegetation and gradually disappears from the vegetation once *C. vulgaris* becomes older than about 7 years (Chapter 3). Canopy structure, which is largely affected by the type of management applied to the vegetation, strongly affects vegetative overwintering of *C. epithymum* and is therefore considered an important factor that determines the viability of its population (Chapter 6). The process of recruitment and further establishment of this species is, however, largely unknown. Variable environmental characteristics created by different management activities may affect each stage in the developmental process differently. In order to optimise the conservation of this endangered parasite it is essential to examine how seed availability, different types of management and subsequent succession of *C. vulgaris* may influence the different stages of the parasite's establishment processes.

More specifically this chapter reports the results of a 2 year field experiment on the process of establishment in *C. epithymum* under various environmental conditions. First, we examined the effect of different seed-densities on seedling recruitment. Second, we investigated the impact of two management activities (mowing and turf-cutting) and the stage of succession of its host plant *C. vulgaris* (pioneer, building and mature phase) on recruitment success and the relative importance of different life-stages on final establishment.

## Materials and methods

### *Establishment scheme of Cuscuta epithymum*

Successful establishment and reproduction of *C. epithymum* requires survival during five life-cycle stages (steps a-e in Fig. 7.1).

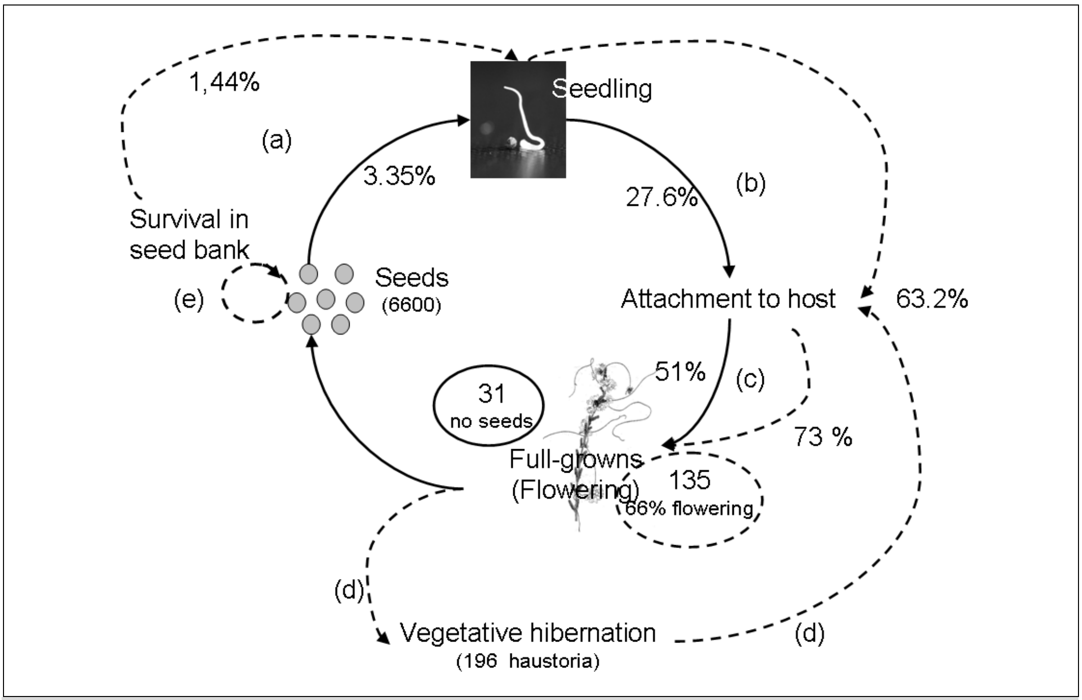


**Figure 7.1.** Establishment diagram for *C. epithymum* during the first 2 years after seed sowing, starting with 6600 seeds and ending up with 135 plants (< 2%), of which 66% flowered. Letters refer to the different steps involved in the process: **a)** emergence of seedlings from seeds produced the previous growing season or from seed bank; **b)** seedling survival, including finding of and attachment to an appropriate host; **c)** growth to a full-grown (flowering) plant; **d)** regeneration by vegetative overwintering via special structures, called haustoria, in the host's tissue and regrowing to a full-grown; **e)** survival of dormant seeds in the seed bank. Processes occurring in the first year after seed shed are represented by solid lines, those taking place in the second year by broken lines. Observed transitions (%) from one stage to another are given next to the arrows. The final 135 full-grown individuals originated both from seeds germinated in the second year (42) and from regrown overwintered haustoria (93). As the latter may result from the same mother plant of which several haustoria overwintered, they are seen as ramets.

Each reproductive plant may produce hundreds to thousands seeds (Gómez, 1994). Controlled germination experiments revealed a combinational dormancy in *C. epithymum* seeds, and it is thought that only part of the seed population exposed to low winter temperatures will germinate at warm spring temperatures (Chapter 4; step **a** in Fig. 7.1). The remaining seeds may form a persistent seed bank (step **e** in Fig. 7.1), allowing germination the following spring(s) (Box 5.1). After germination, seedlings need to infect a suitable host, *C. vulgaris* in our study area, within 14-21 days after emerging or they will die (step **b** in Fig. 7.1). Once a functional connection with the host plant is made by means of the formation of a haustorium, they can further develop to a full-grown or reproductive adult (step **c** in Fig. 7.1). Finally, the capability of *C. epithymum* to overwinter vegetatively through their haustoria may contribute to a durable successful establishment (Chapter 6; step **d** in Fig. 7.1). Each full-grown *C. epithymum* individual (i.e. genet) is capable to produce several haustoria which can overwinter in host stems and develop the next spring in order to fulfil their life-cycle. This may thus result in several plants (ramets) that may each further develop the following growing season.

## Study area

The study was conducted in the Mechelse heide Nature Reserve in north-eastern Flanders (Chapter 3). More specifically, this research was carried out in a dry heath area of 300m × 300m in which *C. epithymum* was absent before the experiment. In this area, the vegetation composition, soil characteristics and topography were homogenous before the onset of the experiment. Local vegetation is typically dominated by *C. vulgaris* (cover: 57 ± 8.3%) with *Molinia caerulea* (1.7 ± 0.7%), *Deschampsia flexuosa* (< 1%), *Carex pilulifera* (< 1%) and *Rumex acetosella* (< 1%). The soil is distinctively sandy and strongly podsolised. Climate has an annual mean precipitation of about 820mm evenly distributed through the year. The winters are mild with an average January-February temperature of around 3.3°C, and the summers are tempered with an average July-August temperature of around 17.7°C; the annual mean temperature is about 10.1°C. The spring period of 2007 was exceptionally warm and dry, especially in April (mean maximum temperature: 21.8 ± 0.99°C with no precipitation).

## Experimental design

Five treatments were applied to unravel the process and limitations of *C. epithymum* establishment and to evaluate the impact of seed-density, management and subsequent heathland succession on the different steps of its establishment. The treatments differed in type of management applied to the vegetation (mowing or turf-cutting) and successional phase (managed in 2005 i.e. pioneer, managed in 2003 i.e. building phase of *Calluna*). The surrounding vegetation belonging to the mature phase (i.e. not managed for more than 10 years) represents the fifth treatment. For this treatment, no structural difference between mowing and turf-cutting could be made. Each of these five treatments has a characteristic canopy structure and height (Table 7.1).

**Table 7.1.** Characteristics of the five different treatments under which *C. epithymum* establishment was studied. Management type and year in which the management is applied, heathland succession stage, mean (± SE) canopy height, % bare ground, % cover of *Calluna vulgaris* (the dominant species of the studied heathland and the main host of *C. epithymum*; Box 2.1, Chapter 3), % cover of bryophytes and the different investigated life-stages per plot (0.04m²).

| *Five treatments£* | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| Management (year†) | Mowing (2005) | Turf-cutting (2005) | Mowing (2003) | Turf-cutting (2003) | No management* |
| Succession stage‡ | Pioneer | Pioneer | Building | Building | Mature |
| Canopy height (cm) (18) | $8.6 \pm 0.9^{a}$ | $6.2 \pm 1.6^{a}$ | $19.8 \pm 1.4^{b}$ | $23.7 \pm 1.4^{b}$ | $54.5 \pm 1.8^{c}$ |
| % bare ground (18) | $24.8 \pm 4.9^{a}$ | $72.3 \pm 7.8^{b}$ | $3.8 \pm 1.4^{a}$ | $26.4 \pm 5.5^{a}$ | $0.1 \pm 0.1^{c}$ |
| % *C. vulgaris* (18) | $25.0 \pm 3.4^{a}$ | $14.3 \pm 5.7^{a}$ | $61.4 \pm 5.4^{b}$ | $54.4 \pm 5.9^{b}$ | $100.0 \pm 0.0^{c}$ |
| % bryophytes (18) | $1.3 \pm 0.6^{ab}$ | $7.2 \pm 4.3^{a}$ | $6.0 \pm 3.5^{ab}$ | $0.7 \pm 0.6^{b}$ | $3.0 \pm 4.9^{ab}$ |
| # seedlings '07 (12) | $1.7 \pm 0.8^{a}$ | $6.7 \pm 3.1^{a}$ | $3.7 \pm 1.3^{a}$ | $2.8 \pm 0.7^{a}$ | $3.4 \pm 1.4^{a}$ |
| # established sdls '07 (12) | $0.7 \pm 0.3^{ab}$ | $2.4 \pm 0.9^{a}$ | $1.4 \pm 0.6^{ab}$ | $0.6 \pm 1.9^{ab}$ | $0.0 \pm 0.0^{b}$ |
| # haustoria '08 (12) | $1.5 \pm 1.0^{ab}$ | $12.7 \pm 4.9^{a}$ | $1.3 \pm 0.8^{ab}$ | $0.9 \pm 0.5^{ab}$ | $0.0 \pm 0.0^{b}$ |
| # seedlings '08 (12) | $0.7 \pm 0.3^{a}$ | $3.3 \pm 1.5^{a}$ | $1.5 \pm 0.9^{a}$ | $2.1 \pm 1.0^{a}$ | $0.4 \pm 0.2^{a}$ |
| # establ.** '08 (12) | $1.4 \pm 0.8^{ab}$ | $9.7 \pm 3.6^{a}$ | $2.2 \pm 1.5^{ab}$ | $2.1 \pm 1.0^{ab}$ | $0.0 \pm 0.0^{b}$ |
| # full-growns '08 (12) | $1.3 \pm 0.8^{ab}$ | $6.7\pm 2.6^{a}$ | $1.7 \pm 1.1^{ab}$ | $1.6 \pm 0.7^{ab}$ | $0.0 \pm 0.0^{b}$ |

The number of studied plots per heathland site, with 18 in total and 12 plots in which seeds were sown are given between brackets. Different letters correspond to significant differences (Tukey's pair-wise mean comparison or Kruskal Wallis post-hoc test, $p < 0.05$). Sdls = seedlings; £: treatments refer to the combination of Management and Succession stage; †: year in which the management application is executed; *: no management for more than 10 years: ‡: growth phases of *C. vulgaris* as described by Gimingham (1972) (see also Fig. 1.1); **:establishments: seedlings + haustoria.

Per treatment, three replicates (patches) were randomly created within the experimental area, resulting in a total of 15 investigated patches of 5m × 5m. Scattered within each patch six permanent plots were laid out (20cm × 20cm; n = 90) in which seeds were sown at two densities (see below). Plots were separated from each other by a minimum buffer of 1m. Before the experiment was started on 11 September 2006, seeds were collected by hand from ripe fruits in three nearby *C. epithymum* populations and mixed to provide a homogeneous seed pool. To determine seed viability, a tetrazolium test was applied to five replicates of 40 seeds (see Chapter 4 for more details). This test revealed that 35 ± 1% of the seeds were not viable, indicating a potential germination percentage of 65%. To evaluate possible effects of seed-density on seedling recruitment success, a total of 6600 seeds were sown at two seed-densities on the soil surface of the plots on 11 September 2006. Seed densities were 20 seeds/ 0.04m² (low density) and 200 seeds/ 0.04m² (higher density) (two replicates per patch, n = 30 for each). The highest seed-density approximates natural seed rain densities in some large and viable nearby *C. epithymum* populations (on average 210 seeds /

0.04m²). Although *C. epithymum* was not found in the immediate neighbourhood, two control plots in which no seeds were sown were created per patch (n = 30), to control for eventual natural seed rain.

## *Data collection*

All the experimental plots in which *C. epithymum* was introduced were surveyed in detail for two subsequent years to obtain an understanding of all the steps involved in the process of establishment. At the time when the first *C. epithymum* seedlings emerged (beginning of April 2007), canopy height (cm) and cover (%) of *C. vulgaris*, bryophytes and bare ground (i.e. area not covered by the vegetation, seen as surrogate for the amount incidence of light) were measured in each patch, to quantify vegetation conditions of each of the five treatments.

To assess seed germination in the first spring after seeds were sown, we counted the number of emerged seedlings per plot every 2 weeks from the 15th March to the 1st of July. Each seedling was marked with a blue string to ensure relocation. All marked seedlings were surveyed during the 1st week of July 2007 to measure infection success, i.e. the number of seedlings that infected a host successfully, and the 1st week of August to determine the number of attached individuals that reached maturity. Only three individuals flowered in their first summer and none of them produced any seeds. All newly-emerging seedlings in the second growing season (2008) thus originated from the initially-introduced seeds. As establishment of reproductive *C. epithymum* individuals was clearly not completed after one growing season, the number of overwintering haustoria (recognised as small, green stem pieces wrapped around the host's stem) and seedlings was counted again in all plots during the second growing season (2008), from 15 March until the 1st of July. In the 1st week of July, the total number of established plants (i.e. sum of established seedlings and sprouted haustoria) was counted per plot. At the last visit (August 2008) final establishment success was measured by counting the total number of (flowering) full-grown plants per plot.

## *Data analysis*

As a first preliminary step, ANOVA followed by a Tukey post-hoc analysis ($p < 0.05$) was used to test whether several canopy measurements (i.e. canopy height, bare ground cover, *C. vulgaris* cover and bryophyte cover) differed significantly between the two management types and three successional phases. The following analyses were performed in two steps, in which first seedling recruitment is investigated and secondly the subsequent establishment steps were considered. The treatment 'mature phase' is not included in the multifactorial analyses, because in this treatment no difference between management applications could be made (see above) and because apart from seedling emergence no further establishment was observed in these plots.

First, the effect of seed-density, management and subsequent succession on seedling recruitment was investigated by using a General Linear Model (GLM: McCullagh and Nelder, 1989). In this model, seed-density (low and high density), type of management (mowing and turf-cutting), heathland succession (pioneer and building) and their interactions were entered as fixed factors, to examine their relative influence on seedling recruitment (total number of seedlings emerged in 2007 and 2008) and on the proportional number of seedlings per added seed. A stepwise backward elimination procedure was used to obtain a final model with the highest adjusted $R^2$. Subsequently, a Kruskal-Wallis test was used to test for differences in seedling emergence between the three successional stages of *C. vulgaris* (mature-, building- and pioneer-phase). Finally, to test if proportional seedling emergence in the two consecutive years was related to plot identity, a Pearson Chi-square test was applied.

In a second step, the effect of management and subsequent heathland succession on the different life-stages following seedling recruitment were evaluated. To investigate the relative importance of management, succession phase, and their interaction, on the different life-stages in the total process of establishment, GLMs were performed. These analyses were applied on the number of individuals per life-stage (haustoria, establishment and adults) proportional to the number of emerged seedlings to eliminate the indirect and predominating effect of a higher seed-density. Further, a Mann-Whitney U test was used to evaluate the differences in canopy height, *Calluna* cover and cover of bare ground between plots where *C. epithymum* established successfully after two growing seasons and plots where *C. epithymum* failed to establish. Additionally, a Pearson Chi-square test was used to evaluate if successful establishment of adult individuals differed between the two seed-densities.

The number of individuals observed in the different life-stages and canopy height were log-transformed and bare ground and bryophyte cover were arcsine-transformed to meet the statistical assumptions. All statistical analyses were performed with SPSS for windows 15.0 (SPSS, 2008).

## Results

First seedlings were observed at the 6th of April 2007, with a germination peak in the second part of May 2007. Also, in the second growing season a substantial number of seeds germinated, with first seedlings appearing at the 1st of May 2008 and no particular germination peak (Fig. 7.2). In both growing seasons, no seedlings were observed in the 30 control plots in which no seeds were sown, so all observed individuals originate from the experimentally sown seeds.

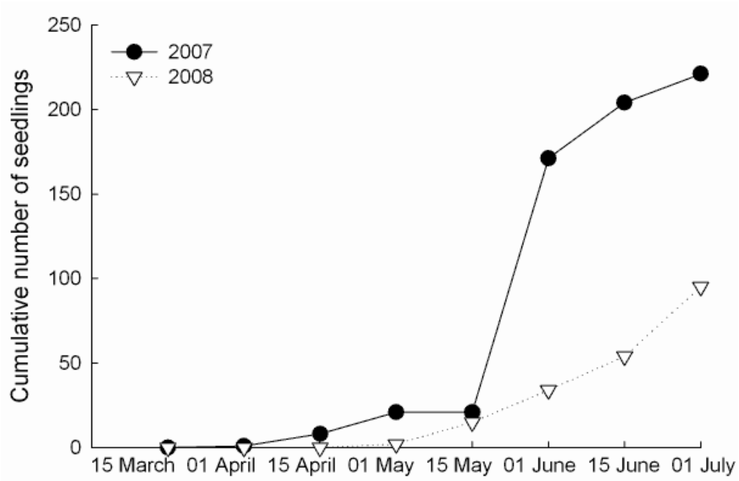


**Figure 7.2.** Cumulative number of *C. epithymum* seedlings found in 60 plots ($0.04m^2$) monitored at biweekly intervals in 2007 (solid lines) and 2008 (broken lines).

*Cuscuta epithymum* seedlings were found in all treatments in both growing seasons (Table 7.1, Fig. 7.3). In the first season 3.35% (221 seedlings) and in the second season another 1.44% (95 seedlings) of all sown seeds germinated (Table 7.1). Seed-density explained a significant part of the observed variation in seedling emergence (Table 7.2). Higher seed-densities resulted in significantly higher germination percentages and a larger number of seedlings per plot compared to the low density plots (mean ± SE: 6.4 ± 1.3 vs. 0.9 ± 0.3 and 3.1 ± 0.7 vs. 0.2 ± 0.1, for 2007 and 2008, respectively, $p < 0.001$). However, when considering the proportion of initially sown seeds that emerged as seedlings, no significant higher emergence success was observed in high seed-density plots compared to the low seed-density plots after 2 years of observation (Table 7.2; Fig. 7.3). Seedling recruitment was not significantly influenced by heathland succession or type of management, although in 2008 the highest number of seedlings was found in high seed-density plots situated in the pioneer turf-cut vegetation (Table 7.1). Furthermore, seedling emergence was not significantly lower in the mature succession phase compared with the pioneer and building phase (Kruskal-Wallis $X^2$ = 1.29; 1.52, for 2007 and 2008 respectively; $p < 0.05$).

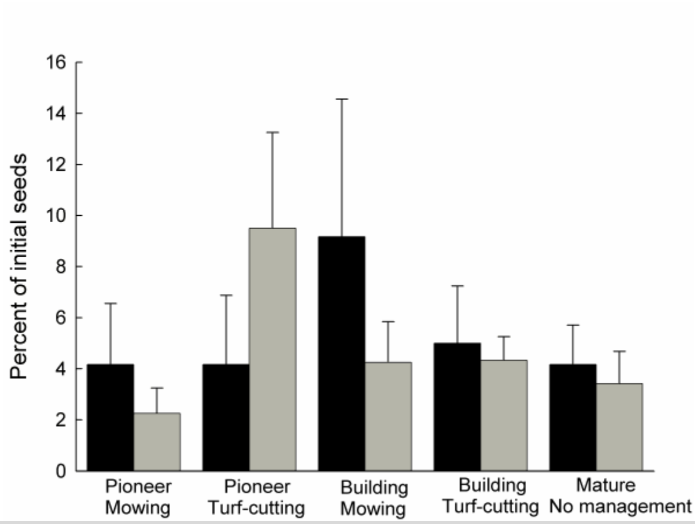


**Figure 7.3.** Mean (± SE) percentage of the sown seeds which emerged as seedling in low (black boxes) and high (grey boxes) seed-density treatments in 0.04m$^2$ plots after a 2-year study period for each treatment. In the mature phase no management is applied for more than 10 years.

**Table 7.2.** Effects of initial seed-density (Dens; i.e low versus high density) and/or phase of the vegetation succession (Succ: i.e. pioneer or building), type of management intervention (Man: i.e. mowing versus turf-cutting) and their interactions on the recruitment success. To exclude the indirect effect of seed-density on the results, the last four life-stages are given proportional towards the number of emerged seedlings.

| | *# Seedlings '07* | | *# seedlings '07/seed* | | *# Seedlings '08* | | *# seedlings '08/seed* | |
|---|---|---|---|---|---|---|---|---|
| | *df (n,d)* | *F* | *df (n,d)* | *F* | *df (n,d)* | *F* | *df (n,d)* | *F* |
| Dens. | 1,43 | 20.32$^{***}$ | 1,42 | 0.98$^{ns}$ | 1,44 | 37.8$^{***}$ | 1,43 | 3.37$^{ns}$ |
| Succ. | 1,43 | 0.70$^{ns}$ | 1,42 | 0.62$^{ns}$ | 1,44 | / | 1,43 | 0.84$^{ns}$ |
| Man. | 1,43 | 1.33$^{ns}$ | 1,42 | 0.20$^{ns}$ | 1,44 | 2.62$^{ns}$ | 1,43 | 0.05$^{ns}$ |
| Dens. × Succ. | 1,43 | / | 1,42 | 2.47$^{ns}$ | 1,44 | / | 1,43 | / |
| Dens. × Man. | 1,43 | / | 1,42 | / | 1,44 | 5.04$^{*}$ | 1,43 | 3.49$^{ns}$ |
| Succ. × Man. | 1,43 | 1.53$^{ns}$ | 1,42 | 2.84$^{ns}$ | 1,44 | / | 1,43 | / |
| Adjusted R$^2$ | 0.30 | | 0.04 | | 0.48 | | 0.07 | |
| | *# establ. '07/sdl* | | *# haust. '08/sdl* | | *# establ. '08/sdl†* | | *# full-gr. '08/sdl†* | |
| | *df (n,d)* | *F* | *df (n,d)* | *F* | *df (n,d)* | *F* | *df (n,d)* | *F* |
| Succ. | 1,44 | 0.53$^{ns}$ | 1,44 | 5.68$^{*}$ | 1,44 | 4.96$^{*}$ | 1,44 | 3.71$^{*}$ |
| Man. | 1,44 | 0.01$^{ns}$ | 1,44 | 4.91$^{*}$ | 1,44 | 4.84$^{*}$ | 1,44 | 2.60$^{ns}$ |
| Succ. × Man. | 1,44 | 0.27$^{ns}$ | 1,44 | 3.64$^{*}$ | 1,44 | 2.74$^{*}$ | 1,44 | 2.19$^{ns}$ |
| Adjusted R$^2$ | 0.05 | | 0.17 | | 0.18 | | 0.11 | |

*F*-values and significant levels from General Linear Models (GLM) are given. /: not present in the model due to backwards elimination; ns: $p > 0.05$; *: $0.01 < p \leq 0.05$; ***: $p \leq 0.001$; df(n,d) = degrees of freedom (numerator, denominator); establ. = establishments; full-gr. = full-growns; sdl = seedling; †=sum of Sdl07 and Sdl08.

The first growing season, only 27.6% of the seedlings (61 in total) succeeded in attaching to a suitable host before dying (Fig. 7.1). At the end of the season, 21 plots (35% of the total) contained full-grown individuals (31 individuals in total), of which only three flowered, but none shed any seeds (Fig. 7.1). In all but two of these plots, hibernation was observed during March 2008 (196 overwintered haustoria in total; Fig. 7.1). Of all overwintering haustoria and new seedlings that emerged during spring 2008, 63.2%

successfully established on a host plant (184 plants, of which 60 (33%) resulted from attached seedlings). In August 2008, 135 successfully established full-grown plants were found, of which 42 (31%) resulted from seeds germinated in the second year and 93 (69%) from overwintering haustoria. Of these 135 plants 66% (89 ramets) flowered and may thus be seen as adult plants.

*Cuscuta epithymum* established successfully as full-grown plants in 32% of the 60 plots where seeds were sown. In 90% of these plots *C. epithymum* also was present in the summer of 2007. The establishment of the parasite in the first year thus significantly predicts additional germination and further establishment of *C. epithymum* the next year (Chi-square test: $X^2 = 36.27$, $p < 0.001$), and new recruitment only appeared in two new plots in 2008. Flowering probability of the ramets originating from overwintered haustoria was 82.8%, in contrast to 20.5% for the individuals that originated from the seedlings stage (9.7% and 28.6% for individuals resulted from the seeds germinated in the 1st and 2nd season, respectively).

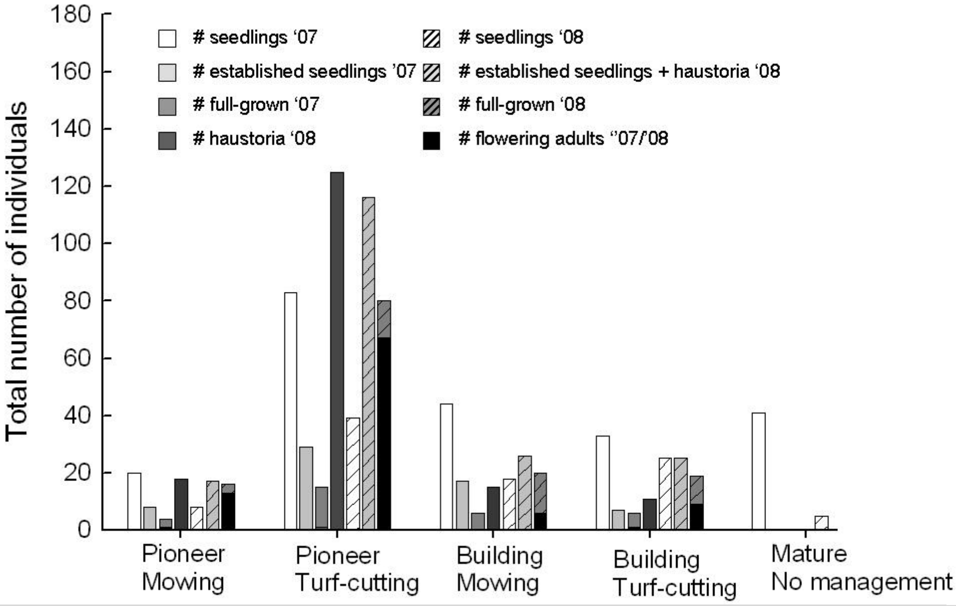


**Figure 7.4.** Sum of all *C. epithymum* individuals found in the 12 plots per treatment for each establishment stage.

Unlike seedling recruitment, attachment to a host plant and the subsequent establishment steps were significantly affected by the succession of *C. vulgaris* (Table 7.2). Infection of a host and subsequent establishment never occurred in mature, unmanaged heathland vegetation (Table 7.1, Fig. 7.4). Although establishment of full-grown individuals is not significantly affected by management type (Table 7.2), the turf-cutted vegetation in the pioneer phase had, however, a significant positive effect on regrowth from overwintering haustoria and seedling establishment in 2008 (Table 7.1, Fig. 7.4). In addition, significantly more flowering adults were found in heathland of the pioneer phase compared to heathland in the building phase (Mann-Whitney U: Z = -2.56; $p < 0.05$; Fig. 7.4). The plots where *C. epithymum* established successfully 2 years after seed sowing were characterised by a significantly lower canopy height, higher percentage of bare ground and a lower cover of *C. vulgaris* than the plots were the parasite did not succeed (Fig. 7.5 and see Table 7.1 for vegetation description of the five heathland sites). Finally, the proportion of plots where *C. epithymum* developed into the full-grown life-stage was significantly higher for high seed-density plots than for low seed-density plots (53% vs. 10%; Pearson Chi-square test $X^2 = 13.02$, $p < 0.001$).

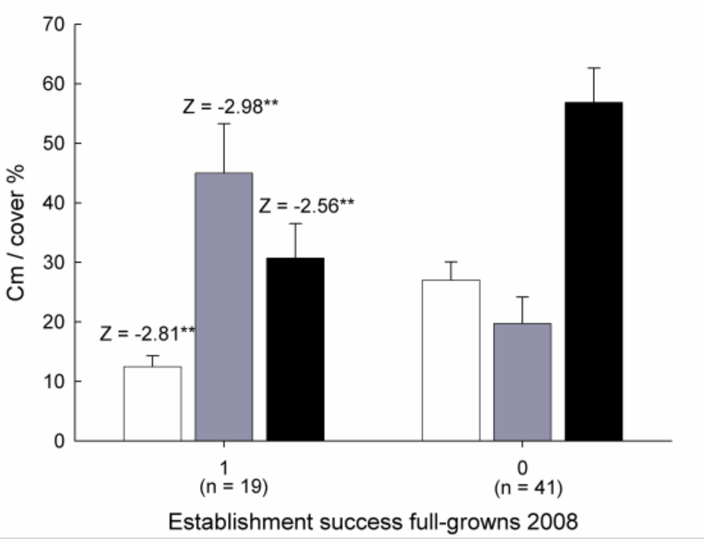


**Figure 7.5.** Mean (± SE) canopy height (cm; white), cover of bare ground (%; gray) and cover of *C. vulgaris* (%; black) in the plots where *C. epithymum* plants were successfully established in summer 2008 (1) and where they did not succeed (0). Only the 60 plots in which *C. epithymum* seeds were sown were included in the analysis. n = number of plots with or without *C. epithymum*. Z-values and significance levels of the Mann-Whitney U tests are given (**: $0.001 < p \leq 0.01$).

## Discussion

Although 65% of the sown seeds were viable (Chapter 4), only a minority (4.79%) emerged as seedlings, which is relatively low compared to the 22% of seeds that germinated under the controlled conditions in the laboratory (Chapter 4). These observations indicate that seedling emergence is an important bottleneck for *C. epithymum* establishment. Since the species is associated with early-successional *Calluna*-stands, we expected *C. epithymum* seeds not to germinate in vegetation belonging to the mature phase. In contrast to our expectation, however, emergence of seedlings was neither related to the management type applied to the vegetation nor to the successional stage of the *Calluna*-dominated heathland. Germination occurred under all treatments, even the unmanaged, dense mature heathland. This can be explained by the fact that *C. epithymum* seeds do not need light or intense temperature fluctuations for successful germination, typically occurring in open places, which corresponds with earlier findings of Chapter 4. So, seeds do not particularly respond to disturbances or the presence of gaps in the vegetation. This observation is in contrasts with many other plant species which particularly occur in young successional vegetation or in open gaps (e.g. Baskin and Baskin, 1998; Jacquemyn et al., 2003). Consequently, the above-mentioned requirements for germination can not be used to explain the observed low germination percentages. Given that seed predation is estimated to cause up to about 75% of seed loss in plants (Fenner, 2000), it may play an important role in *C. epithymum*. Nonetheless, no information concerning the importance of seed predation for seed loss is available for *C. epithymum* seeds or for those of related species. The observation that germination appeared over two successive springs, demonstrates that this species is characterised by a bet-hedging strategy, as proposed in earlier research under laboratory conditions (Chapter 4). This strategy allows germination to be spread over at least two years and thus eliminates the risk that the whole seed bank is lost due to environmental stochasticity (Fenner and Thompson, 2005). A similar strategy is also reported in other species (e.g. *C. campestris*, Dawson, 1965; Costea and Tardif, 2006). In accordance with the results of Hutchinson and Ashton (1980) studying *C. campestris*, the proportion of seeds that emerged as a seedling each year was variable, with 3.4% in the first year, and 1.4% in the second year. As observed under laboratory conditions (Chapter 4), germination occurred once temperature became suitable for seedling establishment in spring. The germination period correlates with the elevation period of winter dormancy in *C. vulgaris* plants, characterised by the cessation of vegetative growth from October until March (Woolhouse and Kwolek, 1981). The germination in April-May is thus strongly synchronised with the availability of suitable hosts (fresh *Calluna* shoots).

Next to a possible pre-germination loss, a remarkable high mortality was observed at the seedling stage, which corroborates the results of many other researchers (e.g. Fenner, 2000; Gómez-Aparicio, 2008). As seedling emergence and survival in *Cuscuta* are very sensitive to drought (Verdcourt, 1948), the extreme dry and hot spring of 2007 may have caused a high percentage of seedling mortality. Seedling loss was indeed considerably lower in the spring of 2008 than in 2007 (72 % vs. 63%, respectively). However, more importantly, the absence of a suitable host in the surrounding vegetation appeared to be a strong bottleneck on seedling settlement. As *C. epithymum* seedlings on average reach 5cm (pers. obs.), a suitable host must be located in the direct proximity before a successful contact can be made (within a radius of 3-8cm; see also Parker and Riches, 1993; Albert et al., 2008 for other *Cuscuta*-spp.). If *C. epithymum* ultimately succeeds in coiling around a host, a prehaustorium must first adhere to the host and a haustorium must penetrate into the host's vascular tissue before attachment is functional (Costea and Tardif, 2006; Albert et al., 2008). This may explain the severe losses of

*C. epithymum* individuals observed between the seedling stage and further adult establishment. In contrast to germination, *C. epithymum* attachment to a host and subsequent establishment always failed in mature, unmanaged heathland. In addition, while seedlings emerged in 68% of all surveyed plots, adult establishment was only completed successfully in 32% of the plots. This indicates that although seeds are able to germinate under variable environmental conditions, successful establishment is, however, much more stringent. This illustrates the importance of the age of the host plants on the successful formation of a functional haustorium. Our data suggest that, in contrast with younger *C. vulgaris* individuals, the older ones show a defence mechanism to prevent penetration and/or development of the haustoria, as it is shown for several host species attacked by *Cuscuta* spp. (e.g. Dawson et al., 1994; Costea and Tardif, 2006; Albert et al., 2006; 2008). Rice plants (*Oryza sativa*) were, for example, resisted against *C. campestris* infestation due to several layers of hard and thick walled sclerenchyma, which prevented the penetration of haustoria (Mishra et al., 2007). In the same way, we expect that the water-resistant protective tissue (phellem) formed at the outside of older woody *C. vulgaris* stems may function as a mechanically barrier against *C. epithymum* attacks. The phellem tissue contains cork cells with suberin in their walls (Mohamed and Giminghma, 1970; pers. obs.), which is shown to impede haustorial penetration in other parasitic species (e.g. López-Curto et al., 2006; Rümer et al., 2007; Albert et al., 2008). Next to such a mechanical barrier, one could also hypothesise that, in contrast with young *C. vulgaris* plants, older host plants defend themselfs by the production of biochemical inhibitors (e.g. Bringmann et al., 1999; Costea and Tardif, 2006), or that older *C. vulgaris* do not emit volatile cues and are therefore not infected, as is recently shown in tomato plants that were infected by *C. pentagona* (Runyon et al., 2006). In addition, it is reasonable that the dense vegetation of older mature heathland may further limit successful attachment of the parasite due to the limited light availability (Parker and Riches, 1993; Haidar et al., 1997; Costea and Tardif, 2006). Altogether this may explain why (reproductive) *C. epithymum* adults were mainly found in turf-cut pioneer heath, characterised by higher numbers of young *C. vulgaris* plants and the highest cover of bare ground, so with the highest light intensities. Our observations indeed indicate that mainly the age of the host plant and associated vegetation conditions have a significant impact on the establishment probability of *C. epithymum*. Furthermore, our data clearly indicate that there is a conflict between the suitability of microsites for seed germination and for subsequent seedling establishment. Similar conflicts between seed and seedling establishment have been obtained in various vegetation types (Schupp, 1995; Gómez-Aparicio, 2008). Although only a small fraction of the available seeds emerges each year, *C. epithymum* will experience notable seedling loss in unsuitable sites because of this conflict between the lack of control over where germination takes place and the stringent attachment requirements.

Although the restricted establishment after the first growing season, new seedlings and overwintering haustoria provided an important second wave of plants the following spring. After two growing seasons, most established full-grown individuals originated from overwintering haustoria (each 1st generation full-grown *C. epithymum* plant resulted on average in 3 full-growns in the 2nd season) and for those ramets, flowering probabilities were much higher compared to individuals originating from seeds germinating in the same growing season. As none of the first generation *C. epithymum* individuals produced seeds, our data suggest that some *C. epithymum* plants need to overwinter as haustoria before they reach the reproductive adult stage, although this may vary from year to year and from local conditions (e.g. second year results and pers. obs. in other study sites). Thus, apart from the importance of overwintering haustoria for extending the survival of the parasite in ageing heath vegetation and the shift from one host to another (Chapter 6), overwintering obviously plays an even more important role in the establishment process by providing additional established reproductive adults.

Although recruitment patterns are mostly determined by the availability of favourable microsites (Bissels et al., 2006), many species, however, have been found to be seed limited (Zobel et al., 2000; Brys et al., 2005). In addition, seed limitation has been assumed to be more common in disturbed (early successional) habitats, such as managed heathland, than in undisturbed ones (Turnbull et al., 2000). In contrast, our results revealed that higher seed-density resulted only in a non-proportional and small seedling increase, suggesting that *C. epithymum* is more establishment limited than seed limited. Persistent seed banks, such as in *C. epithymum* (Box 5.1), further decrease the magnitude of seed limitation because viable seeds in the soil accumulate over time and may recruit into the population even in the absence of reproductive output in a given year (Putwain and Gillham, 1990). In accordance with Clark et al. (2007) and Gómez-Aparico (2008), we can conclude that environmental characteristics (i.e. host quality) more strongly influence the establishment of *C. epithymum* than does seed availability. However, because successful establishment increased with a higher seed-density, the role of seed availability is not totally negligible, which implies that reduced seed output may result in a reduced establishment too.

## Conclusion

While most seed introduction experiments only monitor until seedling survival, our results strongly demonstrated the need of longer-term observations until the self-sustainable reproductive adult stage to obtain clear insights into the process of population establishment via seeds. Our observations further suggest that the rarity of *C. epithymum* may, at least partly, be attributed to seedling emergence at unsuitable growing sites, its stringent requirement for seedling attachment to its host and, to a lesser extent, limited seed availability. Nevertheless, it is shown that under optimal conditions, such as in recently turf-cut or mown heathland, introduction of sufficient *C. epithymum* seeds may lead to some viable populations which could in turn act as new source of populations. Further, our study confirms that the lack of any management of the vegetation is very disadvantageous for the establishment of this species. The presence of an appropriate heathland pioneer phase with sufficient young *C. vulgaris* plants, achieved through cyclical mowing or turf-cutting, is thus of crucial importance to sustain viable *C. epithymum* populations in the long term.

# 8

# Metapopulation viability of an endangered holoparasitic plant in a dynamic landscape

Adapted from: Meulebrouck K., Verheyen K., Brys R., Hermy M. 2009 – Ecography 32: 1-11

Photos: Klaar Meulebrouck (Ziepbeekvallei, 2008)

## Introduction

Disturbance is an important component of many ecological systems, influencing both populations and metapopulations by creating a spatiotemporal mosaic of patches at different successional stages (e.g. Moloney and Levin, 1996). The impact of disturbance on the distribution and dynamics of a species within a specific landscape is expected to depend on both the spatiotemporal disturbance patterns and on the species' demographic characteristics (Moloney and Levin, 1996; Matlack and Leu, 2007). The long-term impact of disturbance on metapopulations of rare species may be crucial for management strategies, but only a limited number of studies have incorporated the auto-ecological characteristics of the focal species with the dynamics of the landscape. This is especially true for plant metapopulations where the majority of the published literature involves theoretical work (see e.g. Husband and Barett, 1996; Higgens and Cain, 2002). Although a few authors have demonstrated the impact of seed emigration and plant life-span on metapopulation viability in disturbed landscapes (e.g. Johst et al., 2002; Verheyen et al., 2004; Bossuyt and Honnay, 2006; Matlack and Leu, 2007), empirical evidence about the effect of seed banks on metapopulation viability is missing. This may be particularly important for early successional species occurring in dynamic landscapes, where establishment of a seed bank may function as an important strategy to buffer populations against local extinctions during unfavourable conditions (e.g. Fenner and Thompson, 2005; Bossuyt and Hermy, 2003; Piessens and Hermy, 2006).

Heathlands in Europe emerged as an anthropogenic landscape about 7000 to 4000 years ago as the result of forest clearance followed by millennia of small-scale management activities, such as grazing, turf cutting, burning and mowing (Webb, 1998; De Blust, 2004; Piessens et al., 2004). As a result, semi-natural heathland systems often provide a habitat for many species relying on particular successional stages, which are the result of heathland management (Goodall and Specht, 1979). During the past century, however, traditional land-use practices have declined causing a degradation and loss of heathland habitat (e.g. Webb, 1998; Piessens and Hermy, 2006). In many heathland areas, conservation strategies are applied now to stop natural succession towards woodland and thus avoid further loss of this habitat (Webb, 1998). However, current nature conservation management is often applied at a large-scale, thus resulting in uniform habitats characterised by homogeneous even-aged vegetation (De Blust, 2004). During last decades, both inadequate management and/or lack of any management are responsible for the severe decline of numerous typical heathland species, such as *Gentiana pneumonanthe* (Oostermeijer et al., 1994), *Diphasiastrum tristachyum* (Muller et al., 2003), *Cirsium dissectum* (Dorland et al., 2003) and *Cuscuta epithymum* (Chapter 2). The early successional short-lived species are especially threatened because their populations may quickly collapse in the absence of continued disturbance. Long-term survival of these early successional species can only be assured when the loss of local populations is counterbalanced by the establishment of new populations through (re)colonisation of newly created patches. Despite the well-known effects of different disturbance regimes on community organisation and diversity (e.g. Bullock and Pakeman, 1997; Vandvik et al., 2005), underlying mechanisms of plant population dynamics have received little attention. Consequently, there is a need to examine the impact of temporal and spatial variation in habitat suitability on individual species performance, (meta)population dynamics and long-term survival.

In this study we focus on the effects of demographic traits and spatiotemporal landscape patterns created by management events on metapopulation dynamics and long-term survival of the early successional holoparasite *C. epithymum*. This endangered species grows on non-woody young heather and typically appears after heathland vegetation has been set back to an earlier successional state (Chapter 3). Like many other species living in similar semi-natural landscapes, *C. epithymum* is generally restricted to young patches of heathland vegetation (mostly *C. vulgaris*), typically with a loss of host-plant quality through natural succession to old, unsuitable heather (Chapters 3, 6 and 7). The combination of directional vegetation change through time, and occasional vegetation disturbance events results in *C. epithymum* showing spatiotemporal dynamics with colonisation in newly created patches of young heather and extinction from patches containing heather growth older than 7 years. The heathland system under study is a rare example of a system in which both the ecology of the focal species and the dynamics of the landscape are well documented. It provides an excellent opportunity to investigate the interacting effects of demographic characteristics and management-induced spatiotemporal landscape dynamics on metapopulation viability. In particular, the following questions were addressed: (1) What are the relative contributions of seed emigration, seed bank persistence and adult life-span to metapopulation survival in a dynamic landscape? (2) What type, frequency and spatial arrangement (including patch size and isolation) of management events are needed to provide a reasonable chance of *C. epithymum* surviving the next 100 years? To answer these questions we used a stage-structured, spatially explicit metapopulation model whose parameters are founded on an extensive data-set originating from 4 years of field observations combined with experimental data collected in four heathland systems.

# Materials and methods

## *Life-cycle diagram*

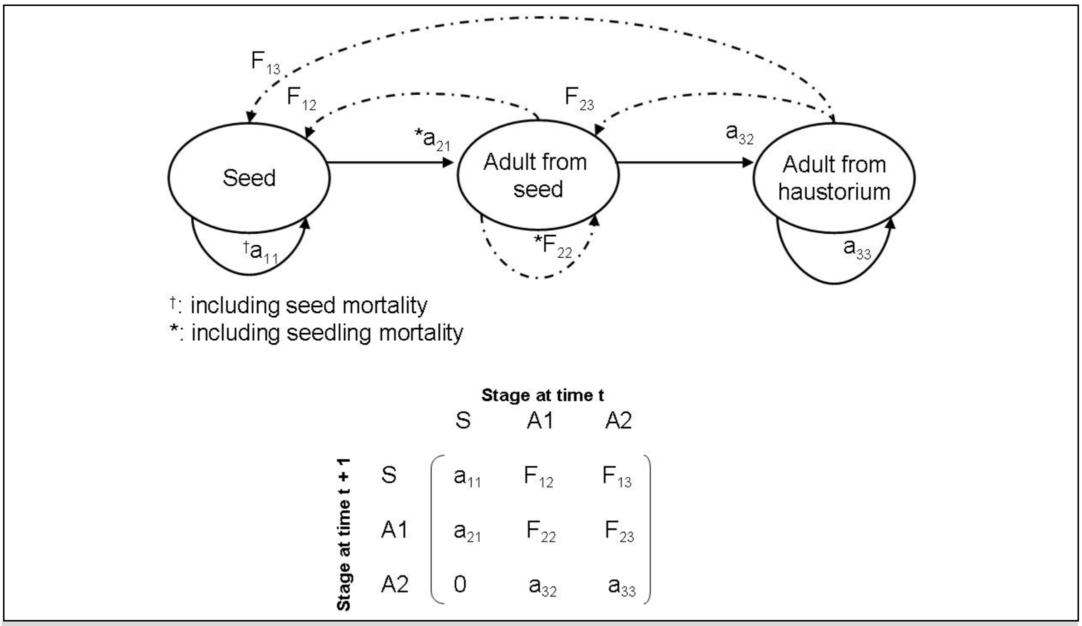


**Figure 8.1.** Life-cycle diagram of *C. epithymum* and the corresponding transition within the matrix: seed bank, adults originating from seeds and adults originating from vegetative overwintering haustoria. Circles indicate life stages, arrows represent the possible transitions among them, and letters refer to the connection between each transition and its matrix entry. Transitions from one stage to another occur in 1-year intervals. Fecundity (F) and fate (a) are represented by arrows with broken and full lines, respectively. $F_{22}$ and $F_{23}$ = germination and establishment of seeds originating from seed rain; $a_{21}$ = germination and establishment of seeds originating from seed bank; $a_{11}$ = dormant seeds staying in seed bank; $a_{32}$ and $a_{33}$ = establishment from overwintering haustoria. Seed (†) and seedling (*) mortality are included in $a_{11}$ and $F_{22}$ and $F_{23}$, respectively. For calculation of the different transition elements see Tables 8.1 and 8.2.

During its life span, *C. epithymum* has three important stages: seeds, adults originating from germinated seeds (Adult from seed) and adults originating from overwintering haustoria (Adult from haustorium; Fig. 8.1). The transition from one stage to another occurs over a 1-year interval (Fig. 8.1). Seeds produced in any one autumn will remain close to the parent plant or disperse to other patches, and they will germinate in the next spring, enter in a persistent seed bank or die. Seedlings observed during spring are assumed to be both the result of germination of seeds in the persistent seed bank or from those dispersed (seed rain) the previous autumn (pers. obs.). To establish as an adult originating from seeds, seedlings only have a maximum of 3 weeks to locate a suitable host or they die. The host is mainly a young *C. vulgaris* individual in dry heathland vegetation and is infected by *C. epithymum* through haustorial connections through which nutrients are taken from the host's vascular tissue (pers.

obs.; see also Chapter 2, 3). Adults can also originate from haustoria that overwinter vegetatively in the host's tissue, sprouting again in the following spring (i.e. Adult from haustorium; Chapter 6). When seedling establishment or haustorial sprouting is successful, adult plants of *C. epithymum* form aggregations of red thread-like stems, with white autogamous flowers during summer (Chapter 2). At the end of the autumn, after seeds are shed, only dried, dead segments of *C. epithymum* stems remain visible. *Cuscuta epithymum* re-establishes each spring from overwintering haustoria and/or germination from the seed stage as long as young *C. vulgaris* individuals are present (Chapter 7). When a disturbance event converts a late-successional heathland patch into a young-successional patch, *C. epithymum* may colonise by germination of recently-dispersed seeds or by recruitment via the seed bank.

### Field populations and plant trait measurements

During 2004-2008, we investigated 108 *C. epithymum* populations in 289 patches in four nature reserves, located in north-eastern Flanders (Belgium; Chapter 3). Distance between these four nature reserves ranges between 2.4km and 12.4km (see Chapter 3, Fig. 3.1). The reserves are seen as isolated heathland fragments among which no interaction occurs. The nature reserves contain dry heathland vegetation, typically dominated by heather (*C. vulgaris*). A rotational management strategy of mainly mowing, sometimes in combination with extensive grazing (cattle or horses), is applied to restore and conserve the heathland vegetation. This rotational strategy creates a dynamic landscape of patches of variable time since last management and has a range of successional stages. Within such heath vegetation, *C. epithymum* typically shows a patchy spatiotemporal distribution. Canopy closure during heathland succession causes population decline and extinction of the above-ground population, although the seed bank may persist. In such dynamic landscapes, metapopulations can be seen as a permanently changing system of suitable patches in which colonisation and extinction of populations change with the local heathland dynamics.

Each summer, we investigated all appropriate *C. epithymum* habitat patches (i. e. recently mown patches typically containing young *C. vulgaris* individuals), allowing us to understand patch dynamics, including changes in host quality (patch suitability), establishment and disappearance of appropriate habitat patches, and population dynamics of *C. epithymum* populations (Chapter 3). In each of the surveyed patches, measurements were made of patch size, patch isolation, characteristics of the vegetation (vegetation height and cover), and the size and fecundity of each *C. epithymum* population (see Chapter 3 for further details). Seed germination and seed sowing experiments were carried out to evaluate germination and establishment rates under both controlled laboratory and field conditions (Chapter 4 and 7, respectively). A seed bag burial experiment was established to quantify seed mortality rates in soil (Chapter 5). Finally, we quantified the amount of overwintering as haustoria in a subsample of the studied populations (Chapter 6).

## Modelling

We modelled the dynamics of *C. epithymum* metapopulations with a spatially explicit, stage-structured stochastic simulation model implemented in RAMAS/Metapop (see Akçakaya, 2002 for further details). This model uses x- and y-coordinates to define the spatial metapopulation structure and captures annual intra-patch population dynamics (Johst et al., 2002). Density dependence based on a ceiling function without the Allee effect is included in the model, which affects vital rates. Environmental stochasticity was included in the model by means of temporal variation in vital rates, which are identical for each patch, but change each time step. Temporal variation in vital rates was achieved by using the coefficient of variation for fecundity ($CV_f = 1.51$) and for survival ($CV_a = 0.82$), derived from observed variation in the study area over a 4-year period. Demographic stochasticity was included in the model by using the demographic stochasticity feature in RAMAS (Akçakaya, 2002). Since such between-years dynamics cannot be captured by single season survey data, between-year stochasticity was achieved by using data from four consecutive years (Peltzer et al., 2008). Each simulation was run for 100 years, with 1000 replications.

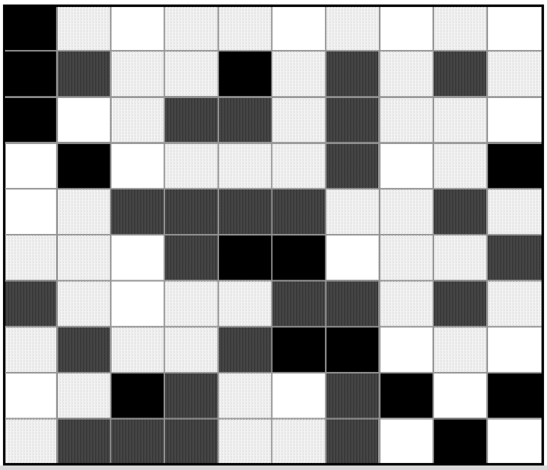


**Figure 8.2.** Hypothetical heathland landscape of 4 hectares in area composed of 100 patches of 20 × 20m$^2$ used in the model. Patch suitability is indicated by black and white squares, while the presence of a persistent seed bank and/or aboveground populations is represented by dotted squares. The spatial distribution of *C. epithymum populations*, suitable and unsuitable patches and initial age of each patch (i.e. successional state; not presented) at the beginning of each simulation are based on real distribution patterns of *C. epithymum* populations and patches of different ages observed during 4-years of research in four heathland reserves. The landscape modelled here is used as a starting point when simulating the variable demographic characteristics. At the beginning of each simulation each of the 100 patches was attributed to one of four different conditions: **1)** □ unsuitable patch without seed bank (n = 20); **2)** ░: unsuitable patch with seed bank (n = 40); **3)** █ suitable patch without seed bank (n = 14); **4)** ▓ suitable patch with seed bank (n = 26).

## *Experimental landscape structure and demographic variables*

The hypothetical 'landscape' used in the model is based on the sizes, spatial arrangement and successional stage of the 289 patches observed during four years of research. It is constructed as a square lattice of 100 cells (further referred as 'patches'). Each of these 100 patches represents a 400m² piece of heathland vegetation, together comprising a 4ha mosaic landscape of adjacent patches with variation in time since the last management event (Fig. 8.2). The used patch size is based on the average (± SE) patch area of 393 ± 48m² found for management patches in the reserve Heiderbos. A 'landscape' of 4ha simulates the size of many heathland fragments in Belgium and other countries in western Europe (e.g. Decleer and De Belder, 1999; Rose et al., 2000). Suitable patches for *C. epithymum* generally arise following management activities (such as mowing), after which host quality and patch suitability for *C. epithymum* gradually declines in time as the heather growths and gets older (i.e. vegetation succession). This changing patch suitability and associated deterministic extinctions was modelled by time-dependent carrying capacity (*K*) of a patch. The latter represents the maximum number of *C. epithymum* adults supported in a patch belonging to a particular succession phase, regardless of the number of seeds present in the seed bank. Following a patch management event, which resets the late-successional patch to a suitable patch containing young *C. vulgaris*, *K* is higher than 0 for a period of 10 years, with a period of possible population growth immediately after intervention followed by a decrease of population size with vegetation ageing (Fig. 8.3).

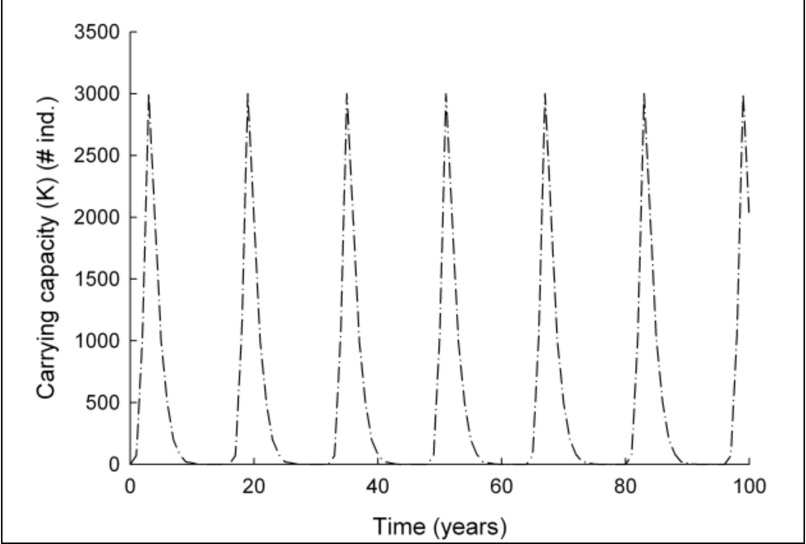


**Figure 8.3.** Modelled change in carrying capacity (*K*, maximum number of adults tolerated per patch) for an individual patch that is been managed at time 0 and at 15-year intervals (mowing frequency = 15 years). For the other simulated mowing frequencies, the presented cycles go faster or slower than the 15-year intervals. The *K* is set at a constant maximum of 3000 individuals, based on our empirical data.

Depending on the frequency of the management intervention, each year a proportion of unsuitable habitat, i.e. late-successional vegetation in the hypothetical 4ha landscape, is converted into suitable heath (newly mown vegetation) and gradually changes towards older heath vegetation. This gradual change of each virtual patch is based on the vegetation succession observed in the study area (see Chapter 3). At time step 0, each patch belongs to one of four conditions, which are suitable or unsuitable for *C. epithymum* growth, in combination with or without a persistent seed bank (Fig. 8.2). Only suitable patches may contain aboveground populations and consequently possess a $K$ higher than 0 at the start of each simulation. A persistent seed bank, on the other hand, can occur in both suitable and currently unsuitable patches.

**Table 8.1.** Parameters used in the *C. epithymum* metapopulation model with their value, calculation mode, study site (All = the four nature reserves, MH = Mechelse Heide) and study period (S = summer, Sp = spring). The column representing parameter values, provides mean ± SE values calculated from our empirical data for the first three values and fixed values used in the model for the last four ones. The number of measurements is given in parentheses. Codes refer to matrix transition elements used in Fig. 8.1.

| *Parameter* | *Value (mean ± SE)* | *Calculation method* | *Location* | *Period* |
|---|---|---|---|---|
| Population size/patch(1) | (1) 224 ± 49 | Mean population size for each vegetation age, including the total number of individuals belonging to stages ‘adult from seed’ and ‘adult from haustorium’ | All | S 2004-‘07 |
| Seed abundance/patch | (2) 21.95 ± 1.55 (n = 570)<br>(3) 2.02 ± 0.07 (n = 228) | Based on population size and mean no. of flowers/individual(2) × mean no. of seeds/flower(3) + taking into account the linear relation between no. of seeds (Y) and vegetation age (X) ($Y = -9.50X + 85.58$; $r^2 = 0.71$; $n = 81$), seed mortality (30%) and germination rate (14%) the year after seed release | All | S 2006-‘07 |
| $F_{22}$ and $F_{23}$ | **2.54** | **No. of reproduced seeds germinating in the following spring per adult;** calculated from fecundity data; including ● | All | S 2006-‘07 |
| $F_{12}$ and $F_{13}$ | **16.30** | **No. of reproduced seeds arriving in the seed bank per adult** considering $F_{23}$, the 43% non-viable + died seeds and fecundity data; including ● | All | S 2006-‘07 |
| $a_{21}$ | **2.38%** | **Annual germination probability from seed bank** discovered from seed burial experiment; including ● | MH | 2006-‘08 |
| ● | **14%** | **Seedling survival** estimated from sowing experiment | MH | Sp 2007 |

For each virtual patch, the initial number of plants per population stage (seed, adult from seed and adult from haustorium) was calculated based on counts performed during 2004 – 2008

(Table 8.1). We used stage matrices to model the individual patch population dynamics. The life-cycle diagram for *C. epithymum* was translated into a transition matrix where the matrix elements $a_{ij}$ and $F_{ij}$ define transition probabilities from stage *j* to stage *i* in 1-year time intervals (Caswell, 2001, Fig. 8.1). The 'fate' or 'survival' transitions ($a_{ij}$) vary between 0 and 1, whereas 'fecundity' transitions ($F_{ij}$) can have higher values. Transition probabilities in Table 8.1 were calculated from field data. For each simulation all 100 populations were attributed to the same transition matrix (Fig. 8.1).

In addition to the above mentioned general baseline of the model, we further applied two distinct steps in the analysis in order to evaluate the impact of three demographic characteristics of the species and to investigate the impact of management interventions in the long-run. We first investigated the possible influence of the three key demographic characteristics mentioned above on metapopulation viability by applying a fixed rotational management of 15 years in each patch as shown in Fig. 8.3. We then modelled the impact of management events on *C. epithymum* metapopulations in the long-term with a fixed and realistic combination of demographic characteristics.

### Simulations of variable demographic characteristics under a fixed rotational disturbance regime

To explore the importance of a persistent seed bank ($a_{11}$ in the transition matrix), life-span ($a_{32} + a_{33}$) and seed emigration on metapopulation viability we ran 36 simulations, each with its own specific transition matrix (Fig. 8.1; Table 8.2). Three different levels of seed bank survival, i.e. percentage of seeds that is able to survive until the next growing season, were used in the simulations: low (7%), moderate (18%) or high (50%) seed survival (Table 8.2). Our seed burial experiment showed that the annual seed bank survival is on average 18% (pers. obs.), and the other two values are chosen to be more extreme scenarios. Research on overwintering haustoria has recently discovered the perennial character of *C. epithymum* (Chapter 6), but the exact proportion of overwintering individuals and the impact on metapopulation dynamics is still not clear. We therefore incorporated variable life-span values of 0, 0.04, 0.10 and 0.25, representing an annual, very short, short or moderately perennial life-span, respectively (Table 8.2). Finally, to evaluate the effect of variable levels of seed emigration on metapopulation dynamics, simulations were performed in which variable levels of dispersal, i.e. variable fractions of all produced seeds leaving a particular patch, were used in the model. We used three fixed values of seed emigration: 4 seeds leaving the patch for every 10 000 seeds produced there, 1 and 3 seeds leaving the patch for every 1000 seeds produced (4/10 000, 1/1000 and 3/1000, respectively). The dispersing seeds were spread over all other patches in a distance-dependent way, following the dispersal function '$M_{ij}$ = a exp (-

$D_{ij}$/b)' available in RAMAS/Metapop (with $M_{ij}$ the number of seeds in source patch *j* that move to patch *i*, b the rate of decline of the number of dispersers with increasing distance, $D_{ij}$ the distance between two patches and seed emigration rate a, see Akçakaya, 2002). Parameter b is set high enough, at 200, to ensure that all patches have a reasonable chance of receiving dispersing seeds, with each successive patch receiving around 50% fewer seeds than the next nearer patch. Low seed emigrations were used because it seems that the majority of *C. epithymum* seeds disperse over very short distances and no clear dispersal event was observed during our field study. Upon arrival in a particular patch, dispersed seeds become part of the seed bank at the site irrespective of successional phase of the patch.

**Table 8.2.** Simulated **a)** three demographic (dem.) characteristics with their parameter values and **b)** various management scenarios used in the different scenarios of the basic model. Codes in parentheses refer to Fig. 8.1.

| a) *Dem. characteristics* | *Modelling method* | *Parameter value range* |
|---|---|---|
| Seed emigration | Fraction of produced seeds leaving the patch | 4/10 000 = low<br>1/1000 = moderate<br>3/1000 = high |
| Life-span ($a_{32}$ + $a_{22}$) | Probability of adult to overwinter via haustoria | 0 = annual<br>0.04 = very short-lived perennial<br>0.10 = short-lived perennial<br>0.25 = moderate-lived perennial |
| Seed bank persistence ($a_{11}$) | % of seeds in the seed bank remaining in the next year. Depends on germination from seed bank and seed survival rate. | 7% = low<br>18% = moderate<br>50% = high |
| b) *Management actions* | *Interpretation* | *Scenarios* |
| Patch distribution | Spatial location of the patches managed in the same year | Clustered = patches in a block<br>Random = random pattern |
| Grazing | Carrying capacity always ≥ 30 individuals | 1 = grazers present in landscape |
| Mowing frequency | No. of years between two consecutive mowing measures, modelled by changes in carrying capacity (no. patches mown/year) | 20 years (5)<br>15 years (7)<br>10 years (10)<br>4 years (25)<br>2 years (50) |

At the start of each of the 36 simulations, the landscape was exactly structured following the pattern shown in Figure 8.2. The initial distribution of seed bank and the *C. epithymum* populations (the four different conditions shown in Fig. 8.2) and change in successional state in each patch (i.e. time after the management intervention is applied to the vegetation, determined by *K*-value) are based on field observations during a 4-year study. In each year six or seven of the 100 virtual patches were set back to initial successional state by mowing which represents a typical heathland management practice in the four studied reserves. This is equivalent to a rotational mowing or mowing frequency of 15 years and is represented in the model by time-dependent carrying capacity (*K,* as mentioned above, Fig. 8.3). The application of a rotational management of 15 years on all patches prescribes the spatial structure of the landscape over the long-term. Finally, for each of the 36 simulations, a different combination of transition elements was used in the transition matrix, to test different combinations of demographic characteristics (Table 8.2).

### *Management scenarios*

To examine the effects of different management regimes, particularly variable mowing frequencies, spatial arrangement of management events, and extensive grazing on *C. epithymum* metapopulation viability, a virtual management experiment was performed. Using five different time-dependent *K*-series (as earlier mentioned for rotational mowing of 15 year; Fig. 8.3), five mowing frequencies were created so that each patch was mown at 20 year, 15 year, 10 year, 4 year or 2 year intervals (Table 8.2). Mowing frequency refers to the frequency at which a particular patch is mown, expressed as the number of years between two consecutive mowing events. The higher the mowing frequency is, the shorter the period between two consecutive mowing events. With the five different mowing frequencies, the number of patches mown each year also fluctuated over time. Consequently, each time interval 5, 6-7, 10, 25 or 50 of the 100 patches were mown, respectively. When mowing frequency changes (by changing the *K*-value), the landscape structure represented in Fig. 8.2, also inevitable changes. For each of these five mowing frequencies, the model was run with a clustered or random spatial arrangement of managed patches. In the clustered arrangement mowing in a particular year was applied to adjacent patches, by which then a larger area of heathland patches with the same successional phase is formed. In the random arrangement patches were randomly mown across the 4ha landscape matrix which tended to create small, isolated patches. In both types of spatial organisation, the number of annually mown patches and so the arrangement of the landscape depends on the mowing frequency. The model was also run with and without extensive grazing to examine the additional impact of grazing on metapopulation viability. Grazing creates young heather shoots and extends patch suitability, so *K* was always held higher than 30 individuals in the grazed situation. This number was based on long-term data of populations growing in the heathland nature reserve 'De Maten', where grazing is used as an additional management tool. For all management scenarios, populations had a transition matrix with the same transition elements, including moderate seed persistence (18%), a short perennial life-span (0.10) and a very low seed emigration (4 seeds emigrating out of every 10000 produced), believed to be a realistic scenario for *C. epithymum* metapopulations in a dynamic landscape with a fixed mowing frequency of 15 years.

To assess metapopulation viability in the different scenarios, extinction probability, time to extinction and the number of individuals in the metapopulation (i.e. metapopulation size) were determined after running the model to simulate 100 years. We used a General Linear Model (GLM: McCullagh and Nelder, 1989) to examine the relative impact of values of the three demographic characteristics and the different management scenarios on extinction probability and metapopulation size after 100 years. This analysis was intended to identify the relative importance of the different scenarios rather than explain all the variation in time.

# Results

## *Demographic characteristics*

Both the extinction probability and the time to extinction of *C. epithymum* metapopulations occurring in patches of 400m² with a fixed mowing frequency of 15 years were decreased significantly with an increasing seed bank persistence and, more importantly with increasing seed emigration (Table 8.3 and 8.4a; Fig. 8.4). The interaction between high seed persistence (50%) and moderate or high levels of seed emigration (1/1000 or 3/1000) significantly increased metapopulation size after 100 years (Table 8.3). To a lesser, but still significant amount, life-span and its interaction with seed emigration reduced extinction probability and time to extinction (Table 8.3 and 8.4a). When seed emigration increased to three seeds per 1000 leaving a patch, a remarkable increase of metapopulation size and decrease in extinction risk was observed (Table 8.3; Fig. 8.4).

**Table 8.3.** Mean number of individuals in the metapopulation after 100 years, for different values of seed emigration, seed persistence and life-span applied in the demographic characteristic simulations. In all simulations a fixed mowing frequency of 15 years is used. The width of the 95% confidence interval is given in parentheses. For exact parameter values, see Table 8.2.

| *Seed emigration* | *Low* | | *Moderate* | | *High* | |
|---|---|---|---|---|---|---|
| **Low seed persistence** | | | | | | |
| Annual | 0 | (0) | 20 | (6.93) | 2831 | (1.73) |
| Very short-lived perennial | 0 | (0) | 39 | (6.00) | 3065 | (1.68) |
| Short-lived perennial | 0 | (0) | 58 | (6.24) | 3362 | (1.66) |
| Moderate-lived perennial | 0 | (0) | 228 | (4.23) | 4273 | (1.59) |
| **Moderate seed persistence** | | | | | | |
| Annual | 0.08 | (0) | 174 | (4.41) | 3906 | (1.70) |
| Very short-lived perennial | 0 | (0) | 254 | (3.84) | 4149 | (1.59) |
| Short-lived perennial | 0.05 | (0) | 369 | (3.38) | 4518 | (1.60) |
| Moderate-lived perennial | 0.12 | (0) | 796 | (2.43) | 5645 | (1.53) |
| **High seed persistence** | | | | | | |
| Annual | 348 | (3.43) | 2573 | (1.71) | 9113 | (1.48) |
| Very short-lived perennial | 401 | (2.97) | 2792 | (1.71) | 9525 | (1.46) |
| Short-lived perennial | 542 | (2.66) | 3213 | (1.67) | 9976 | (1.43) |
| Moderate-lived perennial | 969 | (2.26) | 3992 | (1.59) | 11456 | (1.42) |

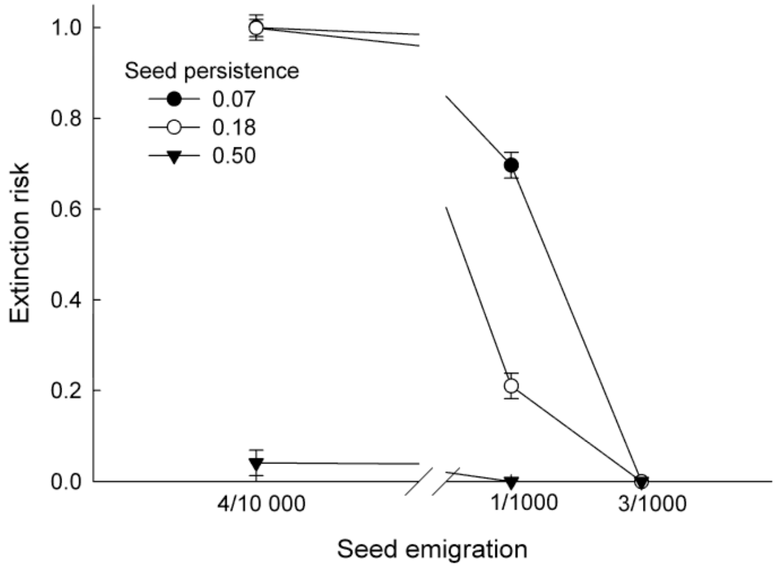


**Figure 8.4.** Change in mean (± SD) extinction probability in the *C. epithymum* metapopulation after a 100 year simulation with increasing seed emigration for three different values of seed bank persistence. Each population is subjected to a rotational management of 15 years. Only the results for short-lived perennials (0.10) are presented. Note the incremental change in levels of seed emigration.

**Table 8.4.** *F*-values, R²adjusted and significance levels of the GLM-analyses testing **a)** the relative influence of seed emigration, seed persistence, and life-span and their interactions on the time to metapopulation extinction and the metapopulation extinction probability after 100 years and **b)** the effect of mowing frequency, spatial management arrangement, grazing and their interactions on time to 50% extinction and metapopulation size after 100 year.

| a) *Variables* | *Time to extinction* | | *Extinction probability* | |
|---|---|---|---|---|
| | *df (n,d)* | *F* | *df (n,d)* | *F* |
| Seed emigration | 2,18 | 505.63*** | 2,18 | 289.06*** |
| Seed persistence | 2,18 | 192.37*** | 2,18 | 190.06*** |
| Life-span | 3,18 | 8.81** | 3,18 | 4.37* |
| Emigr. × Seed pers. | 4,18 | 61.13*** | 4,18 | 70.74*** |
| Emigr. × Life-span | 6,18 | 4.43** | 6,18 | 3.02* |
| R²adj. | 0.98 | | 0.97 | |
| b) *Variables* | *Time to 50 % extinction* | | *Metapopulation size* | |
| | *df (n,d)* | *F* | *df (n,d)* | *F* |
| Frequency | 4,9 | 65792.26*** | 4,9 | 49008.56 |
| Arrangement | 1,9 | 4.64ns | 1,9 | 2.55 |
| Grazing | 1,9 | 3784.85*** | 1,9 | 2905.33 |
| Freq. × Grazing | 4,9 | 769.99*** | 4,6 | 596.71 |
| R²adj. | 1 | | 1 | |

The model with the best *F*-ratio and highest R²-adjusted was retained. *: 0.01 < *p* ≤ 0.05; **: 0.001 < *p* ≤ 0.01; ***: *p* ≤ 0.001; *ns*: not significant; df (n,d) = degrees of freedom (nominator, denominator)

### *Management scenarios*

The virtual management experiment showed that mowing frequency had the greatest affect on the survival probabilities of *C. epithymum* metapopulations (Table 8.4b; Fig. 8.5). Up to a certain point, increasing mowing frequency of a patch (i.e. the shorter the time between two consecutive mowing events), increases the chance that the metapopulation will survive for a period of 100 years, with the species characterised by a fixed short life-span with a moderate seed persistence and a very low seed emigration rate (Table 8.4b; Fig. 8.5). With these demographic characteristics, *C. epithymum* metapopulation is guaranteed to persist over a 100-year time interval, if a mowing frequency of 10 years is applied in which 10% of the landscape is mown annually (Fig. 8.5). Once mowing frequency, however, exceeded 25 patches per year (i.e. 4 years between two consecutive mowing events), the metapopulation size and its long-term survival probability decreased considerably (Fig. 8.5). The interaction of grazing with mowing frequency also explained a significant part of the observed variation in metapopulation abundance and the time to 50% extinction (Table 8.4b). In all management scenarios with the three lowest mowing frequencies (20, 15 or 10 years between two mowing events), metapopulation size was significantly higher in extensively grazed landscapes than in ungrazed situations (Fig. 8.5). For the two lowest mowing frequencies (i.e. mowing frequency of 20 or 15 years), a compensatory effect of grazing was observed, resulting in viable metapopulations after 100 years where without grazing metapopulation extinction occurred (Fig. 8.5). When the frequency at which mowing events are applied increased, less than 10 years between two consecutive mowing events, the effect of grazing was negligible (Fig. 8.5). The spatial arrangement of mowing applications, either random or clustered, had no significant impact on *C. epithymum* metapopulation persistence (Table 8.4b). Metapopulation viability was relatively similar under a clustered or random distribution of the management events, both with and without extensive grazing (Fig. 8.5).

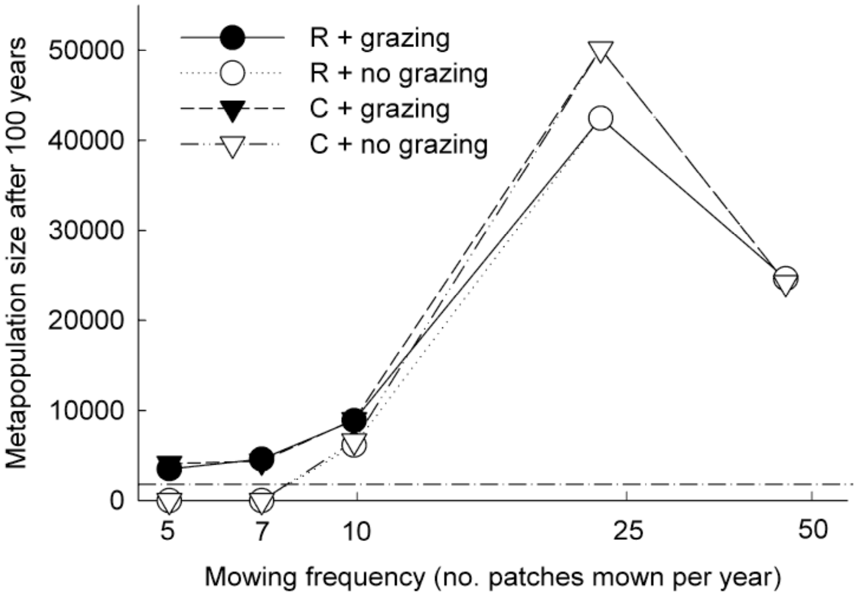


**Figure 8.5.** Change in mean (± SD) number of individuals (metapopulation size) after 100 years with increasing mowing frequency for grazed (black) and ungrazed (white) random (R: circle) and clustered (C: triangle) managed heath landscapes. In this figure mowing frequency is expressed as the number of patches which are mown per year (log scaled). For corresponding periods between two consecutive mowing events see Table 8.2. No difference between grazed and ungrazed treatment was found in landscapes subjected to a mowing frequency > 10 mown patches/ year. Metapopulation viability size is set at 50 individuals (dash-dotted line).

# Discussion

This study modelled the effects of varying demographic characteristics and different management scenarios on the response of a metapopulation of a short-lived early-successional species at the landscape level, and it provides clear insights on how both life-history traits and the spatiotemporal patterns of habitat patches in the landscape affect metapopulation viability in *C. epithymum*.

## *Importance of demographic characteristics*

Early-successional species occurring in dynamic landscapes must be able to colonise new habitats at least as fast as they are lost from existing habitats in order to survive in the long-term. Seed dispersal and the capacity to produce a persistent seed bank are generally viewed as adaptations to deal with this environmental stochasticity, permitting species to escape unsuitable conditions both in time and space and (re)colonise recently disturbed sites (Valverde and Silvertown, 1997; Amarasekare and Possingham, 2001). Although most metapopulation studies only consider seed dispersal in their simulations, the present study demonstrates that both seed dispersal and persistent seed banks may strongly decrease the probability of metapopulation extinction. The combination of a persistent seed bank with occasional long-distance dispersal can be seen as an optimal strategy to cope with the unpredictable availability of suitable habitat patches at the landscape scale. Whilst occasional long-distance seed dispersal increases the chance of colonisation of patches which become available, seed persistence ensures recolonisation of previously occupied patches after a period of unsuitability. Both life-traits additionally offer *C. epithymum* the opportunity to overcome unfavourable periods until the next management event. The interaction of both demographic characteristics may thus have a positive effect on metapopulation viability at the landscape scale (Table 8.4a), and highlights the need to incorporate seed persistence as an important demographic aspect in plant metapopulations.

Although *C. epithymum* seeds are generally categorised as 'unspecialised for long-distance seed dispersal' (Kuijt, 1969) and have no obvious adaptations for dispersal, seed resistance to sheep digestion is reported by several authors (Gaertner, 1950; Kuijt, 1969). Furthermore, Kuijt (1969) and Costea and Tardif (2006) reported evidence that *C. epithymum* seeds may occasionally disperse long-distances via epizoochory and even by means of management instruments (anthropochory). Since most European heathlands have traditionally been managed by large herbivores and mowing for many centuries, it might thus be plausible that livestock or transport in heather cutting, or movement as seeds stuck to tools/machines have played an important role in the dispersal of several early successional species, such as *C. epithymum* (e.g. Webb, 1998; Lennartsson and Oostermeijer, 2001; Couvreur et al., 2005). Our observations stress the importance of occasional long-distance dispersal for establishment of new populations in such highly dynamic landscapes, implying that more attention should be paid to potential long-distance dispersal agents, such as livestock and wild animals or even management machinery (Strykstra et al., 1997; Fenner and Thompson, 2005; Peltzer et al., 2008).

Finally, in contrast to the findings of Bossuyt and Honnay (2006), who investigated plant species in successional dune slacks, our results do not support a strong and positive effect of plant life-span on reducing the risk of metapopulation extinction. This can be attributed to the relatively high patch turnover rates applied in our simulations, and to the inclusion of a persistent seed bank, which may reduce the effect of environmental stochasticity. Nonetheless, previous research revealed that at the population level, vegetative overwintering via haustoria makes *C. epithymum* far less sensitive to local environmental stochasticity and benefits population (re)establishment and successful reproduction shortly after management events (Chapter 6). Overwintering of established individuals plays a far less important role for metapopulation viability and long-term survival than seed persistence and seed dispersal.

### Impact of different management scenarios

Our results clearly indicate that extinction probabilities of *C. epithymum* metapopulations are significantly determined by the frequency at which a management event is applied. The data showed that for a short-lived perennial with moderate seed persistence and very low seed dispersal rates, a mowing frequency with less than 15 years between two consecutive mowing events is needed to ensure a viable metapopulation over a 100-year period. The proportion of occupied patches observed during the long-term study on heathlands, 55 ± 20% of all suitable patches occupied by a *C. epithymum* population, corresponds with the occupancy fraction of the modelled intermediate management scenario with a patch turnover rate between 15 and 10 years, holding an occupancy fraction of 0.04 ± 0.01 and 0.84 ± 0.02, respectively. The results do not reveal any influence of spatial patch arrangement on metapopulation persistence, similar to these reported by Johst et al. (2002), who stressed that the rate of disturbance frequency in a landscape may be more important than spatial patch arrangement. Previous work on natural *C. epithymum* populations also showed no effect of patch size and/or spatial isolation on population size (Chapter 3). In contrast with several other heathland species which do show an area and/or isolation effect (e.g. Piessens et al., 2004), *C. epithymum* can bridge some years of unsuitable conditions by its persistent seed bank. Seeds that arrive into such unsuitable heathland patches become a part of the soil seed bank. The capacity to form persistent soil seed banks may thus explain why spatial patch arrangement is much less important than management frequency for *C. epithymum* metapopulations.

Although *C. epithymum* metapopulations appeared to benefit from any management frequency with a mowing frequency lower than 15 years, our results did demonstrate that an intermediate disturbance frequency generated the best conditions for metapopulation survival in the long-run, and that survival probabilities decreased again once a disturbance frequency threshold of 4 years between two consecutive mowing events was exceeded. The latter can be attributed to the fact that *C. epithymum* needs sufficient young *C. vulgaris* individuals to guaranty establishment. When patches are managed too frequently, there is, however, not enough time for heather to grow back and reach a suitable size to be infested by *C. epithymum*. In that case, the suitable period is often too short for *C. epithymum* to establish and develop into a population that is capable to build up a viable seed bank via its reproductive output. Finally, extensive grazing caused a significantly higher metapopulation survival response and so would compensate for an inadequate mowing frequency. This may be explained by the constant presence of young heather plants that extensive grazing provides. The speed of vegetation succession reduces when heathland is grazed (Vandvik et al., 2005), making mown patches suitable for *C. epithymum* for a longer period than if there was no grazing.

## *Conclusions*

Although it is generally assumed that early successional species invest in a persistent seed bank to buffer local extinction in continuously changing environments (Fenner and Thompson, 2005), to our knowledge, this work is one of the first to demonstrate that the occurrence of seed banks in combination with occasional long-distance dispersal is necessary to ensure the metapopulation viability and long-term survival of an early successional species. If, however, emigration rates are too low, and/or the species fails to produce enough persistent seeds, a sufficiently high management frequency (time between two consecutive mowing events < 15 years) is needed to sustain a viable metapopulation of *C. epithymum*. Cessation of management, or very infrequent management events appear to be very harmful to this short-lived species, whereas extensive grazing can, at least partly, compensate for this lack of management. The results show that, in contrast to the current large-scale management applications on many heaths, the best techniques to maintain viable *C. epithymum* metapopulations are activities such as small-scale cyclical mowing, burning or turf-cutting, in combination with livestock grazing.

The methodology presented in this study is also applicable to other disturbed environments, e.g. flood- or fire-structured communities and semi-natural grasslands. The processes and interactions between life-history traits and management reported in this work will be relevant to metapopulations of many other plants and animals, which live in early successional vegetation. This study thus reveals that the metapopulation approaches used in this work is a useful tool to study plant population dynamics at a landscape scales, and indicates the need for incorporating both spatiotemporal aspects of patch suitability and demographic traits, especially seed persistence, in future metapopulation studies in dynamic landscapes.

# 9

# Conclusions and management guidelines

Photos: Klaar Meulebrouck and Katrien Piessens (Ringkoben, Denmark, 2008; Strabrechtse Heide, The Netherlands, 2005; Mechelse Heide, Belgium, 2005).

Throughout western Europe, dry heathland area has been undergoing a continuous decline during the last decades (centuries), and the remaining heaths are severely fragmented. Consequently, species dependent on a particular phase of the heathland succession have severely declined in abundance and distribution (Chapter 1). One of these species is the endangered holoparasite *C. epithymum*, which typically occurs in the pioneer phase of the successional heathland vegetation (Chapter 2). To preserve *C. epithymum* populations in dry heathland it is necessary to investigate metapopulations of this plant in highly dynamic semi-natural landscapes (Chapter 1). In the first part of this work we gained an understanding of the factors affecting the distribution and dynamics of *C. epithymum* populations (Chapter 3). Important demographic aspects of *C. epithymum*'s life-cycle were discovered (Chapter 4-7). Finally, this knowledge was integrated into a spatially realistic metapopulation model in order to explore the impact of various life-history traits and spatiotemporal landscape dynamics on the long-term survival of *C. epithymum* metapopulations (Chapter 8). The results enabled us to formulate useful guidelines to optimise the conservation of this endangered species and other species with similar habitat requirements and/or life cycles.

## Strongly adapted to dynamic heathlands, but still in danger

*Cuscuta epithymum* can be described as a species that is strongly adapted for living in dynamic heathlands. In particular, it requires conditions typically associated with of young heathland vegetation which belongs to the pioneer phase of the heathland succession. *Cuscuta epithymum* only (re)appears after the vegetation was set back to its initial successional state by vegetation disturbance caused by a management event (Chapter 3). Consequently, it was most frequently found, and had the highest abundances, in young heathland patches. The association of *C. epithymum* with pioneer heathland vegetation is linked with its life-history traits. Suitable primary hosts are required in the immediate proximity of *C. epithymum* for establishment, because it is an obligate holoparasite. In this study, primary hosts were mainly young *C. vulgaris* individuals and/or non-woody parts of this species (Chapter 2, 3 and 7). The results demonstrated that the vegetation structure and host age, strongly affect seedling establishment, and subsequent establishment stages (Chapter 7). Suitable young pioneer vegetation is, however, only a transient phase in the heathland succession, continuing to mature heathland (e.g. Gimingham, 1972). To cope with this dynamic habitat, *C. epithymum* has a number of strategies.

Its seeds have both physical and annual cycling physiological dormancy, creating a double safety strategy (Chapter 4 + 5). The germination of some *C. epithymum* seeds occurs in spring -when conditions are favourable for germination-, but in a portion of the seed crop germination is delayed, avoiding the loss of a whole cohort when heathland is not in the appropriate successional phase (Chapter 4, 5 and 7). This mechanism spreads germination and establishment risks over time (Chapter 7). Only the portion of the seeds containing both non-dormant embryos and water permeable seed coats responds to higher temperatures in spring, and germinates. The remaining dormant fraction remains ungerminated in the soil seed bank until one of the subsequent springs (Chapter 5 and 7). This ability to form a persistent soil seed bank is one of the most crucial life traits of *C. epithymum* living in transient heathland mosaics (Chapter 5). It enables *Cuscuta* to (re)colonise a patch which becomes suitable again after several years of unsuitability.

Also vegetative overwintering by haustoria in the host stem was found to be an important trait for survival in managed environments. Vegetative overwintering guarantees a quicker population establishment and expansion in spring, compared to just seed germination (Chapter 7). Vegetative overwintering means that more flowering adults are present in the population and the seed stock in the soil seed bank is greater, both important for the long-term survival of the species. This overwintering strategy prolongs individual and population survival under less suitable conditions, especially in older *Calluna* vegetation in which seedling recruitment is hampered (Chapter 6). Overwintering therefore increases the population's seed output and spreads this output over several years (Chapter 6 and 7). Overwintering as a haustorium allows *C. epithymum* to 'move' vegetatively from one *C. vulgaris* individual to proximate suitable individuals (Chapter 5). The combination of all these life-history traits demonstrates that a clear match exists between *C. epithymum* and living conditions in dynamic heathlands.

Nevertheless, some findings show the sensitivity of *C. epithymum* to local extinction. *Cuscuta epithymum* requires very specific conditions for all steps in the establishment process (Chapter 7). This high degree of habitat specialisation makes *C. epithymum* particularly susceptible to changing habitat conditions. In addition, the seed viability of *C. epithymum* is found to decrease quite quickly over time (Chapter 5). The annual emergence of seedlings in both suitable *and* unsuitable heathland vegetation accelerates this decline of the seed stock in the soil seed bank (Chapter 7). There is limited seed dispersal, which restricts seed exchange between individual populations and colonisation of new habitats (Chapter 5). Young heathland vegetation is not a climax vegetation and inevitable becomes unsuitable for the growth of *C. epithymum*, so metapopulation survival is strongly dependent on continuation of heathland management measures (Chapter 3-8).

We conclude that *C. epithymum* is still under a high risk of further decline, even if heathland habitats are conserved. The continuation of targeted heathland management, in particular cyclical management, is essential for its long-term conservation.

## Implications for conservation and management

Scientific information and reviews relating to heathland history in western Europe make it clear that up to the 20th century heaths have played a prominent role in traditional agriculture on sandy soils and that heathlands were strongly disturbed by anthropogenic activity (Gimingham et al., 1979; Webb, 1998; Burny, 1999; De Blust, 2004; Kvamme et al., 2004). Heathlands were primarily used as a pasture for grazing animals and were also cut for fodder or litter in the stall, where it could absorb the excrements of the animals. This material was used afterwards to fertilise the fields (Gimingham et al., 1979; Webb, 1998; Burny, 1999; Kvamme et al., 2004). To maintain young heather shoots with an enhanced nutritional value for grazing animals, it was burned regularly (Burny, 1999; Kvamme et al., 2004; Karg 2008). Consequently, near settlements there were extensive areas with young heather of 10 to 15cm height belonging to the (late) pioneer phase of heathland succession (Burny, 1999; Fig. 9.1). It is not surprisingly that many species adapted to the dynamic mosaic landscape created by human activities came to be common in these heaths. For *C. epithymum*, this study clearly proved that it needs managed heathland to guarantee long-term survival. Cessation of management, or very infrequent management, is shown to be very harmful to this early successional species. Despite the alarming state of heathland in Belgium (Hens et al., 2005), there is still limited use of detailed species-specific knowledge and targeted, often small-scaled management measures to ensure the survival of threatened species. In contrast with the current biotope-based management of ecosystems without reference to particular species, the use of species is recommended as a tool for planning conservation measures and evaluating management of semi-natural heathlands (see Maes, 2004). In accordance, this thesis also highlights the importance of species-specific research (demographic data and metapopulation modelling) to underpin heathland management and design, and enabled science based guidelines to improve and prioritise future conservation efforts. In the following paragraphs different aspects of heathland management, which are expected to increase the viability of existing *C. epithymum* populations and which may create new suitable habitat, are discussed. Although this study focuses on heathland management targeted for the conservation of *C. epithymum*, implementation of the recommendations is expected to make a significant contribution to the long-term conservation of other typical heathland species, such as many invertebrates needing similar habitat. Therefore, *C. epithymum* may be represented as a possible target species indicating an adequate management regime and conservation policy for dry *Calluna*-dominated heathlands. It is a Red List species, easily recognisable by non-experts and an attractive species for reserve visitors.

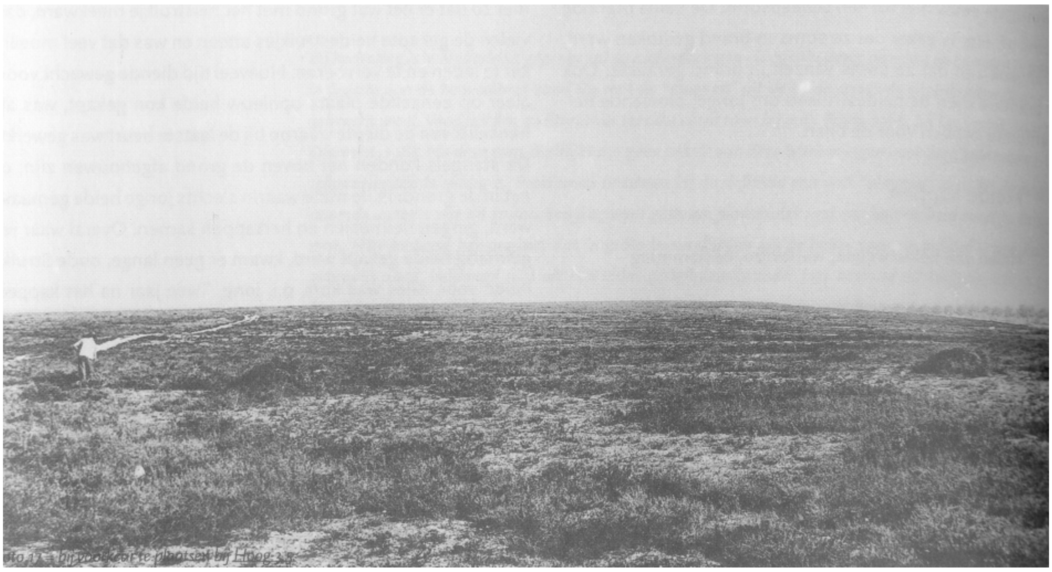


**Figure 9.1.** Extensive open area of intensively managed heathland with farmer (left) nearby Genk (1905, Limburg, Flanders). (From Massart (1912) in Burny, 1999).

## *Management measures*

*Cuscuta epithymum* was mostly found on heathland patches with young growth of *C. vulgaris* of 10-30 cm height, which was up to 7 years after the last management event, and no *C. epithymum* individuals were found in heathland vegetation which had not been managed over 10 years (Chapter 3). This is a guideline for conservation management planning in heathland sites containing *C. epithymum*. In general, all management strategies which lead to a more open vegetation structure with younger growth of *C. vulgaris*, but without removing the topsoil with the persistent seed bank, will promote the populations of this endangered species. Mowing was the most common management method in the four nature reserves in the study, and so *C. epithymum* was predominantly found in mown heathland patches (Chapter 3). Nevertheless, other types of management were also found to be appropriate for *C. epithymum* establishment and growth (Chapter 3, 7, see below).

**Mowing** *C. vulgaris* removes tissue to ground level, and removes the heath cuttings causing a reduction in the soil litter layer. The advantage of mowing heathland vegetation is the rapid regrowth of heather from the rootstocks in the next growing season after winter mowing. If *C. epithymum* seeds are present in the soil seed bank, enough young *C. vulgaris* shoots are available in the growing season after mowing, to enable *C. epithymum* establishment and to allow a rapid population growth. Although in our study area *C. epithymum* was rarely found on controlled **burnt** heather, it is known to successfully spread onto new shoots regrowing from burnt heather plants (Karg, 2008). In the past, controlled burning was commonly used to provide young nutritious heathland for grazing animals, or to fertilise the soil for agriculture (Goodall and Specht, 1981; Kvamme et al., 2004). Regeneration after burning starts after only 1-2 months, and after 6 months the burnt surface may have revegetated and be suitable for *C. epithymum* growth. On some heaths today the vegetation after burning can become dominated by *Deschampsia flexuosa* which is not appropriate for *C. epithymum* growth (pers. obs.). When heather plants are old (mature or degenerative phase, Gimmingham, 1972) the *Calluna* regeneration after controlled burning is relatively slow or even is completely absent (e.g. Mallik and Gimingham, 1985).

Our study demonstrated that **turf-cutting**, which removes the vegetation, leaf litter and the O-layer and a part of the A-horizon of the soil in one operation, also provides optimal conditions for *C. epithymum* establishment (Chapter 7). About 2 or 3 years after turf-cutting, the resultant pioneer heath is characterised by a low canopy height, a high proportion of bare ground and a large number of young *C. vulgaris* plants (mostly seedlings). The development to mature heathland vegetation is observed to be slower than in mown vegetation (pers. obs.; Tanghe, 2007). Throughout this slower growth, the period during which the vegetation is suitable for *C. epithymum* growth is prolonged, which results in larger *C. epithymum* populations and consequently a higher seed output (Chapter 3; pers. obs.). Yet turf-cutting depth is vital. If the turf is cut too deep, too much of the topsoil is removed and, the whole soil seed bank may also be removed. Traditional turf-cutting involved turfs cut by hand (Webb, 1998; Burny, 1999), but now this work is usually performed by specially developed large turf-cutting machines, which may cause negative impacts such as over-deep cutting or soil compaction.

*Cuscuta epithymum* is also observed to rapidly (re)colonise **choppered** heathland patches (pers. obs. in the Ziepbeek reserve). Choppering is a modern heathland measure introduced in the middle of the 1990s and henceforth successfully applied in regenerating dry heaths (Niemeyer et al., 2007). Choppering removes the complete above-ground biomass and the bulk of the O-layer, without affecting the A-horizon (Maes et al., 2004). The result is the creation of bare soil, with only a thin layer of organic material remaining on the surface. The soil structure created by this management technique has similarities with that created by cattle trampling (Maes, 2004). Choppering has two important advantages over turf-cutting for *C. epithymum* establishment. Smaller amounts of the top soil are removed, reducing the risk of damage to the seed bank. Furthermore, the regeneration of the vegetation is faster after choppering than after turf-cutting, allowing *C. epithymum* to establish in the first growing season following choppering.

**Grazing** is also an important management measure (e.g. Williams, 2003; De Blust, 2004; Pakeman and Nolan, 2009). The positive effect of extensive grazing on both the presence of *C. epithymum* populations (Chapter 3b), and the long-term metapopulation viability (Chapter 8), indicates grazing is beneficial and a valuable conservation tool for dry heathlands containing *C. epithymum*. Until the 20th century grazing with sheep, cattle, horses or local breeds of pony had always been a major use of European heathlands (e.g. Webb, 1998; Burny, 1999; Alonso et al., 2001; Kvamme et al., 2004; Karg, 2008). However, during the 20th century heathland grazing for agriculture almost ceased as the profitability of agricultural production on heathland declined to practically zero (Webb, 1998; Burny, 1999). At present, grazing is increasingly promoted as a feasible and sustainable means of conservation management to maintain a diverse heathland vegetation (e.g. Bullock and Pakeman, 1997; Bokdam and Gleichman, 2000; Kvamme et al., 2004; Piessens et al., 2006). Although heathland vegetation was found to be suitable for the growth of *C. epithymum* for longer periods when it is grazed (Chapter 3, 8), the *C. epithymum* populations were much smaller on grazed heath if no additional management events were applied (Chapter 3b). Based on these findings, grazing management alone is not sufficient to maintain viable populations of *C. epithymum*, at least not with the grazing regime in the studied reserve (De Maten, extensive grazing by Galloways from May to September at a density of about 0.1 cow ha-1). Additional management methods or higher grazing densities are necessary for *C. epithymum* conservation. This is similar to traditional farming management where grazing was combined with other techniques such as controlled burning and/or mowing (e.g. Webb, 1998; Burny, 1999; Kvamme et al., 2004). The response of *C. epithymum* populations is expected to strongly depend on the grazing intensity (type of livestock, stocking rate, grazing season, the proportion of grazed heathland), as has been shown for other heathland species (Bullock and Pakeman, 1997). Common recommended stock densities are a maximum of one cow or horse per 5-6ha per year and a maximum of five sheep per ha per year (Williams, 2003). The grazing densities used in Flemish heathland nature reserves containing *C. epithymum* (see Chapter 2) are not sufficient to keep heather at the optimal succession phase if no additional management is provided. Although some areas tend to be grazed very strongly, most of the heather was not grazed enough to be able to sustain viable *C. epithymum* populations (pers. obs.).

The metapopulation model used in this study emphasises the importance of occasional long-distance dispersal for establishment of new *C. epithymum* populations (Chapter 8). Livestock and management machinery can act as long-distance dispersal agents not only within one heath but also between heaths, for example when moving livestock or machinery between nature reserves.

## Management frequency and spatial planning

Management frequency is critical for the long-term maintenance of *C. epithymum* (Chapter 8), which indicates the importance of proper management planning. For *Cuscuta*, heathland managers should promote relatively short management cycles because of the decline in seed density and viability with time (Chapter 5). There is an optimum of 7-10 years and should be a maximum of 15 years between two consecutive management events in any one spot, depending on the type of management. The optimal interval between two mowing events is, for example shorter than the optimal interval for turf-cutting. This recommended management frequency is roughly similar to traditional management intervals (Burny, 1999; Kvamme et al., 2004). For example, historically the heather plants were burnt before they reached the mature phase (between 10 and 15 years old, Kvamme et al., 2004). A significant percentage of the heathland area should be managed every year. The resultant mosaics of heathland patches of different phases in the heathland succession will not only promote an increased *C. epithymum* metapopulation size, but will also provide habitat for a wide range of heathland animals and plants (e.g. Maes, 2004; Maes et al., 2004; Kvamme et al., 2004). It is unlikely that heathland managers will have the resources or intention to manage too frequently (< 4 years) which is shown to negatively effect *C. epithymum* population size (Chapter 8; see Gimingham, 1972 for effects on heathland vegetation). By analogy, a too high grazing pressure converts the dwarf-shrub dominance to grass dominance (e.g. Pakeman and Nolan, 2009).

No influence of spatial patch arrangement was found on metapopulation survival (Chapter 8), but the location of management events should still be planned. Management efforts should be concentrated on habitats where *C. epithymum* currently grows or has grown in the recent past, and so recolonisation from the seed bank is expected, but should also aim to facilitate a natural spread of the species. A cyclic management strategy applied on rather small areas of heathland will provide a mosaic of vegetation in which sufficient young heathland patches may be available for occasional seed dispersal. Large-scale management, which creates large areas of homogeneous heathland, affects *C. epithymum* negatively (and many other species) and should be avoided. Management should be executed as a priority in the immediate vicinity of existing *C. epithymum* populations because of its limited dispersal capacity (Chapter 5). Dispersing seeds or the growth from *C. epithymum* threads from the surrounding older vegetation (late pioneer or building phase) onto the newly created pioneer vegetation will be facilitated.

## Restoration and reintroduction

Heathlands are still being lost or deteriorating in quality across western Europe, so there is a desire to preserve and improve our remaining heathlands, and also to restore them in areas where they have recently been lost (e.g. Bakker et al., 1996; Bossuyt and Hermy, 2003). Sites where *C. epithymum* was historically present have high potential for restoration of heathland with it, as recorded in several Flemish restoration projects (Chapter 3, 5). Nevertheless, knowledge of the historical distribution *C. epithymum* is often poor, mostly on a coarse scale rather than at detailed site-level (e.g. Duvigneaud, 1945; Van der Veken et al., 2004; Piessens, 2006), which makes it difficult to find previous *C. epithymum* populations. In addition, the time since abandonment is an important factor for restoration success of heathland with *C. epithymum*, because the *C. epithymum* seed viability declines quite rapidly over time (Chapter 5). Corresponding, the seed density of other heathland species, such as *C. vulgaris*, declines with time in the soil seed bank (Bossuyt and Hermy, 2003). Restoration of heathland on sites abandoned or forested for several decades is thus strongly seed limited. Further, the former use of the sites to be restored will determine restoration success to a considerable extent (e.g. Bossuyt and Hermy, 2003). If the former heathland was converted to arable land or industrial sites restoration of heathland is expected to be problematic due to a faster decline in the seed bank owing to ploughing and the high nutrient levels resulting in a high density of nontarget species. Nutrient lowering techniques (e.g., top soil removal or intensive mowing) are then necessary before restoration is likely to be successful (e.g. Marrs and Britton, 2000; Pywell et al., 2002), although restoration projects on these sites have varying success (Hens et al., 2005).

If a *C. epithymum* seed bank is believed to be absent from a heathland restoration site, reintroduction may be considered. As demonstrated by spreading infected *C. vulgaris* stems (Chapter 4) or by sowing seeds in recently managed heathland (Chapter 5), reintroduction in sites with suitable habitats or where the habitat can soon become suitable is relatively simple. Whilst preservation and spread of existing populations should remain the priority, reintroduction should, in our opinion, be seriously considered. Reintroduction projects should be combined with heathland restoration measures to create suitable patches of *C. vulgaris* to (re)-create a viable *C. epithymum* metapopulation in the long-term, and the establishment of the populations should be recorded and closely monitored.

## Additional remarks

It should be noted that the requirements for *C. epithymum* conservation are not necessarily met by simply copying traditional management techniques, since considerable changes in environmental quality (e.g. nitrogen deposition) and landscape context (fragmentation, see e.g. Piessens, 2006) have occurred in the last few decades. Therefore, management of lowland heathland containing *C. epithymum* must be tailored to fit the conditions prevailing at individual sites, rather than following a standard prescription throughout Flanders or even Europe. Habitat management for *C. epithymum* should not be considered alone, but integrated into a holistic long-term conservation plan where the local site conditions and the needs of a number of plants and animals are considered (i.e. multispecies approach, Maes, 2004; Hens et al., 2005). Monitoring of current *C. epithymum* populations is recommended, to identify the responses of *C. epithymum* to the implemented management measures, to evaluate if targets are being achieved, and to adjust conservation management if necessary.

Next to the lack of management, poor environment quality forms an additional threat to heathland and its inhabitants (e.g. Barker et al., 2005; Hens et al., 2005; Härdtle et al., 2006). Since heathlands are restricted to nutrient poor, relatively acid soils, the current high rates of atmospheric nitrogen deposition causes that the management measures described above will not always be sufficient to sustain *C. epithymum* populations. The high nitrogen depositions often results in dense tall vegetation dominated by grass species (e.g. Aerts and Berendse, 1988; Hens et al., 2005; Niemeyer et al., 2007), which is not suitable for *C. epithymum* establishment. Therefore, additional measures and policy efforts for improving environmental quality are highly recommended to ensure sustainable heathlands with *C. epithymum*.

## Some limitations of our research

### *Limitation of research on one species*

In our study we focused on a single dry heathland indicator species, *C. epithymum*. The objective of this thesis was to discover some of the key factors that are essential to ensure long-term survival of this endangered species. We also wanted to study the metapopulation dynamics of a plant species with a seed bank in a landscape where its habitats are transient. The choice of only one species to study gave the opportunity to look at many aspects of *C. epithymum*'s basic biology and its interaction with the environment. Only through this detailed research we may become aware of the many difficulties that *C. epithymum* faces.

However, this single-species approach has some limitations. It does not always allow the extrapolation of management recommendations to other heathlands where *C. epithymum* is absent, or to management recommendations where other species may have different needs (see 'implications for conservation and management'). The recommendations for the conservation of *C. epithymum* are expected to benefit many other heathland species, and there are extrapolation potentials to other poorly studied heathland species or other *Cuscuta* (weed) species. However, other heathland species may be harmed by the implementation of the recommended management. Sand lizards (*Lacerta agilis*), for example, do not tolerate large fires (Bullock and Webb, 1995) and the management frequency suggested for *C. epithymum* may be too high for some lichens (Matt and Legg, 2008). The use of management recommendations for one single species or one taxonomic group therefore does not necessarily result in the conservation of all other species or taxonomic groups as well. Therefore, we recommend that heathland managers consider our management recommendations in the context of each individual heath in combination with ecological knowledge about other species that are indicators for the different habitats in the biotiope (Maes, 2004).

## Limitation of seed dispersal quantification

Dispersal events are biologically very important for plants, as they affect colonisation probabilities, (meta)population structure and persistence (Ouburg et al., 1999). For many plant species, dispersal is thought to be very difficult to investigate because of their low frequency (Bullock et al., 2002). Whilst aiming at a full survey of population dynamics, including colonisation and extinction (chapter 3), or quantifying small-scale dispersal dynamics (Box 5.1), several problems were encountered. As *C. epithymum* possesses a persistent seed bank, it was not possible to distinguish between colonisation from seed dispersed from other populations (spatial dispersal) or recolonisation from the seed bank (dispersal in time). To avoid this problem we separated a study of seed bank processes (chapter 5) from a study of spatial dispersal (Box 5.1). For the spatial dispersal experiment, the absence of a persistent seed bank of *C. epithymum* was the main criterion for site selection. Despite that, we were not able to quantify a seed dispersal event (see Box 5.1).

There were also some problems during the introduction of viable source populations. A limited germination of the introduced seeds and the absence of sufficient flowering individuals forced us to reduce the study period to one year. This reduction of the observation time, and the very intensive but unpredictable results achieved with catching seeds with seed traps, meant that we decided to drop that part of the research where we had intended to measure dispersal distances by trapping seeds at various distances from the source population with seed traps. These issues partly caused the poor results for this part of the study. It is possible that some seeds dispersed to suitable patches, but did not manage to germinate in the first spring after seed set, or some seeds might have germinated, but did not succeed in attaching to a suitable host. Some seeds may have landed in the unsuitable, old heathland, and may have entered a persistent seed bank. Further, the time frame for this research was too short. It is clear that only one year of dispersal monitoring is inadequate to be able to draw final conclusions. Finally, this experiment investigated short-distance dispersal but not long-distance dispersal within a heathland site or between two heathland fragments. Knowledge about long-distance dispersal would have been extremely useful, because it would allow an evaluation of *C. epithymum*'s dispersal limitation at the metapopulation scale.

One possible way to investigate dispersal of *C. epithymum* is to offer suitable growing conditions in patches at different distances from the existing population. When performing such experiment it is necessary to know all the exact locations where *C. epithymum* had been present in the past, or had previously the ability to disperse seeds into, to take into account the possible influence of surviving populations in persistent seed banks. We expect that it will be even harder to estimate long-distance dispersal and that underestimation possibly can occur (Cain et al., 2000). Another more promising way for assessing seed dispersal between populations could be the use of molecular markers (see 'suggestions for future research').

The results presented in Box 5.1 and those of the sowing experiments (Chapter 7) clearly suggest that seed dispersal limits the spatial expansion of *C. epithymum*, especially dispersal at larger distances. For a population inhabiting an isolated patch we can conclude that colonisation of new sites will be exceptional, which has to be taken into account in management planning. To overcome this limitation, strategies which strengthen local populations and also promote long-distance dispersal should be used (see 'management measures' and 'management frequency and spatial planning'). Most European heathlands with *C. epithymum* populations were traditionally managed by grazing livestock, mowing and/or turf-cutting for many centuries, so it is likely that long-distance dispersal of seeds by livestock, transport in heather cuttings and/or turf, or as seeds stuck to management equipment have played a much larger role in the past (e.g. Webb, 1998; Couvreur et al., 2005; Lindborg, 2006). With the loss of traditional management, most of these long-distance dispersal agents disappeared. Based on evidence of dispersal limitation and the fragmentation of heaths in Flanders, it is likely that local populations of *C. epithymum* confined to habitat fragments separated by several kilometers are isolated from each other (if no artificial transport occurs between nature reserves, see for example Couvreur et al., 2005 for zoöchory). It is uncertain whether (meta)populations currently occurring in Flanders (see Fig. 2.4) are still viable or if some of them may be considered as functionally extinct. The isolated, relic populations in the provinces West-Vlaanderen and Vlaams Brabant (see Fig. 2.4) are good examples of possibly functionally extinct populations, as other relict (meta)populations in Flanders may be. In accordance with this observation, Piessens and Hermy (2006) found relatively low numbers of extinction for heathland species in comparison with the high area losses, but expect that more future extinctions unless environmental conditions are improved.

The lack of recruitment and reduced output in small populations of *C. epithymum* (Chapter 3), and thus the lack of seed bank replenishment, the relatively quick depletion of the persistent seed bank (chapter 7) and the deficiency of other source populations may cause small populations to decline to extinction. This suggests that *C. epithymum* will not automatically return to heaths where both *C. epithymum* populations and its persistent seed bank have disappeared, which has important consequences for restoration of heathlands (see 'restoration and introduction'). To understand more about this it would be advisable to study the genetic variation and so discover the gene flow between (meta)populations in Flanders (see 'suggestions for future research').

## *Limitations associated with metapopulation modelling*

This research clearly showed that a metapopulation concept and the models that build on this concept can be is a useful tool for evaluating plant populations in a dynamic landscape with transient habitats. Yet our metapopulation model (as the majority of models) still has some important restrictions.

A first set of limitations is related to the parameter estimations. Parameter estimation is always a critical step in the use of any metapopulation model for predictive purposes. The parameters used in our metapopulation model are founded on an extensive dataset originating from 4 years of field observations combined with experimental data collected in four heathland reserves (Chapter 8). The length of the study period for collecting sufficient data was an important restriction and also the one that was most difficult to improve. Like many researchers studying ecological processes we were confined to the 4 years available for PhD research. Few conservation biologists have the ability to conduct long-term assessments of population demography, population dynamics and patch dynamics. More detailed information would have been collected and more accurate parameter estimations would have been made if the study period had been more extensive. For example, during our research no complete colonisation-extinction cycle in which *C. epithymum* individuals appear, increase and subsequently decrease in numbers and finally disappear, could be monitored, because this process takes 10 years on average (Chapter 3b). It is assumed that populations will repeatedly show the trajectory as presented in Fig. 8.3, depending on management frequency. This problem is partly overcome by studying 108 populations in patches belonging to different successional stages (i.e. time since last management) in four nature reserves. The results obtained must be viewed with some caution, because short-term variability does not necessarily represent the variation that is relevant for longer time periods.

In addition, to test the importance of some demographic characteristics and management scenarios, the initial number of plants per population stage (seed, adult from seed and adult from haustorium) was needed. For each of these values, some problems arose. Firstly, *C. epithymum* appears in the vegetation as a snarl of red threads and therefore, it was difficult to distinguish individual plants. In addition, vegetative overwintering of one plant may result in several separate ramets, which complicates the calculations. Finally, the exact number of seeds in the seed bank must also be estimated. To overcome this problem and to improve precision, seed abundance was based on a combination of population size and seed production, taking into account vegetation age, seed mortality and germination rate and is thus based on some generalisations. It, however, was never our intention to predict the exact population size after 100 years, so the absolute values of the outcome must be approached with some caution.

Another problem encountered during our modelling process was the absence of some data. We were unable to determine the maximum period of seed bank viability (Chapter 5) and we had not managed to quantify seed dispersal distances (Box 5.1; see above). The seed burial experiment (Chapter 5) did give us an idea of seed survival, but the lack of dispersal distances was a more important gap in our knowledge when considering metapopulations. The dispersal of seeds, and the spatial limits on this process, have huge effects on dynamics at all scales, from local processes such as gap colonisation to landscape processes such as metapopulation dynamics (Bullock et al., 2002b). For our metapopulation model, the lack of information implied that we needed to base extrapolations on existing dispersal curves. We assumed that colonisation is a function of distance from the nearest existing population and that short-range dispersal is the main factor in the establishment of new populations. We also assumed that dispersal is constant among different environments. Nevertheless, the surrounding vegetation structure of a patch and its location in the landscape affect wind speed, turbulence and direction, and thus may modify dispersal patterns (Bullock et al., 2003).

An understanding of dispersal rate and variation in dispersal patterns among different environments is needed to have a better notion of *C. epithymum* metapopulation dynamics. If seed dispersal is as limited as expected, *C. epithymum* is likely to have difficulties in occupying newly available, suitable habitats. It must be stressed that extremely rare successful dispersal events are believed to have, in the long-term, a dominant role in *C. epithymum* migration (see Chapter 8). It is thus important that managers bear these dispersal limitations in mind when planning their management (see 'implications for conservation and management'). Further, future conclusive research on metapopulation dynamics of plants needs to concentrate on quantifying dispersal at different scales.

The final concern regards the interpretation of the metapopulation concept. Among ecologists, there is an ongoing debate about the applicability of the metapopulation concept for plants (see Chapter 1). We agree that studying the regional dynamics of plants may cause some problems, but when one is interested in processes acting at different spatial and temporal scales within a dynamic landscape, we firmly believe that a metapopulation approach is the most useful way to study these processes. Most criticisms of metapopulation concept for plants are based on very restricted interpretations of metapopulation theory. In this thesis large-scale spatial *C. epithymum* population structure is considered as a metapopulation structure in the broad sense (Hanski and Gilpin, 1997).

Furthermore, it could be argued that the low level of dispersal events between *C. epithymum* populations obstructs the use of the metapopulation concept. However, there are ways by which *C. epithymum* seeds can cross unsuitable habitats (seed persistence, transport by herbivores or management tools, etc.), and so local populations of *C. epithymum* are potentially connected to other populations. Many studies have shown that even a very limited amount of dispersal can have a profound effect upon the recipient population (Hanski and Gilpin, 1997). The results of our metapopulation model have shown that a surprisingly small number of dispersing seeds per year allow metapopulations to persist in a dynamic landscape where they otherwise would go extinct (Chapter 8). Recognition of the potential importance of metapopulation structure for *C. epithymum* dynamics has important implications for conservation efforts (see before), but will also make conservation more likely to be succesful in the long term.

Finally, the scale on which *C. epithymum* shows evidence of a metapopulation structure is open to discussion. In this thesis, metapopulations are examined at the scale of heathland sites belonging to one nature reserve (max. 700 ha, see Chapter 3a). In these systems, *C. epithymum* inhabits distinct habitat patches (managed heathland) surrounded by areas of unsuitable old heathland. When, however, *C. epithymum* is considered at a larger scale, such as the province of Limburg, the dynamics within and between the different heathland sites must be considered. At this larger scale, we think that there is hardly any exchange between the different sites and so the migration rates come close to zero. This is an alarming conclusion, because these (meta)populations will behave as isolated elements from where no newly created or restored heathland sites will be colonised and which exhibit no extinction/recolonisation dynamics. Anthropogenic activity can partly compensate for this shortcoming. For example, the use of the same mowing machine in different heathland sites might transport adhering seeds between different sites, as has been shown for *Rhinanthus* species (e.g. Ameloot, 2007) and several coastal dune plants (Couvreur et al., 2006). In the worst case, reintroduction should be seriously considered (see 'restoration and introduction').

## Suggestions for future research

After four years of research there are still some gaps in the ecological knowledge of *C. epithymum*. The results of the metapopulation model clearly highlight the importance of long-distance dispersal events for the metapopulation viability of *C. epithymum* (Chapter 8). However, because of the low frequency of these dispersal events there are no field records of long-distance seed dispersal, which is a problem inherent in seed dispersal research (see Cain et al., 2000). The commonly used methods of quantifying dispersal, such as seed traps and wind tunnel experiments, often underestimate long-distance dispersal (Cain et al., 2000). Genetic research is suggested as a good method to investigate long-distance seed dispersal, as dispersal contributes to the gene flow between populations and thus influences the genetic variation within/between populations (Ouburg et al., 1999; Cain et al., 2000; Honnay et al., 2009). By studying molecular markers that are inherited through seeds (e.g. mitochondrial and chloroplast DNA), dispersal distances can be estimated. Genetic studies can also investigate genetic differentiation between *C. epithymum* populations, and for investigating the relative importance of seed dispersal and pollination on gene flow. For this purpose the comparison of differentiation in nuclear versus cytoplasmic molecular markers is usually used (Ouburg et al., 1999). Seed dispersal is then equivalent to overall gene flow between two populations minus the genetic contribution due to cross-pollination. *Cuscuta epithymum*'s persistent seed bank (Chapter 5), may have various genotypes accumulated in the soil (Honnay et al., 2008), so genetic research could also explicitly incorporate the effects of a persistent seed bank on the genetic diversity of *C. epithymum*. A population genetic approach is expected to uncover some important ecological processes.

The long-term persistence of *C. epithymum* may partly depend on pollination processes (Chapter 2) as for many other plant species (e.g. Brys et al., 2008). *Cuscuta epithymum* populations in Flanders are often limited to very small and isolated heathland relics (Chapter 1, 2). Pollination success, and consequent seed set and the long-term survival of plant populations, is often reduced in fragmented habitats (Hermy et al., 2007; Honnay and Jacquemyn, 2007). Populations may become separated by a distance greater than the foraging range of pollinators, causing reduced inter-population pollination (Kearns and Inouye, 1997). Small plant populations may be less attractive for insects, and hence receive less pollinator visits. Consequently these populations are less frequently pollinated (Jacquemyn et al., 2002). The relative importance of direct and indirect effects of fragmentation on pollination efficiency, and hence reproductive success, can be expected to vary greatly between species, depending on the degree of dependence on pollinators for successful reproduction and on the breeding system. More empirical research is needed to fully understand the possible impact of reduced pollination success on *C. epithymum* population persistence. The exact reproductive processes still remain unclear. It is possible that *C. epithymum* has a mixed mating system, in which floral traits promote outcrossing, but allows self-pollination (Box 2.1), but scientific confirmation is lacking. More empirical research is needed to obtain an understanding of the exact functioning of *C. epithymum*'s breeding system, the implications of self- and cross-pollination on seed production and fitness of the progeny, and the impact of population size and isolation on pollination efficiency.

Although *C. epithymum* has a broad host range, our results showed that *C. epithymum* has some degree of selectivity in host use (Chapter 2, 3). Further, it is shown that some plants use active defence mechanisms to prevent successful penetration or development of *C. epithymum* haustoria. The attachment of haustoria on older *C. vulgaris* is, for example, most probably prevented by the presence of the protective periderm tissue containing cork cells (Chapter 7). Nonetheless, many exciting questions about the interaction of *C. epithymum* with compatible host plants and defence mechanisms against *C. epithymum* infection remain. What are the effective hosts of *C. epithymum*, excluding those where a haustorial initiation occurs without penetration of the epidermis (e.g. grasses; see Chapter 2, Costea and Tardif, 2006). Why are some hosts preferred and how does *C. epithymum* selects its host (volatile cues, nitrogen content,…For a review see e.g. Costea and Tardif, 2006; Albert et al., 2008; Pennings and Simpson, 2008)? How the formation of the haustorial connection of *C. epithymum* occurs at molecular level (cell-to-cell communication mechanisms between the parasite and the host)? Which active defence mechanisms are used to prevent *C. epithymum* infection (mechanical and/or biochemical) and how penetration is exactly impeded? What is the exact role of the periderm development in *C. vulgaris* in preventing *C. epithymum* penetration and overwintering? What are the consequences of these host-parasite interactions for the natural communities they inhabit (community structure, diversity, nutrient dynamics see Ameloot et al., 2008, ..)? It would be interesting to research the compatible and incompatible interactions of *C. epithymum* with different hosts, to study the attachment and penetration process in detail and to recover the influence of *C. epithymum* on ecosystem processes. Microscopic examination of infected hosts (stem sections) and experimental (physiological) studies would be fruitful for these purposes.

The guidelines above for sustainable heathland conservation are underpinned by ecological knowledge of a heathland indicator species, although several aspects and underlying processes still remain unknown. How will, for example, species of different taxonomic groups (plants, vertebrates, invertebrates) respond to restoration projects and the different types and intensities of heathland management recommended in this research (for example, sand lizards *Lacerta agilis* do not recover after large fires; Bullock and Webb, 1995)? What is the effect of nitrogen deposition on some understudied heathland species, including *C. epithymum*? How will heathlands respond to the likely changes in climate and how should management anticipate these changes? Only a thorough understanding of different aspects of the whole biotope, and communication between practitioners and scientists can result in the sustainable conservation of this valuable ecosystem. Further scientific research should be carried out on the responses of heathland as biotope, and more specifically heathland species, to management applications and environmental factors, with a systematic collection of field observations and in particular, a continual transfer of knowledge between researchers and heathland managers.

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# Publications

## Peer reviewed international articles

- Meulebrouck K., Ameloot E., Verheyen K. and Hermy M. (2007). Local and regional factors affecting the distribution of the endangered holoparasite *Cuscuta epithymum* in heathlands. *Biological Conservation* **140**: 8-18. (doi: 10.1016/j.biocon.2007.07.017)
- Meulebrouck K., Ameloot E., Van Assche J., Verheyen K. and Hermy M. (2008). Germination ecology of the holoparasite *Cuscuta epithymum*. *Seed Science Research* **18**: 25-34. (doi: 10.1017/S0960258507871133)
- Meulebrouck K., Ameloot E., Brys R., Tanghe L., Verheyen K. and Hermy M. (2009). Hidden in the host: Unexpected vegetative hibernation of the holoparasite *Cuscuta epithymum* (L.) L. and its implications for population persistence. *Flora* **204**: 306-315. (doi:10.1016/j.flora.2008.03.005)
- Meulebrouck K., Verheyen K., Brys R. and Hermy M. (2009). Metapopulation viability of an endangered holoparasitic plant in a dynamic landscape. *Ecography* **32**: 1-11. (doi:10.1111/j.1600-0587.2009.05861x)
- Meulebrouck K., Verheyen K., Brys R. and Hermy M. (2009). Limited by the host: Host age hampers establishment of holoparasite *Cuscuta epithymum*. *Acta Oecologica*: **35**: 533-540. (doi: 10.1016/j.actao.2009.04.002)

Work in progress:

- Meulebrouck K., Verheyen K. and Hermy M. Will the sleeping beauties wake up? Seasonal dormancy cycles in seeds of the holoparasite *Cuscuta epithymum*. Submitted to ‘Seed Science Research’.

## Local publications

- Meulebrouck K., Ameloot E., Verheyen K., Van Assche J. and Hermy M. (2006). Klein warkruid ontrafeld. Een ecologische studie in vier heidegebieden. *Natuur.Focus* **5** (1): 10-16.
- Meulebrouck K., Ameloot E., Verheyen K. and Hermy M. (2008). Wat kronkelt daar in de heide? De ecologie van Klein warkruid in Limburgse heidegebieden. *Likona Jaarboek* **17**: 40-49.

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## Proceedings

- Meulebrouck K., Ameloot E., Verheyen K. and Hermy M. (2005). Metapopulation dynamics of the parasitic *Cuscuta epithymum* in heathlands. 9th European Heathland Workshop (14.09.2005-18.09.2005), Belgium.
- Meulebrouck K., Ameloot E., Verheyen K. and Hermy M. (2007). Local and regional factors affecting the distribution of the endangered holoparasite *Cuscuta epithymum* in heahtlands. 10th European Heathland Workshop (24.06.2007-01.06.2007), Central to Northern Norway.
- Meulebrouck K., Ameloot E., Van Assche J.A., Verheyen K., Hermy M. and Baskin C.C. (2008). To germinate or not to germinate, that is the question! Germination ecology of the holoparasite *Cuscuta epithymum*. In: Abstracts of the young botanists day 2007: “Plant conservation in theory and practice”. A meeting held in Bailleul (France) on 23 november 2007. *Belgian Journal of Botany* **141** (2), pp. 196-197.

## Undergraduate dissertation

- Meulebrouck K. (2005). Ecologie van *Cuscuta epithymum* een studie in heidegebieden te Midden-Limburg. Eindverhandeling, K.U.Leuven, Leuven.