Regulation of Meadow Saffron (*Colchicum autumnale* L.) in non-intensively managed grasslands

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Dissertation for the degree of
Doctor agriculturae (Dr. agr.)

submitted to the
Faculty Agricultural Sciences, Nutritional Sciences
and Environmental Management at
Justus-Liebig-University Giessen

submitted by
Dipl.-Biol. Linda S. Jung

Giessen, June 2014

Dean of Faculty 09: Prof. Dr. Dr. Peter Kämpfer

Accepted on the recommendation of
Prof. Dr. Dr. Annette Otte, examiner
Prof. Dr. Bernd Honermeier, co-examiner

List of publications

The thesis 'Regulation of Meadow Saffron (*Colchicum autumnale* L.) in non-intensively managed grasslands' is based on the following three papers:

1. Jung L.S., Winter S., Eckstein R.L., Kriechbaum M., Karrer G., Welk E., Elsässer M., Donath T.W., Otte A. (2011). Biological flora of Central Europe: *Colchicum autumnale* L. Perspectives of Plant Ecology, Evolution and Systematics 13: 227-244.
2. Jung L.S., Eckstein R.L., Otte A. & Donath T.W. (2012). Above- and below-ground nutrient and alkaloid dynamics in *Colchicum autumnale*: optimal mowing dates for population control or low hay toxicity. Weed Research 52: 348-357.
3. Winter S., Jung L.S., Eckstein R.L., Otte A., Donath T.W. & Kriechbaum M. (2014). Control of the toxic plant *Colchicum autumnale* in semi-natural grasslands: effects of cutting treatments on demography and diversity. Journal of Applied Ecology 51: 524–533.

Author's contribution:

In paper 1, the first two authors contributed equally doing the main literature collection, survey and writing of the paper and incorporated results from own field experiments. The co-author E. Welk generated the distribution map and interpreted it. Other co-authors helped clarifying controversial points and contributed with valuable ideas and suggestions.

In paper 2, I did the main field work, data analysis and paper writing. The co-authors initiated the study, planned the design and improved the paper with their criticism and comments.

In paper 3, the first two authors contributed equally. They conducted the main part of the field work, data analysis and writing. Co-authors provided the idea and design for the study, helped with field work and data analysis and gave valuable comments to the paper.

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1. General introduction

European semi-natural grasslands are habitats with high biodiversity and nature conservation value (Pärtel *et al.*, 2005). They also provide important ecosystem services as e.g. food production, carbon sequestration, purification of water and air, enhancement of biodiversity, medicinal resources or recreational services (Lemaire *et al.*, 2011). Nowadays, these grasslands are threatened by afforestation, management intensification, abandonment (European Environment Agency, 2010), conversion to arable land and atmospheric nitrogen deposition (Duprè *et al.*, 2010). These changes lead to a dramatic loss of species richness (Fuller, 1987; Green, 1990).

The EU Habitats Directive (European Commission, 1992/1995) aims at ensuring the preservation and restoration of valuable habitats. Most grassland types listed in annex I of the Habitats Directive depend on traditional, extensive management. Consequently, extensive grassland management is promoted by EU agri-environmental programs (Regulation No 1698/2005; European Commission, 2005), which offer compensation payments for a late first cut and/or reduced fertiliser input. However, prescribed extensive management of EU agri-environmental programs may promote the occurrence and frequency of toxic plants like *Senecio aquaticus* (Suter and Lüscher, 2008), *Equisetum palustre* (Čop *et al.*, 2009) and *Colchicum autumnale* (Briemle, 2003; Winter *et al.*, 2011).

Colchicum autumnale is a well-known toxic grassland weed (Wehsarg, 1929; Davies, 1964; Briemle, 2006; Winter *et al.*, 2011) occurring in many parts of Central Europe (chapter 5: Fig. 1). The high toxicity results from several alkaloids (mainly colchicine, demecolcine and colchicoside), which are present in all plant parts (Vicar *et al.*, 1993; Poutaraud and Girardin, 2002). Due to its relatively broad ecological amplitude, *C. autumnale* is a characteristic species for the class Molinio-Arrhenatheretea in Germany (Oberdorfer, 1983; Dierschke, 1997) and typically found in constantly or periodically wet grasslands which are mown or grazed once or twice a year. The good adaptation of the perennial geophyte to extensive management results from its extraordinary yearly life-cycle (Wehsarg, 1929; Franková *et al.*, 2003): in mid-August, the plant consists of a large nutrient-filled mother corm. From a bud of this corm, a new shoot grows and summer dormancy ends. Between August and November flowers develop and appear consecutively above-ground (Muntean *et al.*, 1981; Jäger and

Werner, 2005). After the flowering period, foliar leaves, stem, and capsules develop below-ground and grow just until the soil surface. Their development until about November consumes the largest part of the mother corm's reserves (Franková, 2003). Subsequently, the plant enters winter dormancy until the beginning of March. Leaf growth in spring is accompanied by a further strong decrease of reserves, especially starch content, in the shrinking mother corm (chapter 6: Fig. 5;Franková, 2003). As the plant continues growing, photosynthetic products exceeding the demand for the formation of new plant tissue are stored in the base of the growing shoot and a new daughter corm forms. From the middle of April to the middle of June/end of July, size (Wehsarg, 1929) and starch content (chapter 6: Fig. 5; Franková, 2003) of the daughter corm increase steadily. At the end of May, the mother corm is completely exhausted. The capsules ripen between the middle of June and the end of July. At this time programmed senescence of above-ground plant parts starts (Wehsarg, 1929) which become successively yellow, brown, and dry. Capsules open and seeds are released after the middle of June (Muller-Schneider, 1986). The daughter corm becomes the new mother corm and the plant enters summer dormancy for 2–8 weeks (Godet, 1987). During its annual life-cycle, *C. autumnale* thus completes its photosynthetically active period until first mowing in extensively managed grasslands takes place in mid-June.

In recent years *C. autumnale* has become an increasing problem in some Central European regions where it occurs in high densities (Briemle, 2006; Winter *et al.*, 2011). Some authors (Briemle, 2003) attribute this observation to the extensification of grassland management with the development of EU agri-environment measures since the 1990s (European Commission, 2005). Farmers with high *C. autumnale* population densities have faced increasing difficulties to market their hay as purchasers fear intoxications of their life-stock (Winter *et al.*, 2011). Indeed, cases of acute poisoning or even death of horses, cattle and sheep due to accidental *C. autumnale* consumption have been reported (e.g. Chizzola and Janda, 2002; Chareyre *et al.*, 1989; Kamphues and Meyer, 1990; Kupper *et al.*, 2010). Yet, it is often ignored that life-stock generally avoids the plant in hay and on the pasture (Wehsarg, 1929; Stebler and Schröter, 1981). Therefore intoxications are relatively rare, despite the regionally high occurrence of *C. autumnale* (Winter *et al.*, 2011). However, in order to reduce *C. autumnale* in the fodder there is a risk that farmers intensify management, as *C. autumnale* is sensitive to an increased cutting frequency, or completely abandon management. Both would inevitably

lead to high losses of biodiversity. The same problem persists when applying traditionally recommended measures to control *C. autumnale* densities, e. g. cutting followed by fertilisation with slurry (Wehsarg 1929; Rauschert, 1961), the use of non-specific herbicides (e.g. Davies, 1964) or ploughing up grasslands (Braungart, 1899). The only 'soft' control measure suggested in the literature with a potentially acceptable, i.e. low, impact on biodiversity is an early cut without additional fertilization (e.g. Wehsarg, 1929; Briemle and Elsässer, 2008). However, the effect of such management on biodiversity has not yet been investigated and suggestions for the best cutting time are contradictive. For instance, according to Briemle and Elsässer (2008), *C. autumnale* should be mulched at 10 cm height (i.e. about mid-April) or when *Taraxacum officinale* sheds its seeds (i.e. end of April/beginning of May). The very beginning of May is also recommended by Wehsarg (1929), with the restriction that the exact date varies with the climatic conditions of a region. In contrast to the previous authors, Stebler and Schröter (1981, Luzern and Waadt, Switzerland) regard mid-May as the best time to remove leaves, i.e. when plant capsules already start ripening.

One approach when deciding on the optimal mowing point is to address plant metabolism (Nkurunziza and Streibig, 2011). In general, perennial plants mobilise carbon, nitrogen and phosphorus from their storage organs at the beginning of the vegetation period, when growth is most rapid (Chapin *et al.*, 1990). On the other hand, these nutrients may be diverted from growth early in the vegetation period to form new storage reserves (Lambers *et al.*, 1998). Therefore, from a physiological point of view, the best time to reduce vitality of *C. autumnale* by removing above-ground plant parts is when storage reserves in the corm are low and nutrient contents of leaves and capsules are high, guarantying that a large enough portion of plant resources is removed. Up to date, only two studies have investigated the temporal change of two storage compounds, i.e. protein and starch content (Franková, 2003; , 2006) in the corms of *C. autumnale* and no study has documented changes of nutrient content simultaneously in above- and below-ground plant parts.

Studies of *C. autumnale* metabolism can also be used to determine when alkaloid content in above-ground parts of *C. autumnale* is low in order to reduce hay toxicity. The main function of alkaloids is plant defence, yet they may also serve the storage or transport of nitrogen (Wink, 1987). In agreement with this interpretation is the decrease of alkaloid content in *C. autumnale* leaves and capsules at the end of the

growing season (Poutaraud and Girardin, 2002; Vicar *et al.*, 1993). Although there is a number of studies on alkaloid content in leaves and seeds (e. g. Mróz, 2002; Poutaraud and Girardin, 2002; Vicar *et al.*, 1993) no one has yet documented alkaloid changes in shorter than monthly time intervals or compared several populations.

In addition to studies on physiological plant processes, the optimal mowing point for *C. autumnale* reduction can be determined with mowing experiments. A modern and widely applied method to evaluate management effects on populations of perennial plant species as *C. autumnale* is the collection of population biological data and their analysis with matrix population models (e. g. Lennartsson and Oostermeijer, 2001; Brys *et al.*, 2004; Jongejans *et al.*, 2006; Dauer *et al.*, 2012). Such an approach provides results on population growth rates and gives insight in underlying mechanisms by allowing detailed analyses of demographic processes or transitions between life stages (Caswell, 2001). Besides testing the direct effect of cutting on *C. autumnale*, mowing experiments also allow for studying the effect of cutting on the surrounding vegetation and plant biodiversity. Mowing experiments and analyses of physiological plant processes are therefore an ideal combination to develop appropriate management schemes that address high densities of *C. autumnale* without compromising the high nature conservation value of the respective grasslands.

2. Objectives

The general aims of this thesis were (i) to assemble and critically review information on the species *C. autumnale*, fill information gaps and gain a better understanding of the species, (ii) to investigate nutrient and alkaloid dynamics of *C. autumnale*, (iii) to study the effect of different mowing treatments on the population biology of *C. autumnale* and plant species diversity. Results of (i) – (iii) are utilized to derive management schemes to reduce toxicity in hay from grasslands with high *C. autumnale* densities with the smallest possible negative impact on the co-occurring vegetation and thus plant species richness.

2.1. Characterization of *Colchicum autumnale* (chapter 5)

In the first study, we collected and processed information on a variety of aspects characterizing *C. autumnale* as e.g. taxonomy, morphology, distribution, biology, physiology, biochemistry, and genetics. To this end, we thoroughly reviewed the existing

literature. The information was condensed and summarized. Contradictive results between studies were clarified as far as possible by the help of experts and own investigations. Wherever possible, existing information gaps were filled with results from own experiments. Special emphasis was placed on morphology, germination and the response of *C. autumnale* to competition and management.

2.2. Nutrient and alkaloid dynamics of *Colchicum autumnale* (chapter 6)

In the second study, a physiological approach was used to find the optimal mowing point in order to either reduce populations of *C. autumnale* or to reduce only toxicity in hay. We recorded the quantitative change of nutrient and alkaloid contents in different plant parts of *C. autumnale* over the vegetation period. To generate valid results for different climatic conditions, the study was conducted in two biogeographical regions.

The specific objectives were: (i) to investigate whether and how the temporal pattern of nutrient and alkaloid content differs between biogeographical regions, (ii) to determine, on the basis of nutrient dynamics, the point when cutting impairs *C. autumnale* growth the most, and (iii) to determine the mowing point for low toxicity levels of hay with *C. autumnale* on the basis of alkaloid dynamics in above-ground parts of *C. autumnale*.

2.3. Control of *Colchicum autumnale* in semi-natural grasslands (chapter 7)

The third study dealt with the effect of mowing dates and intensities on *C. autumnale* density and plant species diversity in extensively managed grasslands. To this end, mowing experiments were conducted over four-years on 16 extensively managed grassland sites with dense *C. autumnale* populations in two countries, Germany (study conducted by us) and Austria (study conducted by Silvia Winter from the University of Natural Resources and Life Sciences, Vienna). Effects on *C. autumnale* populations were evaluated with matrix population models. Responses of plant species diversity were judged on the basis of vegetation surveys and multivariate data analyses.

The three main points of interest in this study were: (i) to find the cutting treatment which most effectively reduces the abundance and population growth rate of *C. autumnale*, (ii) to find out how the vital rates of *C. autumnale* are influenced by the different cutting treatments, and (iii) to determine the effect of the different treatments on the co-occurring grassland vegetation.

3. Study area

The study was conducted in three biogeographical regions in Germany, Federal state of Hesse (Fig. 1). Within each region, three study sites, were selected for mowing experiments (chapter 7); for studies on nutrient and alkaloid dynamics, study sites of only two regions were considered (chapter 6; Fig. 1).

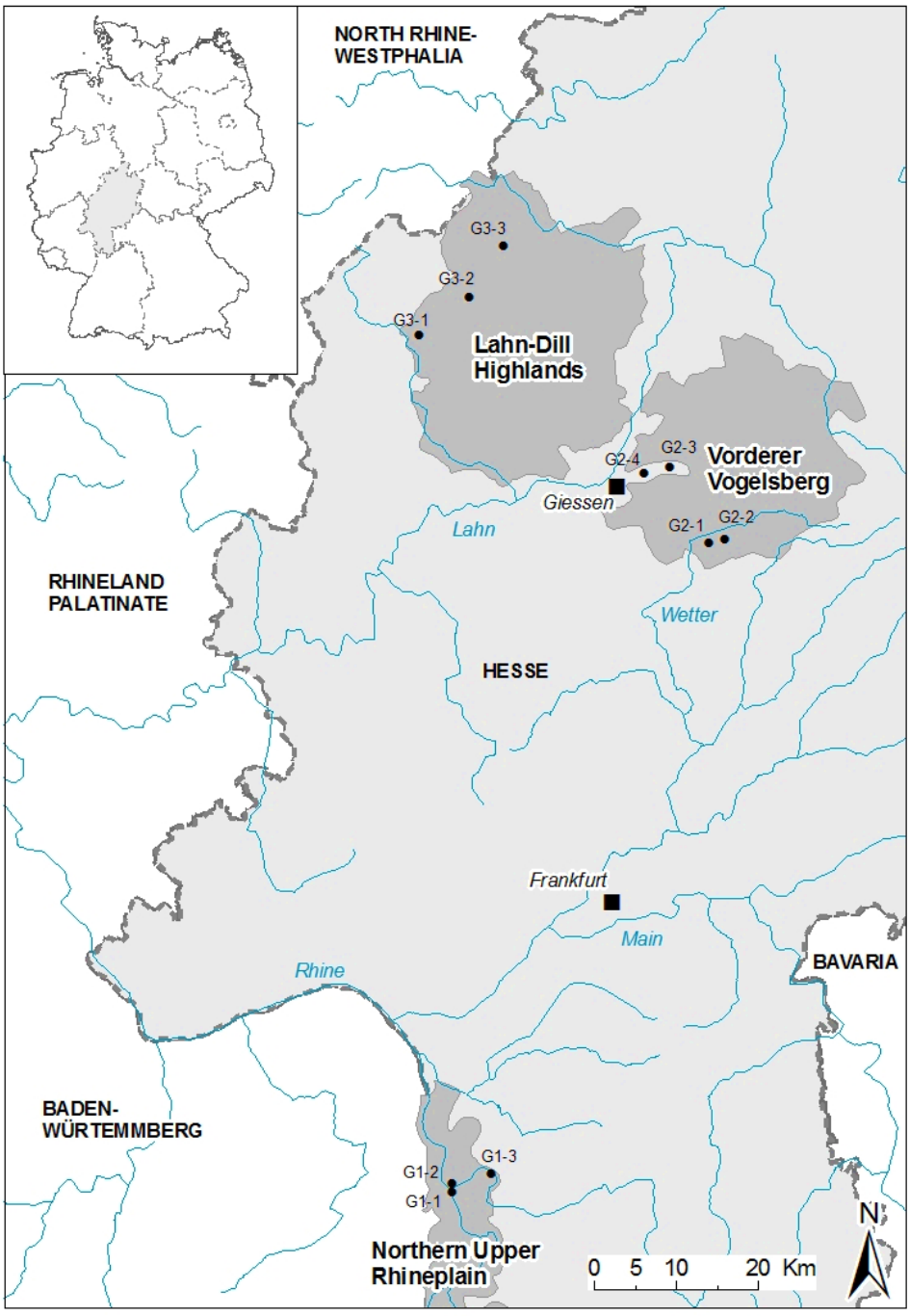

Fig. 1. Study area. Grey = Federal state of Hesse, dark grey = biogeographical regions, black dots = study sites with populations of *Colchicum autumnale*.

The regions are situated along a climatic gradient with respect to mean annual daily temperatures and precipitation (data from HLUG, 2009) and the average annual vegetation period (days > 5 °C; data from Deutscher Wetterdienst, 1981). The study sites in the northernmost of the regions, the Lahn-Dill Highlands (LDH) are characterized by the highest annual precipitation sums of 900 - 1000 mm, the lowest annual temperatures of 7 - 8 °C per year and the shortest vegetation period (days with mean daily temperature ≥ 5 °C) of 120 - 220 days. The study sites in the second region, Vorderer Vogelsberg (VV), which is situated in vicinity of the LDH in south-eastern direction, have lower annual precipitation sums, 600 - 700 mm, while daily temperatures are higher than in the LDH with an annual mean of 9 - 10 °C resulting in a longer vegetation period of 220 - 230 days. The regions LDH and VV are both located in the mid-western part of Hesse, whereas the third region, the Northern Upper Rhineplain (UR) is located in the south of Hesse. It is the warmest and driest of the regions. The study sites in this region are characterized by an annual mean daily temperature of 10 - 11 °C, a vegetation period of > 250 days and a mean annual precipitation of 550 - 650 mm.

Besides different climatic conditions, biogeographical regions vary in geological and edaphic conditions, resulting in different land use and landscapes. The LDH, covering 1270 km², comprise the largest diversity of geologic and edaphic conditions of the three study regions. Typical for the region are the devonian and carbonian sediments (BfN, 2012). The numerous soil types form a small-parcelled mosaic; the main soil types are shallow rankers and regosols on hill tops, brown soils on upper and middle slopes, pseudogleys on lower slopes and gleys in alluvial plains (Harrach, 1998). Given the rough weather conditions and short vegetation period, the region is relatively unfavourable for cultivation (Frede and Bach, 1999). Accordingly, with over 60%, forest is the dominating land use type (BfN, 2012). The agrarian structure is characterised by a heterogeneous small-parcelled mosaic of arable fields, grassland and fallow land (Simmering, 2006).

The 554 km² large area of the VV is a basalt covered landscape dissected by gullies, especially in the west and southwest. Soils originate mainly from basalt or loess and show high fertility, representing favourable conditions for agriculture (HLUG, 2009). Soil types range from pseudogleyed soils in areas above 350 m a.s.l., over rankers and brown earths on steeper slopes to parabrown soils in flat areas (Müller, 1984). Arable fields

represent the main type of land use with about 40%, followed by grassland and forest with about 30%, respectively (HLUG, 2009).

The RH, an area of 852 km², was formed by historical inundations in the meander zone of the Rhine, but is now mostly canalized due to the correction of the Rhine in the 19th century (BfN, 2012). On gravel and sand sediments, fine-grained calcareous alluvial soils have formed (Böger, 1991). Forest management is common along the margins of the Rhine, while most of the landscape is dominated by agricultural land in areas less influenced by the water dynamics of the river (BfN, 2012). Almost half of the RH represents areas under nature protection. The largest one in Hesse with 2,370 ha is the Nature Reserve "Kühkopf-Knoblochsaue" harbouring the three study sites of this biogeographical region.

All study sites are extensively managed grasslands, i.e. unfertilised with one or two cuts per year, showing high abundances of *C. autumnale* (Table 1). Investigated grasslands belonged to the alliances Arrhenatherion elatioris, Bromion erecti, Polygono-Trisetion, Violion caninae, and Molinion caerulae (Ellenberg, 2009). The exact location and vegetation type of the study sites is shown in Table 1.

Table 1. Location and characterisation of grassland sites for studies on *Colchicum autumnale* (HLUG, 2009).

Region	Study Site	Initial plant density/m² (thereof seedlings)	Location and altitude (m.a.s.l.)	Vegetation type according to Ellenberg, 2009) and habitat types listed in annex I of the Habitats Directive (European Commission, 1992/1995)
Northern Upper Rhineplain (G1)	G1-1	59.1 (20.7)	8°46'48" 49°83'08", 90	Cnidion dubii/ Alluvial meadows of river valleys of the *Cnidion dubii* 6440
	G1-2	60.3 (8.3)	8°39'85" 49°81'05", 90	Cnidion dubii /Alluvial meadows of river valleys of *the Cnidion dubii* 6440
	G1-3	27.6 (1.6)	8°39'8" 49°81'97", 90	Arrhenatherion elatioris/ Lowland hay meadow 6510
Vorderer Vogelsberg (G2)	G2-1	16.4 (0.1)	8°83'2" 50°52'7", 177	Arrhenatherion elatioris/ Lowland hay meadow 6510
	G2-2	16.2 (5.1)	8°86'02" 50°53'02", 177	Arrhenatherion elatioris/ Lowland hay meadow 6510
	G2-3	39.8 (5.8)	8°76'49" 50°60'99", 159	Arrhenatherion elatioris/ Lowland hay meadow 6510
	G2-4	Not recorded	8°72'11" 50°60'29", 230	Arrhenatherion elatioris/ Lowland hay meadow 6510
Lahn-Dill Highlands (G3)	G3-1	47.2 (3.4)	8°32'89" 50°75'35", 298	Arrhenatherion elatioris/ Lowland hay meadow 6510
	G3-2	32.3 (0.3)	8°41'61" 50°79'51", 444	Polygono-Trisetion/ Mountain hay meadow 6250
	G3-3	19.9 (1.8)	8°47'43" 50°85'22", 330	Polygono-Trisetion/ Mountain hay meadow 6250

4. Methods

4.1. *Colchicum autumnale* sampling and preparation - *chapter 6*

For studies on nutrient and alkaloid dynamics, *Colchicum autumnale* plants were collected in 2009 from six populations in two biogeographical regions in Hesse, Germany: the LDH and VV. Sampling started on 30 March, when *C. autumnale* plants were about 6 cm high, and was carried out every 14 days until 27 April. From that date onwards, samples were collected every ten days until plant leaves had turned brown and dry and the species entered summer-dormancy (15 July in VV and 3 August in LDH). At each sampling date, we measured leaf length of at least five plants prior to digging out 15 plants per population. Primarily, plants with three leaves and one capsule were collected, and only exceptionally plants with four leaves or two capsules were sampled. Plants were separated into three fractions: (1) leaves plus capsules, (2) old corm and (3) new corm. After capsules had opened (25 June in VV and 5 July in LDH), fraction (1) was analysed as two separate fractions leaves plus capsules with (1.1) and without (1.2) seeds. For each fraction, all plants from the same population were pooled. Old and new corms were cut in the middle, resulting in two samples of 15 corm halves for each corm type per population. One sample of corm halves of each corm type and the fraction 'leaves plus capsules' were dried at 60 °C for about 40 hours, weighed, coarsely ground with a cutting mill (SM 300, Retsch, Haan, Germany) and then finely ground using a vibratory disc mill (T 100, Siebtechnik GmbH, Mülheim/Ruhr, Germany). The ground samples were analysed for concentrations of total nitrogen, phosphorus and potassium and the quantity of the alkaloids colchicine, demecolcine and colchicoside. The other 15 halves of each corm were frozen, freeze-dried for about 70 h, weighed, and finely ground. In these samples, starch content was determined. Until conducting the analyses, all samples were stored dry at -20 °C.

4.2. Nutrient and alkaloid analyses - *chapter 6*

For analysing total phosphorus and potassium contents, 500 mg of each sample were ashed in a muffle furnace at 550 °C for 18 hours, cooled, and spiked with dilute nitric acid. The solution was boiled and filtered into a beaker, which was filled up to 50 ml with distilled water. The concentration of total phosphorus was determined by the Vanadate-Molybdate-method (Kitson & Mellon, 1944; spectral photometer used: PM7, ATG GmbH, Oberkochen, Germany). Potassium analysis was carried out in an atomic

absorption spectrometer (220 FS, Varian, Melbourne, Australia) at wavelength 404.4 nm and slit 0.5 nm. Total nitrogen concentration of the ground plant tissue was determined using an elemental analyser (CE instruments, EA 1110, Italy; CE Instruments, 1996). Starch concentration was analysed applying a starch-UV-test kit (Company Boehringer Mannheim GmbH/R-Biopharm, Germany). This test starts with an enzymatic starch digestion and determines starch content indirectly via the content of free glucose molecules. As corms of *C. autumnale* contain free glucose besides starch, glucose quantity was determined prior to starch hydrolysis and later subtracted. Since the old corm withered with the advancing vegetation period, starch analysis was stopped after 5 June; nutrient analyses continued until 5 July in VV and 15 July in LDH.

Alkaloid analysis was based on the method of Körner & Kohn (2005) and modified as follows: 0.1875 g of a ground plant part sample were extracted with a 25 ml methanol-water mixture (50:50, v:v), containing 0.01 mM EDTA, in an ultrasonic bath for 2 x 15 min and subsequently filtered through a 0.45 µm PTFE filter. The extract was assayed by HPLC with a Merck Superspher 100 RP-18e (250 mm x 4 mm). The mobile phase consisted of buffer (A) (KH2PO4 50 mM, EDTA 3 mM, pH 6.0 adjusted with NaOH) and methanol (B). The following gradient was used at 1 ml min^{-1} and 40 °C: 0 - 9 min: 70% A, 10 - 15 min: 50% A, 18 - 23 min: 70% A; detection wavelength was 355 nm. Colchicine (AppliChem GmbH, Germany), demecolcine (Molekula Deutschland Limited, Germany) and colchicoside (LGC GmbH, Germany) were used as reference standards. Nutrient and alkaloid concentrations were multiplied with the dry weight of the corresponding sample divided by the number of plants contributing to that sample, resulting in the quantity of nutrient/alkaloids per plant part. For statistical analyses of the nutrients N, P, and K, we calculated the ratio between nutrient quantity in leaves plus capsule divided by the sum of the quantity in the two corms. Starch content of old and new corm was summed, representing the total quantity of this storage compound per plant.

4.3. Management experiments - *chapter 7*

The effects of management on *C. autumnale* were investigated in cooperation with Silvia Winter and her colleagues of the University of Natural Resources and Life Sciences Vienna who conducted the management experiments in Austria, while I and the working group from Giessen University performed the experiments in Germany.

In 2008, management experiments were established in seven *C. autumnale* populations in Austria and nine populations in Germany, located in three geographical regions per country. At each study site, three to four cutting treatments were conducted (Table 2) on permanently marked 4 m^2 plots within an area of about 400 m^2.

Table 2. Overview of cutting treatments in Austria and Germany.
Abbreviations in columns 2 and 3 are explained in the first column. Mid = ca. day 15th of a month, early = first decade of a month, late = third decade of a month.

Treatment names (cutting dates)	Austria	Germany
Control (June/July)	C	C
Late May cut (late May and mid-June in Austria)	LM	LM
Early May cut (early May, mid-June)	EM	EM
Repeated May cut (early May, late May)*		RM
Repeated flower removal in autumn (June/July)	F	

* in region G2 only (see Table 1)

Each treatment was applied to four (Germany) or five plots per study site (Austria, except for site A2-2 (see Table S1) where $n = 3$). Plots were arranged in a randomised block design. Control plots were cut at the traditional mowing date in the middle of June or July. The LM ("late May cut") treatment was cut in late May at a plant height of approximately 35 cm. EM ("early May cut") and RM ("repeated May cut") treatments were first cut in late April or early May (except in Germany 2008, where the first cut in the EM treatment was conducted in late May), when plants were approximately 25 cm high. The reason for bringing the first cut forward in the EM treatment in Germany was that *C. autumnale* did not regrow after a cut in late May 2008 as it was desired in order to test the effect of two cuts. The second cut in the EM treatment was carried out in June and in late May in the RM treatment. Treatment F was cut in June/July and flowers were removed every ten days in autumn. Depending on vegetation regrowth and farmer's management, plots of all treatments at the same site were cut additionally in autumn. Vegetation was cut to a height of ca. 5 - 10 cm with lawn mowers, hedge shears or brush cutters and the cut material was removed.

The position, leaf, capsule and flower numbers of each *Colchicum* individual were recorded within the central 1 m^2 of the permanently marked plots from 2008 until 2011. All recorded individuals of *C. autumnale* were assigned to one of six life stages (Fig. 1): i) 'seedling' (S) with one primary leaf ≤ 1 mm width (which could include 1 (2) year old plants as they are indistinguishable from seedlings), ii) 'small vegetative' (L1) with one leaf, which was classified as seedling in the previous year or with a leaf width > 1 mm,

iii) 'medium vegetative' (L2) with two leaves, iv) 'large vegetative' (L3) with three or more leaves, v) 'generative' (G) with capsules, and vi) 'dormant' plant (D), i.e. plants that did not appear above ground during the photosynthetically active period. For details on the classification of life stages see Appendix S1.

The soil seed bank of *C. autumnale* is classified as transient by Thompson *et al.* (1997), therefore, we did not include the life stage 'seed'.

4.4. Transition matrix model and population dynamics - *chapter 7*

Based on the frequency distribution of recorded life stages, a 6 x 6 transition matrix was constructed for each population, treatment and year (Table S2; for calculation details see Appendix S2). Each matrix element (a_{ij}) was calculated from the number of individuals in stage *j* in year *t* that passed into stage *i* in year *t+1*, divided by the column total of stage *j* (Caswell 2001).

The population growth rate was calculated from the averaged matrices of the population transition matrices for each treatment, country and year. A 95% confidence interval was established by bootstrapping the data (10000 iterations) for each treatment, year and country. For bootstrapping, matrix elements representing transition probabilities were replaced by a binomial random distribution and then resampled. Fertility values were bootstrapped by resampling (with replacement) average fertility values calculated per region and treatment.

Life-table response experiments (LTREs) were conducted using matrices based on vital rates (Tables S3 and S4) to analyse the contribution of different vital rates to the difference in the population growth rate λ (Δλ) between each treatment and the control of each country (Caswell, 2001). Each matrix element is a product of the lower-level vital rates: survival (σ_j), stasis ($\gamma_{i=j}$), growth ($\gamma_{i>j}$), retrogression ($\gamma_{i<j}$), generative reproduction (Φ_{ij}) and vegetative reproduction (K_{ij}; Franco & Silvertown 2004, see Appendix S3 for details). Before conducting the LTRE, we separated the averaged transition matrices per treatment into one matrix excluding vegetative reproduction and one matrix including only vegetative reproduction in order to calculate the contribution of vegetative reproduction (K_{ij}) to Δλ. For details on the calculation of the LTRE based on vital rates see Auestad *et al.* (2010). All analyses were performed with the programme Poptools version 3.0 (Hood, 2008).

4.5. Vegetation data - *chapter 7*

To record effects of management experiments on the vegetation, the abundance of all vascular plant species within the central 1 m^2 in permanent plots was recorded according to Braun-Blanquet (1932) each year during the study period (2008 - 2011). Due to logistic constraints, vegetation data were only collected in Germany. To investigate species diversity, we calculated Shannon Index and Evenness (McCune *et al.*, 2002), measured as Pielou's *J*, for each permanent plot. To investigate whether species composition fluctuated stronger in the treatments compared to the control plots, the temporal species turnover-rate for each population and treatment was calculated as follows: $(NR + D)/(n_t+n_{t+1})$ (Mühlenberg 1989), where NR = no. of species per plot that were newly recorded in year *t+1* but did not occur on the plot in year *t*, D = no. of species that had disappeared during the transition from year *t* to year *t+1*, and n_t and n_{t+1} denoting the species numbers in year *t* and *t+1*.

4.6. Statistical analyses - *chapter 6 and 7*

For data evaluation, we applied parametric statistical analyses, multivariate methods and matrix population models. Parametric analyses were used to derive optimal cutting dates from (i) nutrient and alkaloid dynamics, and management effects on (ii) survival and fertility of *C. autumnale*, and (iii) vegetation. Vegetation data were furthermore analysed with multivariate methods. Matrix population models served the analysis of population dynamics of *C. autumnale* under different types of management.

In order to investigate whether the temporal pattern of nutrient and alkaloid content differed between biogeographical regions and to determine the best mowing point, we fitted three different models to the untransformed data of (i) the above-ground to below-ground ratio of N, P, and K, (ii) the above-ground alkaloid content, and (iii) starch content of the corms.

A gamma distribution was fitted to the ratio data of N, P, and K: $\Gamma = \alpha x^{\beta} \exp(-\gamma x)$, where Γ represents the ratio of N, P, or K, and x is the date. As we were interested in the point of maximal ratio, the equation was differentiated with respect to x in order to obtain the parameter x^{max}; x^{max} was then introduced into the model, resulting in:

$$\Gamma = \exp(\log(\alpha) + \gamma x^{max} \log(x) - \gamma x).$$

In order to compare x^{max} between regions, curves for both regions were fitted simultaneously by the joint model 1:

$$\Gamma = \exp(\log(\alpha_i) + \gamma_i x_i^{max} \log(x) - \gamma_i x), \quad [1]$$

where *i* is the *i*-th region. The parameters for the second region were re-parameterised as:

$\alpha_2 = \alpha_1 + \delta_\alpha$,

$\gamma_2 = \gamma_1 + \delta_\gamma$,

$x_2^{max} = x_1^{max} + \delta_x^{max}$,

where numbers 1 and 2 denote the respective region. The dummy variable *g* was created to encode the regions by the values 1 and 0, respectively. The parameters in the joint model were then calculated as:

$\alpha_2 = \alpha_1 + g * \delta_\alpha$,

$\gamma_2 = \gamma_1 + g * \delta_\gamma$,

$x_2^{max} = x_1^{max} + g * \delta_x^{max}$.

Differences between regions in the temporal change of alkaloid content in above-ground parts were determined in an identical way, by fitting a quadratic function of the form:

$y = \alpha * x^2 + \beta * x + \gamma$,

where y represents alkaloid content and *x* the date. Re-parameterising the equation and joining the models resulted in model 2:

$$y = -(\beta_i / (2 * x_i^{max})) * x^2 + \beta_i * x + \gamma_i. \quad [2]$$

The introduction of a dummy variable and parameter calculation was done as in model 1.

Data of starch content in the storage organs showed a sigmoid pattern. We fitted two linear slopes to determine the point when the slope of starch content increases, i.e. the change point. The linear slope model of Schabenberger & Pierce (2002) was applied:

$y = (\beta0 + \beta1 * x) * (x <= \alpha1) + (\beta0 + \beta1 * \alpha1 + \beta2 * (x - \alpha1)) * (x > \alpha1)$,

where α1 indicates the change point. Joining the models for the two regions resulted in model 3:

$$y = (\beta0_i + \beta1_i * x) * (x <= \alpha1_i) + (\beta0_i + \beta1_i * \alpha1_i + \beta2_i * (x - \alpha1_i)) * (x > \alpha1_i). \quad [3]$$

A dummy variable was introduced and parameters calculated for each region as in model 1. In each of the models 1-3, unknown parameters were determined by fitting a non-linear regression model via least squares as implemented in the nls procedure in the statistical programme R. Our null hypothesis for model 1 and 2 was H_0: $x_1^{max} = x_2^{max}$ and for model 3 H_0: $\alpha1_1 = \alpha1_2$. Given the equations for $x_2^{max} = x_1^{max} + \delta_x^{max}$ and for $\alpha1_2 = \alpha1_2 + \delta_{\alpha1}$, the null hypothesis was accepted when $\delta_x^{max} = 0$, or $\delta_{\alpha1} = 0$, which was

tested in a t-test. To test when alkaloid content significantly decreased in above-ground plant parts after the peak (x^{max}), we first selected the sampling date closest to the peak. With reference to that date, we conducted a one-way ANOVA (separately for each region) followed by Tukey's HSD (Honestly Significant Difference) post-hoc test, to determine until when alkaloid content had significantly decreased.

Effects of mowing experiments on *C. autumnale* and the surrounding vegetation were evaluated by an analysis accounting for repeated measures data according to von Ende (2001). Within the analysis, we tested the effects of treatments and time on (i) the survival probability of *C. autumnale*, (ii) the probability of *C. autumnale* individuals to become generative in the next year, and (iii) the Shannon Index and Evenness of the vegetation data. For the between-subjects factors region, treatment and stage (and their interactions), a three-way ANOVA was calculated. The effect of the within-subject factor year (and its interactions) was assessed by a MANOVA. Before analyses, data from the study plots were pooled per population and per treatment to obtain enough plants per transition and thus robust estimates. We excluded the life stages 'seedlings' and 'generatives' from analyses (i) and (ii) due to missing values. Dormant plants were also excluded, as by definition they are not able to die during dormancy. To identify differences among treatments, regions, and life stages, Tukey's HSD (Honestly Significant Difference) post-hoc test was used. We further expand on the rationale behind this statistical approach in Appendix S4.

Using a two-way ANOVA, vegetation surveys of 2008 and 2011 were compared concerning species turnover rates and the proportion of declining Red List species between treatments and regions. We also checked visually which Red List species declined from 2008 to 2011 in treatments versus the control. Region G2 was analysed separately as only this region included the repeated May cut (RM) treatment.

Prior to multivariate statistical analyses of vegetation data, cover values of the extended Braun-Blanquet scale were converted to the 1-9 ordinal scale of van der Maarel (1979: 100). Preliminary analyses of these data showed no general differences in species composition and abundance between regions; therefore, data were analysed at the study site level. In order to determine whether different treatments led to a vegetation differentiation between plots, an MRPP (multi-response permutation procedures; McCune *et al.*, 2002) was carried out for each year 2008-2011. We tested

differences between plots for each year with treatment as grouping factor and Sørensen distance measure for community data.

Requirements of ANOVA and MANOVA, i.e. heteroscedasticity of data and normal distribution of residuals, were investigated using the Bartlett test (Bartlett, 1937) and by plotting the errors against the standard normal deviates, respectively. When necessary, data were arcsine transformed or a Box-Cox transformation (Box & Cox, 1964) was conducted; Box-Cox for data on probability of survival and capsule production was done after adding 0.001 to each data value, due to zero-values.

Analyses on nutrient and alkaloid dynamics were performed in Statistica 9 (2009; StatSoft Inc., Tulsa, Oklahoma, USA) and R, version 2.10.1 (R Development Core Team, 2008). ANOVA and MANOVA of *C. autumnale* population data were calculated with Statistica 10 for Windows (2010; StatSoft Inc., Tulsa, Oklahoma, USA). MRPP were conducted with PC-ORD 5.3 (McCune *et al.*, 2002). Results were regarded significant at $P < 0.05$, except for MRPP, where Bonferroni correction (Sokal and Rohlf, 2007) was applied to correct for multiple testing, changing significance level to $P < 0.0019$.

5. Biological flora of Central Europe: *Colchicum autumnale* L.

Jung L. S.* and Winter S.*, Eckstein R. L., Kriechbaum M., Karrer G., Welk E., Elsässer M., Donath T. W., Otte A. (2011). Perspectives in Plant Ecology, Evolution and Systematics 13(3): 227-244.

* contributed equally

Perspectives in Plant Ecology, Evolution and Systematics 13 (2011) 227–244

Contents lists available at ScienceDirect

Perspectives in Plant Ecology, Evolution and Systematics

journal homepage: www.elsevier.de/ppees

Biological Flora of Central Europe

Colchicum autumnale L.

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ARTICLE INFO

Article history:
Received 9 April 2010
Received in revised form 18 January 2011
Accepted 11 April 2011

Keywords:
Colchicaceae
Grassland
Species biology
Management

ABSTRACT

Colchicum autumnale is a perennial hysteranthous geophyte, which is native to Europe. It is characteristic of periodically wet to moderately moist grasslands but also occurs in alluvial forests. This article gives an overview of the taxonomy, distribution, life cycle, and population biology of *C. autumnale* and puts special emphasis on its morphology, germination and its response to competition and management.

Taxonomy and morphology

Taxonomy

Colchicum autumnale L. Sp. Pl. (1753): 341–Herbst-Zeitlose, Zeitlose, autumn crocus, meadow saffron.
Homotypic synonyms: *Bulbocodium autumnale* (L.) Lapeyr.
Heterotypic synonyms: *Colchicum borisii* Stef.; *C. bulgaricum* Velen.; *C. commune* (L.) Neck., nom. superfl.; *C. crociflorum* Sims; *C. drenowskii* Degen & Rech. f. ex Kitan.; *C. orientale* Friv. ex Kunth; *C. pannonicum* Griseb. & Schenk; *C. polyanthon* Ker Gawl.; *C. praecox* Spenn.; *C. rhodopaeum* Kov.; *C. transsilvanicum* Schur; *C. vernale* Hoffm.; *C. vernum* (Reichard) Georgi; *C. vranjanum* Adamovic ex Stef., des. inval.

The genus *Colchicum* L. belongs to the Colchicaceae, a family that was in the past included in the Liliaceae s.l. Members of the Colchicaceae are perennial herbs with a subterranean corm or rhizome and hypogynous flowers with six tepals (Bowles, 1924; Nordenstam, 1998). The phylogeny of the family has been reconstructed using plastid (Rudall et al., 2000; Vinnersten and Reeves, 2003) and mitochondrial DNA (Fay et al., 2006).

According to a recent synopsis (Persson, 2007), the genus *Colchicum* (incl. *Merendera* Ramond and *Bulbocodium* L.) comprises 99 species. Most of these are confined to small areas, from northern Africa, southern Europe, and the Middle East through western Asia to the borders of central Asia. *C. autumnale* is one of the few more widespread species and the only species to extend to northern Europe and Great Britain (Persson, 1993). While Stefanoff (1926) placed *C. alpinum* DC. in a different subgenus (Archicolchicum), Bowles (1952) identified it as one of the next relatives of *C. autumnale*, together with *C. neapolitanum* Ten. and *C. corsicum* Baker. The Iberian *Colchicum multiflorum* Brot. is also very closely related to *C. autumnale*. According to Meusel et al. (1965), most *Colchicum* species of southwestern Europe are closely related to *C. autumnale*.

Flora Europaea (Brickell, 1980) accepted 39 *Colchicum* species of which *C. rhodopaeum* Kov., *C. drenowskii* Degen & Rech. f. and *C. borisii* Stef. were subsequently relegated to the synonymy of *C. autumnale* (Persson, 1993, 2007). Brickell (1980) proposed a "*C. autumnale* group" including *C. autumnale*, *C. neapolitanum*, *C. lusitanum* Brot., *C. lingulatum* Boiss. & Spruner and *C. parnassicum* Sart., Orph. & Heldr. ex Boiss., while noting that this group is not fully understood and in need of further study. This statement might apply to the genus in general and in particular to species with leaves and flowers appearing in different seasons (i.e. hysteranthous). In

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doi:10.1016/j.ppees.2011.04.001

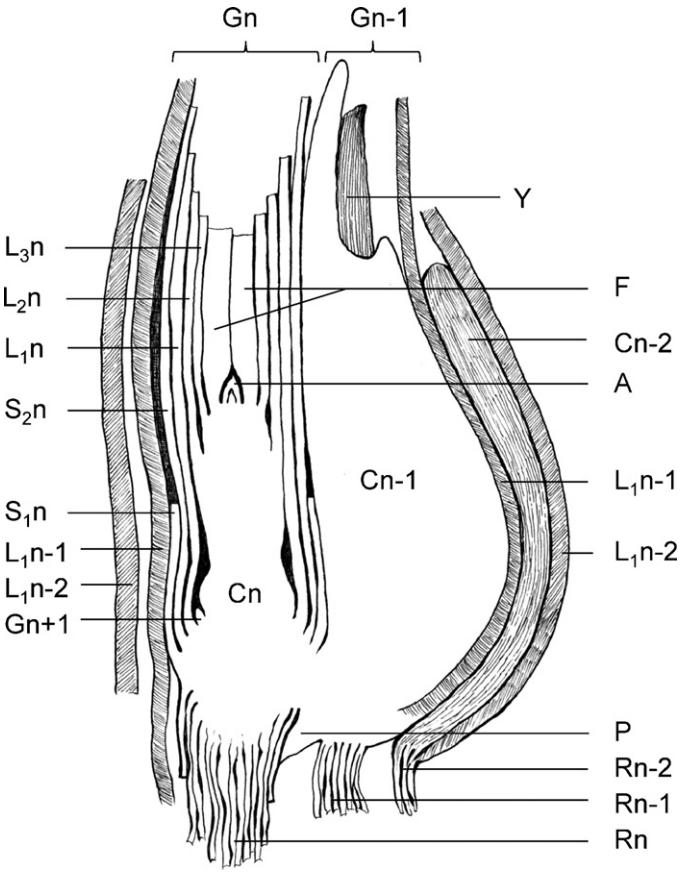

Fig. 1. Longitudinal section through the base of a flowering *Colchicum autumnale* plant (drawing by S. Rosner after Irmisch, 1850, modified). C = corm, S = sheath leaf, L = foliar leaf, F = flowers, A = axis, R = roots, Y = last year's remaining shoot, P = protuberance, G = shoot generation, n + 1 = next year, n = current year, n − 1 = one year old, n − 2 = two years old, subscript numbers indicate sequence.

addition, many herbarium specimens consist of flowering material only (Brickell, 1980). Subsequent to the taxonomic treatments of Baker (1879) and Stefanoff (1926), Persson (2007) provides a nomenclatural synopsis of the genus *Colchicum* but a comprehensive revision is still missing.

A hybrid between *C. autumnale* and *C. alpinum* was reported by Perrenoud and Favarger (1971) from the French Alps.

Morphology

Axis

C. autumnale is a geophyte with an irregular shaped corm[2] of 3.5–7.0 cm in diameter; one corm side is flattened, the opposite one convex (Heimann-Winawer, 1919). At its lower end the flattened corm side ends in a more or less distinct beak-shaped structure, called protuberance (Fig. 1, P) by Jaehn et al. (1985). According to their analysis, it is formed by the prophyll and the hypopodium (see the 'Leaves' section) by asymmetric abaxial growth (Jaehn et al., 1985). The prophyll is developed as a leaf primordium and can only be detected in an early developmental stage. Other authors like Irmisch (1850) and Loew and Kirchner (1934) did not detect this prophyll and regarded the protuberance of the daughter corm as an extension of the mother corm. The internode between the prophyll and the first sheath leaf (Fig. 1, S_1n) is compressed and serves for the insertion of the roots. The next two internodes are erect and even shorter. A node follows carrying the first foliar leaf (Fig. 1, L_1n). The latter is succeeded by a somewhat elongated internode which develops the future corm. Jaehn et al. (1985) did not count the internode preceding the prophyll; consequently they stated that the "fourth internode" develops the future corm. We suggest to count the hypopodium as the first internode (following Irmisch, 1850; Troll, 1937), consequently, the corm is built by the fifth internode. A bud (Fig. 1, Gn+1) at the bottom of the corm, in the axil of the first foliar leaf, develops into the innovation shoot of the next year (Irmisch, 1850; Wehsarg, 1929; Troll, 1937; Jaehn et al., 1985). Exceptionally, Karrer and Winter (pers. obs.) found plants with the regular innovation bud positioned in the axil of the second sheath leaf (Fig. 1, S_2n). This so called "renewal" (Loew and Kirchner, 1934) or "regular" bud (Franková et al., 2003a) replaces the old corm. A second innovation bud is situated near the top of the corm in the axil of the second foliar leaf (Fig. 1, L_2). It develops into a separate new corm when the mother corm has stored enough reserves to multiply clonally or when the regular bud is destroyed. The names "reserve" (Loew and Kirchner, 1934) and "irregular" (Franková et al., 2003a) buds are derived from this function. Sometimes, a third bud is developed in the axil of the third foliar leave (Fig. 1, L_3n) (Irmisch, 1850). This bud can be induced by the injury of the shoots formed by the renewal bud (Wehsarg, 1929, 1935).

The protuberance of young individuals grows vertically into the soil, enabling the corm to penetrate into greater depth. In the case of adults that have reached a depth of 15–20 cm, the protuberance elongates horizontally. This ability is probably based on the mechanical properties of the sclerenchymatic tissue of the protuberance (Franková et al., 2003a). The closer the mother corm is located to the soil surface, the more the protuberance elongates (Irmisch, 1850). Storage tissue of the corm comprises parenchyma rich in starch and filled with many vascular bundles (Loew and Kirchner, 1934; Franková et al., 2003a). The innovation shoots stay connected to the mother corm by their protuberances for several years (Wehsarg, 1929).

Roots

The unbranched root system of *C. autumnale* is characterised by two different root types. On adult plants, up to 250 (Winter, pers. obs.; Rimbach, 1897: 200) 0.5–1 mm thick and up to 30 cm long roots develop in September (Rimbach, 1897). A root profile is given in Kutschera and Lichtenegger (1983). Furthermore, 1–2 roots, showing a thick, swollen basal part, appear in April and May (Rimbach, 1897). Both root types may occur on plants of all ages. However, the development of spring roots depends on corm depth in the soil and does not occur in corms that have reached a depth of 15–20 cm (Rimbach, 1897; Franková et al., 2004). According to Kutschera and Lichtenegger (1983), September roots have a single layer exodermis, which is followed by 6–7(–8) layers of parenchyma cells. The inner parenchyma consists of large intercellulares which fall apart in older roots (Rimbach, 1897). Consequently, the vascular bundles detach from the extern parenchyma. Cells in the outermost layer of the inner parenchyma are filled with raphide bundles (Raunkiær, 1895). The central vascular bundle of September roots mostly shows a triradiate structure and only rarely one of higher order (Rimbach, 1897; Tillich and Jung, pers. obs.; Kutschera and Lichtenegger, 1983: two to four rays; but Raunkiær, 1895: five to six rays). The vascular bundle in spring roots is of tetrarch or pentarch structure and possesses more than twice the diameter of that in September roots (Rimbach, 1897). These

[2] Bell (1991) defines a corm as "a short swollen stem of several internodes and nodes" which is generally replaced by one or several new corms during the growing season. In contrast, a tuber "forms by the swelling of the distal end of a slender underground rhizome" and "normally survives longer than the main plant".

roots are characterised by a thick inner parenchyma and show small intercellulares (Rimbach, 1897). Slight undulation of the endodermis in the swollen basal part indicates a contractile function, although contraction itself could not be observed (Rimbach, 1897). Rimbach (1897) and our own observations (Karrer, pers. obs.) lead to the conclusion that the translocation of the plant into larger depth is a result of protuberance growth. Wave-like structures of endodermis or hypodermis are completely missing in the September roots.

Leaves

Irmisch (1850) was the first to describe two distinct sheath leaves developed along the basal part of the innovation shoot of *C. autumnale* (Fig. 1). The first leaf (Fig. 1, S_1n), also called ephemeral sheath leaf, is fleshy and 1 cm long. It is formed like an elongated hood, consisting of a thin white membrane, and lacks any vascular bundles (Irmisch, 1850; Heimann-Winawer, 1919; Wehsarg, 1929). When the shoot elongates, the first leaf tears open at the abaxial side of the top suggesting an addorsed position like a prophyll (Irmisch, 1850; Jaehn et al., 1985; Karrer and Winter, pers. obs.). The whitish second sheath leaf (Fig. 1, S_2n) has a green upper brim, is about 10 times longer than the first sheath leaf (Irmisch, 1850), and terminates in a little tip. The presence of only few vascular bundles at the base of the second sheath leaf is compensated by large intercellulares in the sheath. Irmisch (1850) and Troll (1937) noticed that the spatial position of those sheath leaves was not in accordance with the regular phyllotaxy of monocotyledon prophylls as both were said to be addorsed. In a detailed morphological analysis, Jaehn et al. (1985) detected an additional first leaf (prophyll) in correct addorsed position, which can be recognized only in early developmental stages. The position of the main vascular bundles of the following first sheath leaf indicates its abaxial insertion. Consequently, the first three leaves show a distichous phyllotaxy.

The leaves following the second sheath leaf develop a lamina and represent the green, photosynthetically active leaves (Fig. 1, L_1n–L_3n). The sheath of the second foliar leaf (Fig. 1, L_2n) is almost totally connate to the following internode. The insertion line of the second foliar leaf (Fig. 1, L_2n) is bent down in median position. Consequently, the reserve bud is positioned a little bit below the general insertion line of the subtending second foliar leaf. While the sheath leaves and the first foliar leaf (Fig. 1, L_1n) are arranged more or less distichous, the second and following foliar leaves change to alternate phyllotaxis with a divergence angle of 140–150° (Irmisch, 1850; 144° in Wehsarg, 1929). Except for the internode between the first and second foliar leaves, all internodes are short and the leaves are arranged in a loose rosette (Bowles, 1952). A single corm develops up to six foliar leaves (Bornemann, 1920; Jaehn, 1984). Only the first foliar leaf possesses a long, tube-like sheath, which originates from the bottom of the corm and completely encloses it. The following foliar leaves arise from the nodes above the corm (Irmisch, 1850; Loew and Kirchner, 1934).

Both leaf-types, i.e. sheath and foliar leaves, possess an epidermis with cuticle. Epidermis cells contain large elongated proteinoplasts (Thaler, 1953). The epicuticular wax belongs to the *Convallaria* type and consists of parallel arranged platelets (Barthlott and Theisen, 1998). However, sheath leaves lack palisade cells, and their vascular bundles contain a reduced number of xylem cells; in some of the vascular bundles, the xylem is not developed (Thomas, 1900). In the foliar leaves, the epidermis is followed by one layer of short palisade cells and another layer of chlorophyll containing cells, while chlorophyll-free tissue fills the leaf centre (Loew and Kirchner, 1934). A hypodermis is located below the epidermis on the edge of the leaf and occasionally in the middle of the leaf's reverse side (Loew and Kirchner, 1934). Vascular bundles in the foliar leaves are strongly differentiated and contain a high number of xylem cells (Thomas, 1900). Stomata are distributed equally on both sides of the leaf (Loew and Kirchner, 1934). Vascular bundles of both leaf types are arranged in parallel (Loew and Kirchner, 1934). Foliar leaves reach a length of 35–65(–100) cm and a width of 1–6.5 cm (Muntean et al., 1979; Winter, pers. obs.).

Flowers

Flowers are arranged in an umbel-like (Schumann, 1904; Wehsarg, 1929) stout raceme (Persson, 1993) along the upper part of the annual stem. The lowermost flower generally develops in the axil of the third foliar leaf (except when a third bud is present), whereas additional flowers originate from the axils of the following leaves, which are rarely foliar leaves but scales (Wehsarg, 1929). Instead of a terminal flower, the inflorescence ends with a ceasing terminal meristem (Irmisch, 1856). At the onset of blooming, every flower is attached to the inflorescence axis by a short pedicel of few millimetres length, which elongates at the end of the flowering period (Wehsarg, 1929). The maximum number of fully developed flowers per plant ranges from four (Irmisch, 1850; Loew and Kirchner, 1934) to seven (Hegi, 1910; Jung et al., unpubl. data) or even nine (Muntean et al., 1983), and a few (Irmisch, 1856; Karrer, pers. obs.), in some cases up to 11 (Wehsarg, 1929), additional rudimentary flowers.

The perianth of the flowers consists of 6 connate petaloid tepals, forming a narrow 20–35 cm long tube, and 6 thin tepal lobes with rounded tips. Tepal lobes can reach 40–50 mm in length (Loew, 1908; Heimann-Winawer, 1919). Once flower tissue is exposed to sunlight, tepal colour changes from white to light-purple or purple. However, some flowers stay white (Hegi, 1910; Wehsarg, 1929), representing one of the several varieties known for the species (Bowles, 1952). The pentacyclic trimeric flower organ arrangement corresponds to that of the Liliaceae. The two outermost circles are composed of three exterior tepals and three slightly smaller interior tepals. Flowers are thus actinomorphic.

Subsequent to the tepals, there follow two circles of respectively three dithecal stamens, 11–24 mm long (Loew, 1908). The stamens are fixed by short filaments to the tepal tube – the three inner ones higher up than the outer ones. At the outer (dorsal) side of each filament's basis (for longitudinal section see Leins and Erbar, 2008: 117), an orange tissue produces nectar that is secreted into a hair-covered furrow of the respective tepal (Wehsarg, 1929). The yellow linear anthers, 5–8 mm long, are dorsifixed. Pollen is released along two lateral fissures of the anthers (Heimann-Winawer, 1919).

In the centre of the flower, three distinct styles form the innermost circle of the flower. They are 20–25 cm long, white, and originate from the top of the syncarpous ovary (Heimann-Winawer, 1919). Each style ends in a 3 mm long hooked stigma, including a groove that is covered with small 136–144 μm long papillae for pollen reception (Heimann-Winawer, 1919; Loew and Kirchner, 1934). Within the styles, this groove turns into a channel, which connects to the ovary, providing the route for the pollen tubes. Anomalies from the described flower organisation, e.g. connations, are common (Loew and Kirchner, 1934) and especially found in flowers appearing between February and May. These spring flowers regularly lack carpels and sometimes also stamens (Wehsarg, 1929). Often, their tepal tissue is green and resembles that of green foliar leaves containing chloroplasts and palisade cell as well as a higher number of stomata and thicker mesophyll layers (Harder and Lorenzen, 1966). These green flowers are, however, not genetically determined but represent the response to unsuitable weather conditions in autumn (Loew and Kirchner, 1934 – see the 'Response to abiotic factors' section).

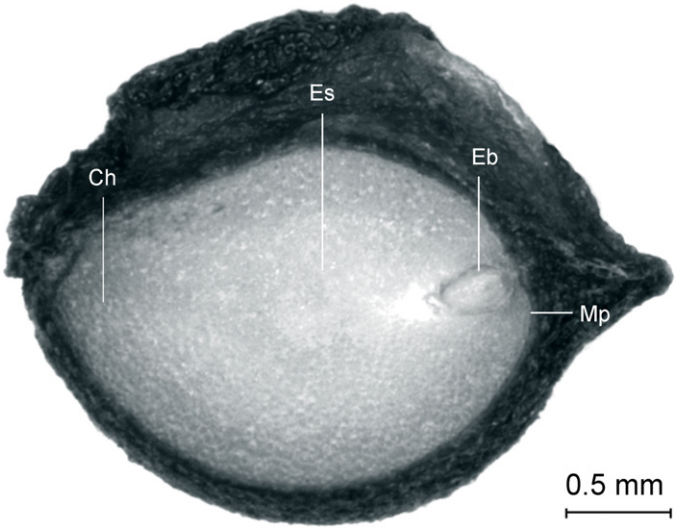

Fig. 2. Longitudinal-section through a ripe seed of *Colchicum autumnale*. Ch = chalaza, Es = endosperm, Eb = embryo, Mp = micropyle. Photo by Markus Kasnitz.

Ovary and seeds

Flowers are hypogynous with ovaries located underground, just above the new corm. One ovary consists of three connate carpels, each forming a separate loculus with axillary placentation. The ovary develops into a beaked oval capsule of 2–7.5 cm length and 1–2.5 cm width (Muntean et al., 1979), which is sub-divided by three septa. In each loculus, numerous half-anatropous ovules are arranged in two placenta rows in axillary position (Wehsarg, 1929; Karrer, pers. obs.). The capsules appear above-ground in the middle of the leaf rosette after significant elongation of the internode between the second and third foliar leaves (Troll, 1937). They are attached to the stem by short pedicels. At maturity, the capsules open from the top and disconnect septicidally in their upper third.

Seeds are roundish, brown, and have a large white proliferation, which extends on one side of the seed between micropyle and chalaza (Loew and Kirchner, 1934). The endotesta is composed of two layers of narrow, cutinized cells, containing brown pigment and oil as well as aleurone grains (Heimann-Winawer, 1919). The exotesta consists of 2–3 layers of thin-walled parenchymatic cells with starch grains and one layer of epidermis cells (Heimann-Winawer, 1919). The parenchymatic cells of the funiculus are also filled with starch grains and form the proliferation (Heimann-Winawer, 1919), called strophiole. Misleadingly, most authors denote it as "caruncula" (cf. Heimann-Winawer, 1919; Nordhagen, 1933; Loew and Kirchner, 1934), which, however, is by definition an excrescence of the exotesta at the micropyle (Wagenitz, 2003). During seed maturation, the starch in the exotesta of seed and funiculus is transformed into sugar compounds that are emitted on the exterior of the seed (Nordhagen, 1933). As a consequence, cells shrink and the strophiole, the place where sugar excretion is highest, shrivels (Nordhagen, 1933). The tiny embryo is located laterally to the micropyle (Fig. 2).

Distribution and habitat requirements

Geographical distribution

C. autumnale is a Middle European species. The native distribution extends from Ireland and N-England in the Northwest over N-France, S-Belgium, and Central Germany to S-Poland in the Northeast. From there it reaches southwards over westernmost Ukraine and the Romanian Carpathians to SE-Bulgaria. The southern distribution limit stretches from N-Greece and S-Albania over the N-Apennines in Italy to the Eastern Pyrenees, the Asturias, and the Sierra de Gredos in Spain, where the species supposedly has its south-westernmost occurrences (Fig. 3).

The distribution range of *C. autumnale* corresponds to ranges of the submediterranean/montane-middle European distribution range type 8 (Meusel and Jäger, 1992). This range type consists predominantly of species of the temperate deciduous forest flora. Within the *Hippocrepis-comosa-subtype 8.9*, the range of *C. autumnale* can be regarded as an anthropogenic extended representative, while species like *Cirsium eriophorum* (L.) Scop., *Phyteuma orbiculare* L. s.l., or *Tilia platyphyllos* L. (*Astrantia-major-subtype 8.8*) are more strongly confined to montane regions in their native ranges.

The amended distribution formula after Jäger and Werner (2005) can be given as (m/mo)-sm/demo-stemp·$c_{1\text{-}4}$EUR.[3]

Distribution limits

Within the continuous continental European distribution range, *C. autumnale* is largely missing in a large gap in the Great Hungarian Plain, where precipitation is low and soils are partly salinized. A further distribution gap is located in the Bavarian/Bohemian Forest region of the Czech Republic, Austria and Germany, and in the Eastern Central Alps between the Ötztaler Alps and the Lower Tauern (Hendrych, 1985; Chytrý and Rafajová, 2003; Niklfeld and Schratt-Ehrendorfer, 1967–2005; BIB, 2010). A smaller gap is located in Central France in the Sologne (Dept. Loir-et-Cher, Arrond. Romorentin-Lantenay). Supposedly, these latter gaps are due to the dominant occurrence of infertile, acidic soils in these regions, as *C. autumnale* prefers base rich, fertile soils (Butcher, 1954; Oberdorfer, 1994). The so-called Hercynian gap was interpreted by Hendrych (1985) to point to a Carpathian origin of the northern Czech populations. Generally, the species very rarely occurs in upper subalpine and alpine Mountain belts, resulting in a further gap in the Western Alp's high altitudes. However, single occurrences are reported from up to 2227 m in the Inner Alps, Riffelalp/Zermatt (Schroeter in Jaccard, 1895). The increase in altitude towards dryer, southern latitudes is illustrated in Fig. 4.

At its southern distribution limit, *C. autumnale* does not reach the mountains of Sterea Ellas but has its southern boundary in N-Greece (Strid, 1996), namely in the Rhodope-Orvilos Mountains and the N-Pindos. Here, the species is confined to montane to subalpine habitats where mean May precipitation is above 60 mm. Although several times reported to occur in the European part of Turkey, no occurrences have been confirmed (Brickell, 1980; Persson, 1999b, 2000; Akan and Eker, 2005). Furthermore, the occurrences in lowland SE-Bulgaria, given by Bondev (1995) might be regarded doubtful, as Persson (pers. comm., 2010) has never seen the species in material from there.

In Italy, the delimitation of the distribution is not well documented. According to Zanetti (1997) the species is quite common in

[3] It reads: *C. autumnale* has few montane occurrences in the meridional floristic zone. More regular populated are the regions in the adjacent submeridional floristic zone, where *C. autumnale* is partly confined to mountain forelands surrounding hilly landscapes (demontane distribution). In the southern temperate floristic zone, the species occurs also in edaphically suitable lowland habitats. The geographical distribution is confined to Europe, where the species occurs from oceanic to suboceanic regions (zones 1–4 of Jäger's phytogeographical continentality zones, Jäger, 1968).

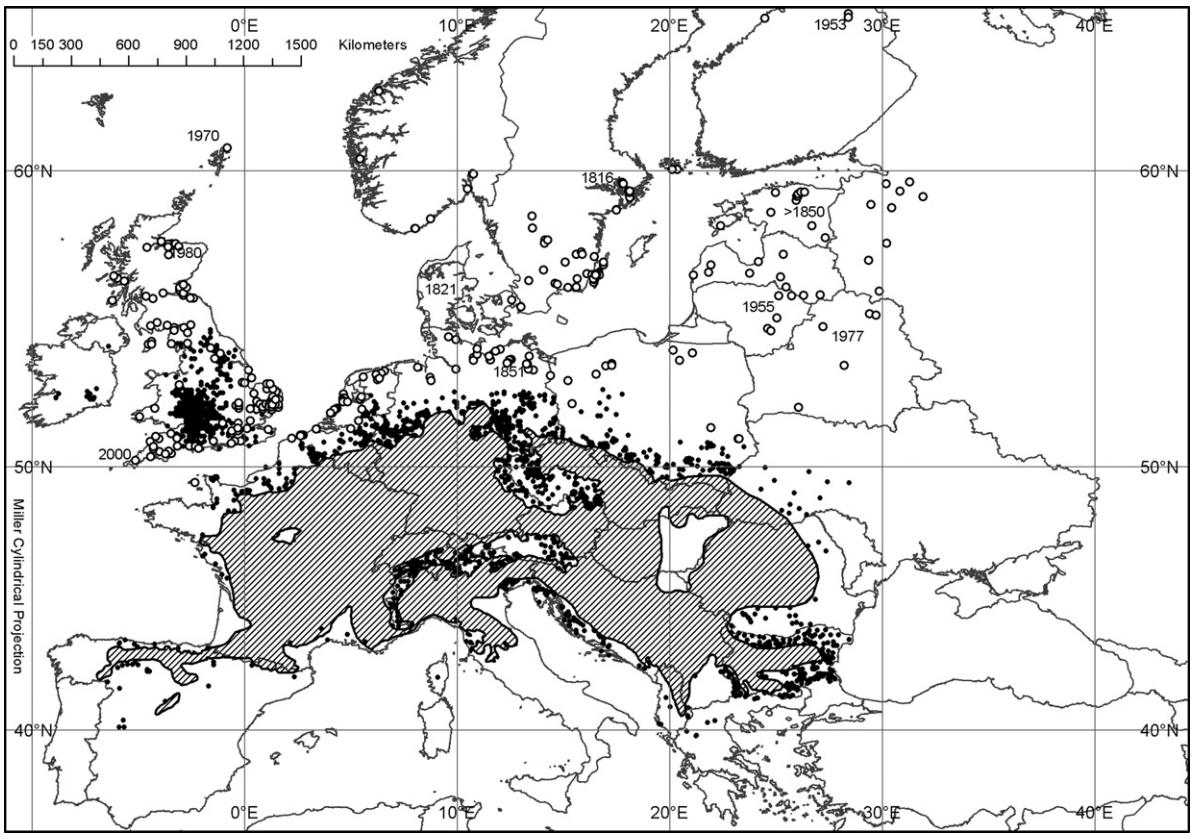

Fig. 3. The geographical distribution of *Colchicum autumnale* L. compiled by E. Welk. Hatched black areas indicate the continuous distribution range; black dots indicate single native, and white dots single synanthropic occurrences. For countries and regions with synanthropic occurrences, the respective year of the oldest record is given. The original distribution map is based on data compiled in Meusel et al. (1965). In addition, a wide range of additional data sources was used to compile an up-to-date distribution data set for *C. autumnale*. A list can be provided upon request from the author of the map E. Welk.

the inner arc of the Alps, while it is much more sparsely distributed in lowland Padania and the Northern Apennines.

In S-France, *C. autumnale* is missing in the Mediterranean department Gard (Association Tela Botanica, 2009). There, and on Corsica, the species is replaced by the similar *C. neapolitanum* (TEN.) TEN. (Jeanmonod and Gamisans, 2007; Rameau, 2008). The southern distribution limit in Spain seems to be primarily caused by climate (late spring water balance), and secondarily by edaphic reasons. The species is missing from climatically suitable regions of the eastern Pyrenees probably because par-

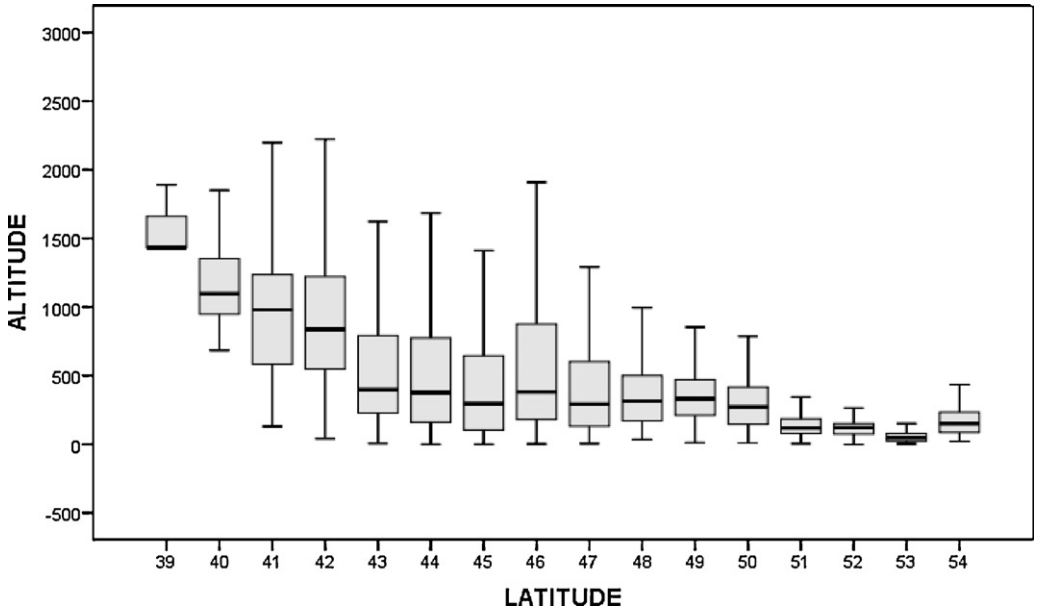

Fig. 4. Box and whisker plots of the altitudinal distribution of *Colchicum autumnale* along latitude classes of one degree. Indicated are the lower and upper quartiles (box), the median (bold line), and the minimum and maximum values.

ent material consists largely of acidic granite and gneissose rocks.

The distribution records from more southern parts of Spain and Portugal seem doubtful since floristic publications concerning Andalusia (Valdés, 1987; Blanca et al., 2009) do not report this species from Southern Spain. However, the situation cannot be easily judged since many south-western European floristic sources include *C. multiflorum* BROT. as a synonym into *C. autumnale* s.l. Also the westernmost outpost (Prov. Galicia, Pontevedra, Faro) listed in Proyecto Anthos (Castroviejo et al., 2006) is not confirmed by Buján and Inmaculada (2008).

C. autumnale reaches its northwestern distribution limit in the Nore Valley in SE-Ireland. Because the very restricted distribution seems to be spatially associated with the presence of ancient monasteries, the native status of the populations was debated repeatedly (Smith and Waldren, 2010). Recent genetic analyses revealed high levels of genetic diversity that are likely to reflect a long period of occupation in Ireland. This suggests that younger introduction is unlikely to have occurred (Smith and Waldren, 2010).

The northern native distribution limit in England reaches the counties Westmoreland and Yorkshire (Preston et al., 2003). While the regional occurrence and abundance of the species in England are generally correlated with base rich and moist, yet well drained soils (Butcher, 1954), it seems limited towards north by mean May temperatures below 10 °C. In contrast, the continental northern distribution limit, reaching from S-Brittany to S-Normandy, is spatially correlated with the occurrence of acidic metamorphic rocks. Also, the northern distribution limit in S-Netherlands, Central Germany, and S-Poland seems not correlated with climatic factors. Here, the native distribution might be limited mainly edaphically due to acidic infertile soils of pleistocene origin.

Wehsarg (1929) explains the native northern distribution limits by reproduction problems (delayed seed ripening and flowering). In contrast, Meusel et al. (1965) assume that *C. autumnale* has not reached its maximum extension and is still spreading subspontaneous on improved cultivated grasslands to the North. This tendency is obvious also in the compiled distribution data (Fig. 3).

Northward range expansion

Since the species is popular in gardens and becomes naturalized when planted or discarded into suitable habitats, naturalized occurrences are to be found along the northern distribution limit reaching from Inverness in Scotland and a single naturalized occurrence from the Shetlands (BSBI, 2010), to coastal Norway (max. 62°N), the Uppsala region in Sweden (60°N), and SW-Finland (Åland/Ahvenanmaa). Judged from Google Earth aerial photographs (accessed 08/2010), the northernmost (~64°N) occurrences in the Oulu province (near Raahe [1990], near Hyrynsalmi [1953]) might be situated in urban park meadows. (Furthermore, the older record was provided by a Pharmacist, who might have herbarised cultivated plants.) The status of all these occurrences is doubtless non-native since the oldest records are dated from the 19th century (see Fig. 4). Synanthropic occurrences are also reported from the US: Kentucky, Massachusetts, Maryland, North Carolina, New Hampshire, New York, Oregon, Utah, and Vermont (USDA, NRCS; 2010).

Frequency and abundance of *C. autumnale* in the northern lowland regions and at the southern distribution limit are lower than in central parts of its distribution area (D'Amato, 1955; Pignatti, 1982; Oberdorfer, 1994).

Habitat

According to Oberdorfer (1980), *C. autumnale* originates from open deciduous and alluvial forests, from where it could spread to adjacent meadows and pastures. Such open or semi-open landscapes might have been created by mega-herbivores (cf. Bradshaw and Mitchell, 1999; Gerken and Görner, 1999, 2001).

C. autumnale is characteristic of seasonally wet to moderately moist grasslands (Oberdorfer, 1994), river banks, waterside meadows (Butcher, 1954; Preston et al., 2003), and wet grasslands (Antonetti et al., 2006) but avoids permanently water-logged sites (Wehsarg, 1929; Winterhoff, 1993). It also inhabits semi-dry to periodically wet calcareous (e.g. short-time flooded) grasslands (Mróz, 2006) and gorse heath (Antonetti et al., 2006). Furthermore, it occurs next to dirt roads (Preston et al., 2003; Mróz, 2006), forest margins or clearings (Adriaens et al., 2009), and in alluvial forests (Oberdorfer, 1994).

C. autumnale tolerates moderate shade (≥12% relative illuminance; Ehrendorfer, 1998), but prefers direct sunlight (Ellenberg et al., 1992). It is the only species of the genus that sustains low temperatures of −20 °C (Nordenstam, 1998). Preferably, it occurs in regions with moderate temperate climate on basic or slightly acidic soils that are well imbued but not constantly wet (Oberdorfer, 1994). *C. autumnale* favours moderate nutrient rich and deep soils (Seybold, 1998). It is frequently found on brown soils (Zoller, 1954) but also on alluvial soils or soils influenced by groundwater like pseudogleys (Winter et al., unpubl. data).

Typical pH-values of soils preferred by *C. autumnale* vary between 4.8 and 8.0 (upper soil layer) according to data from Poland (Mróz, 2008), Austria (Bassler et al., 1998; Winter et al., unpubl. data), Switzerland (Zoller, 1954), and France (Poutaraud and Girardin, 2006). Available phosphorus content was on average 39.7 mg/kg soil in 25 polish populations (Mróz, 2008 – acetic acid extraction) and 15.4 mg/kg in 25 Austrian sites (Winter et al., unpubl. data – CAL extraction). The average available potassium contents reached values of 46.4 mg/kg in Poland (Mróz, 2008 – acetic acid extraction) and 113.6 mg/kg in Austria (Winter et al., unpubl. data – CAL extraction, according to Schüller, 1969). With respect to the Ellenberg indicator value for nutrients/nitrogen (Ellenberg et al., 1992) the species is indifferent, however, *C. autumnale* is missing at extremely nutrient rich and poor sites (see the 'Communities' section). Total nitrogen values of soils varied between 0.37% and 1.08% in 44 populations in Austria (Bassler et al., 1998; Winter et al. unpubl. data).

Communities

Due to its relatively broad ecological amplitude, *C. autumnale* is listed as character species for the class Molinio-Arrhenatheretea in Germany (Oberdorfer, 1980; Dierschke, 1997) together with widespread species like *Festuca pratensis* or *Ranunculus acris*. Schubert et al. (2001) classify *C. autumnale* as character species of the order Molinietalia caeruleae. This order contains constantly or periodically wet grasslands, including floodplain meadows, which are mown or grazed once or twice a year. Furthermore, it comprises tall-herb communities along ditches and water-banks in moist forests and recently abandoned grasslands. *C. autumnale* prefers nutrient rich soils from lower altitudes up to the submontane zone. Thus, within the Molinietalia caeruleae, *C. autumnale* is characteristic of the regularly mown and widespread *Trollio europaei–Cirsietum oleracei* (KUHN, 1937) OBERD. 1957 (Schubert et al., 2001). It is a typical species of the *Sanguisorbo officinalis–Silaetum silai* (KLAPP 1951) VOLLR. 1965, a grassland community on loamy to clayey soils with seasonally varying moisture conditions in moderate temperate floodplains (Schubert

et al., 2001). *C. autumnale* also occurs in the lower herb layer of the *Filipendulo ulmariae–Geranietum palustris* W. KOCH 1926, a tall-herb community, which develops after the abandonment of agricultural management (Mayer, 1939). In a synsystematic compendium of French meadows, the suballiance *Colchico autumnalis–Arrhenatherenion elatioris* FOUCAULT 1989, comprising mesohygrophilous grasslands on alluvial and colluvial soils, is listed (Géhu, 2001).

C. autumnale separates as differential species subtypes of grassland communities on somewhat richer soils from the main subtype on poor soils, or subtypes of grassland communities on poorer soils from the main subtype on rich soils. It is also used to differentiate between syntaxa on soils with seasonally varying water regime from those with constant moisture conditions. This illustrates the intermediate habitat preference of the species. For example, in the Belgian Ardennes, Dumont (1979) describes a subass. colchicetosum (mainly characterised by Arrhenatheretalia species) within the Alchemillo-*Trisetetum flavescentis* HORVAT 1931 (Horvat, 1931), and a subass. colchicetosum within the Crepido-*Juncetum acutiflori* OBERD. 1957 (characterised by Molinietalia species, like *Juncus acutiflorus* or *Scorzonera humilis*).

In meadows on sandstone and marl in the Vienna Woods and adjacent parts of the Flysch zone, *C. autumnale* grows in association with species that indicate seasonally varying moisture (Ellmauer and Mucina, 1993). Such meadows have been classified as *Filipendulo vulgaris*–Arrhenatheretum HUNDT et HÜBL 1983. Steinbuch (1995) used *C. autumnale* to distinguish the subass. narcissetosum radiiflori at warm and base rich sites with varying moisture conditions within the *Festuco pratensis–Alopecuretum pratensis* STEINBUCH 1995. Furthermore, *C. autumnale* is characteristic of the *Betonica officinalis–Narcissus radiiflorus* community on loamy, periodically wet and basic soils in northwestern Styria/Austria (Bassler et al., 2000).

Where *C. autumnale* occurs in grassland communities of relatively dry habitats, e.g. in the *Onobrychido viciifoliae*–Brometum T. MÜLLER 1966 (Mucina and Kolbek, 1993), it indicates sufficient soil moisture for at least some part of the growing season. Zoller (1954) describes a Colchico-Mesobrometum ZOLLER 1954 from the calcareous Jura mountains on deep brown soils with a dense moss layer and a high frequency of *Arrhenatheretalia* species. The Colchico-*Festucetum pratensis* DUVIGNEAUD 1958 can be found on alluvial soils and includes species of semidry grasslands like *Bromus erectus* and *Salvia pratensis*, but also species of moist grasslands such as *Ranunculus repens* and *Filipendula ulmaria* (Grévilliot and Muller, 1995).

Besides grassland communities, *C. autumnale* can also be found in alluvial forests of the alliance Alnion incanae on nutrient rich and deep soils that are periodically flooded (Wallnöfer et al., 1993; Lazowski, 2001).

Response to abiotic factors

Plants at higher altitudes, e.g. near the southern distribution limits in Greece or southern Bulgaria, show smaller and fewer foliar leaves (sometimes only two). These plants also flower earlier (Schroeter, 1908; Persson, 1999a).

C. autumnale develops flowers and leaves simultaneously in spring, when it has been hindered to flower in autumn, e.g. by early snowfall, flooding or massive barriers, such as silage bales or piles of wood (Ascherson and Graebner, 1905–1907; Bornemann, 1920). Frost in spring can result in blackened leaf tips (Butcher, 1954). In a Polish study (Mróz, 2006), the number of fruits and seeds of *C. autumnale* correlated positively with soil pH and concentrations of Ca and Mg in soil; a negative correlation was detected for N and K concentrations in soil. In contrast, leaf number in vegetative adults correlated positively with soil N and K concentrations, but negatively with concentrations of Cu. Furthermore, leaf Ca content and number of fruits per *C. autumnale* plant can be influenced by soil type (Mróz, 2008). Germination seems to be especially sensitive to drought since only small numbers of seedlings can be found after dry years (Wehsarg, 1929). However, also long periods of water logging can cause the death of populations (Wehsarg, 1929).

Abundance

The local abundance of *C. autumnale* is influenced by several factors, i.e. type of grassland management (Winter et al., unpubl. data), stand height (Smith, 2004), and location within the distribution area (Hengeveld and Haeck, 1982). Average cover percentages of *C. autumnale* are mostly 1–5% but also reach 6–25% (Bassler et al., 1998). In moist meadows it can cover up to 50% of the total vegetation (Stebler and Schröter, 1891; Braungart, 1899). In grasslands in England, *C. autumnale* is usually abundant or sub-dominant but never forms closed stands (Butcher, 1954).

Life cycle and biology

Life cycle

C. autumnale is a polycarpic perennial geophyte. Its life cycle can be divided into seven developmental stages (Fig. 5): seed (not shown), seedling, small vegetative with one leaf (foliar leaf), medium vegetative with two leaves, large vegetative with three or more leaves, generative plant, and dormant plant. The arrows of Fig. 5 indicate possible transitions between these stages (see Caswell, 2001), e.g. a seedling either becomes a small vegetative plant with one leaf or it dies. A small vegetative plant remains several years in the same stage (e.g. on average 38.0% ± 28.9 SD of all small vegetatives remain within their stage from one to the next year, $n = 16$ populations, number of plants = 395) before it may develop two leaves or even two leaves and a capsule (generative plant). The developmental stages do not correspond to certain plant ages, but it can be assumed that generative plants are in most cases older than small vegetatives with one leaf. Fig. 5 indicates frequent transitions (transition probabilities greater than 30%) with thick, rare transitions (transition probabilities smaller than 5%) with dashed lines.

Data on the species' life cycle provided by the authors in this paper are based on nine German and eight Austrian *C. autumnale* populations in extensively managed grasslands of the class Molinio-Arrhenatheretea (Jung et al., 2010 and unpubl. data). Corm size and leaf number increase with plant age (Wehsarg, 1929; Rosenthal, 1963) as well as corm weight. The latter, however, varies greatly between plants of the same stage: small vegetatives have an average corm weight of 103 mg (SD = 151.0, $n = 33$), medium vegetatives of 295 mg (SD = 178.4, $n = 30$) and large vegetatives of 559 mg (SD = 241.6, $n = 10$) (Jung et al., unpubl. data). Based on corm depth in the soil and number of corm sheaths, plants are estimated to reach ages of at least 15–20 years (Rimbach, 1897; Franková et al., 2004). In the Botanical Gardens of the Martin-Luther-University Halle-Wittenberg plants have reached an age of more than 50 years (Jäger, pers. comm., 2010).

Survival rates of all life stages except seedlings range between 41.8 and 98.2% (average 82.8% ± 15.2 SD, $n = 16$; Jung and Winter, unpubl. data). Seedling survival averages 72.1% ± 30.8 SD ($n = 16$ populations, number of seedlings = 571; Jung and Winter, unpubl. data). It takes a seedling at least four to six years (Poutaraud and Girardin, 2006), sometimes up to 20 years (Loew and Kirchner, 1934), to become a flowering individual. Usually, individuals flow-

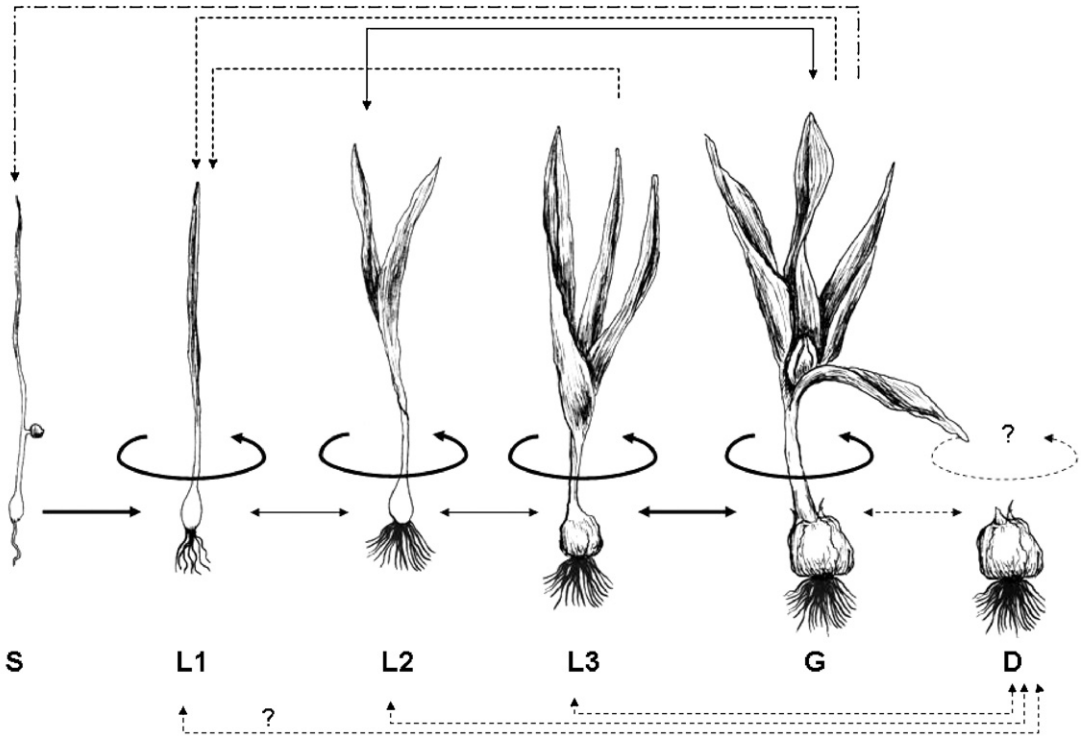

Fig. 5. Life cycle graph of *Colchicum autumnale*. S = seedling, L1–L3 = vegetative plant with one, two and three or more leaves, G = generative plant with capsules, D = dormant plant. Arrows indicate possible transitions between stages, the dash-dotted line represents fecundity, and the question mark doubtful transitions. Fat lines show transition probabilities greater than 30%, thin lines less than 30% and more than 5% and dashed lines less than 5%. Drawing by S. Rosner.

ering in autumn show three or more leaves in springtime and rarely only one or two (see Fig. 5). In a four-year-survey from 2006 to 2009 in Lower Austria, 14% of all plants flowering in 2006 did not flower again, 31% flowered once thereafter, 38% twice, and 17% flowered every year (n = 106, Winter et al., unpubl. data).

The old plant is replaced by a new shoot every year (Irmisch, 1850). As the innovation shoots are renewed completely every year, the life cycle of *C. autumnale* can be called pseudoannual. Plants with two or more leaves may reproduce vegetatively (Jaehn, 1984; Winter and Jung, unpubl. data; but cf. Wehsarg, 1929: three and more leaves), when the corm has a minimum size of 20–30 mm and thus has stored enough reserves (Godet, 1987). However, the proportion of vegetative reproduction depends on the site conditions. Generally, 0–30% of the plants in populations reproduce vegetatively (Godet, 1987). It Vegetative reproduction sometimes comprises only 5% (Butcher, 1954) but may increase to 47% if conditions are very favourable, as it is the case in cultivation (Poutaraud and Girardin, 2003). According to Mróz (2006), 9% of all individuals in meadows and 26% along dirt roads and shrubs develop two corms in the following year.

In England (Butcher, 1954) and Ireland (Smith, 2004), population growth seems to depend mostly on vegetative reproduction. This appears to be also true in tall grass vegetation in Austria whereas vegetative reproduction is low in short grass meadows (Winter et al., unpubl. data).

On average, adult plants generate 1.32 ± 1.19 SD (n = 16 populations, number of generative plants = 1015) seedlings per year (Winter and Jung, unpubl. data). Dormancy, i.e. the intermission of leaf development for one (or more?) years, occurs at different developmental stages: medium vegetatives with two leaves, large vegetatives with three or more leaves and generative plants (Smith, 2004; Winter et al., unpubl. data). It is still uncertain, whether also small vegetatives may become dormant. Dormancy percentages range between 1.8% and 11.7% of the vegetative and generative plants (Smith, 2004, n = 322; Winter et al., unpubl. data, n = 239). Calculated annual population growth rates vary between 0.7 and 1.2 (Smith, 2004: on average 1.01 ± 0.05 SD, n = 4 populations, number of plants = 1033; Jung et al., 2010: on average 0.99 ± 0.20 SD, n = 16 populations, number of plants = 2685) and may reach 1.41 (Winter and Kriechbaum, 2009) due to a high germination percentage.

Table 1
Range of alkaloid contents [% of dry mass] in different parts of *Colchicum autumnale*; n.s. = not specified (Petitjean et al., 1978; Muntean et al., 1981; Vicar et al., 1993; Wolf, 1995; Poutaraud and Girardin, 2002).

Alkaloid/plant part	Corm	Leaves	Flowers	Seeds
Colchicine [%]	0.12–1.9	0.02–1.42	0.15–0.85	0.14–1.2
Demecolcine [%]	0.18–0.37	0.08	n.s.	n.s.
Colchicoside [%]	n.s.	0	0	0.48–0.1

Spatial distribution of plants within populations

Spatial distribution of *C. autumnale* on meadows varies with population age and management practice. According to Wehsarg (1929), cluster formation can be promoted by a late mowing date (end of June or later) and a high population age, because most clusters originate from vegetative reproduction. This type of reproduction requires large nutrient reserves and is thus only common in older plants (Wehsarg, 1929; Godet, 1987). Additionally, tall vegetation may favour cluster formation, because seedling establishment is suppressed (Smith, 2004). Some clusters may also arise from seeds of one cohort, which germinated at one microsite (Wehsarg, 1929). According to Wehsarg (1929), a scattered distribution of individuals is predominant in grasslands with short

Table 2
Population size, area and plant density of *Colchicum autumnale* across Europe – the first row shows the lowest and the second row the highest plant density reported in the respective source. Number of plants per population in Belgium and Poland were estimated based on the counts of flowering individuals and the overall mean proportion of flowering plants; *n* = number of populations analysed; n.s. = not specified.

Country	Habitat type	*n*	Number of plants per population	Area (m^2)	Plant density/m^2	Source
Ireland	Meadow	4	45,741 270	30,000 50	1.52 5.40	Smith (2004)
Belgium	Meadow	17	65 3247	5300 5900	0.01 0.55	Adriaens et al. (2009)
Poland	Meadow	10	2063 290	22,500 400	0.09 0.73	Mróz (2006)
Poland	Verge	5	111 792	300 470	0.37 1.69	Mróz (2006)
Germany	Meadow	9	n.s. n.s.	n.s. n.s.	17.6 99.7	Jung et al. (unpubl. data)
Austria	Meadow	44	16,600 >1,000,000	2920 32,400	5.7 427 (1760)[a]	Graiss (unpubl. data)

[a] Plant density including seedlings.

vegetation, young populations, or populations mown when seeds are mature but leaves are still green. In the last case, nutrient storage in the plant corm might be impeded and thus vegetative recruitment prevented. Therefore, a scattered distribution may result from a generative rather than a vegetative reproduction (Wehsarg, 1929). Typical plant densities in natural populations range from 1 to 4 plants/m^2 (Poutaraud and Girardin, 2006) but in dense populations an average of 15–57 plants/m^2 is common (Jung et al., unpubl. data) with a maximum of 427 plants/m^2 without seedlings (Graiss, unpubl. data; see also Table 2).

Phenology

The seasonal development of *C. autumnale* is clearly structured into an autumnal period, lasting from the middle of August until the middle of November, and a photosynthetically active period, starting in March and finishing at the end of June (Franková et al., 2003a). In mid-August, the plant consists of the small macerated old corm, which is connected to the large nutrient-filled new mother corm. At this time, shoot development and the appearance of the second sheath leaf above the soil surface start, and thus summer dormancy ends. Between August and November flowers arise consecutively above-ground from the centre of the sheath (Muntean et al., 1981; Jäger and Werner, 2005). In mild winters, e.g. in England, flowers can even be found until February (Wehsarg, 1929). The flower anlage is already developed between the first week of May and beginning of June (Schumann, 1904; Heimann-Winawer, 1919). The outermost flowers develop at first and are consequently the first ones to appear above-ground; the innermost flower flourishes at last. In mid-July, cell division of the archespor in the anthers starts, resulting in microsporocytes (Heimann-Winawer, 1919). Meiosis takes place at the beginning of August (Godet, 1987). During flowering, roots arise from the root disc of the daughter corm. Root development can continue until January (Irmisch, 1850; Godet, 1987). At the flowering stage, the corm is only 2 mm high and hardly thicker than the stem (Wehsarg, 1929). After the flowering period, foliar leaves, stem, and capsules develop below-ground and grow just until below the soil surface. Their development until about November consumes the largest part of the corm reserves (Franková et al., 2003a). Then, the plant enters winter dormancy until the beginning of March. However, if weather conditions are suitable, leaves may continue growing during winter but remain inside the soil (Wehsarg, 1929). Springtime leaf appearance is accompanied by a strong decrease of starch content in the shrinking mother corm, whose reserves are mobilised for the growth of new leaves and capsules (Franková et al., 2003a). Additionally, newly photosynthesised assimilates are used for tissue development. In the middle of April/beginning of May, capsules are lifted above the ground and turn green. Young seeds are coloured white (Nordhagen, 1933). From mid-April to the middle of June/end of July, size (Wehsarg, 1929) and starch content (Franková et al., 2003a) of the daughter corm increase steadily. At the end of May, the former mother corm is completely exhausted, consisting only of its sheaths. The capsules ripen between the middle of June and the end of July, at the time when the programmed senescence of leaves, stem, and capsules starts (Wehsarg, 1929). Successively, all above-ground organs become yellow, brown, and dry. Roots disappear and seeds turn brown (Wehsarg, 1929). Capsules open and seeds are released after mid-June (until early August in the mountains; Müller-Schneider, 1986). The daughter corm becomes the new mother corm and the plant enters summer dormancy for 2–8 weeks (Godet, 1987). The sheath of the first foliar leaf remains, enclosing the corm as a brown dry tunic (Fig. 1, L_1n-1), which may persist several years (Wehsarg, 1929). This tunic forms a stiff, hollow tube above the corm, called the cap, which provides a passage for flowers and leaves. During ageing, the persisting sheath remains turn black.

Reproduction

C. autumnale is a hermaphrodite. A plant may flower for the first time at an age of four to six years (Poutaraud and Girardin, 2006). Plants originating from vegetative reproduction are already able to flower the following year (Mróz, 2006).

Flowers of *C. autumnale* are proterogynous and were long thought to be heterostylous (Knuth, 1899; Kerner von Marilaun, 1913). In fact, stamens grow gradually during anthesis so that each flower passes through three style phases, which were described by Heimann-Winawer (1919). At the beginning of flowering, stamens are much shorter than the styles (long style phase). Some days thereafter, flowers pass into the middle style phase as stamens extend without reaching the stigmas yet. In the final stage, the short style phase, the stamens grow up to or even above stigma level. Xenogamy is promoted in the early flowering stage, while autogamy is facilitated later (Heimann-Winawer, 1919). The diporate (Chester and Raine, 2001) and irregularly shaped pollen grains are up to 55 μm long and 32 μm wide (Heimann-Winawer, 1919). Outside they are yellow and oily with verrucous structures,

and inside they are filled with starch grains all over (Heimann-Winawer, 1919). Fertilisation of the ovule takes place 7–11 days after pollination (Hofmeister, 1861; Heimann-Winawer, 1919). Self-pollination leads on average to a smaller number of seeds per capsule (33.6 ± 5.03 SD) compared to cross-pollination (82.5 ± 5.23 SD; Muntean et al., 1981).

Important pollinators are *Bombus hortorum* and the honey bee (*Apis mellifera*), which already visit flowers that are just about to open. The bumblebee pollinates flowers by touching stigmas and stamens with the front of its body (Loew and Kirchner, 1934). Honey bees crawl down to the bottom to collect nectar without touching the anthers (Loew and Kirchner, 1934), but may become dusted with pollen, which has fallen onto the hairs of the nectar furrow (Butcher, 1954). They also crawl between the styles to collect pollen, thereby causing pollen transfer (Loew and Kirchner, 1934). Nectar-sucking butterflies like *Vanessa io* and *V. urticae* (Knuth, 1899), *Autographa gamma*, and *Macdunnoughia confusa* (Poschlod et al., 2003), but also a number of pollen-eating hover flies (*Eristalis tenax*, *Syritta pipiens*, *Syrphus arcuatus*, *S. corollae*, *S. pyrastri*, *S. ribesii*), feed directly on the anthers. They touch the stigmas only from time to time, but may still act as pollinators (Knuth, 1899). Small slugs and snails may cause contact between the stigmas and stamens when eating the petals (Hegi, 1910; Wehsarg, 1929). *Musca domestica* and other small muscides (Knuth, 1899; Hegi, 1910), as well as several thrips species, i.e. *Taeniothrips stratus*, *T. vulgatissimus*, *Thrips major*, *T. physapus* and *Frankliniella intonsa*, visit flowers and in that way may pollinate them (Butcher, 1954).

Percentage of flowering plants per population (considering only plants with more than one leaf) ranged from 5.0 to 72.3% in nine German populations of *C. autumnale* (mean 37.9% ± 18.6 SD, number of plants = 914; Jung et al., unpubl. data). Fruit set averaged 70.1% of all flowering individuals, varying between 42.3% and 88.6% in two Austrian populations over three years (SD = 17.4, *n* = 6; Winter et al., unpubl. data).

Diaspores are seeds, ripening inside the capsules. One single plant develops 1–6 capsules (on average 1.9 ± 0.9 SD, *n* = 770; Winter et al., unpubl. data). The number of seeds per capsule varies between 6 and 203, with an average of 74 ± 50.1 SD seeds (*n* = 40; Winter et al., unpubl. data). Seed production per plant ranges from 7 to 526 (average: 136 ± 108.6 SD, *n* = 40; Winter et al., unpubl. data). The following seed measurements are based on pooled material from four German populations. Mean seed length is 2.18 mm (SD = 0.33, *n* = 100; Jung et al., unpubl. data). Seed height and width average 1.77 mm (SD = 0.24, *n* = 100) and 2.03 mm (SD = 0.25, *n* = 100), respectively. Seed mass was measured on seeds from four Austrian populations and averaged 5.23 mg (SD = 1.30, *n* = 71 capsules; Winter et al., unpubl. data).

Seed release occurs under dry conditions (xerochasy; Müller-Schneider, 1986) as capsules open (Wehsarg, 1929), and is facilitated by wind (Loew and Kirchner, 1934). Seeds most often fall to the ground from a height of 0.05–0.4 m (Butcher, 1954; Jäger and Werner, 2005). During fall, the seeds accelerate up to 2.45 m/s (Tackenberg, 2001) or even 6.27 m/s (Maurer and Stöcklin, unpubl. data in Poschlod et al., 2003). Capsules still contain many seeds when peduncles and stem weaken and come to lie on the ground. Such basicarpy and the presence of a yellowish-white elaiosome are characteristics of ant dispersal (myrmecochory), which is the main way of seed dispersal in *C. autumnale* (Nordhagen, 1933; Persson, 1993). Ant species dispersing *C. autumnale* seeds are *Formica rufa*, *Myrmica rubra*, *Lasius niger*, and *Lasius emerginatus* (Müller-Schneider, 1986). Besides, seeds are dispersed by epi- as well as endozoochory. Especially grazing livestock transports the sticky seeds either on its hooves (Nordhagen, 1933) or inside its intestinal tract (Poschlod et al., 2003). Several human activities largely contribute to seed dispersal (agochory), e.g. by hay making, sowing grassland by the use of hay transfer of fresh plant material, and applying manure that contains *C. autumnale* seeds (Bornemann, 1920; Wehsarg, 1929). Capsules float and seeds may be transported long distances if capsules fall into streams (hydrochory; Wehsarg, 1929). Some seeds are not dispersed and germinate next to the parent plant.

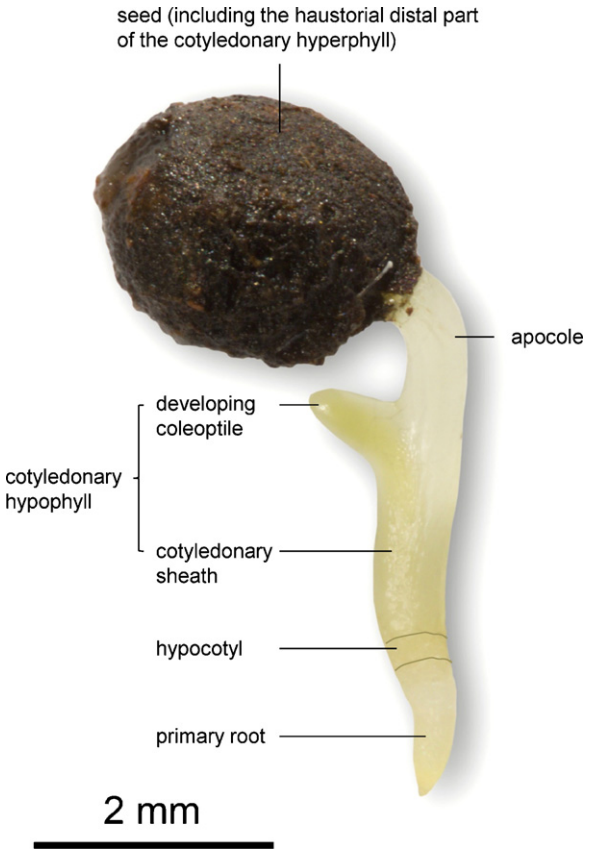

Fig. 6. Germinating seed of *Colchicum autumnale*. Photo by Josef Pennerstorfer.

Germination

Germination takes place in the autumn following seed shed (Irmisch, 1856; Loew and Kirchner, 1934) or one year later (Butcher, 1954; Jaehn, 1984). Germination percentage was 11–19% in common garden experiments, determined by the number of seedlings that appeared in spring following sowing (Rosenthal, 1963; Muntean et al., 1983). Higher germination of 48% was documented for preselected large seeds (diameter >1.6 mm), which were treated for 5 min in 3% NaOCl prior to sowing in the field (Kasnitz, 2010). Under lab conditions it is difficult to trigger germination (Jaehn, 1984; Poutaraud and Champay, 1995). Of the seeds from four pooled German populations stored for four months at room temperature, only 8.4% (SD = 4.85, *n* = 8, number of seeds = 440) germinated after a warm (alternating temperature: 25/15 °C) followed by a colder (15/5 °C) incubation temperature under dark conditions (Kasnitz, 2010). Treatment time until germination started, was 12 weeks. When seeds were disinfected for 5 min with 3% NaOCl, germination rates increased to 78.9% (SD = 8.24, *n* = 8, number of seeds = 440; Kasnitz, 2010). Seeds from seven Austrian populations that had been stored at room temperature for six months showed a mean germination rate of 32.8% (SD = 13.70, *n* = 28, number of seeds = 1400; Winter et al., unpubl. data) after a consecutive incubation in darkness at 15/25 °C,

20/10 °C, and 5 °C after five months. Scarification of seeds with sand paper did not influence germination (Winter et al., unpubl. data).

Existing data show that seed dormancy of *C. autumnale* is still not well understood. Physical dormancy can be excluded because seeds quickly absorb water without any special treatment (Jung et al., unpubl. data). Morphophysiological dormancy is characteristic of the closely related Liliaceae family (Baskin and Baskin, 2001; Fay et al., 2006). The underdeveloped linear embryo in mature seeds of *C. autumnale* (Fig. 2, Heimann-Winawer, 1919; Jaehn, 1984) and our experimental data support this interpretation. The seed bank of *C. autumnale* was classified as transient by Thompson et al. (1997), based on two studies (Von Borstel, 1974; Poschlod and Jackel, 1993) recording the seeds that germinated in soil samples taken from natural vegetation. In these studies, *C. autumnale* was found in the above-ground vegetation, but not in the soil samples. However, the apparent absence of *C. autumnale* in the seed bank may also be an artifact, if (i) no seeds were captured with the soil sample or (ii) germination conditions were not suitable for the species. Results of two burial experiments point at a short-term persistent seed bank of *C. autumnale*. In one experiment, an average of 1.6% ± 1.39 SD seeds ($n = 24$, 1200 seeds mixed from seven German populations) were still alive (tetrazolium test) after 1 year and 3 months burial in the soil (Jung et al., unpubl. data). One year later, this number had declined to 0.2% ± 0.67 SD. In another burial experiment at seven Austrian sites (seeds from those respective sites), 27.8% ± 20.9 SD seeds ($n = 35$, 1750 seeds) were classified viable by the pressure test after two years of burial (Winter et al., unpubl. data). Rosenthal (1963) observed germination of *C. autumnale* seeds in a field experiment over five years. Germination reached values between 11 and 22% in the first two years and around 1–2% in the fourth and fifth years after sowing.

Germination is hypogeal. The radicle appears first, followed by the cotyledonary sheath and the basal part of the cotyledonary hyperphyll (apocole). The distal part of the hyperphyll remains hidden inside the seed and has haustorial function (Loew and Kirchner, 1934; Jaehn, 1984; Fig. 6). After the cotyledon has reached its final length, the primary root commences elongation growth, and the coleoptile develops; its length is dependent on the depth of seed position. The first primary leaf emerges from the base of the sheath-like coleoptile but will not grow above the soil surface until next spring. The primary root only dies back when the seedling enters summer dormancy (Irmisch, 1856).

The narrow lineate primary leaf of the seedling is built by a sheath and a lamina, both with (5–)7(–9) parallel nerves (Irmisch, 1856; Wehsarg, 1929). Inside the sheath, a bud develops into a 2–3 mm thick corm with one renewal and one reserve bud (Wehsarg, 1929). The primary leaf and the renewal bud are separated by an angle of 140–150° (Irmisch, 1850; 144° in Wehsarg, 1929). This is a distinct difference to adult plants, where the renewal bud is located in the axil of the first foliar leaf. Besides the primary leaf, a scale-like or 2.5 cm long thread-like leaf may be present at the base of the primary corm in altered position (180°) with respect to the primary leaf (Irmisch, 1856; Loew and Kirchner, 1934). With the development of the renewal bud into a shoot, the monopodial structure of the axis changes into a sympodial structure, which is maintained throughout the plants' life, as the axes of several years remain connected (Wehsarg, 1929; Fig. 1).

Response to competition and management

Management measures, like late mowing or grazing, have positive effects on populations of *C. autumnale* (Mróz, 2006; Adriaens et al., 2009). Grazing animals create open sites, which may favour seedling establishment. The sticky seeds are dispersed to adjacent grasslands by endo- and epizoochory (Kerner von Marilaun, 1898; Müller-Schneider, 1948). Livestock mostly avoids grazing *C. autumnale* because of its toxicity. This selective grazing promotes its establishment and population growth on pastures. According to Smith (2004), population size is negatively correlated to vegetation height. Furthermore, the percentage of clumped individuals is higher in tall than in low grasslands, indicating a reduction of generative for the benefit of vegetative reproduction. In a cultivation experiment, a decline of the thousand-seed dry weight in clumps of five or more plants was observed, implying intraspecific competition (Poutaraud and Girardin, 2006). Plant size is related to the height of the surrounding vegetation; it can reach even 1 m at the verge of alluvial forests (Winter, pers. obs.).

Competition for light reduces the number of flowers per plant and fruit set (Bornemann, 1920; Godet, 1987; Mróz, 2006). In grasslands that have been reforested with conifers, *C. autumnale* disappears gradually after canopy closure (Bornemann, 1920). Nevertheless, the plant species can survive for a long time in grasslands afforested with deciduous trees, like poplars, oaks, or alders (Bornemann, 1920; Winterhoff, 1993; Van Landuyt et al., 2006; Karrer, pers. obs.).

C. autumnale is a well-known toxic grassland weed in Central Europe, and control measures were published already 80–100 years ago (e.g. Braungart, 1899; Bornemann, 1920; Wehsarg, 1929; Korsmo, 1930). After Second World War until the late seventies, it lost its importance as grassland weed in the course of the Green Revolution (industrialised agriculture). Recently, in several extensively managed Central European regions, populations of *C. autumnale* have increased again (Briemle, 2003). Due to its high toxicity, farmers are faced with severe problems. Considering the yearly life cycle, an early cut (in April or at the beginning of May) may weaken *C. autumnale* populations. By then, the daughter corm has not yet stored many reserves, and the starch reserves of the mother corm have been consumed for the growth of leaves and capsules (Franková et al., 2003a). If leaves are cut or damaged, *C. autumnale* is not able to produce new leaves in the same vegetation period. However, if only the tips of just emerging leaves are removed in late April or early May, the leaves continue growing (Winter, pers. obs.). In the years following such an early cut, large vegetative and generative plants produce fewer and often smaller leaves. Consequently, only a negligible number of plants will flower and reproduce (Winter and Jung, unpubl. data). Already Krašan (1873) showed that the artificial disruption of the photoactive period in May can impede flower development.

According to Klapp and Stählin (1936), the proportion of *C. autumnale* in harvested biomass decreases when grasslands are fertilised with phosphorus, potassium and nitrogen, as fertilisation promotes the growth of other more competitive plants. The intensive application of liquid manure after the first cut or after grazing in May leads to corm decay, followed by plant death (Bornemann, 1920; Wehsarg, 1935; Rauschert, 1961; Jürgens et al., 1968). Fertilisers with corrosive attributes like calcium cyanamide or kainite also damage the plant effectively (Fürst, 1926). On the other hand, fertilisation can stimulate *C. autumnale* to grow taller and stronger (Braungart, 1899; Zimmer et al., 2001). In general, intensified management with an early first cut or grazing in May, followed by the application of fertilisers, weakens the plants (Fürst, 1926; Diercks and Junker, 1959; Rauschert, 1961; Stählin, 1969; Elsäßer et al., 2009). This is also due to the lack of generative reproduction and to the failure of seed dispersal as a consequence of haymaking (Braungart, 1899; Bornemann, 1920).

C. autumnale rarely appears in grasslands that are grazed intensively in May, owing to the trampling effect (Bornemann, 1920; Korsmo, 1930; Wehsarg, 1935; Krause, 1955). Cattle must have grazing experience in order to avoid intoxications (Stählin, 1969; Briemle, 1996). The extent of *C. autumnale* intake, however, dif-

fers between cattle breeds. In a grazing experiment in Germany, it was observed that Galloway cattle fed on *C. autumnale* without any obvious damage to health, whereas the German breed "Hinterwaelder" avoided it nearly totally (Elsäßer, 2008).

According to an analysis of 1882 grassland relevés in Austria, *C. autumnale* occurs most frequently in meadows which are mown once a year and late in the season or are extensively grazed (Bassler et al., 2000, 2003; Bohner and Sobotik, 2000; Lichtenecker et al., 2003).

Herbicides can reduce (Reglone; Küthe, 1969), or totally eradicate (Paraquat; Davies, 1964) *C. autumnale* plants, but some herbicides like MCPB or MCPA do not show any significant effects (nowadays, the application of Paraquat, Reglone and MCPB is forbidden in Germany). The use of Glyphosate is not really effective against *C. autumnale* (Elsäßer, pers. obs.). Nevertheless, herbicides like Paraquat or Glyphosate also harm the herbage, and in this way force farmers to reseed their grasslands, a measure which is not always effective.

Herbivores and pathogens

Two basidiomycetes, *Urocystis colchici* (Schltdl.) Rabenh. (leaf smut) and *Uromyces colchici* Mass. (*Colchicum* rust), attack *C. autumnale* (Butcher, 1954).

Leaf smut (Ustilaginales) produces greyish blister-like sori filled with a large number of dark-brown spores on the leaves of the plant (Mordue and Ainsworth, 1984). Spores are released when the leaf epidermis is cracking. The fungus probably spreads through ustilospores that germinate from infected plant remains inside the soil (Mordue, 1988). *Urocystis colchici* is distributed in Europe as well as in Canada, the USA, Russia, Japan, India, and Turkey (Mordue, 1988). Infection with leaf smut leads to reduced colchicine content (Zogg, 1985) and necrosis of leaves (Butcher, 1954). Although large outbreaks may occur, in most cases the smut is of minor or only local importance (Mordue, 1988).

Colchicum rust belongs to the Uredinales and produces blackish-brown sori on both sides of the leaf-blade (Massee, 1892) as well as on the tunic, i.e. the leaf sheath enclosing the corm (Boerema, 1961). After being released, the pale brown teleutospores that remain on the plant or in the soil need to hibernate before being able to infect other plants (Massee, 1899). This rust fungus occurs in the Caucasus region (on *Colchicum speciosum*, Kuprevich and Ul'janishchev, 1975) and sporadically throughout entire Europe (Gäumann, 1959). It is regarded as a rare disease (Boerema, 1961). The host range of both basidiomycetes is restricted to the genus *Colchicum* (Boerema, 1961; Mordue, 1988).

Further plant pathogenic fungi on *Colchicum* are the imperfect (mitosporic) species *Ascochyta juelii* Bubák, *Septoria colchici* Pass., *S. gallica* Sacc. & P. Syd., and the rust fungus *Uredo colchici – autumnalis* Guyot & Massenat (Brandenburger, 1985).

Recently, Meadow saffron breaking virus (MSBV), a potyvirus on *C. autumnale*, was discovered and described by Poutaraud et al. (2004). The virus occurs in corms, leaves and flowers, but not in seeds, and spreads rapidly between plants, probably through aphids as vectors. Infection leads to yellowing, deformation, and necrosis of leaves as well as to petal break of flowers. The infection rate was specified in one natural population to be 70%, and the severity of symptoms indicated ecological importance. The only record of MSBV originates from Wasserbourg in France. The host range of the virus seems to be limited to *C. autumnale*, but closely related families, i.e. Liliaceae, remain to be tested.

Small slugs and snails feed on *C. autumnale*, but rather help the plant to self-pollinate than cause any damage (Hegi, 1910; Wehsarg, 1929). Slug species of the genus *Arion* (*A. lusitanicus* or *A. rufus*), however, may reduce fertility and assimilation rate of single plants when feeding on flowers and leaves (Jung, pers. obs.). Larvae of the moths *Cnephasia pasiuana* and *C. stephensiana* (det. P. Buchner, 2008) also role up leaves and feed on these, thus reducing the photosynthetic surface (Winter and Jung, pers. obs.). *Cnephasia* species are not specialised on *C. autumnale*, but they are polyphageous on different herbaceous plants, e.g. *Antirrhinum*, *Bromus*, *Cardaminopsis*, *Helianthemum*, *Lychnis*, and *Pimpinella* (Hering, 1957). Larvae of an unknown moth species feed on the corm of *C. autumnale* and may consume a large amount of storage compounds of single plants (Jung, pers. obs.).

Mycorrhiza

C. autumnale possesses a vesicular-arbuscular mycorrhiza (Poschlod et al., 2003) of the Paris-quadrifolia-type, which was described in detail by Birgel (1953). The mycorrhiza is restricted to certain cell-layers and characterised by an intracellular mycelium with assembled arbuscules and sporangioles. The exterior mycelium is strongly developed and may thus promote plant growth and nutrient supply. Intensive mycorrhiza formation occurs in natural *C. autumnale* populations on almost every soil type, e.g. sand-loess, loess, and sand. No mycorrhiza was observed on loess-clay and peat (Birgel, 1953). Even on soil which lacked vegetation for several years, mycorrhiza developed, pointing at a saprophytic or persistent phase in the life cycle of the mycorrhiza.

Physiological data

C. autumnale conducts the C3-pathway of photosynthesis (Poschlod et al., 2003). The corm of the plant mainly consists of starch (~50%) and includes 8% proteins, 5% free sugars (sucrose, glucose, fructose), and 3% lipids (Franková et al., 2003b, 2005a). Seeds contain 20% protein in form of aleurone, lipids (8%), and, furthermore, phytosterol, sugar, gallic acid, and starch (i.e. in the endosperm; Wehsarg, 1929).

Starch and protein metabolism are key processes during the seasonal development of *C. autumnale* and were described in detail by Franková et al. (2003a, 2006). Starch degradation in the mother corm characterises the autumnal phase and the phase of leaf development in spring. It is governed by the enzymes α-amylase, β-amylase, and α-glucosidase. The formation of starch in the daughter corm starts in autumn and is the result of nutrient transition from the mother corm. Starch content then increases rapidly from the end of April until mid-June, when photosynthetic assimilates are produced. During winter, high levels of sucrose in both corms probably serve cryoprotection.

Proteins in the daughter corm are formed from the pool of free amino acids in autumn. During this time, protein content in the mother corm stays constant. The free amino acids in the daughter corm may originate from protein turn-over in both corms, as indicated by high proteolytic activity. Roots do not provide nitrogen during autumn, as indicated by their low nitrate reductase activity at this time of the year (Franková et al., 2005b). The period from winter until the beginning of plant development in March is characterised by high proteolytic activity, resulting in decreased protein content in both corms (Franková et al., 2006). Responsible enzymes for proteolysis are five exopeptidases (L-Ala-AP, L-Leu-AP, Z-Glu-Tyr-CP, Z-Glu-Phe-CP, and Gly-L-Pro-DPP) and two endopeptidases (Suc-Phe-EP and BAPA-ase). In March and April, nitrate reductase reaches maximum activity in the roots, suggesting that nitrogen supplied by the mother corm does not satisfy the demand of the growing plant (Franková et al., 2005b). Although nitrate reductase is mainly active in the roots, it also occurs in leaves and stem and to a small amount in the two corms.

Biochemical data

Around 30 tropolone alkaloids have been discovered in *C. autumnale* (Poutaraud and Girardin, 2003). Main alkaloids are colchicine, demecolcine, 3-demethylcolchicine, and colchicoside (Liebenow and Liebenow, 1981; Poutaraud and Champay, 1995). Each plant part contains alkaloids to different percentages; ranges are given in Table 1 (Petitjean et al., 1978; Vicar et al., 1993; Poutaraud and Girardin, 2002). The alkaloid content varies in the course of the vegetation period in corms, leaves, capsules, and seeds (Vicar et al., 1993; Poutaraud and Girardin, 2002), and during the anthesis of flowers (Poutaraud and Girardin, 2002). Furthermore, the alkaloid content may be influenced by soil mineral composition (Mróz, 2002; Poutaraud and Girardin, 2005) as well as the time of day, weather conditions, or exposition (Seifert, 1979). The fermentation process in silage fodder over eight months leads to a decrease in colchicine content of *C. autumnale* plants, e.g. from 0.249% to 0.11–0.19% (Chizzola, unpubl. data). According to Wehsarg (1929), the alkaloid content in hay does not decrease with storage time. Chizzola (unpubl. data) found that colchicine content declined by 2.2–72.4% (mean = 15.3%, SD = 19.1, $n = 12$) in dried and grounded plant materials after one year storage. In hay with *C. autumnale* contents of 2.4–16.7% (mean = 10.59, SD = 13.74, $n = 9$), colchicine concentrations ranged from 0.008–0.092% (mean = 0.046, SD = 0.033, $n = 9$; Chizzola, unpubl. data).

Colchicine and its derivatives are highly toxic alkaloids, which lead to death after ingestion of only a few mg/kg body weight (median lethal dose: LD_{50} of colchicine in cattle: 1 mg/kg; Althaus, 2010). Thus, *C. autumnale* is regarded as one of the most important and dangerous grassland weeds in Central Europe (Hegi, 1910; Bornemann, 1920). In general, livestock neither consumes *C. autumnale* in fresh nor dried condition (Wehsarg, 1929; Stebler and Schröter, 1891), but every now and then intoxications occur in cattle, horses, sheep, and pigs, sometimes leading to death (Chareyre et al., 1989; Lohner and Gindele, 1989; Cooper and Johnson, 1998; Chizzola and Janda, 2002). Main reasons for intoxications are chaffed fodder and food scarcity, e.g. during pasturing in spring (Stebler and Schröter, 1891; Wehsarg, 1929).

Colchicine possesses a bitter taste (Autenrieth, 2008) and acts as feeding deterrent on *Locusta* spp., polyphagus *Syntomis* larvae, *Agelaius* (Wink, 1993), and livestock (Wehsarg, 1929). The alkaloid thus protects the plant from herbivores and might also prevent virus, bacterial or fungal infection, as is generally presumed for alkaloids (Wink, 1992; Fattorusso and Taglialatela-Scafati, 2008). An auxiliary function of colchicine could be the transport and storage of nitrogen (Wink, 1992). Allelopathic effects were demonstrated by the inhibition of seedling development in *Lepidium* (median effective dose: ED_{50} = 0.01%; Wink, 1993).

Genetic data

C. autumnale individuals from Bulgaria, France, Italy, Spain, Switzerland (Persson, 2009), and Austria (Dobes and Hahn, 1997) contain 36 chromosomes ($2n = 4x = 36$). Six chromosomes are 3.7–5 μm long, whereas the length of the others is about 1–2 μm (Perrenoud and Favarger, 1971). Different chromosome numbers in other publications ($2n = 38$: Levan, 1940; Levan and Steinegger, 1947; Muntean et al., 1987) seem to be due to aneuploidy, methodical difficulties (fragmentation of chromosomes), the misinterpretation of multiple constrictions, or the reunion of short chromosomes (Fridlender et al., 2002). *C. autumnale* is a tetraploid plant with a 2C genome size (DNA) of 5.89 pg (= 5.7×10^9 bp; Fridlender et al., 2002). The majority of *Colchicum* species (about 75%) show variable levels of polyploidy, e.g. *C. corsicum* has 24 times ($2n = 216$) the basis genome of *C. autumnale* (Persson, 1993).

Intraspecific genetic variation in *C. autumnale* was analysed by Smith and Waldren (2010) based on AFLP data of 20 populations from Ireland, Britain, France, and Spain. In total, 90% of the AFLP loci were polymorphic. The proportion of polymorphic loci in single populations varied between 0.039 and 0.394. Nei's gene diversity, i.e. the average genetic diversity across all loci, ranged from 0.055 to 0.222 among populations. Total gene diversity was 0.305, and average within-population gene diversity was 0.142. Analysis of molecular variance showed that population differentiation was high (fixation index: $F_{ST} = 0.683$; Smith and Waldren, 2010).

There exist 10 DNA sequence entries in the EMBL-GenBank (19.03.2010), most of them derived from parts of the chloroplast genome (Vinnersten and Reeves, 2003: atpB-rbcL intergenic spacer, tRNA-Leu gene, trnL-trnF intergenic spacer, rps16 gene; Case et al., 2008: atpB-rbcL intergenic spacer, rbcL gene, ndhF gene; Sramkó et al., 2008: cpITS2, 4.5 S gene, cpITS3; Hahn, 1999, unpubl.: atpB gene). Only three entries exist for ribosomal DNA: 26 S ribosomal DNA gene (Neyland, 2000, unpubl.), 18 S rDNA gene (Soltis et al., 1997), and nuclear encoded ribosomal DNA ITS1 (Table 2) (Sramkó et al., 2008).

Hybrids

There exists one record of natural hybrids between *C. autumnale* and *C. alpinum* in the French Alps (Perrenoud and Favarger, 1971). These hybrids show intermediate chromosome numbers of $2n = 46–47$ (*C. autumnale*: $2n = 36$, *C. alpinum*: $2n = 56$) and exhibit rudimentary pollen production as well as poor pollen germination rates (Perrenoud and Favarger, 1971).

Status of species

C. autumnale is not threatened in the centre of its distribution area. However, it can be found in Red Data Books of several countries at the distribution limits: Great Britain, Ireland, The Netherlands, Luxembourg, Lithuania, Estonia, Belarus, Ukraine, Albania, and northern federal states of Germany (Table 3).

A clear decline of *C. autumnale* sites is reported from Poland (Zarzycki et al., 2002 cited in Mróz, 2006) and the Ukraine (Sheliah-Sosonka, 1996). Intensification of agricultural production, draining, ploughing, as well as re-seeding of grasslands (Mennema et al., 1985; Hardtke and Ihl, 2000; Adriaens et al., 2009; Smith and Waldren, 2010), seem to be the most important threats to *C. autumnale*. The collection of flowers and bulbs of this medicinal plant are mentioned as further reasons for its decline in the Ukraine, Serbia, and Poland (Sheliah-Sosonka, 1996; Piękoś-Mirkowa and Mirek, 2003 cited in Mróz, 2006; Dajić, 2004). It is forbidden to collect seeds or bulbs in Italy and Poland (Vender and Fusani, 2004; Węglarz and Geszpyrch, 2004). Hydrological alterations also appear to influence the decline or the increase of *C. autumnale* (flood control measures can increase the abundance due to shorter periods of flooding; Wehsarg, 1929). In its core distribution area, e.g. Germany and Austria, in extensively managed grasslands, populations of *C. autumnale* seem to have increased during the last years (Briemle, 2003; Winter and Kriechbaum, 2009). In some regions, agri-environmental programmes support low input agriculture, and may in this way promote population growth of *C. autumnale*.

C. autumnale has been used in medicine for over 2000 years (Hartung, 1954) and its alkaloids possess anti-inflammatory, myorelaxant, and analgesic properties (Forni and Massarani, 1977). Over the last decades, colchicine and its congeners have been applied in traditional medicine (Le Hello, 2000) as well as in homeopathy (Gessner, 1974) in a great variety of fields, e.g. dermatology,

Table 3
European countries and federal states of Germany which include *Colchicum autumnale* in their Red lists.

Country	IUCN status	Source
Ireland	Critically Endangered	Preston et al. (2003), Kingston (2005)
Albania	Endangered	Vangjeli et al. (1995)
Lithuania	Endangered	Balevicius and Ladyga (1992)
Luxembourg	Endangered	Colling (2005)
Belarus	Vulnerable	Maximovitch (1993)
Estonia	Vulnerable	Lilleleht (2001–2002)
Ukraine	Vulnerable	Sheliah-Sosonka (1996)
Great Britain	Near threatened	Preston et al. (2003) and Cheffings and Farrell (2005)
The Netherlands	Extremely rare[a]	Van der Meijden et al. (2000)
Germany	Not listed for the entire country	Korneck et al. (1996)
Brandenburg	Critically endangered	Ristow et al. (2006)
Saxony	Critically endangered	Schulz (1999)
Lower Saxony and Bremen	Endangered	Benkert et al. (1996), Garve (2004)
Northrhine-Westphalia	Endangered	Wolff-Straub et al. (1999)
Saxony-Anhalt	Endangered	Benkert et al. (1996), Frank et al. (2004)
Mecklenburg-Western Pomerania	Extremely rare[a]	Voigtländer and Henker (2005)

[a] IUCN status missing.

rheumatology, cardiology, phlebology, and hepatogastroenterology (Le Hello, 2000). Their therapeutic effect for most indications is based on the depolymerization of microtubules (Levy et al., 1991). Today, colchicine is an approved treatment for gout, familial Mediterranean fever, amyloidosis, sarcoidosis, Behçet's syndrome, and scleroderma (Ghosh and Jha, 2008). However, due to the high toxicity and low therapeutic index of colchicine, its practical application is limited (Ghosh and Jha, 2008). In plant breeding it is used to induce polyploidy in crop plants (Roberts and Wink, 1998).

The late flowering time and pretty flowers make *C. autumnale* an appreciated ornamental plant. Bowles (1952, 161) lists four different varieties of *C. autumnale*: variety *album* with white flowers, variety *striatum* "*with irregularly striped pink and white flowers*", variety *alboplenum* with double white flowers, and variety *pleniflorum* with "*double lilac flowers*".

Acknowledgements

We thank H. Niklfeld for information on distribution, W. Holzner for his help with the 'Communities' section, H.-J. Tillich for his help to clarify morphological contradictions, the description of seedling morphology and germination, M. Kasnitz and J. Pennerstorfer for the photographs, S. Rosner for the drawings, G. Bassler and A. Lichtenecker for data on pH-values and cover percentages of *C. autumnale* (the 'Abundance' and 'Habitat' sections) and R.J. Smith and S. Waldren for information on Ireland, E. Jäger and K. Marhold for literature on germination and chromosomes, P. Buchner for the determination of *Cnephasia* spp., M. Kühlmann for collecting data on seed morphology, J. Scholz vom Hofe for logistic help with many experiments, and the following people who provided information about the Red List Status of *C. autumnale*: A. Clemente (P), M. Chytry (CZ), Z.D. Stevanovic (RS), V. Feráková and K. Hegedusova (SK), F. Prosser (Trentino), L. Popescu (RO), T. Nantsou, T. Constantinidis and G. Kamari (GR), T. Wilhalm (Südtirol), V. Matevski (MK), and P. Wind (DK). For constructive suggestions and discussion we thank A. Bohner, W.B. Dickoré, J. Greilhuber, E. Hübl, J. Kosa and M. Kropf. We thank the reviewers (E. Jäger and one anonymous reviewer) for his/her thorough review and highly appreciate the comments and suggestions, which significantly contributed to improving the quality of the publication. We are grateful for the financial support provided by the Deutsche Bundesstiftung Umwelt (ref. no. 25073-33/0), the Federal Ministry of Agriculture, Forestry, Environment and Water Management in cooperation with federal states of Austria, Jubiläumsfonds der Österreichischen Nationalbank, Österreichische Bundesforste, and Biosphärenpark Wienerwald Management GesmbH.

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6. Above- and below-ground nutrient and alkaloid dynamics in *Colchicum autumnale*: optimal mowing dates for population control or low hay toxicity

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Jung L. S., Eckstein R. L., Otte A., Donath T. W. (2012). Weed Research 52: 348-357.

WEED RESEARCH An International Journal of Weed Biology, Ecology and Vegetation Management

DOI: 10.1111/j.1365-3180.2012.00923.x

Above- and below-ground nutrient and alkaloid dynamics in *Colchicum autumnale*: optimal mowing dates for population control or low hay toxicity

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Received 28 April 2011
Revised version accepted 30 March 2012
Subject Editor: Cesar Fernandez-Quintanilla, CSIC, Madrid

Summary

In some Central European regions, the conservation of seminatural grasslands is jeopardised by management intensification or abandonment, caused by high densities of the toxic weed *Colchicum autumnale*. We investigated two possibilities to deal with *C. autumnale*: (i) reducing population densities by mowing when nutrient contents are high in leaves and capsules and low in the storage organs, that is, when the ratio between both is highest, or (ii) reducing alkaloid content in hay by mowing when alkaloid content of leaves and capsules of *C. autumnale* is low. To identify the optimal mowing point, we analysed the dynamics of nutrients, starch and alkaloids of naturally grown plants in two biogeographical regions. In the colder region, the maximum nutrient ratio between above-ground and storage organs, as well as alkaloid content in leaves and capsules, occurred significantly later. Compared with the common first mowing date (15 June), alkaloid content decreased significantly until 5 July in both regions. On both dates, it was on average 1.8 times higher in the colder region. Our results suggest the following time for the two management options: (i) mowing at about 25 cm plant height (late April/early May) to reduce *C. autumnale* densities or (ii) delayed mowing in late June/early July when the plant has turned brown and dry and alkaloid content has declined.

Keywords: Meadow saffron, toxic weed, management, grassland, nitrogen, resource depletion, colchicine, physiology.

JUNG LS, ECKSTEIN RL, OTTE A & DONATH TW (2012). Above- and below-ground nutrient and alkaloid dynamics in *Colchicum autumnale*: indications for optimal mowing dates for population control or low hay toxicity. *Weed Research* **52**, 348–357.

Introduction

Colchicum autumnale L. (meadow saffron, autumn crocus) is a well-known toxic grassland weed in several parts of Europe (Davies, 1964; Briemle, 2006; Winter *et al.*, 2011). Horses, cattle and sheep generally avoid the plant in hay and on the pasture (Wehsarg, 1929; Stebler & Schröter, 1981), but occasionally, intoxications are reported, sometimes leading to death (Chareyre *et al.*, 1989; Panariti, 1996; Kupper *et al.*, 2010). During recent decades, *C. autumnale* has become an increasing problem in extensively managed seminatural grasslands of some Central European regions (Briemle, 2006; Winter *et al.*, 2011). Farmers with high population densities of the plant have increasing difficulties to market their hay. As *C. autumnale* is sensitive to an increased cutting frequency, there is a risk that farmers intensify or completely abandon management. However, seminatural

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grasslands are amongst the most species-rich ecosystems in Europe (Pärtel *et al*., 2005), and many grassland types are endangered habitats (Council of Europe, 2010). As seminatural grasslands are only maintained by low-input agriculture, there is an urgent need for management schemes to address high densities of *C. autumnale* without compromising the high nature conservation value of these meadows.

Colchicum autumnale is a perennial geophyte with a corm as storage organ that is replaced every year. Given its extraordinary yearly life cycle (Wehsarg, 1929; Franková *et al*., 2003), *C. autumnale* profits from extensive management. After summer dormancy, a new shoot with flowers develops from the plant corm in autumn. Subsequent to flowering, leaves and capsules develop below ground before winter, allowing the plant to start its growth early next spring. During spring development, storage reserves of the old primary corm are strongly reduced. As the vegetation period progresses, excess photosynthetic products are stored in the growing new plant corm. Seed shed and senescence of above-ground plant parts start around mid-June. Thus, the plant is able to complete its photosynthetically active period before the first mowing in extensively managed grasslands takes place.

As an effective method to reduce high densities of *C. autumnale*, where single-plant removal is not feasible, an early cut of leaves and capsules is promoted (Wehsarg, 1929: Lower Bavaria, Germany; Briemle & Elsässer, 2008: southwest Germany). However, the best time for an early cut is not clearly indicated in the literature. For instance, Briemle and Elsässer (2008) suggest mulching the plant at a height of 10 cm (in about mid-April) or when *Taraxacum officinale* sheds its seeds (end of April/beginning of May). The very beginning of May is also recommended by Wehsarg (1929), although he mentions that the date may vary between climatically different regions. Stebler and Schröter (1981, Luzern and Waadt, Switzerland) favour mid-May as the time to remove leaves, that is, when plant capsules already start ripening. As the corm of adult plants is located at 15–20 cm soil depth and leaves and capsules develop inside the soil before winter, a large part of the plant is still located below ground in early spring. Therefore, a too early cut only removes the leaf tips, so that leaves will still grow to large size after the cut and capsules develop normally (LS Jung, pers. obs.). Only if the vegetative part removed is large enough, will *C. autumnale* plants become smaller (fewer leaves) in subsequent years or disappear, resulting in a lower population growth rate and fewer flowering plants (Jung *et al*., 2010). Flower removal in autumn hardly reduces the viability of *C. autumnale* populations (Jung *et al*., 2010) for several reasons: only about 40% of the individuals with more than one leaf produce flowers (Jung *et al*., 2011), plants flower successively from August until October (necessitating several cuts to remove all flowers) and flower tissue is very delicate and short-lived (few plant resources are removed with flowers).

Plant metabolism is crucial to address when deciding on the optimal mowing point (Nkurunziza & Streibig, 2011). In general, perennial plants mobilise carbon, nitrogen and phosphorus from their storage organs at the beginning of the vegetation period, when growth is most rapid (Chapin *et al*., 1990). On the other hand, these nutrients may be diverted from growth early in the vegetation period to form new storage reserves (Lambers *et al*., 1998). Therefore, from a physiological point of view, the best time to reduce vitality of *C. autumnale* by removing above-ground plant parts is when storage reserves in the old and new corm are low and nutrient contents of leaves and capsules are high, guaranteeing that a large enough plant part and enough plant resources are removed. Up to date, only two studies have investigated the temporal change of two storage compounds, that is, protein and starch content (Franková *et al*., 2003, 2006), in the corms of *C. autumnale,* and no study has regarded changes in nutrient content simultaneously in above- and below-ground plant parts. As leaf removal reduces *C. autumnale* populations much more effectively than flower removal, we investigated *C. autumnale* only during its photosynthetically active period. Our first objective was to compare the temporal variation of nitrogen, phosphorous and potassium content in above-ground parts (leaves and capsules) and corms and, additionally, starch in the corms (as the major storage compound, Franková *et al*., 2003), between two biogeographical regions. Based on these data, our second aim was to identify the optimal point for leaf removal of *C. autumnale* in each region. In the following, we will use the term 'mowing' equivalent to 'leaf removal'. We use both expressions as neutral concerning the utilisation of the cut biomass, which thus is not necessarily used as fodder.

The high toxicity of *C. autumnale* results from several alkaloids (mainly colchicine, demecolcine and colchicoside), which are present in all plant parts (Vicar *et al*., 1993; Poutaraud & Girardin, 2002). Although the main function of alkaloids is plant defence, they may also serve for the storage or transport of nitrogen (Wink, 1987). Consequently, alkaloid content in *C. autumnale* leaves and capsules decreases at the end of the growing season (Vicar *et al*., 1993; Poutaraud & Girardin, 2002). Although there are several published studies on alkaloid content in leaves and seeds (e.g. Vicar *et al*., 1993; Mróz, 2002; Poutaraud & Girardin, 2002), no one has yet documented alkaloid changes in shorter than monthly

time intervals or compared several populations. Thus, our third study objective was to investigate when and to what extent alkaloid content (measured as the sum of colchicine, demecolcine and colchicoside content) decreases in above-ground parts during senescence. Because of the flowering biology of *C. autumnale* described earlier and the low biomass of the delicate flower tissue, there is little alkaloid-containing material in hay from an autumn cut. Therefore, this cut can generally be marketed without problems, and we did not include flowering plants into alkaloid analysis.

We addressed the following questions:

- Does the temporal pattern of nutrient and alkaloid content differ between biogeographical regions?
- When is the optimal time for mowing as a control measure, that is, when is the ratio of nutrient content between above-ground and below-ground parts at its highest?
- When and to what extent does alkaloid content in above-ground plant parts decrease?

Materials and methods

Study sites

Colchicum autumnale plants were collected from six populations, which were located in two biogeographical regions in Hesse (Germany). The study region Lahn-Dill Highlands (LDH) is located in the west of Hesse and characterised by average yearly precipitation sums of 900–1000 mm (HLUG, 2009), a mean daily air temperature of 7–8°C per year and a vegetation period (days with mean daily temperature ≥5°C) of 120–220 days (Deutscher Wetterdienst, 1981). Populations in this region grow at an altitude of 250–450 m. The *C. autumnale* populations investigated in the second region, Vorderer Vogelsberg (VV), are located at 180 m above sea level. This region (situated in the centre of Hesse), has lower annual precipitation sums of 600–700 mm, a warmer climate with an annual mean daily temperature of 9–10°C (HLUG, 2009) and a longer vegetation period of 220–230 days (Deutscher Wetterdienst, 1981).

At each sampling site, two data loggers (TG-4080 Tinytag Transit) were buried at 2 cm below soil surface to measure soil temperature at 1-hour intervals from January until December 2009. Mean daily soil temperature was then calculated across all populations from one region. The annual mean daily soil temperature in 2009 was 9.4°C ± 0.53 SD in LDH and 10.5°C ± 0.67 SD in VV (Fig. 1). A mean daily soil temperature equal to or above 5°C was found in 2009 on 270 days ± 9.1 SD in VV, but only on 249 days ± 1.0 SD in LDH.

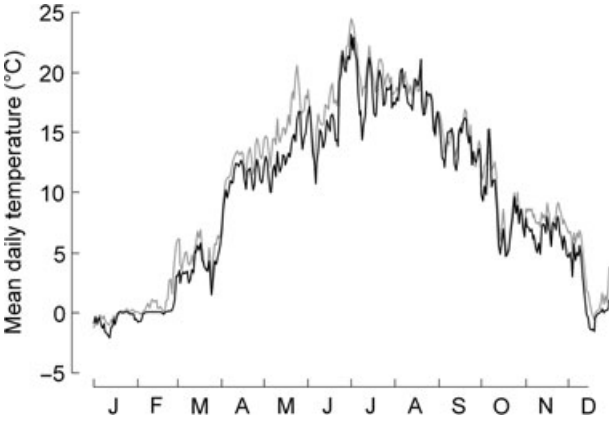

Fig. 1 Mean daily temperature at 2 cm below soil surface in 2009 in two regions in Hesse, Germany. Grey line = region Vorderer Vogelsberg (VV), black line = region Lahn-Dill Highlands (LDH).

Plant sampling and preparation

Plant samples were collected in 2009. Sampling started on 30 March, when *C. autumnale* plants were about 6 cm high, and was carried out every 14 days until 27 April. From that date onwards, samples were collected every 10 days until plant leaves had turned brown and dry and the species entered summer dormancy (15 July in VV and 3 August in LDH). At each sampling date, we measured leaf length of at least five plants prior to digging out 15 plants per population. Primarily, plants with three leaves and one capsule were collected, and only as an exception, plants with four leaves or two capsules were sampled. Plants were separated into three fractions: (1) leaves plus capsules, (2) old corm and (3) new corm. After capsules had opened (25 June in VV and 5 July in LDH), fraction (1) was analysed as two separate fractions (1.1) leaves plus capsules without seeds and (1.2) seeds. For each fraction, all plants from the same population were pooled. Old and new corms were cut across the middle, resulting in two samples of 15 corm halves for each corm type per population. One sample of corm halves of each corm type and the fraction 'leaves plus capsules' were dried at 60°C for about 40 h, weighed, coarsely ground with a cutting mill (SM 300; Retsch, Haan, Germany) and then finely ground using a vibratory disc mill (T 100; Siebtechnik GmbH, Muelheim/Ruhr, Germany). The ground samples were analysed for concentrations of total nitrogen, phosphorus and potassium, and the quantity of the alkaloids colchicine, demecolcine and colchicoside. The other 15 halves of each corm were frozen, freeze-dried for about 70 h, weighed and finely ground. In these samples, starch content was determined. Until being analysed, all samples were stored dry at −20°C.

Nutrient and alkaloid analyses

For analysing total phosphorus and potassium contents, 500 mg of each sample was ashed in a muffle furnace at 550°C for 18 h, cooled and spiked with dilute nitric acid. The solution was boiled and filtered into a beaker, which was filled up to 50 mL with distilled water. The concentration of total phosphorus was determined by the vanadate–molybdate method (Kitson & Mellon, 1944; spectral photometer used: PM7; ATG GmbH, Oberkochen, Germany). Potassium analysis was carried out in an atomic absorption spectrometer (220 FS; Varian, Melbourne, Australia) at wavelength 404.4 nm and slit 0.5 nm. Total nitrogen concentration of the ground plant tissue was determined using an elemental analyser (CE instruments, EA 1110, Italy; CE Instruments, 1996). Starch concentration was analysed applying a starch-UV-test kit (Company Boehringer Mannheim GmbH/R-Biopharm, Germany). This test starts with an enzymatic starch digestion and determines starch content indirectly via the content of free glucose molecules. As corms of *C. autumnale* contain free glucose besides starch, glucose quantity was determined prior to starch hydrolysis and later subtracted. As the old corm withered with the advancing vegetation period, starch analysis was stopped after 5 June; nutrient analyses continued until 5 July in VV and 15 July in LDH.

Alkaloid analysis was based on the method of Körner and Kohn (2005) and modified as follows: 0.1875 g of a ground plant part sample was extracted with a 25 mL of methanol–water mixture (50:50, v:v), containing 0.01 mM EDTA, in an ultrasonic bath for 2×15 min and subsequently filtered through a 0.45-μm PTFE filter. The extract was assayed by HPLC with a Merck Superspher 100 RP-18e (250 mm × 4 mm). The mobile phase consisted of buffer (A) (KH_2PO_4 50 mM, EDTA 3 mM, pH 6.0 adjusted with NaOH) and methanol (B). The following gradient was used at 1 mL min^{-1} and 40°C: 0–9 min, 70% A; 10–15 min, 50% A; 18–23 min, 70% A; detection wavelength was 355 nm. Colchicine (AppliChem GmbH, Germany), demecolcine (Molekula Deutschland, Germany) and colchicoside (LGC GmbH, Germany) were used as reference standards. Nutrient and alkaloid concentrations were multiplied with the dry weight of the corresponding sample divided by the number of plants contributing to that sample, resulting in the quantity of nutrient/alkaloids per plant part. For statistical analyses of the nutrients N, P and K, we calculated the ratio between nutrient quantity in leaves plus capsule divided by the sum of the quantity in the two corms. Starch content of old and new corm was summed, representing the total quantity of this storage compound per plant.

Statistical analyses

To investigate whether the temporal pattern of nutrient and alkaloid content differed between regions and to determine the best mowing point, we fitted three different models to the untransformed data of (i) the above-ground to below-ground ratio of N, P and K, (ii) the above-ground alkaloid content and (iii) starch content of the corms.

A gamma distribution was fitted to the ratio data of N, P and K: $\Gamma = \alpha x^{\beta} \exp(-\gamma x)$, where Γ represents the ratio of N, P or K, and x is the date. As we were interested in the point of maximal ratio, the equation was differentiated with respect to x in order to obtain the parameter $x^{\max}$; $x^{\max}$ was then introduced into the model, resulting in:

$$\Gamma = \exp(\log(\alpha) + \gamma x^{\max} \log(x) - \gamma x).$$

To compare $x^{\max}$ between regions, curves for both regions were fitted simultaneously by the joint model 1:

$$\Gamma = \exp(\log(\alpha_i) + \gamma_i x_i^{\max} \log(x) - \gamma_i x), \qquad (1)$$

where i is the ith region. The parameters for the second region were re-parameterised as:

$$\alpha_2 = \alpha_1 + \delta_\alpha,$$

$$\gamma_2 = \gamma_1 + \delta_\gamma,$$

$$x_2^{\max} = x_1^{\max} + \delta_{x^{\max}},$$

where numbers 1 and 2 denote the respective region. The dummy variable g was created to encode the regions by the values 1 and 0 respectively. The parameters in the joint model were then calculated as:

$$\alpha_2 = \alpha_1 + g * \delta_\alpha,$$

$$\gamma_2 = \gamma_1 + g * \delta_\delta,$$

$$x_2^{\max} = x_1^{\max} + g * \delta_{x^{\max}}.$$

Differences between regions in the temporal change of alkaloid content in above-ground parts were determined in an identical way, by fitting a quadratic function of the form:

$$y = \alpha * x^2 + \beta * x + \gamma,$$

where y represents alkaloid content and x the date. Re-parameterising the equation and joining the models resulted in model 2:

$$y = -(\beta_i/(2 * x_i^{\max})) * x^2 + \beta_i * x + \gamma_i. \qquad (2)$$

The introduction of a dummy variable and parameter calculation was made as in model 1.

Data for starch content in the storage organs showed a sigmoid pattern. We fitted two linear slopes to

determine the point when the slope of starch content increases, that is, the change point. The linear slope model of Schabenberger and Pierce (2002) was applied:

$$y = (\beta 0 + \beta 1 * x) * (x \leq \alpha 1) + (\beta 0 + \beta 1 * \alpha 1 + \beta 2 * (x - \alpha 1)) * (x > \alpha 1),$$

where α_1 indicates the change point. Joining the models for the two regions resulted in model 3:

$$y = (\beta 0_i + \beta 1_i * x) * (x \leq \alpha 1_i) + (\beta 0_i + \beta 1_i * \alpha 1_i + \beta 2_i * (x - \alpha 1_i)) * (x > \alpha 1_i). \quad (3)$$

A dummy variable was introduced and parameters calculated for each region as in model 1. In each of the models 1–3, unknown parameters were determined by fitting a non-linear regression model via least squares, as implemented in the nls procedure in the statistical program R. Our null hypothesis for model 1 and 2 was H_0: $x_1^{max} = x_2^{max}$ and for model 3 H_0: $\alpha 1_1 = \alpha 1_2$. Given the equations for $x_2^{max} = x_1^{max} + \delta_{x^{max}}$ and for $\alpha 1_2 = \alpha 1_2 + \delta_{\alpha 1}$, the null hypothesis was accepted when $\delta_{x^{max}} = 0$, or $\delta_{\alpha 1} = 0$, which was tested in a *t*-test. To test when alkaloid content significantly decreased in above-ground plant parts after the peak (x^{max}), we first selected the sampling date closest to the peak. With reference to that date, we conducted a one-way ANOVA (separately for each region) followed by Tukey's honestly significant difference (HSD) *post hoc* test, to determine until when alkaloid content had significantly decreased. Prior to the ANOVA, data on alkaloid content were tested for heteroscedasticity using the Bartlett test (Bartlett, 1937). Heteroscedasticity and non-normality of residuals were investigated by plotting the errors against the fitted values and against the standard normal deviates respectively. When necessary, data were transformed applying Box–Cox transformation (Box & Cox, 1964). All analyses were performed in R, version 2.10.1 (R Development Core Team, 2008) and Statistica 9 for Windows (2009; StatSoft, Tulsa, OK, USA). Differences were considered significant at $P < 0.05$.

Results

Differences between biogeographical regions

Nutrient contents

Non-linear regression showed that the point of the highest ratio of N, P and K and the point where starch content in the corms started to increase differed between regions (Table 1, Fig. 2). This difference was significant for N, P and K. The maximum (x^{max}) for all nutrients and the change point ($\alpha 1$) for starch were on average reached 13.6 days earlier in VV compared with LDH. Averaging x^{max} of N, P and K and $\alpha 1$ of starch for each region, resulted in the date of 22 April ± 11.2 days SE for VV and 6 May ± 8.3 days SE for LDH.

Alkaloid content

Non-linear regression revealed a significant difference between regions in the point of the highest alkaloid content (x^{max}; Table 1, Fig. 3). In accordance with results from nutrient analyses, x^{max} was reached earlier in VV (24 May) compared with LDH (7 June). The Tukey's HSD test showed that alkaloid content significantly decreased from 26 May until 25 June by 43% in

Table 1 Results of non-linear regression analyses for the temporal change of nutrient and alkaloid contents in *Colchicum autumnale* of two regions, Germany (n = 3 populations per region)

	VV	LDH	R^2	δ	$P\,\delta$	Plant part	Model
Nitrogen							
x^{max}	27 April	7 May	83.6	10.9	<0.001	Lf/Corms	1
95% CI	24 April–30 April	3 May–11 May					
Phosphorus							
x^{max}	3 May	12 May	79.0	9.3	0.002	Lf/Corms	1
95% CI	29 April–8 May	7 May–18 May					
Potassium							
x^{max}	13 April	7 May	70.8	23.1	<0.001	Lf/Corms	1
95% CI	8 April–19 April	30 April–13 May					
Starch							
$\alpha 1$	16 April	27 April	90.7	11.13	0.35	Corms	3
95% CI	30 March–4 May	4 April–26 May					
Alkaloids							
x^{max}	24 May	7 June	73.8	14.3	<0.001	Lf	2
95% CI	21 May–28 May	2 June–13 June					

VV, Vorderer Vogelsberg; LDH, Lahn-Dill Highlands; Alkaloids, sum of colchicine, demecolcine and colchicoside; x^{max}, maximum value; $\alpha 1$, osculation point between two linear slopes; δ, difference between regions in x^{max} or $\alpha 1$; $P\,\delta$, P-value for H_0: δ, 0, CI, confidence interval; Lf, Leaves plus capsule with seeds. For model number see text.

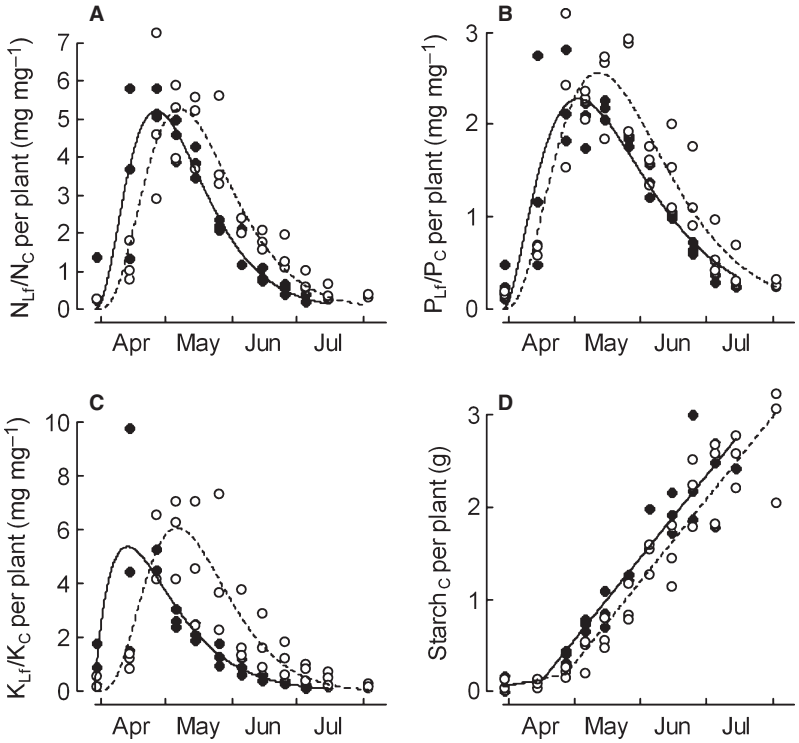

Fig. 2 Ratios of nitrogen (A), phosphorus (B) and potassium (C) between above-ground and below-ground plant parts, and (D) starch content in below-ground plant parts of *Colchicum autumnale* in two regions of Germany. Subscripts denote plant organs where nutrient content was measured. Lines are fitted gamma functions (A–C) and the linear slope model of Schabenberger and Pierce (2002) (D). Lf, leaves plus capsule with seeds; C, sum of old and new corm; black points and line, region Vorderer Vogelsberg; white points and dashed line, region Lahn-Dill Highlands.

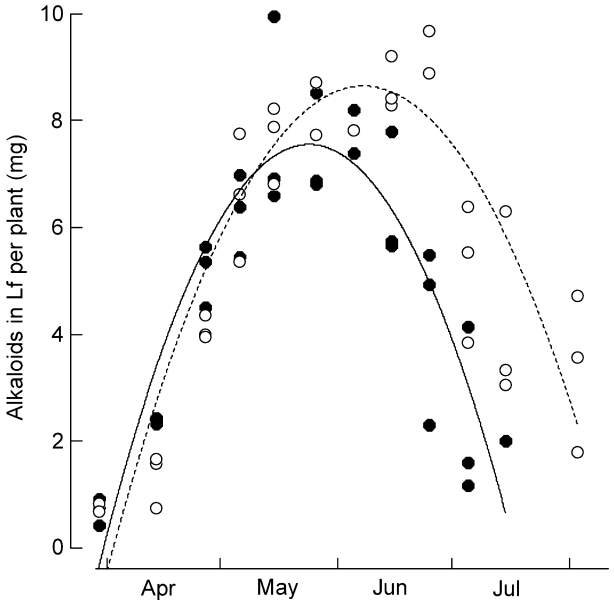

Fig. 3 Alkaloid content (colchicine, demecolcine, colchicoside) in above-ground parts of *Colchicum autumnale* in two regions of Germany. Lines are quadratic functions fitted through non-linear regression. Lf, leaves plus capsule with seeds; black points and line, region Vorderer Vogelsberg; white points and dashed line, region Lahn-Dill Highlands.

VV and from 5 June until 5 July by 46% in LDH (Table 2). In relation to 15 June, the common first mowing date, a significant decrease occurred in both regions until 5 July. During this period, the percentage decrease was 64% in VV and 38% in LDH (Table 2). Mean toxicity in LDH was on both dates 1.3 and 2.3 times higher than in VV. Analyses of fractions (1.1) and (1.2) showed much higher alkaloid contents in the seeds than in the leaves and capsules without seeds (Table 3). While in VV, alkaloid content of leaves had decreased to zero by 5 July, in LDH, alkaloids in leaves had only disappeared by 3 August.

The mean nutrient and alkaloid contents are presented separately for above-ground parts (Fig. 4), old and new corm (Fig. 5) and mean dry weights (Fig. 6).

Table 2 Mean alkaloid content ± SD and mean dry weight ± SD of *Colchicum autumnale* plants of two regions in Germany (n = 3 populations per region) at different dates

Region	Date	Mean dry plant weight (g) ± SD	Mean alkaloid content per plant (mg) ± SD
VV	26 May	2.0 ± 0.11	7.4 ± 0.97
	15 June	1.9 ± 0.34	6.4 ± 1.21
	25 June	1.8 ± 0.51	4.2 ± 1.70
	5 July	1.5 ± 0.43	2.3 ± 1.60
LDH	5 June	2.4 ± 0.66	9.8 ± 1.51
	15 June	2.2 ± 0.45	8.6 ± 0.49
	5 July	2.0 ± 0.15	5.3 ± 1.30

Alkaloids, sum of colchicine, demecolcine and colchicoside; VV, Vorderer Vogelsberg; LDH, Lahn-Dill Highlands.

Fraction region	Date	Mean alkaloid content per fraction in mg ± SD			
		Leaves + capsules without seeds		Seeds	
		VV	LDH	VV	LDH
	5 July	0.0 ± 0.00	1.9 ± 0.95	2.3 ± 1.60	3.4 ± 0.40
	15 July	0.0 ± 0.00	0.3 ± 0.58	2.01	3.9 ± 1.22
	3 August	n.a.	0.0 ± 0.00	n.a.	3.4 ± 1.47

Alkaloids, sum of colchicine, demecolcine and colchicoside; VV, Vorderer Vogelsberg; LDH, Lahn-Dill Highlands; n.a., not analysed.

Table 3 Mean alkaloid content ± SD of two plant fractions of *Colchicum autumnale* plants from two regions of Germany (n = 3 populations per region except for 15.7. in VV, where n = 1)

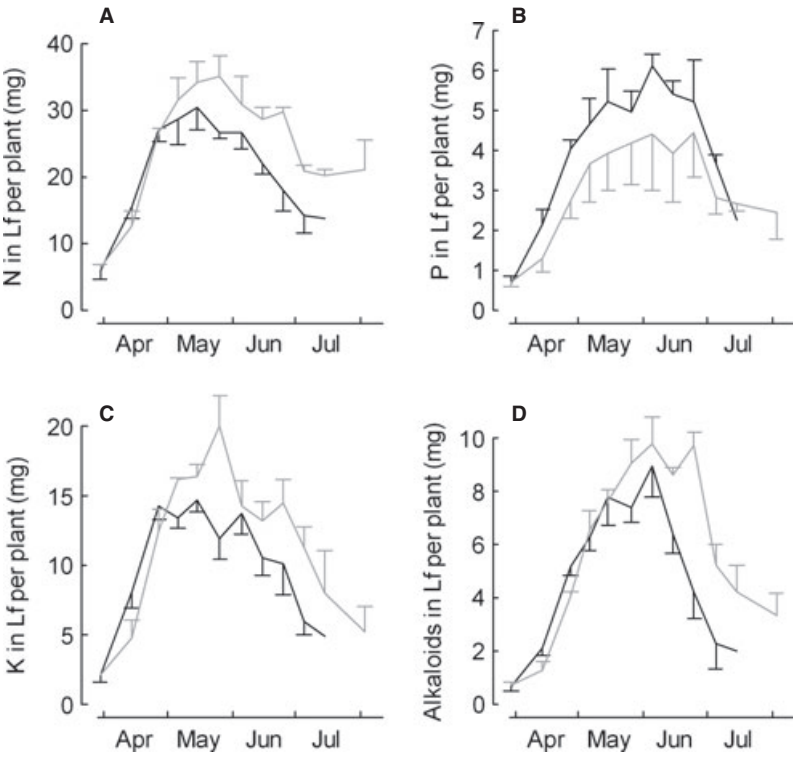

Fig. 4 Temporal course of mean content ± SE of nutrients nitrogen (N), phosphorus (P), potassium (K) and alkaloids (sum of colchicine, demecolcine and colchicoside) in above-ground parts of *Colchicum autumnale* in two regions of Germany [n = 3 populations per region except for 15.7. in region Vorderer Vogelsberg (VV), when n = 1]. Lf, leaves plus capsule with seeds; black line, region VV; grey line, region Lahn-Dill Highlands.

Discussion

Differences between biogeographical regions

Temperature is regarded the most influential factor for plant phenology in temperate regions (Diekmann, 1996; Wielgolaski, 1999). Thus, the shorter and later starting vegetation period in LDH as compared with VV is directly linked to the lower mean annual daily temperature. Because of the climatic characteristics, we expected a delayed temporal change of nutrient and alkaloid contents in LDH. This delay in LDH was observed for alkaloid content and the nutrients N, P and K. However, besides temperature, other factors may influence plant phenology, for example, exposition, nutrient concentrations in soil (Dahlgren *et al.*, 2007) or genetic predisposition (McMillan & Pagel, 1958). However, the similar maximum nutrient concentrations for N, P and K in *C. autumnale* parts between regions (data not shown) indicate very similar soil nutrient concentrations in both regions. Besides, most authors agree with Caprio (1966) that the influence of soil on plant phenology is usually smaller than the influence of climate. This suggests that differences in nutrient dynamics between regions were the result of climatic differences between regions.

To account for the variation of environmental factors, which may influence plant phenology and physiology, temporal and spatial replicates of the study units are generally recommended. This study was replicated spatially (two regions and six populations), but not temporally, as it was only conducted during 1 year. However, as the environmental factors varied between (climatic) regions and populations, our spatial replicates may partly account for the missing temporal replication (years with different weather conditions).

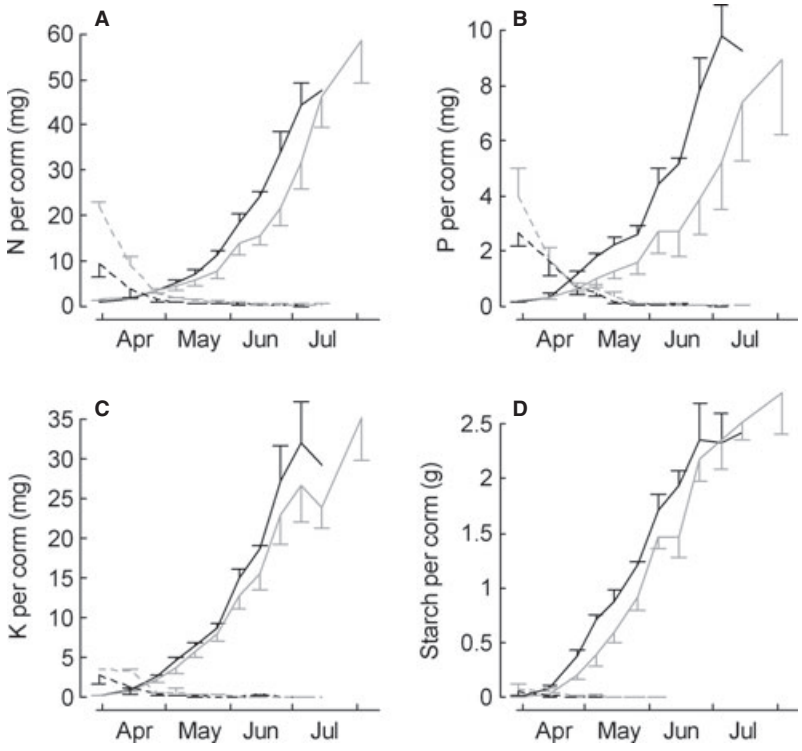

Fig. 5 Temporal course of mean content of nutrients ± SE in storage organs of *Colchicum autumnale* in two regions of Germany [$n = 3$ populations per region except for 15.7. in region Vorderer Vogelsberg (VV), where $n = 1$]. N, nitrogen; P, phosphorus; K, potassium; black line, region VV; grey line, region Lahn-Dill Highlands; continuous line, new corm; broken line, old corm.

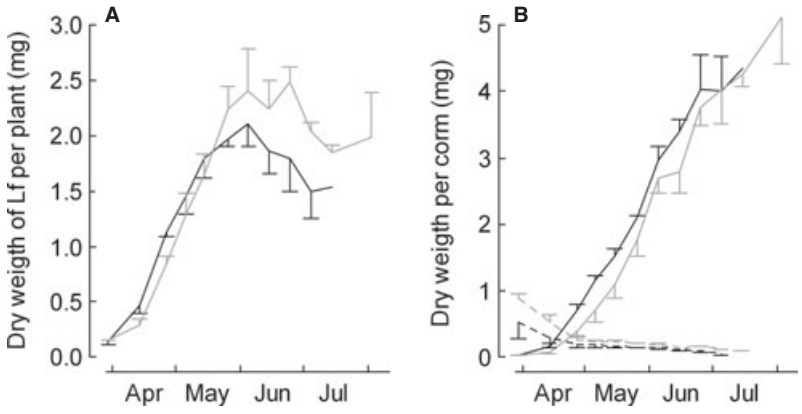

Fig. 6 Temporal course of mean dry weight ± SE in above-ground parts and storage organs of *Colchicum autumnale* in two regions of Germany [$n = 3$ populations per region except for 15.7. in region Vorderer Vogelsberg (VV), where $n = 1$]. Lf, leaves plus capsule with seeds; black line, region VV; grey line, region Lahn-Dill Highlands; continuous line, new corm; broken line, old corm.

Therefore, we expect our results to be strong enough to be generalised in space and time.

Management option 1: Reduction in population density of Colchicum autumnale

Our results indicate that, in 2009, most nutrients would have been removed from the plant and an optimal reduction of *C. autumnale* vitality achieved on 22 April in VV and 6 May in LDH. These dates corresponded to the following phenological development of the plants in 2009: average leaf length (measured from soil surface) in VV on 27 April was 26 cm ± 3.7 SD and in LDH on 6 May 26 cm ± 6.0 SD. Capsules were visible with their top located at 8–10 cm above ground. First results from an experiment testing the effect of different mowing dates on the vitality of *C. autumnale* support the indicated dates as the best mowing time (Jung *et al.*, 2010).

At the recommended mowing time, that is, the end of April/beginning of May, the alkaloid content in above-ground parts is already high (Fig. 3), but the sward height of the meadow is still low. Making fodder at this time is therefore not reasonable for the farmer because: (i) biomass from plant species other than *C. autumnale* hardly dilutes toxicity in the fodder and (ii) fodder quantity is very small and does not recompense the work for processing it. As mulching (cutting and leaving the clippings on the ground) is a cheaper type of management and the mulch can serve as fertiliser, we suggest removing leaves by mulching at this phenological plant stage. The disadvantage of this management option is a

yield loss in fodder gained in June. Besides, this yield loss would need to be budgeted for over an uncertain number of years, as it is unclear how fast and effectively an earlier cut reduces *C. autumnale* density. Effects on the plant community are also unknown.

Another management approach with potentially little negative effects on species richness is grazing sites with high *C. autumnale* densities, leading to plant damage through trampling (for details and references see Jung *et al.*, 2011).

Final results of the aforementioned mowing experiments are in progress; yet, studies on the effect of grazing on *C. autumnale* densities are still scarce. Other management options, for example, use of herbicides, fertilisation, etc., with associated deleterious effects on biodiversity, are summarised in Jung *et al.* (2011).

Management option 2: Decreased alkaloid content in Colchicum autumnale

Our results suggest postponing the first cut from mid-June to a later date, in this study 5 July, to achieve a significantly reduced alkaloid content in above-ground parts of *C. autumnale* and thus in the fodder. The actual alkaloid content at this time, however, depends on the region and phenological plant development; for example, while in VV, plant leaves had turned yellowish brown and dry on 5 July, in LDH, plants were just turning yellow. Besides the delayed development, higher mean dry plant weight in LDH was responsible for the higher alkaloid content in plants of this region (Table 2). In both regions, seed shed reduced total plant weight and thus alkaloid content in *C. autumnale* after 15 June. However, even at the end of the vegetation period, alkaloids were still detectable. The reason was the high alkaloid content of seeds, whereas leaves contained no alkaloids when they had turned brown and dry. This was also observed by Poutaraud and Girardin (2002) and indicates that vegetative plants may lose alkaloid content completely at the end of the vegetation period, while generative plants will only become alkaloid free, when they have shed all seeds. Compared with management option 1, there is no loss of fodder yield. Although there is a risk of enhancing population growth of *C. autumnale* by allowing seed shed, this risk is low in dense populations, as these are generally stable and do not increase anymore (Jung *et al.*, 2010).

It must be pointed out that the actual alkaloid content in hay/fodder harvested on a certain date cannot be easily derived from numbers of *C. autumnale* plant parts reported in this article, as numerous factors must be considered: (i) site (climate, soil characteristics, etc.), (ii) size of *C. autumnale* plants and status (vegetative versus generative), (iii) *C. autumnale* density, (iv) *C. autumnale* distribution, as a patchy distribution may cause strongly varying alkaloid contents in hay bales from the same grassland site and (v) biomass production. Besides alkaloid content, the actual risk of intoxication also depends on the extent to which animals typically select *C. autumnale* from the fodder (Wehsarg, 1929; Stebler & Schröter, 1981, pers. comm. with farmers). In summary, we suggest that feeding fodder from a delayed cut in July or August, when *C. autumnale* has turned brown and shed its seeds, reduces the chances of intoxication.

Acknowledgements

We thank two anonymous reviewers for their constructive comments, which considerably improved the manuscript. Furthermore, we are much indebted to H.-P. Piepho for invaluable help with the statistical analyses. J. Scholz-vom Hofe, M. Kasnitz, J. Fischer and C. Franken took part in data collection. We thank C. Hartwig and R. Düring (Institute of Soil Science and Soil Conservation, Giessen University) for skilfully improving and applying the analytical methodology for alkaloids determination. A. Allerdings and D. Steffens, Institute of Plant Nutrition, University Giessen, and R. Allerdings (Institute of Agronomy and Plant Breeding, Giessen University) analysed plant nutrients. The project was funded by Deutsche Bundesstiftung Umwelt (Ref. no. 25073-33/0).

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7. Control of the toxic plant *Colchicum autumnale* in semi-natural grasslands: effects of cutting treatments on demography and diversity

Winter S.* and **Jung L. S.***, Eckstein R. L., Otte A., Donath T. W. & Kriechbaum M. (2014). Journal of Applied Ecology 51 (2): 524–533.

* contributed equally

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Journal of Applied Ecology 2014, **51,** 524–533 doi: 10.1111/1365-2664.12217

Control of the toxic plant *Colchicum autumnale* in semi-natural grasslands: effects of cutting treatments on demography and diversity

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Summary

1. Semi-natural grasslands are important habitats for the conservation of biodiversity in Europe. High population densities of toxic *Colchicum autumnale* in these grasslands may cause problems for livestock and the marketing of hay. Consequently, farmers may either intensify grassland management to reduce *C. autumnale* in the fodder or abandon the land; both practices will lead to a loss of biodiversity. Previous studies suggesting early cutting to control *C. autumnale* did not consider population dynamics and the effects on plant diversity.

2. We conducted a four-year experiment in six regions within Austria and Germany, applying five cutting treatments in 16 *C. autumnale* populations to test the effects of cutting date and frequency on *C. autumnale* and co-occurring vegetation. Demographic data were evaluated with matrix population models, life-table response experiment (LTRE), ANOVA and MANOVA. Vegetation data were analysed with multiresponse permutation procedures (MRPP), ANOVA and MANOVA.

3. Population growth rate was significantly reduced in plots cut in early and late May compared to plots cut in June (control).

4. Plants cut in late April or early May showed the lowest survival probability. Significantly fewer large vegetative plants developed capsules in the following year when cut in early or late May. LTRE analysis showed that differences in the population growth rate between the control and early cut treatments were mainly the result of a reduced survival and growth and an increased retrogression to smaller stages.

5. Multiresponse permutation procedures revealed no differences in vegetation composition between treatments except for one site in 2011. There were no differences in Shannon index, evenness or species turnover rate within any year.

6. *Synthesis and applications*. The greatest reduction in vitality of *Colchicum autumnale* was observed in grasslands cut in late April or early May. After three years of early cutting, no reduction in plant species diversity was observed. The second cut should be postponed to July to enable seed shed of plants. Grassland management decisions to control toxic *C. autumnale* must be made in close cooperation with nature conservation authorities to consider site characteristics and requirements of endangered species.

Key-words: biodiversity, dormancy, extensive grassland management, grassland weed, LTRE, matrix population models, MRPP, population dynamics

Introduction

European semi-natural grasslands are habitats with high biodiversity and nature conservation value (Pärtel, Bruun & Sammul 2005). They also provide important ecosystem

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services (Lemaire, Hodgson & Chabbi 2011). Nowadays, these grasslands are threatened by afforestation, management intensification, abandonment (European Environment Agency 2010), conversion to arable land and atmospheric nitrogen deposition (Duprè *et al.* 2010). These changes lead to a dramatic loss of species richness (Fuller 1987; Green 1990).

The EU Habitats Directive (European Commission 1992/1995) aims at ensuring the preservation and restoration of valuable habitats. Most grassland types listed in annex I of the Habitats Directive depend on traditional, extensive management. Consequently, extensive grassland management is promoted by EU agri-environmental programmes (Regulation No 1698/2005), which offer compensation payments for a late first cut and/or reduced fertilizer input. However, management according to these programmes only partly reflects traditional management occurring 50–100 years ago (Lennartsson & Oostermeijer 2001). Back then, farmers cut smaller areas over a longer time period and spent more time on labour-intensive weed control measures (e.g. Braungart 1899). Prescribed extensive management of agri-environmental programmes may promote the occurrence and frequency of toxic plants like *Senecio aquaticus* (Suter & Lüscher 2008), *Equisetum palustre* (Čop, Vidrih & Hacin 2009) and *Colchicum autumnale* (Briemle 2003; Winter, Penker & Kriechbaum 2011).

Recently, *Colchicum autumnale* has received increasing attention because farmers managing grasslands with high densities of this species have difficulties using and selling their hay (Winter, Penker & Kriechbaum 2011). Consequently, farmers may either intensify or abandon grassland management to reduce *C. autumnale* in the fodder; both will cause biodiversity loss. Although intoxications with *C. autumnale* are very rare, given its regionally high occurrence (Winter, Penker & Kriechbaum 2011), cases of acute poisoning of sheep, cattle and horses from Austria (Chizzola & Janda 2002), Germany (Kamphues & Meyer 1990) and Switzerland (Kupper *et al.* 2010) have been reported. The reports were followed by calls to reduce this toxic species. Measures traditionally recommended for a reduction in *C. autumnale* are cutting followed by fertilization with slurry (Wehsarg 1929; Rauschert 1961), the use of herbicides (e.g. Davies 1964) or ploughing up grasslands (Braungart 1899). However, all of these measures have negative impacts on overall species richness.

Photosynthesis and reserve formation of *C. autumnale* are limited to the short period between April and June (Franková *et al.* 2003; Jung *et al.* 2012). A cut during the photosynthetically active period will disrupt the formation of new reserves and thus reduce the survival probability of *C. autumnale*. A reduction in *C. autumnale* abundance by cutting grasslands early in spring was reported by some authors (e. g. Wehsarg 1929); however, these studies were geographically limited and did not consider population dynamics. In order to produce valid results across regions and to understand the effects of different cutting dates and frequencies on the population biology of *C. autumnale*, we conducted five different cutting treatments in 16 *C. autumnale* populations located in Austria and Germany (three regions in each country).

We used matrix population models, which are well suited for the evaluation of population dynamics under different management regimes (e.g. Jongejans, Sheppard & Shea 2006; Dauer, McEvoy & Van Sickle 2012).

The objective of this transnational study was to find the optimal date for grassland management with a maximal reduction in *C. autumnale* and minimal impact on co-occurring vegetation.

We addressed the following research questions:

1. Which cutting treatment is most effective in reducing the abundance and population growth rate of *C. autumnale*?

2. How are the vital rates of *C. autumnale* influenced by the different cutting treatments?

3. What is the effect of the different treatments on the co-occurring grassland vegetation?

Materials and methods

STUDY SPECIES

Colchicum autumnale (Colchicaceae) is a perennial geophyte native to Central Europe. The English name 'autumn crocus' refers to its unusual life cycle: in autumn (August–October), a new shoot develops from the innovation bud of the corm and flowers appear above ground. Subsequent to flowering, leaves and capsules develop below ground and appear above ground in the following spring (March–April). During the photosynthetically active period, the old corm withers and is replaced by a new corm (Loew & Kirchner 1934). The plant remains underground during dormancy periods in summer (July–August) and winter (November–March). Fruits appear in May; seed ripening and seed shed take place in June. In addition to the annual replacement of the old corm by a new one, another corm may develop from a second innovation bud, resulting in vegetative reproduction (Jung *et al.* 2011). Seeds germinate below ground in autumn and display their first foliar leaf in spring (Loew & Kirchner 1934).

Colchicum autumnale mainly occurs in grasslands; it is a character species of the class Molinio-Arrhenatheretea (Oberdorfer 1983), which represents mesophilous grasslands of medium to extensive agricultural utilization intensity. In litter meadows, *C. autumnale* is less problematic as it is not used for fodder production, but only for litter production from a cut after mid-September. At this time, above-ground parts already have decomposed and are unlikely to harm cattle (Jung *et al.* 2012). Although *C. autumnale* is not rare or threatened in the centre of its distribution area, it is listed in the Red Data Books of several countries at its distribution limits (Jung *et al.* 2011).

STUDY SITES AND MANAGEMENT DESIGN

In 2008, we established management experiments in seven *C. autumnale* populations in Austria and nine in Germany, located in three geographical regions per country (see Table S1, Supporting Information). Investigated grasslands belonged to the alliances Arrhenatherion elatioris, Bromion erecti, Polygono-Trisetion,

Violion caninae and Molinion caerulae (Ellenberg 2009). They showed high abundances of 38.6 ± 4.5 SE *C. autumnale* plants per m² (excluding seedlings) and were managed extensively (no fertilizer, 1–2 cuts year^{-1}).

At each study site, we conducted three to four cutting treatments (Table 1) on permanently marked 4-m² plots within an area of about 400 m². Each treatment was applied to four (Germany) or five plots per study site [Austria, except for site A2-2 (see Table S1, Supporting Information) where $n = 3$]. Plots were arranged in a randomized block design. Control plots were cut at the traditional mowing date in mid-June or July. The LM ('late May cut') treatment was cut in late May at a plant height of approximately 35 cm. EM ('early May cut') and RM ('repeated May cut') treatments were first cut in late April or early May (except in Germany 2008: first cut in late May), when plants were approximately 25 cm high. The second cut in the EM treatment was carried out in June and in late May in the RM treatment. Treatment F was cut in June/July and flowers were removed every ten days in autumn. Depending on vegetation regrowth, plots of all treatments at the same site were cut additionally in autumn. Vegetation was cut to a height of ca. 5–10 cm with lawn mowers, hedge shears or brush cutters, and the cut material was removed.

The position, leaf, capsule and flower numbers of each *Colchicum* individual were recorded within the central 1 m^2 of the permanently marked plots from 2008 until 2011. All recorded individuals of *C. autumnale* were assigned to one of six life stages (Fig. 1): (i) seedling (S) with one primary leaf ≤1 mm in width [which could include 1 (2)-year-old plants as they are indistinguishable from seedlings], (ii) small vegetative (L1) with one leaf, which was classified as seedling in the previous year or with a leaf width >1 mm, (iii) medium vegetative (L2) with two leaves, (iv) large vegetative (L3) with three or more leaves, (v) generative (G) with capsules and (vi) dormant plant (D), that is, plants that did not appear above ground during the photosynthetically active period. For details on the classification of life stages, see Appendix S1 (Supporting Information).

The soil seed bank of *C. autumnale* is classified as transient by Thompson, Bakker & Bekker (1997); therefore, we did not include the life stage 'seed'.

Table 1. Overview of cutting treatments in Austria and Germany

Treatment names (cutting dates)	Austria	Germany
Control (June/July)	C	C
Late May cut (late May and June in Austria)	LM	LM
Early May cut (early May, June)	EM	EM
Repeated May cut (early May, late May)*		RM
Repeated flower removal in autumn (June/July)	F	

*In region G2 only (see Table S1, Supporting Information).

TRANSITION MATRIX MODEL AND POPULATION DYNAMICS

Based on the frequency distribution of recorded life stages, a 6x6 transition matrix was constructed for each population, treatment and year (Table S2, Supporting Information; for calculation details, see Appendix S2, Supporting Information). Each matrix element (a_{ij}) was calculated from the number of individuals in stage j in year t that passed into stage i in year $t + 1$, divided by the column total of stage j (Caswell 2001). The population growth rate was calculated from the averaged matrices of the population transition matrices for each treatment, country and year. A 95% confidence interval was established by bootstrapping the data (10 000 iterations) for each treatment, year and country. For bootstrapping, matrix elements representing transition probabilities were replaced by a binomial random distribution and then resampled. Fertility values were bootstrapped by resampling average fertility values calculated per region and treatment.

Life-table response experiments (LTREs) were conducted using matrices based on vital rates (Tables S3 and S4, Supporting Information) to analyse the contribution of different vital rates to the difference in the population growth rate ($\Delta\lambda$) between each treatment and the control of each country (Caswell 2001). Each matrix element is a product of the lower-level vital rates: survival (σ_j), stasis ($\gamma_{i=j}$), growth ($\gamma_{i>j}$), retrogression ($\gamma_{i<j}$), generative reproduction (Φ_{ij}) and vegetative reproduction (K_{ij}; Franco & Silvertown 2004; see Appendix S3, Supporting Information for details). Before conducting the LTRE, we separated the averaged

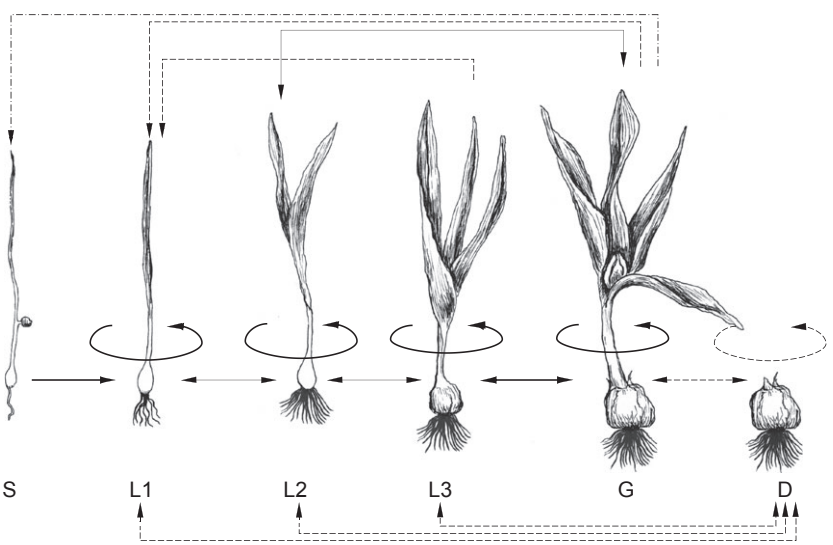

Fig. 1. Life stages of *Colchicum autumnale*. S, seedling; L1–L3, vegetative plant with one, two and three or more leaves; G, generative plant with capsules; D, dormant plant. Arrows indicate possible transitions between stages; the dash-dotted line represents fecundity. Bold lines show transition probabilities >30%, thin lines 5–30% and dashed lines <5% (modified from Jung *et al.* 2011).

transition matrices per treatment into one matrix excluding vegetative reproduction and one matrix including only vegetative reproduction in order to calculate the contribution of vegetative reproduction (K_{ij}) to $\Delta\lambda$. For details on the calculation of the LTRE based on vital rates, see Auestad *et al*. (2010). All analyses were performed with the program POPTOOLS version 3.0 (Hood 2008).

VEGETATION DATA

The abundance of all vascular plant species within the central 1 m² in permanent plots was recorded according to Braun-Blanquet (1932) each year during the study period (2008–2011). Due to logistic constraints, annual vegetation data were only collected in Germany. To examine species diversity, we calculated Shannon index and evenness (McCune, Grace & Urban 2002), measured as Pielou's *J*, for each permanent plot. To investigate whether species composition fluctuated stronger in the treatments compared to the control plots, the temporal species turnover rate for each population and treatment was calculated as $(NR + D)/(n_t+n_{t+1})$ (Mühlenberg 1989). NR denotes the number of species per plot that were newly recorded in year $t + 1$ but did not occur on the plot in year t, D is the number of species that had disappeared during the transition from year t to year $t + 1$, and n_t and n_{t+1} denote the species numbers in year t and $t + 1$, respectively.

STATISTICAL ANALYSES

An analysis accounting for repeated-measures data according to von Ende (2001) was conducted to analyse the effects of treatments and time on (i) the survival probability of *C. autumnale*, (ii) its probability to become a generative plant in the next year and (iii) the Shannon index and evenness of the vegetation data. For the between-subjects factors region, treatment and stage (and their interactions) a three-way ANOVA was calculated. The effect of the within-subject factor year (and its interactions) was assessed by a MANOVA. Before analysis, data from the study plots were pooled per population and treatment to obtain enough plants/transitions and thus robust estimates. We excluded the life stages seedlings and generatives from analyses (i) and (ii) due to missing values. Dormant plants were also excluded, as by definition they are not able to die during dormancy. Data were arcsine-transformed, or a Box–Cox transformation (Sokal & Rohlf 2007) was conducted after adding 0·001 to each data value. To identify differences between the treatments, regions and life stages, Tukey's HSD (honestly significant difference) post hoc test was used. We further expand on the rationale behind this statistical approach in Appendix S4, Supporting Information.

Prior to statistical analyses of vegetation data, cover values of the extended Braun–Blanquet scale were converted to the 1–9 ordinal scale of van der Maarel (1979: 100). Preliminary analyses showed no general differences in species composition and abundance between regions; therefore, data were analysed at the study site level. In order to determine whether different treatments led to a vegetation differentiation between plots, MRPP (multiresponse permutation procedures; McCune, Grace & Urban 2002) were carried out for each year 2008–2011. We tested differences between plots for each year with treatment as grouping factor and Sørensen distance measure for community data. ANOVA and MANOVA were calculated with Statistica 10 for Windows (2010; StatSoft Inc., Tulsa, OK, USA). MRPP were conducted with PC-ORD 5.3 (McCune, Grace & Urban 2002). ANOVAS were used to compare vegetation surveys of 2008 and 2011 concerning species turnover rates and the proportion of declining Red List species between treatments and regions. We also checked visually which Red List species declined from 2008 to 2011 in treatments vs. the control. Region G2 was analysed separately as only this region included the repeated May cut (RM) treatment. Results were regarded significant at $P < 0{\cdot}05$, except for MRPP, where Bonferroni correction (Sokal & Rohlf 2007) was applied to correct for multiple testing, changing significance level to $P < 0{\cdot}0019$.

Results

SURVIVAL PROBABILITY

Plant survival was significantly influenced by the factors region, stage and treatment (the latter only in Austria, in Germany $P = 0{\cdot}051$; Table 2). There was a general decrease in survival (7–18%) in each treatment (except RM) during the study period (Fig. 2). EM and RM treatments, which were cut in early May, resulted in the lowest survival probabilities (66% in EM and 67% in RM in Germany; 75% in EM in Austria) (Fig. 2 and S1, Supporting Information). A significant 9% difference in the EM treatment compared to the control was only detected in the final study year in Austria (Fig. 2).

TRANSITION TO GENERATIVE PLANTS

The probability of a plant to become generative was significantly affected by treatment, stage, their interaction and the year in both countries (Table S5, Supporting Information). Large vegetatives were 5–78 times more likely to become generative in the control and 3–39 times in the flower removal treatment F, than those in the early (EM, RM) or late May cut treatments (LM, Fig. S2, Supporting Information). Transition probabilities in the LM and EM treatments were 68–74% and 59–100% lower compared to the control in all transition periods (Fig. 3). Excluding the transition period 2008–2009 which had a first cut in late (instead of early) May for the EM treatment in Germany, transition probability in the EM treatment was reduced by 97–100%.

POPULATION GROWTH RATE

Population growth rates (λ = lambda) for each *C. autumnale* population and treatment varied from 0·36 to 1·68 in Germany and from 0·48 to 1·29 in Austria (Table S6, Supporting Information). In Austria, λ of the averaged matrices decreased in the LM and EM treatments by 27 and 33%, respectively, over the study period (Fig. 4). Lambda was always smaller than 1, indicating population decline. Lambda was significantly (non-overlapping confidence intervals) smaller (by 16–29%) in LM and EM treatments than in the control and F treatment in the last

Table 2. Effects of between-subject (BS) factors (R = three regions per country; T = four (Austria) or three (Germany) treatments, see Table 1; St = three life cycle stages, L1, L2, L3, see Fig. 1) and within-subject (WS) factors (Y, year and its interactions) on survival probability of *C. autumnale* in two countries. Between-subject effects were determined by ANOVA, whereas within-subject effects were determined by MANOVA. d.f., degrees of freedom; MQ, mean sum of squares

Austria	Source of variation	d.f.	MQ		*F*	*P*
BS	R (region)	2	0·28		3·68	0·033
	T (treatment)	3	0·23		3·05	0·038
	St (stage)	2	0·74		9·82	0·0003
	R × T	6	0·04		0·54	0·77
	R × St	4	0·04		0·52	0·72
	T × St	6	0·04		0·52	0·79
	R × T × St	12	0·02		0·25	0·99
	Error	45	0·07			
	Source of variation	Wilks' Lambda	$d.f._{Hypothesis}$	$d.f._{Error}$	*F*	*P*
WS	Y (year)	0·35	2	44	40·78	<0·0001
	Y × R	0·61	4	88	6·16	0·0002
	Y × T	0·73	6	88	2·45	0·031
	Y × St	0·49	4	88	9·27	<0·0001
	Y × R × T	0·81	12	88	0·83	0·62
	Y × R × St	0·81	8	88	1·21	0·30
	Y × T × St	0·66	12	88	1·69	0·08
	Y × R × T × St	0·56	24	88	1·22	0·25

Germany	Source of variation	d.f.	MQ		*F*	*P*
BS	R (region)	2	3·55		36·09	<0·0001
	T (treatment)	2	0·31		3·12	0·051
	St (stage)	2	2·49		25·28	<0·0001
	R × T	4	0·10		1·02	0·41
	R × St	4	0·03		0·25	0·91
	T × St	4	0·04		0·42	0·79
	R × T × St	8	0·02		0·2	0·99
	Error	52	0·10			
	Source of variation	Wilks' Lambda	$d.f._{Hypothesis}$	$d.f._{Error}$	*F*	*P*
WS	Y (year)	0·41	2	51	36·59	<0·0001
	Y × R	0·93	4	102	0·99	0·42
	Y × T	0·97	4	102	0·40	0·81
	Y × St	0·82	4	102	2·62	0·04
	Y × R × T	0·95	8	102	0·32	0·96
	Y × R × St	0·93	8	102	0·51	0·85
	Y × T × St	0·94	8	102	0·40	0·92
	Y × R × T × St	0·77	16	102	0·88	0·59

two transition periods (Fig. 4; Table S6, Supporting Information). Lambdas in treatment F and the control were similar. In Germany, λ of the EM and RM treatments was significantly lower (24% and 45%, respectively) than in the control in the second transition period, but not in the final year. The significant decrease in λ in the EM treatment from the first to the second transition period may be the result of the shifted cutting date. There was no difference between the LM treatment and the control. The RM treatment exhibited the lowest population growth rate (0.52) of all treatments in the second transition period. In both countries, population growth rates varied greatly with time: in the last transition period 2010–2011, population growth rates of all treatments (except RM) were between 11 and 33% lower than in the first period.

LIFE-TABLE RESPONSE EXPERIMENTS

The LTRE analyses showed that differences in the population growth rate (Δλ) between each treatment and the control were mainly the result of a reduction in growth and survival, and an increasing retrogression [Fig. 5; Tables S7, Supporting Information (Austria) and S8, Supporting

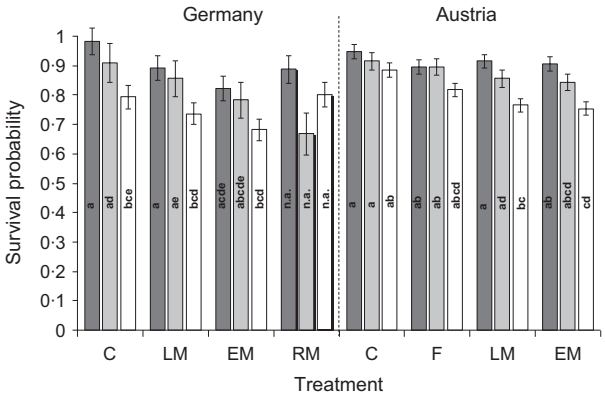

Fig. 2. Mean survival probability ± SE of *Colchicum autumnale* under different treatments in Germany (n = 9 populations, except for RM where n = 3, therefore n.a., not analysed) and Austria (n = 7 populations) from 2008–2011. Treatments: C, control; F, flower removal; EM, early May cut; LM, late May cut; RM, repeated May cut; dark grey bars = transition 2008–2009; light grey bars = 2009–2010; white bars = 2010–2011. Bars with different combinations of lowercase letters indicate significant differences at the 5% level (Tukey's test) separately for each country.

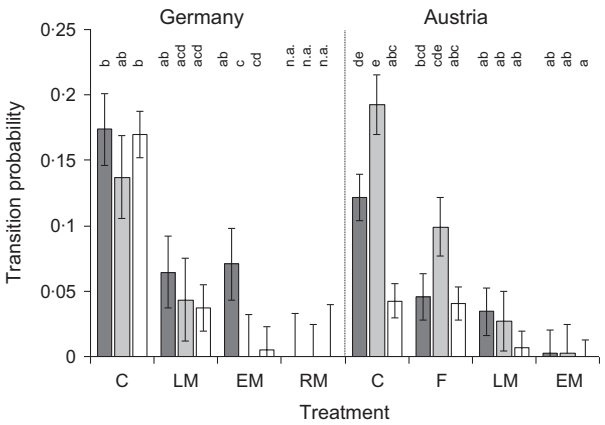

Fig. 3. Mean transition probability to a generative plant ± SE of *Colchicum autumnale* under different treatments for 3 years of transition in Germany (n = 9 populations, except for RM where n = 3, therefore n.a., not analysed) and Austria (n = 7 populations). Bars with different combinations of lowercase letters indicate significant differences at the 5% level (Tukey's test) separately for each country. Treatments: C, control; F, flower removal; EM, early May cut; LM, late May cut; RM, repeated May cut; dark grey bars = transition 2008–2009; light grey bars = 2009–2010; white bars = 2010–2011.

Information (Germany)]. Survival (σ_j) decreased most in the earliest cut plots (EM and RM treatments) and has continually decreased in all treatments over the study period in Austria. In Germany, a reduced stasis was only found for the first two transition periods of the EM and RM treatments, whereas stasis decreased during the whole study period in Austria. Generative and vegetative reproduction had a relatively small impact on $\Delta\lambda$. The largest contribution of all treatments and transition periods to $\Delta\lambda$ was the reduced growth of the RM treatment in Germany from 2008–2009.

ANALYSES OF VEGETATION DATA

The MRPP showed that vegetation composition did not differ significantly between the treatments except for one site in 2011 (in region G2; data not shown). This difference disappeared when excluding the RM treatment, which was only conducted in region G2.

The analyses of the Shannon index (Table S9, Supporting Information) and evenness (not shown) by ANOVA and MANOVA revealed no differences between the treatments. Although the interaction between year and treatment was significant for the Shannon index, there were no differences between treatments in the same year. A separate analysis of region G2 (which included the RM treatment) showed no differences in Shannon index or evenness between treatments either. Similarly, the ANOVAS on species turnover rate and the proportion of declining Red List species showed no difference between the treatments (excluding the RM treatment) and the control (data not shown). The same results were found including only region G2 and the RM treatment (data not shown). According to the visual data check, two species increased in the control, but declined in the LM and EM treatments: *Luzula campestris* decreased in abundance (only in region G2; the species did not occur in region G1) and *Saxifraga granulata* disappeared (the species only occurred in G1).

Discussion

EFFECTS OF TREATMENT REGIMES ON *COLCHICUM AUTUMNALE*

The earliest first cutting date, that is, late April or early May, had the strongest negative effect on population growth of *C. autumnale* through a reduction in survival and growth and an increase in retrogression. Furthermore, the transition probability to the generative stage was significantly reduced. Physiological data suggest that at the time of an early cut in May, *C. autumnale* has mobilized most of its storage reserves for leaf and capsule development, whereas the formation of new reserves from photosynthetic products is just about to start (Franková *et al.* 2003; Jung *et al.* 2012). Although a later cut in May (LM treatment) removes more leaf material, it also allows the plant to accumulate more storage compounds, increasing its vitality (Jung *et al.* 2012).

The flower removal treatment F was least effective in reducing survival and population growth rate of *C. autumnale*. Plants flower successively over several weeks, and only about 40% of large plants actually bloom (Jung *et al.* 2011). Therefore, only a small part of the population is affected by flower removal. According to Wehsarg (1929), flower removal may even enhance plant vitality by preventing capsule formation and thus saving energy, which might have contributed to the high λ values of treatment F. According to our results, the dormant stage

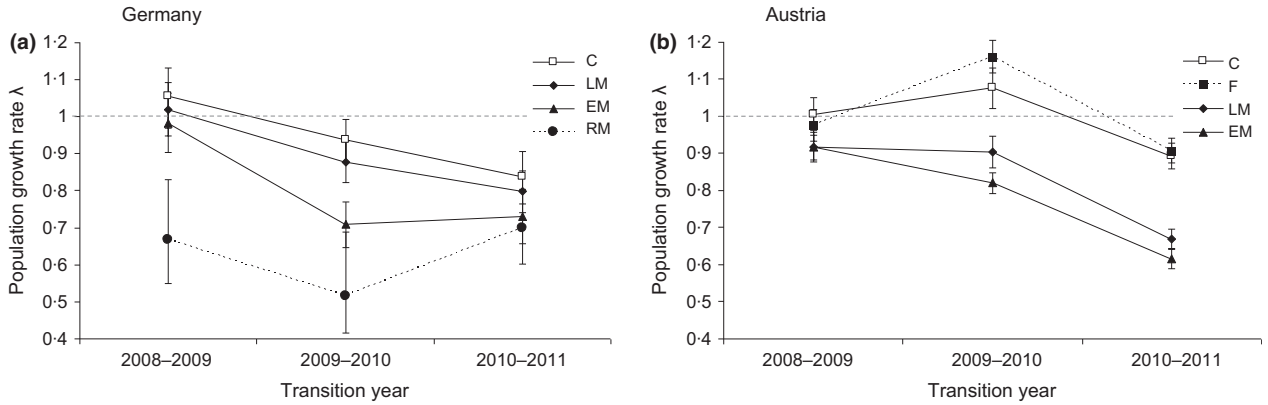

Fig. 4. Mean population growth rates (λ) with 95% confidence intervals of *Colchicum autumnale* for three transitions under different treatments in a) Germany (n = 9 populations per treatment, except for RM where n = 3 populations) and b) Austria (n = 7 populations per treatment). Treatments: C, control; F, flower removal; EM, early May cut; LM, late May cut; RM, repeated May cut. Open symbols denote controls; broken horizontal line indicates stable population growth (λ = 1).

in the life cycle of *C. autumnale* only plays a minor role (cf. Smith 2004) and is therefore negligible regarding the effects of the cutting treatments.

Besides the negative effects of the early cutting treatments on population dynamics, the Austrian study area experienced cumulative effects exhibited by the continuously declining population growth rates and retrogression of generatives and large vegetatives. In Germany, no cumulative effect was observed. One reason might be that cutting dates were not properly adapted to phenology in each year. For example, due to the late start of the growing season in 2010, plants were cut at a smaller size than in the previous years. Consequently, less plant tissue and resources were removed, resulting in equal or higher population growth rates in 2010–2011. The extremely dry spring period in 2011 might have resulted in an overall decline of λ in Germany, whereas the cold and wet period in May 2010 could be responsible for the decline in the number of generative plants in Austria in 2011. The suggested influence of external factors as weather conditions on *C. autumnale* is also supported by the differences in survival and generative reproduction between years and regions and the annual fluctuation of λ in the control. Vital rates of perennial herbs respond differently to temporal and spatial variation (Jongejans & de Kroon 2005) with strong effects of temporal variation on growth. Extreme weather conditions, such as an unusual hot or dry summer, affect population growth rates through immediate effects on vital rates (Lennartsson & Oostermeijer 2001; Hüls, Otte & Eckstein 2007).

EFFECTS OF TREATMENT REGIMES ON PLANT SPECIES RICHNESS AND COMPOSITION

After three years, there was no clear change in vegetation composition and plant species diversity in treatments vs. control. From all statistical analyses, only MRPP indicated that two cuts in May can alter vegetation composition. According to the visual data check, two German Red List species (*Luzula campestris* and *Saxifraga granulata*) declined in abundance after an early or late cut in May. In other studies, negative effects of advancing the first cut were observed for species such as *Pimpinella saxifraga,* where an early cut in June or early grazing in May led to a reduced fertility (Auestad *et al.* 2010). The proportion of *Primula veris* flowering individuals also declined with grazing in early May (Brys *et al.* 2004). A cut in early May, as the best management to reduce *C. autumnale* according to our results, conflicts with current agri-environmental schemes for species-rich grasslands (Regulation No 1698/2005) recommending first cutting dates in mid-June or July.

An increase in cutting frequency or an earlier first cut, combined with an increase in fertilizer application, are key driving factors for declining species diversity in grasslands (e.g. Zechmeister *et al.* 2003; Dietschi *et al.* 2007). However, without fertilization, the effect of an increased cutting frequency on species diversity is much weaker (Oomes & Mooi 1981; Kirkham & Tallowin 1995; Čop, Vidrih & Hacin 2009). In grasslands of intermediate nutrient supply, species diversity may not or be minimally affected by a two- or three-cut management regime (Oomes & Mooi 1981; Čop, Vidrih & Hacin 2009), while nutrient-poor grasslands may show stronger effects (Kirkham & Tallowin 1995; Čop, Vidrih & Hacin 2009). A recent meta-analysis showed that postponing the mowing date from May to July or later had positive or neutral effects on plant species diversity in European meadowland (Humbert *et al.* 2012).

In Central European grasslands, a spring grazing period from late February until April, followed by a first cut in late June, was common in traditional management regimes until 1850 (Kapfer 2010). In such a management scheme, possible negative impacts of the early biomass removal on species diversity may be compensated by a reduced biomass of competitive grasses in the aftermath, leaving more space for less-competitive species. Many plant species show an intrinsic ability for compensatory

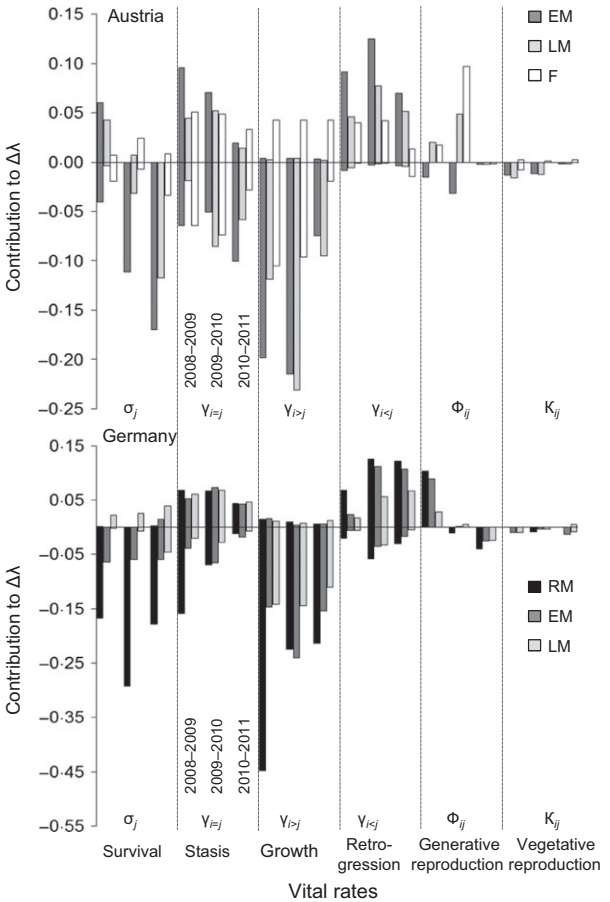

Fig. 5. Contribution of vital rates to the difference in population growth rate (Δλ) between the control and other treatments (F, flower removal; EM, early May cut; LM, late May cut; RM, repeated May cut) in *Colchicum autumnale* populations of Germany and Austria, as determined by LTRE (life-table response experiment). Bar sections above zero display the summed positive contributions, and bar sections below zero the summed negative contributions. The bar-triplets show the different transitions for 2008–2009, 2009–2010 and 2010–2011.

regrowth, which is due to higher relative growth rates early in the season (Strauss & Agrawal 1999). Although seed shed before the first cut is prevented, many species are able to develop ripe seeds until the second cut in June or September (Kirkham & Tallowin 1995; Jung, pers. obs.). The vast majority of grassland species are long-lived perennials that strongly rely on vegetative reproduction (Ellenberg 2009). Therefore, negative effects of an early May cut without fertilization on plant species diversity should be small, although this needs to be verified in long-term experiments.

Nevertheless, a change in management will affect some species negatively, for example annual or short-lived species that rely exclusively on generative reproduction (e.g. *Rhinantus* spp.; Kirkham & Tallowin 1995; Čop, Vidrih & Hacin 2009). Species without the ability to resprout after an early cut, such as orchids, are also likely to decrease when their infructescence is repeatedly removed. Possible negative impacts on fauna, such as ground-breeding birds (Gruebler *et al*. 2008), also need to be considered.

CONCLUSIONS

The results of our study suggest that an early first cut in late April or early May (depending on region and vegetation development) reduces survival, reproduction and the population growth rate of *C. autumnale* effectively and will lead to its decline. At the time of cutting, plants should be approximately 25 cm high. This guideline should therefore be widely applicable as this is the phenological state at which *C. autumnale* is most vulnerable to the removal of above-ground biomass in all climatic regions involved in the study. Biomass yield is very low and alkaloid content of *C. autumnale* high at this time (Jung *et al*. 2012), which makes a harvest unappealing to farmers. Being the cheapest type of management, mulching seems most appropriate for an early cut. For mulching, it is important to chop vegetation into small pieces to guarantee rapid decomposition of the resulting litter layer and thus enable seed germination of light-demanding species. After an advanced first cut, the second cut should be postponed for four weeks compared to the traditional mowing date of a grassland site (i.e. from mid-June to mid-July) to facilitate seed shed of re-flowering grassland species. As the '*Colchicum* problem' does not concern litter meadows (see Methods), there is no need for management changes in this grassland type. An early cut has negative effects on some species of high conservation value like orchids and some annual species and may also alter the species composition. Therefore, the risk of species loss through early cutting must be weighed carefully against the risk of biodiversity loss through management intensification or abandonment. Decisions should be made on a site-specific basis and only in close cooperation with the nature conservation authorities.

In order to meet nature conservation goals and to ensure long-term extensive grassland management, compensation payments from agri-environmental schemes are essential. However, more flexibility concerning the date of the first cut is needed when toxic plant species such as *C. autumnale* put grassland management at risk.

Acknowledgements

We thank the Deutsche Bundesstiftung Umwelt (Ref. no. 25073-33/0), the Federal Ministry of Agriculture, Forestry, Environment and Water Management (BMLFUW) in cooperation with federal states, Jubiläumsfonds der Österreichischen Nationalbank, Österreichische Bundesforste and Biosphärenpark Wienerwald Management GesmbH for financial support. We are grateful to Inger Auestad for detailed advice on the implementation of the LTRE based on vital rates, Bernhard Spangl for the help with the statistical analysis and the anonymous reviewers whose comments significantly improved this manuscript. Furthermore, we thank the farmers and nature conservation administration that allowed us to conduct the management experiments on their grasslands and all people who helped during the project: W. Holzner, K. Graiss, P. Kieninger, I. Auberger, J. Scholz-vom Hofe, R. Baumgärtel, M. Ernst, J. Busse and all students.

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Received 9 July 2012; accepted 23 December 2013
Handling Editor: Danny Hooftman

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Classification of *C. autumnale* into life stages.

Appendix S2. Transition matrix model construction.

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Fig. S2. Transition probability to the generative stage of *Colchicum autumnale* under different treatments.

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Table S3. Vital rate values for the averaged matrices of the Austrian treatments.

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Table S5. Effects of region, treatment, stage and year on transition probability of life stages to the generative stage.

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8. General discussion

8.1. Characterization of *Colchicum autumnale*

Colchicum autumnale has attracted the attention of people in Europe for more than 150 years. This is not surprising, as there is hardly any plant species with such controversial and extraordinary characteristics. One distinction from most other plant species is the separate appearance of leaves and flowers which has resulted in the great number of common names for the plant and may have offered the incentive for the numerous studies on its morphology (Irmisch, 1850; Wehsarg, 1929; Jaehn *et al.*, 1985). Due to its toxicity, *C. autumnale* is known and feared as an agricultural weed since the end of the 19th century because it can cause severe intoxications and death in life-stock (Braungart, 1899; Wehsarg, 1929; Rauschert, 1961). On the other hand, *C. autumnale* has been valued as medical plant for more than 2000 years (Hartung, 1954; Le Hello, 2000). It has furthermore played an important role in plant breeding (Roberts and Wink, 1998) and as an ornamental plant in gardens (Bowles, 1952).

The variety of aspects, interests and conflicts with *C. autumnale* is reflected in the large amount of existing literature found during the literature review (chapter 5). Besides a great deal of knowledge, the literature contained contradictive information and information gaps. In the review article we clarified several of these ambiguities, especially concerning *C. autumnale* morphology, i.e. the origin of the protuberance, the number of internodes, which internode forms the new corm, the question of roots helping the translocation of the plant into larger depth, and the correct definition for the proliferation of the seed. Information gaps were filled with our own data. We contributed data on *C. autumnale* population biology, e. g. the life cycle, morphological characterization of life stages, probability of survival, flowering, generative reproduction, dormancy, and annual population growth rates. The distribution map of *C. autumnale* from Meusel et al. (1965) was updated by E. Welk with a detailed analysis of regional conditions and occurrence. Despite many germination studies of *C. autumnale* (Muntean *et al.*, 1981; Jaehn, 1984; Zimmer *et al.*, 2001; Smith, 2004), germination requirements of *C. autumnale* are hardly understood. Our experiments generated the highest germination rates ever reported under laboratory conditions and we suggest the type of seed dormancy to be either morphological or morphophysiological. Our garden experiments led us to a correction of

the previous definition of the seed bank of *C. autumnale* as 'transient' (Thompson *et al.*, 1997) to 'short term persistent' which has impacts on *C. autumnale* management.

The review of recommended management measures to control *C. autumnale* revealed the long history of combating *C. autumnale* as a toxic weed (Bornemann, 1920; Braungart, 1899; Wehsarg, 1929). It became evident that none of these measures aimed at a selective control and most of them would strongly reduce overall plant diversity. Most control measures originate from the beginning and middle of the 19th century when nature conservation did not play any role. This situation has changed. The combination of agriculture and nature conservation is of increasing importance today. A control of toxic *C. autumnale* should therefore not contradict biodiversity protection. To fulfill these requirements, the development of adopted management schemes for *C. autumnale* control is essential.

8.2. Differences between biogeographical regions

As temperature is regarded the most influential factor for plant phenology in temperate regions (Diekmann, 1996; Wielgolaski, 1999; Ellenberg, 2009), we expected that the climatic differences between the biogeographical regions LDH and VV would be reflected in the nutrient and alkaloid dynamics of *C. autumnale* (chapter 6). We presumed that the lower mean annual daily temperature and shorter, later starting vegetation period in LDH as compared to VV leads to a delayed temporal change of nutrient and alkaloid contents in LDH. This delay in LDH was observed for alkaloid content and the nutrients N, P, and K. Besides temperature, other factors may influence plant phenology, e.g. exposition, nutrient concentrations in soil (Dahlgren *et al.*, 2007), or genetic predisposition (McMillan & Pagel, 1958). Exposition was similar at all study sites and maximum nutrient concentrations for N, P, and K in *C. autumnale* parts (data not shown) indicated very similar soil nutrient concentrations in both regions. Therefore, these factors cannot explain differences in alkaloid and nutrient dynamics between regions. Besides, the influence of soil on plant phenology is generally regarded to be smaller than the influence of climate (Caprio, 1966). This suggests that differences in nutrient dynamics between regions were indeed the result of climatic differences rather than the result of other factors.

8.3. Implications of nutrient and alkaloid dynamics for *Colchicum autumnale* management

Analyses of nutrient dynamics (chapter 6) showed that most nutrients are removed from *C. autumnale* when the plant is cut at about 25 cm average leaf length, which corresponds to the end of April/beginning of May in the study regions. At this point the ratio of nutrient content between above-ground and below-ground parts is highest. *C. autumnale* has mobilised most of its storage reserves for leaf and capsule development, whereas the formation of new reserves from photosynthetic products is just about to start (see also Franková *et al.*, 2003). At this developmental stage, a cut removes most nutrients with the leaves while leaving the plant with only few storage compounds in the corm. The plant's vitality is then reduced the most and reduction of population densities should be greatest.

The findings on alkaloid dynamics of *C. autumnale* (chapter 6) indicated that alkaloid contents in hay are significantly reduced when postponing the mowing date for three weeks from mid-June, the common first mowing date, to the beginning of July. The reasons for this reduction are the wilting and drying of plant parts and the seed shed.

However, the actual alkaloid content in hay/fodder gained at a certain date cannot directly be derived from *C. autumnale* numbers at a grassland site, for it is affected by numerous factors which can widely differ between sites: (i) site characteristics (climate, soil characteristics, exposition etc.), (ii) phenological development of *C. autumnale*, (iii) size of *C. autumnale* plants and status (vegetative versus generative), (iv) *C. autumnale* density, (v) *C. autumnale* distribution, as a patchy distribution may cause strongly varying alkaloid contents in hay balls from the same grassland site, and (vi) biomass production. The influence of factors (i) and (ii) was reflected by different alkaloid contents of *C. autumnale* on 5 July in the two study regions. In the study region with the warmer climate, plant leaves had turned yellowish-brown and dry at this date, while plants in the colder study region were just turning yellow.

A stage of *C. autumnale* when alkaloid content in plants with capsules is reduced to zero could not be determined. Although leaves contained no alkaloids when they had turned brown and dry at the very end of the vegetation period of *C. autumnale*, alkaloid content of seeds remained high. This was also observed by Poutaraud & Girardin (2002) and indicates that vegetative plants may lose alkaloid content completely at the end of the vegetation period, while generative plants will only become alkaloid-free when they

have shed all seeds. Therefore, a risk of intoxication of life stock consuming hay with *C. autumnale* from a very late cut, is highly reduced but not absolutely excluded.

8.4. Effects of cutting regimes on population development of *C. autumnale*

The findings from our mowing experiment (chapter 7) are in accordance with conclusions drawn from *C. autumnale* nutrient dynamics for controlling the plant species (chapter 6). The earliest first cutting date, i.e. late April or early May, had the strongest negative effect on population growth of *C. autumnale*. It resulted in the reduction of the demographic processes survival and growth and an increase in retrogression. Furthermore, the transition probability to the generative stage was significantly reduced. Although a later cut in May (LM treatment) removed more leaf material and thus nutrients, it also allowed the plant to accumulate more storage compounds, increasing its vitality. An earlier cut is also not advisable, as it removes only the leaf tips. Only if the vegetative part removed is large enough, *C. autumnale* plants become smaller (fewer leaves) in subsequent years or disappear, resulting in a lower population growth rate and a lower number of flowering plants (Jung & Winter, pers. obs.).

The flower cutting treatment, which was only conducted in Austria, showed that regular flower removal is least effective in reducing survival and population growth rate of *C. autumnale*. Plants flower successively over several weeks, and only about 40% of large plants actually bloom (Jung *et al*. 2011). Therefore, only a small part of the population is affected by flower removal. Furthermore, flower tissue is very delicate and short-lived and only few plant resources are removed with flowers. According to Wehsarg (1929), flower removal may even enhance plant vitality by preventing capsule formation and thus saving energy, which might have contributed to the high λ values of this treatment.

The dormant stage in the life-cycle of *C. autumnale* was shown to play only a minor role (cf. Smith 2004) and is, therefore, probably negligible regarding the effects of the cutting treatments. Besides the immediate effects of the cutting treatments on survival probability, generative reproduction and population growth rate, there was a cumulative effect in Austria during the study period, indicated by the continuously declining population growth rate and the retrogression of generatives and large vegetatives. In Germany, no cumulative effect was observed. One reason might be that cutting dates were not properly adapted to phenology in each year. For example, due to

the late start of the growing season in 2010, plants were cut at a smaller size than in the previous years. Consequently, less plant tissue and resources were removed, resulting in equal or higher population growth rates in 2010-2011. The extremely dry spring period in 2011 might have resulted in an overall decline of λ in Germany, whereas the cold and wet period in May 2010 could be responsible for the decline in the number of generative plants in Austria in 2011. The suggested influence of external factors as weather conditions on *C. autumnale* is also supported by the differences in survival and generative reproduction between years and regions and the annual fluctuation of λ in the control. Vital rates of perennial herbs respond differently to temporal and spatial variation (Jongejans & de Kroon 2005) with strong effects of temporal variation on growth. Extreme weather conditions, such as an unusual hot or dry summer, affect population growth rates through immediate effects on vital rates (Hüls *et al.*, 2007; Lennartsson and Oostermeijer, 2001).

8.5. Effects of treatment regimes on plant species richness and composition

After the application of different mowing treatments for three years (chapter 7), there was no clear change in vegetation composition and plant species diversity in treatments versus control. From all statistical analyses, only MRPP indicated that two cuts in May can alter vegetation composition. According to the visual data check, two German Red List species (*Luzula campestris* and *Saxifraga granulata*) declined in abundance after an early or late cut in May. In other studies, negative effects of advancing the first cut were observed for species such as *Pimpinella saxifraga,* whereas an early cut in June or early grazing in May led to a reduced fertility (Auestad *et al.* 2010). The proportion of *Primula veris* flowering individuals also declined with grazing in early May (Brys *et al.*, 2004). A cut in early May, was indicated by our results as the best management period to reduce *C. autumnale*. This conflicts with current agri-environmental schemes for species-rich grasslands in the European Union (Regulation No 1698/2005) recommending first cutting dates in mid-June or July.

An increase in cutting frequency or an earlier first cut, combined with an increase in fertiliser application, are key driving factors for declining species diversity in grasslands (e.g. Zechmeister *et al.*, 2003; Dietschi *et al.*, 2007). However, without fertilization, the effect of an increased cutting frequency on species diversity is much weaker (Oomes and Mooi, 1981; Kirkham and Tallowin, 1995; Čop *et al.*, 2009). In grasslands of intermediate nutrient supply, species diversity may not or be minimally

affected by a two- or three-cut management regime (Oomes and Mooi, 1981; Čop *et al.*, 2009), while nutrient-poor grasslands may show stronger effects (Kirkham and Tallowin, 1995; Čop *et al.*, 2009). A recent meta-analysis showed that postponing the mowing date from May to July or later had positive or neutral effects on plant species diversity in European meadowland (Humbert *et al.* 2012).

In Central European grasslands, a spring grazing period from late February until April, followed by a first cut in late June was common in traditional management regimes until 1850 (Kapfer, 2010). In such a management scheme, possible negative impacts of the early biomass-removal on species diversity may be compensated by a reduced biomass of competitive grasses in the aftermath, leaving more space for less-competitive species. Many plant species show an intrinsic ability for compensatory regrowth, which is due to higher relative growth rates early in the season (Strauss and Agrawal, 1999). Although seed shed before the first cut is prevented, many species are able to develop ripe seeds until the second cut in June or September (Kirkham and Tallowin, 1995; Jung, pers. obs.). The vast majority of grassland species are long-lived perennials that strongly rely on vegetative reproduction (Ellenberg, 2009). For these reasons, negative effects of an early May (late April) cut without fertilization on plant species diversity should be of small magnitude, although this needs to be verified in long-term experiments.

Nevertheless, a change in management will affect some species negatively, e.g. annual or short-lived species that rely exclusively on generative reproduction (e.g. *Rhinantus* spp.; Kirkham and Tallowin, 1995; Čop *et al.*, 2009). Species without the ability to resprout after an early cut, such as orchids, are also likely to decrease in the long-term when their infructescence is repeatedly removed. Possible negative impacts on fauna, such as ground breeding birds (Gruebler *et al.*, 2008), also need to be considered.

8.6. Conclusions

There are two management options to reduce alkaloid content in hay from meadows with *C. autumnale*: (option 1) cutting to reduce the population density of *C. autumnale* and (option 2) cutting when alkaloid content in above-ground parts of *C. autumnale* is low.

For management option 1, results of our study suggest an early first cut in late April or early May (depending on region and vegetation development). Cutting at this

point reduces survival, reproduction, and the population growth rate of *C. autumnale* effectively and will lead to its decline. At the time of cutting, plants should be about 25 cm high. This is the phenological state at which *C. autumnale* is most vulnerable to the removal of above-ground biomass in all climatic regions of this study, and this guideline should therefore be widely applicable. Biomass yield is very low and alkaloid content of *C. autumnale* is high at this time (chapter 6; Jung *et al.*, 2012), which makes a harvest unappealing to farmers. Being the cheapest type of management, mulching seems most appropriate for an early cut. For mulching, it is important to chop vegetation into small pieces to guarantee rapid decomposition of the resulting litter layer and thus enable seed germination of light demanding species. The disadvantage of early mulching is a loss in fodder yield. In order to enable the regrowth of enough biomass and to facilitate seed shed of re-flowering grassland species, the second cut should be postponed by four weeks compared to the traditional mowing date of a grassland site (i.e. from mid-June to mid-July). An early cut has negative effects on some species of high conservation value like orchids and some annual species and may also alter the species composition, although we did not detect any strong decline of species diversity at our study sites after three years of management. However, the risk of species loss when regulating *C. autumnale* through early cutting must be weighed carefully against the risk of biodiversity loss through management intensification or abandonment. Consequently, decisions should be made on a site-specific basis and in close cooperation with the nature conservation authorities and agricultural offices. Besides their expertise, flexibility of the public authorities is needed to change existing management of grassland sites which are subject to restrictions from agri-environment schemes or regulations from protected areas. The early-cut management causes additional costs for mulching and losses of fodder yield to the farmer. At grassland sites where this management is favored to regulate high *C. autumnale* numbers, it is therefore suggested to financially compensate farmers.

For management option 2, we suggest postponing the first mowing date for six weeks, e.g. from mid-June to the beginning of July, or, for lowest toxicity levels in hay, to the point when *C. autumnale* leaves are brown and dry. Due to high seed alkaloid content, the toxicity level in *C. autumnale* does not decline to zero. Actual alkaloid contents in hay with *C. autumnale* cannot easily be derived from phenological plant stage and average plant density as they are influenced by numerous further factors. Therefore, postponing the first mowing date can highly reduce the risk of intoxication of

life stock consuming hay with *C. autumnale* but the remaining risk cannot be reliably assessed. Although there is a risk of enhancing population growth of *C. autumnale* by allowing seed shed, this risk is low in dense populations, as they are generally in a stable stage and do not increase anymore (Jung & Winter *et al.*, 2010).

9. Summary

During the last decades, the toxic grassland species *C. autumnale* has become an increasing problem in extensively managed semi-natural grasslands of some Central European regions (Briemle, 2006; Winter *et al.*, 2011). Farmers with high population densities of the plant have increasing difficulties to market their hay. As *C. autumnale* is sensitive to an increased cutting frequency, there is a risk that farmers intensify or abandon management. This would inevitably lead to high losses of biodiversity as semi-natural grasslands are amongst the most species-rich ecosystems in Europe (Pärtel *et al.*, 2005). The same hazard persists when applying traditionally recommended measures to control *C. autumnale* (Wehsarg 1929; Braungart, 1899). A measure with a potentially acceptable impact on biodiversity is an early cut without additional fertilization, but respective studies (e.g. Wehsarg, 1929; Briemle & Elsässer, 2008) did not consider effects on plant diversity and are contradictive in the best cutting time.

Given this background, the main objective of this thesis was to derive management schemes to reduce toxicity in hay from grasslands with high *C. autumnale* densities with the smallest possible negative impact on the surrounding vegetation and thus plant species richness. We used three approaches: (i) the collection, critical review, and addition of general information on *C. autumnale* (chapter 5), (ii) the investigation of the temporary nutrient and alkaloid dynamics of *C. autumnale* (chapter 6), (iii) the study of the effect of different mowing treatments on the population biology of *C. autumnale* and plant species diversity (chapter 7).

Nutrient and alkaloid dynamics were studied in two biogeographical regions in Germany. Plants from different *C. autumnale* populations were collected over the course of the vegetation period in 2009 and analysed. Mowing treatments were conducted over four years in six regions in Austria (by Silvia Winter, University of Natural Resources and Life Sciences, Vienna) and Germany (by us). We applied four (Austria: five) cutting treatments in 16 *C. autumnale* populations to test effects of cutting date and frequency on *C. autumnale* and co-occurring vegetation. We collected data on the population biology of *C. autumnale* and conducted vegetation surveys.

For statistical analyses of data on nutrient and alkaloid dynamics, we fitted different models and compared the date of the curve maximum or change point of the curve. Demographic data were evaluated with matrix population models, life-table

response experiment (LTRE), ANOVA and MANOVA. Vegetation data were analysed with multi-response permutation procedures (MRPP), ANOVA and MANOVA.

The literature review revealed some contradictive information and information gaps about *C. autumnale*. We clarified ambiguities with emphasis on *C. autumnale* morphology. We filled information gaps with our data on the population biology of *C. autumnale*, distribution range, germination requirements, and seed bank classification.

Nutrient and alkaloid dynamics showed a clear dependence on climatic conditions. In the populations located in the cooler biogeographical region, the ratio of the maximum nutrient content between above-ground plant parts and storage organs, as well as alkaloid content in leaves and capsules occurred significantly later. The ratio between the nutrient content in above-ground plant parts and the storage organ peaked at the end of April/beginning of May, at about 25 cm leaf length of *C. autumnale*. At this developmental stage, a cut removes most nutrients from the plant and leaves it with few storage compounds in the corm. Compared to the common first mowing date (15 June), alkaloid content decreased significantly until 5 July in both study regions. It was about twice higher in plants from the colder region to those in the warmer one.

Mowing experiments showed a significant decrease of the population growth rate (λ) and the number of large vegetative plants developing capsules in the following year for plots cut in late April/early and late May compared to plots cut in June (control). Repeated flower removal in autumn had no effect on λ. Differences in the population growth rates ($\Delta\lambda$) between each treatment and the control were mainly the result of a reduction in growth and survival and an increasing retrogression as shown by LTRE. A cut in late April/early May resulted in the lowest survival probability. Differences in vegetation composition between treatments and the control were detected for the treatment “early plus late May cut”. After three years of early cutting, no reduction in plant species diversity was observed for any treatment.

In summary, results of our studies suggest the following two management options to reduce toxicity in hay from grasslands with *C. autumnale*: option 1) mowing at about 25 cm plant height of *C. autumnale* (ca. late April/early May) to reduce its population density or option 2) delayed mowing in late June/early July when the plant has turned brown and dry and alkaloid content has declined. For option 1, the second cut should be postponed to July to enable seed shed of plants. Any grassland management decisions to control toxic *C. autumnale* must be made in close cooperation with nature conservation authorities to consider site characteristics and requirements of endangered species.

10. Zusammenfassung

In den letzten Jahrzehnten wird die giftige *C. autumnale* in einigen Regionen Mitteleuropas auf extensiv bewirtschafteten halbnatürlichen Grünlandflächen zunehmend als problematisch betrachtet (Briemle, 2006; Winter *et al.*, 2011). Bei hohen Populationsdichten haben Landwirte vermehrt Heuvermarktungsprobleme. Da *C. autumnale* sensibel auf erhöhte Schnittfrequenzen reagiert, besteht die Gefahr der Bewirtschaftungsintensivierung oder -aufgabe betroffener Flächen. Dies würde zu hohen Biodiversitätsverlusten führen, da halbnatürliches Grünland zu den artenreichsten Ökosystemen in Europa zählt (Pärtel *et al.*, 2005). Dieselbe Gefahr besteht bei traditionellen Bekämpfungsmaßnahmen (Wehsarg 1929; Braungart, 1899). Die einzige Maßnahme mit potentiell akzeptabler Wirkung auf die Artenvielfalt ist ein früher Schnitt ohne zusätzliche Düngung, aber betreffende Studien (z. B. Wehsarg, 1929; Briemle & Elsässer, 2008) geben keine Auskunft über die Auswirkung auf die Pflanzenartenvielfalt und widersprechen sich im empfohlenen Schnittzeitpunkt.

Vor diesem Hintergrund war das vorrangige Ziel dieser Doktorarbeit, Bewirtschaftungsmaßnahmen für einen reduzierten Giftgehalt in Heu von Grünlandflächen mit hohem *C. autumnale* Besatz abzuleiten. Der negative Einfluss auf die Begleitvegetation sollte dabei so gering wie möglich sein. Es wurden drei Ansätze genutzt: (i) Sammlung, kritische Bewertung und Ergänzung von Informationen zu *C. autumnale* (Kapitel 5), Untersuchung (ii) der zeitlichen Veränderung von Nährstoff- und Alkaloidgehalten (Kapitel 6) und (iii) Evaluierung des Einflusses verschiedener Mahdregime auf *C. autumnale* und die Pflanzenartenvielfalt (Kapitel 7).

Der zeitliche Verlauf der Nährstoff- und Alkaloidgehalte wurde in zwei Naturräumen Deutschlands untersucht. In definierten Zeitintervallen wurden Pflanzen verschiedener *C. autumnale* Populationen in der Vegetationsperiode 2009 analysiert. Mahdexperimente erfolgten 2008-2011 in je drei Naturräumen Österreichs (durch Silvia Winter, BOKU Wien) und Deutschlands (durch uns). Wir untersuchten vier (Österreich: fünf) Mahdvarianten in 16 *C. autumnale* Populationen bezüglich des Effektes von Schnittzeitpunkt und -häufigkeit auf *C. autumnale* und die Begleitvegetation.

Die Auswertung zur Nährstoff- und Alkaloiddynamik erfolgte durch den Vergleich verschiedener Modelle bezüglich des Zeitpunkts der Kurvenmaxima bzw. change points. Populationsbiologische Daten wurden mit Matrixpopulationsmodellen, Life-table

response experiments (LTRE), ANOVA und MANOVA analysiert, Vegetationsdaten mit multi-response permutation procedures (MRPP), ANOVA und MANOVA.

Die Literaturauswertung zeigte Widersprüche und Informationslücken zu *C. autumnale*. Wir klärten v. a. morphologische Zweideutigkeiten, z. B. Ursprung, Funktion und Definition bestimmter Organe. Wir füllten Informationslücken mit eigenen Daten zu Populationsbiologie, Verbreitung, Keimungsansprüchen und Samenbankklassifizierung.

Der zeitliche Verlauf der Nährstoff- und Alkaloidgehalte in *C. autumnale* zeigte eine klimatische Abhängigkeit und war im kälteren Naturraum verzögert. Der Quotient aus den Nährstoffgehalten der über- und unterirdischen Pflanzenteile erreichte sein Maximum Ende April/Anfang Mai bei etwa 25 cm Blattlänge von *C. autumnale*. In diesem Stadium werden der Pflanze durch Schnitt die meisten Nährstoffe entzogen – bei nur geringen Mengen an Reservestoffen in der Knolle. Verglichen mit dem üblichen ersten Schnitttermin (15. Juni), nahm der Alkaloidgehalt in beiden Untersuchungsregionen signifikant zum 5. Juli ab. Er lag bei den Pflanzen im kühleren Naturraum etwa doppelt so hoch wie bei denen im wärmeren.

Die Mahdexperimente zeigten eine signifikante Abnahme der Populationswachstums- (λ) und Reproduktionsrate bei einem Schnitt Anfang oder Ende Mai verglichen mit einem Junischnitt (Kontrolle). Eine wiederholte Blütenentfernung im Herbst hatte keinen Effekt auf λ. Unterschiede in λ zwischen Behandlungen und Kontrolle lagen laut LTRE v. a. in einer Reduktion der Wachstums-, Überlebensrate und Pflanzengröße begründet. Die geringste Überlebenswahrscheinlichkeit zeigte sich bei einem frühen Schnitt Ende April/Anfang Mai. Unterschiede in der Vegetationszusammensetzung zwischen Behandlungen und Kontrolle wurden nur für die Variante „früher plus später Maischnitt“ verzeichnet. Auswirkungen auf die Pflanzenartenvielfalt wurden nicht festgestellt.

Zusammenfassend lassen sich folgende zwei Bewirtschaftungsempfehlungen ableiten, um den Giftgehalt in Heu von Grünland mit *C. autumnale* zu reduzieren: Option 1) Mahd von *C. autumnale* bei 25 cm Größe (ca. Ende April/Anfang Mai) zur Reduktion der Populationsdichte; Option 2) Verschiebung des Mahdtermins auf Ende Juni/Anfang Juli, wenn die Pflanze braun und vertrocknet ist und der Alkaloidgehalt reduziert. Bei Option 1 sollte der zweite Schnitt auf Juli verschoben werden, um ein Aussamen der Begleitvegetation zu ermöglichen. Bewirtschaftungsentscheidungen zur Kontrolle der giftigen *C. autumnale* müssen in enger Zusammenarbeit mit Naturschutzbehörden erfolgen, um Standorteigenheiten und Ansprüche gefährdeter Arten zu berücksichtigen.

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12. Acknowledgements

I would like to express my gratitude to everyone who helped and supported the realisation of this thesis.

First of all I thank Prof. Dr. Dr. Annette Otte for providing the valuable opportunity to join her working group, her supervision, suggestions, and guidance during the thesis. I thank Prof. Dr. Honermeier for being the second referee of this thesis.

I am much indebted to PD Dr. Tobias Donath for the development of this research project and to him and Prof. Dr. Lutz Eckstein for their valuable supervision, support with statistical issues, discussion and interpretation of results, and their assistance with any upcoming problems. I very much thank Josef Scholz-vom Hofe for his help with all technical tasks, ideas on the practical implementation of experimental set-ups, willingness to work overtime to guarantee the successful fieldwork and delicious self-made pizza and cake. For the extensive laboratory analyses of samples I would like to thank Prof. Dr. Rolf Düring and Christoph Hartwig (Institute of Soil Science and Soil Conservation, Giessen University) who skilfully improved and applied the analytical methodology for alkaloids determination and Prof. Dr. Diedrich Steffens and A. Allerdings, Institute of Plant Nutrition, Giessen University, and R. Allerdings (Institute of Agronomy and Plant Breeding, Giessen University) who conducted the plant nutrient analyses. Prof. Hans-Jürgen Tillich, Department of Systematic Botany and Mykology of the University of Munich, provided invaluable help to clarify morphological contradictions of *C. autumnale*. For their statistical help I thank Prof. Hans-Peter Piepho from the Bioinformatics Institute of the University of Hohenheim and Prof. Dr. Gabriel Schachtel, Institute of Biometry and Population Genetics, Giessen University.

I thank all students who helped with the collection of the innumerable field data (in alphabetical order): Fabian Bischoff, Janina Fischer, Christoph Franken, Tim Gattinger, Vera Hakmann, Linda Hilgers, Tobias Houska, Fritz Kinderman, Melanie Kühlmann, Theresa Pfaff, Sirrka Rausche, Lena Tomm, and Carola Zenke. I am grateful to all my collegues for their support, exchange of ideas and the pleasant working atmosphere (in alphabetical order): Dr. Sandra Burmeier, Oliver Ginzler, Markus Kasnitz, Sascha Keiner, Christiane Lenz-Kuhl, Dr. Arben Mehmeti, Dr. Birgit Reger, Ralf Schmiede, Benjamin Schulz, Dr. Dietmar Simmering, Martina Spenner, Dr. Nicole Voß, PD

Dr. Rainer Waldhardt, Bianka Zelle. Thanks to Beatrix Mattonet and Pia Wagner for their help with administrative issues.

Special thanks go to the farmers who provided a part of their grassland for our experiments and shared their experiences with *Colchicum autumnale*: Heinz Artz, Manfred Büttner, Karl und Jens Michel, Hartmut Schneider, Wolfgang Klein, Lothar Hild, Werner Bonn. The implementation of the mowing experiments would not have been possible without the good cooperation with the executive employees of the responsible authorities: Ralph Baumgärtel (Hessisches Forstamt Groß Gerau), Jürgen Busse (Regierungspräsidium (RP) Giessen), and Dr. Mathias Ernst (RP Darmstadt). They issued the necessary permits and helped with the selection of suitable grassland sites (Ralph Baumgärtel).

My special gratitude goes to Silvia Winter and her supervisors from the University of Natural Resources and Life Sciences in Vienna for the invaluable and enjoyable cooperation, intensive discussions and exchange of ideas on *C. autumnale*, which resulted in several joint publications.

I particularly appreciate the Deutsche Bundesstiftung Umwelt (DBU) for providing financial support without which the project could not have been conducted.

I would like to express my special thanks and love to my family and friends for supporting and encouraging me. And last but not least I deeply thank my boyfriend Daniel for his understanding of me often working on public holidays, and his unfailing support and patience during the whole period of the thesis.

13. Appendix

Appendix S1. Classification of *C. autumnale* into life stages

Small plants are easily overlooked in the field. If plants with one leaf of ≤ 3 mm width were not recorded during one year, but (again) the year thereafter, they were added in the dataset for the previous year. As dormant plants depend on their storage reserves, we presumed that plants with one leaf ≤ 3 mm could not be dormant. The length of the study period permitted the detection of only three transitions. Therefore, we assumed a maximum dormancy period of two years. Plants with one leaf > 3 mm and ≤ 1 cm were classified as dead after two years without appearance aboveground and plants with at least one leaf > 1 cm were considered dead when they did not appear aboveground for three years.

Appendix S2. Transition matrix model construction

Each matrix element (aij) was calculated from the number of individuals in stage j in year *t* that passed into stage *i* in year *t+1*, divided by the column total of stage *j* (Caswell 2001). Vegetative offspring were not added to the column total of stage *j* in year *t.* Therefore, the column total of stage *j* could exceed 1. Fecundity was calculated by dividing the number of seedlings in the year *t+*1 by the number of generative plants of the year *t*.

The percentage of plants in a certain life stage remaining dormant for two years could only be calculated from the data for the transition period 2008-2009. Therefore, these percentages for two-year dormancy were added in the matrices 2009-2010 and 2010-2011. Furthermore, in the matrix 2010-2011, the percentage for one-year dormancy had to be added. This percentage was calculated as the average percentage observed for each stage and treatment in the matrices 2008-2009 and 2009-2010. Any additions for the transition to dormancy in a matrix were only done until the percentage of dead plants reached zero. Dormant plants could per definition not die, as this transition could not be observed in the field. Therefore, the added percentage for dormant plants remaining dormant in the transition period 2010-2011 could not be subtracted from the dormant plants that died and was subtracted proportionately from each of the other transitions for dormant plants. Newly emerged plants that appeared directly adjacent to vegetative or generative individuals were considered offspring

through vegetative reproduction. Initial plant numbers ranged from 16.2 to 121.3 plants per square metre (Table S1).

Appendix S3. LTRE construction

Survival (σ_j) is the percentage of plants in stage *j* in year *t* that survived until year *t+1*, growth ($\gamma_{i>j}$) is the probability that an individual in stage *j* in year *t* grew to a larger life stage *i* (incl. dormant to non-dormant stage) in year *t+1* or went from a vegetative to the generative stage, retrogression ($\gamma_{i<j}$) accounted for the percentage of individuals decreasing in size or passing from the generative to a vegetative stage or rather to the dormant stage, and stasis ($\gamma_{i=j}$) accounted for the proportion of individuals without changes in stage class (see Tables S3-S4).

Appendix S4. Rationale behind statistical analyses

Before data analysis, we aggregated the data of our four/five 1 m^2 plots per treatment and per population. This was necessary because plots were relatively small compared to the size of the investigated populations and turned out to differ strongly concerning the population structure of *C. autumnale*, i.e. the number of individuals per life stage. As a result, not the single sampling plots were regarded as replicates, but the different populations entered the analyses as replicates. Prior to the analyses, data were arc-sine transformed (cf. Quinn & Keough 2002) and then subsequently fulfilled the assumptions of the ANOVA (homogeneity of variances, normal distribution of residuals). We analysed the between-subjects interactions (region, treatment, stage) with a univariate ANOVA according to von Ende (2001). To explore the impact of the within-subject time (year and all possible interactions) we used the MANOVA since this method does not depend on the assumption of sphericity and compound symmetry. These assumptions are rarely met by repeated counting data. MANOVA is an accepted method "*to produce multivariate test statistics in the context of repeated-measures ANOVA"* (Field, Miles & Field 2012, 554).

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Figure S1. Survival probability of *Colchicum autumnale* under different treatments

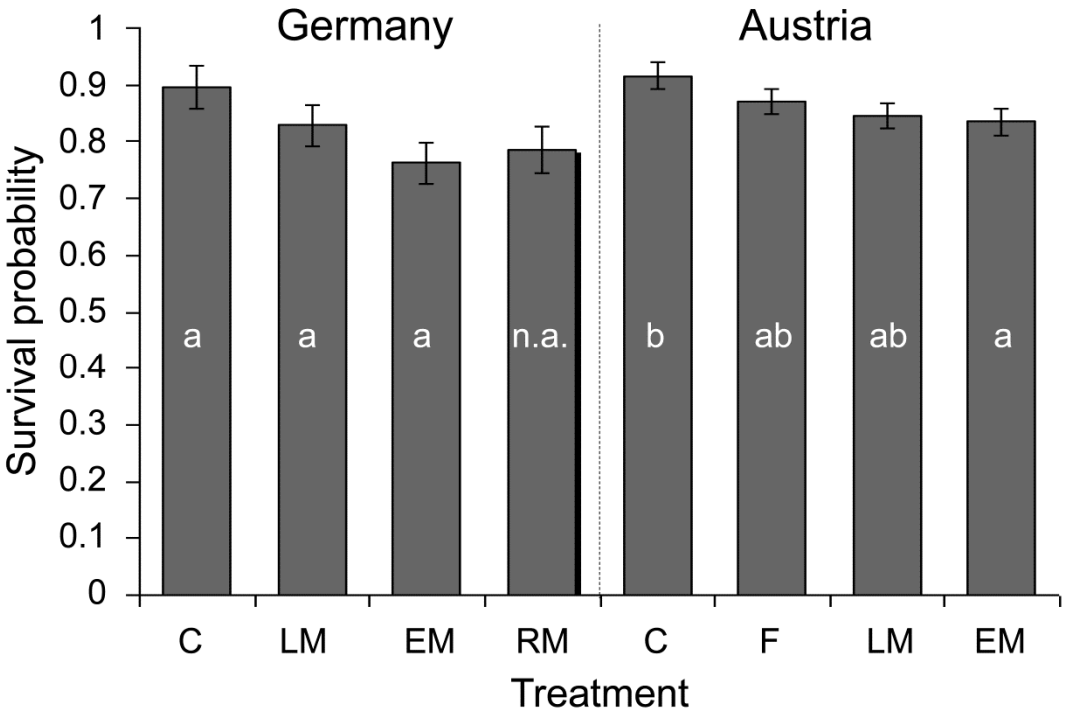

Fig. S1. Mean survival probability ± SE of *Colchicum autumnale* under different treatments for 3 transition periods in Germany (n = 9 populations, except for RM where n = 3 – therefore n.a. = not analysed) and Austria (n = 7 populations). Treatments: C = control, F = flower removal, EM = early May cut, LM = late May cut, RM = repeated May cut. Bars with different combinations of lowercase letters indicate significant differences at the 5% level (Tukey's test) separately for each country.

Fig. S2. Transition probability to the generative stage of *Colchicum autumnale* under different treatments.

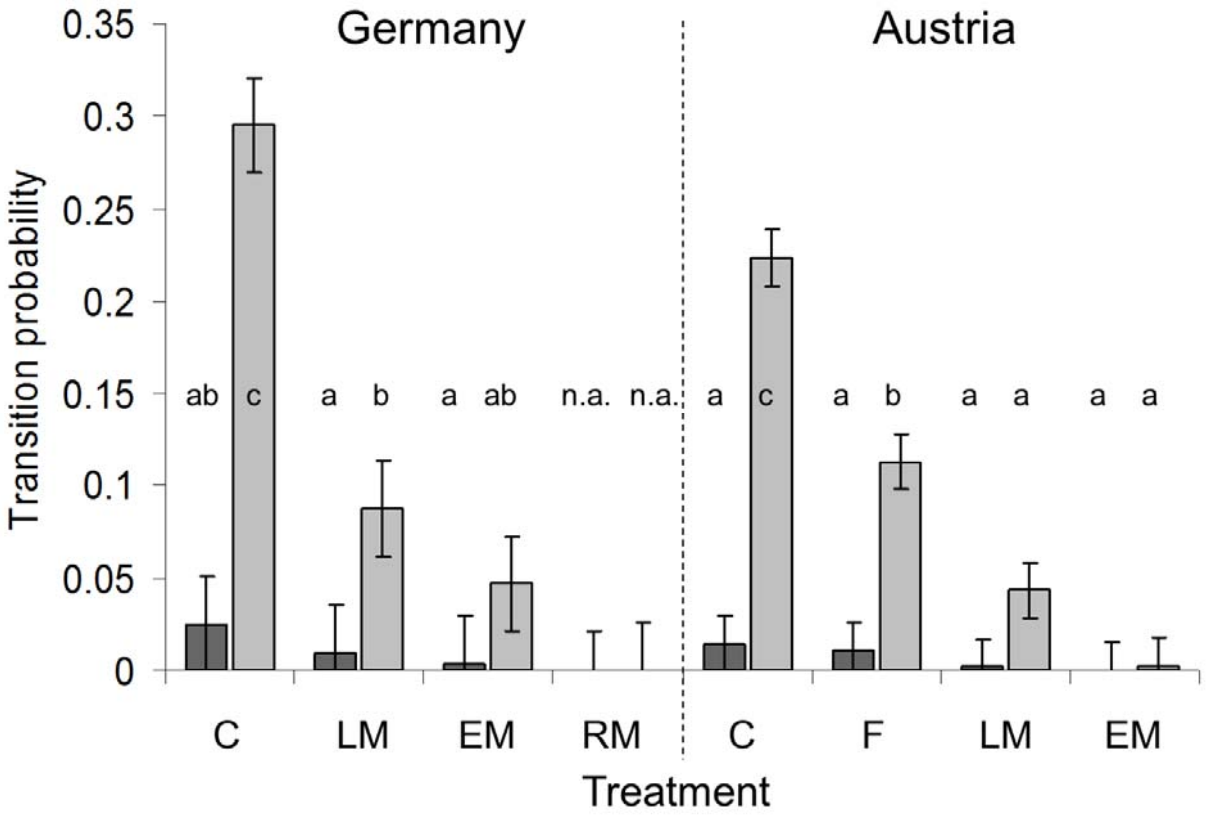

Fig. S2. Mean transition probability to a generative plant ± SE of medium (L2) and large vegetatives (L3) (see Fig. 1) under different treatments in Germany (*n* = 9 populations, except for RM where *n* = 3 – therefore n.a. = not analysed) and Austria (*n* = 7 populations). Treatments: C = control, F = flower removal, EM = early May cut, LM = late May cut, RM = repeated May cut; dark grey bar = medium vegetative (L2), light grey bar = large vegetative (L3). Bars with different combinations of lowercase letters indicate significant differences at the 5% level (Tukey's test) separately for each country.

Table S1. Climatic characteristics of geographical regions and characterisation of grassland sites in Austria (data were interpolated with the Austrian version of DAYMET (Hasenauer *et al.*, 2003) and Germany (HLUG, 2009).

Country/ Region	Mean annual precipitation (mm)	Mean annual temperature (°C)	Study Site	Initial plant density/m² (therefrom seedlings)	Location and altitude (m.a.s.l.)	Vegetation type according to Ellenberg, 2009) and habitat types listed in annex I of the Habitats Directive (European Commission, 1992/1995)
Austria/ Vienna Woods (A1)	740-750	9.7-9.8	A1-1	25.2 (0.6)	16°05'25'' 48°04'05'', 415	Arrhenatherion elatioris/ Lowland hay meadow 6510
			A1-2	26.6 (0.2)	16°04'35'' 48°12'43'', 428	Arrhenatherion elatioris/ Lowland hay meadow 6510
Austria/ Schneeberg territory (A2)	930	8.8-8.9	A2-1	65.7 (8.8)	15°57'39'' 47°52'20'', 600	Bromion erecti/ Semi-natural dry grasslands and scrubland facies on calcareous substrates 6210
			A2-2	121.3 (50.8)	15°58'58'' 47°49'51'', 600	Arrhenatherion elatioris/ Lowland hay meadow 6510
Austria/ Southern Waldviertel (A3)	730-780	7.5-7.9	A3-1	77.4 (16.2)	15°25'12'' 48°24'26'', 686	Arrhenatherion elatioris/ Lowland hay meadow 6510
			A3-2	28.8 (0.5)	15°17'32'' 48°25'30, 750	Arrhenatherion elatioris/ Lowland hay meadow 6510
			A3-3	76.6 (7.4)	15°17'37'' 48°25'29'', 750	Violion caninae/ Species-rich *Nardus* grasslands, on silicious substrates in mountain areas 6230
Germany/ Northern Upper Rhineplain (G1)	550-650	10-11	G1-1	59.1 (20.7)	8°46'48" 49°83'08", 90	Cnidion dubii/ Alluvial meadows of river valleys of the *Cnidion dubii* 6440
			G1-2	60.3 (8.3)	8°39'85" 49°81'05", 90	Cnidion dubii /Alluvial meadows of river valleys of *the Cnidion dubii* 6440
			G1-3	27.6 (1.6)	8°39'8" 49°81'97", 90	Arrhenatherion elatioris/ Lowland hay meadow 6510
Germany/ Vogelsberg Foothills (G2)	600-700	9-10	G2-1	16.4 (0.1)	8°83'2" 50°52'7", 177	Arrhenatherion elatioris/ Lowland hay meadow 6510
			G2-2	16.2 (5.1)	8°86'02" 50°53'02", 177	Arrhenatherion elatioris/ Lowland hay meadow 6510
			G2-3	39.8 (5.8)	8°76'49" 50°60'99", 159	Arrhenatherion elatioris/ Lowland hay meadow 6510
Germany/ Lahn-Dill Highlands (G3)	900-1000	7-8	G3-1	47.2 (3.4)	8°32'89" 50°75'35", 298	Arrhenatherion elatioris/ Lowland hay meadow 6510
			G3-2	32.3 (0.3)	8°41'61" 50°79'51", 444	Polygono-Trisetion/ Mountain hay meadow 6250
			G3-3	19.9 (1.8)	8°47'43" 50°85'22", 330	Polygono-Trisetion/ Mountain hay meadow 6250

Hasenauer, H., Merganicova, K., Petritsch, R., Pietsch, S.A. & Thornton, P.E. (2003) Validating daily climate interpolations over complex terrain in Austria. *Agricultural and Forest Meteorology*, **119**, 87-107.

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Table S2. Transition matrices of *Colchicum autumnale* of Austria (*n* = 7 populations) and Germany (*n* = 9 populations) calculated by averaging transition matrices across populations of one country during three time intervals (2008–2009, 2009–2010, 2010–2011). Each matrix element (a_{ij}) was calculated from the number of individuals in stage *j* in year *t* that passed into stage *i* in year *t+1*, divided by the column total of stage *j* (Caswell, 2001). Vegetative reproduction was incorporated in the corresponding matrix cell. S = seedlings, L1 = small vegetatives, L2 = medium vegetatives, L3 = large vegetatives, G = generatives with capsules and D = dormant plants. Treatments: C = control, F = flower removal, EM = early May cut, LM = late May cut, RM = repeated May cut.

2008-2009								2009-2010							2010-2011					
Austria																				
Treatment C																				
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	1.23	0.00		0.00	0.00	0.00	0.00	3.72	0.00		0.00	0.00	0.00	0.00	3.20	0.00
L1	0.90	0.55	0.10	0.01	0.01	0.08		0.42	0.72	0.05	0.00	0.00	0.13		0.65	0.72	0.13	0.01	0.01	0.29
L2	0.00	0.23	0.72	0.14	0.12	0.47		0.00	0.12	0.61	0.05	0.05	0.34		0.00	0.06	0.70	0.17	0.17	0.29
L3	0.00	0.00	0.07	0.60	0.46	0.09		0.00	0.00	0.20	0.58	0.29	0.11		0.00	0.00	0.06	0.69	0.57	0.23
G	0.00	0.00	0.01	0.24	0.43	0.01		0.00	0.00	0.03	0.35	0.61	0.00		0.00	0.00	0.00	0.07	0.18	0.00
D	0.00	0.00	0.03	0.02	0.00	0.35		0.00	0.00	0.01	0.01	0.01	0.42		0.00	0.00	0.02	0.02	0.00	0.19
Treatment F																				
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	2.04	0.00		0.00	0.00	0.00	0.00	13.6	0.00		0.00	0.00	0.00	0.00	2.80	0.00
L1	0.66	0.64	0.10	0.01	0.01	0.15		0.45	0.63	0.04	0.01	0.01	0.17		0.51	0.60	0.10	0.00	0.01	0.46
L2	0.00	0.17	0.61	0.18	0.11	0.33		0.00	0.17	0.61	0.06	0.07	0.34		0.00	0.05	0.59	0.13	0.22	0.36
L3	0.00	0.00	0.14	0.70	0.65	0.15		0.00	0.00	0.22	0.76	0.56	0.17		0.00	0.00	0.12	0.77	0.63	0.07
G	0.00	0.00	0.01	0.09	0.18	0.01		0.00	0.00	0.01	0.18	0.40	0.02		0.00	0.00	0.01	0.06	0.11	0.00
D	0.00	0.01	0.07	0.03	0.05	0.37		0.00	0.02	0.02	0.01	0.01	0.29		0.00	0.02	0.04	0.02	0.02	0.12
Treatment LM																				
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	2.82	0.00		0.00	0.00	0.00	0.00	9.25	0.00		0.00	0.00	0.00	0.00	1.10	0.00
L1	0.80	0.72	0.18	0.00	0.02	0.23		0.44	0.66	0.08	0.01	0.00	0.40		0.32	0.62	0.19	0.01	0.01	0.45
L2	0.00	0.15	0.71	0.39	0.34	0.49		0.00	0.12	0.75	0.28	0.29	0.29		0.00	0.01	0.60	0.64	0.34	0.32
L3	0.00	0.00	0.03	0.49	0.45	0.09		0.00	0.00	0.07	0.57	0.58	0.04		0.00	0.00	0.01	0.21	0.17	0.05
G	0.00	0.00	0.00	0.06	0.17	0.00		0.00	0.00	0.01	0.04	0.09	0.01		0.00	0.00	0.00	0.01	0.00	0.00
D	0.00	0.00	0.02	0.02	0.01	0.19		0.00	0.00	0.01	0.01	0.01	0.26		0.00	0.00	0.01	0.02	0.00	0.18
Treatment EM																				
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
L1	0.92	0.89	0.24	0.02	0.01	0.19		0.00	0.76	0.15	0.00	0.00	0.46		0.00	0.58	0.33	0.02	0.00	0.21
L2	0.00	0.03	0.60	0.59	0.26	0.50		0.00	0.03	0.70	0.49	0.00	0.67		0.00	0.02	0.43	0.64	0.14	0.35
L3	0.00	0.00	0.01	0.33	0.48	0.07		0.00	0.00	0.02	0.39	0.14	0.00		0.00	0.00	0.01	0.09	0.00	0.00
G	0.00	0.00	0.00	0.00	0.02	0.00		0.00	0.00	0.00	0.01	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.01	0.02	0.01	0.10		0.00	0.00	0.01	0.00	0.00	0.01		0.00	0.00	0.01	0.01	0.00	0.01

to be continued…

Table S2. – Continued.

Germany																			
Treatment C																			
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	7.41	0.00		0.00	0.00	0.00	0.00	2.57	0.00		0.00	0.00	0.00	0.00	2.82	0.00
L1	0.36	0.45	0.03	0.00	0.00	0.08		0.35	0.55	0.05	0.01	0.01	0.16		0.32	0.45	0.02	0.01	0.00	0.19
L2	0.00	0.22	0.67	0.07	0.07	0.41		0.00	0.12	0.56	0.11	0.10	0.61		0.00	0.08	0.50	0.10	0.05	0.29
L3	0.00	0.00	0.13	0.47	0.50	0.08		0.00	0.01	0.17	0.52	0.46	0.14		0.00	0.01	0.20	0.47	0.29	0.23
G	0.00	0.01	0.01	0.35	0.35	0.04		0.00	0.00	0.02	0.25	0.24	0.04		0.00	0.00	0.04	0.30	0.17	0.13
D	0.00	0.03	0.05	0.04	0.03	0.38		0.00	0.01	0.10	0.04	0.03	0.06		0.00	0.02	0.04	0.03	0.02	0.05
Treatment LM																			
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	10.70	0.00		0.00	0.00	0.00	0.00	3.17	0.00		0.00	0.00	0.00	0.00	0.58	0.00
L1	0.40	0.55	0.03	0.01	0.01	0.05		0.33	0.69	0.07	0.02	0.01	0.18		0.27	0.41	0.08	0.01	0.00	0.05
L2	0.00	0.18	0.69	0.14	0.11	0.35		0.00	0.03	0.64	0.21	0.22	0.71		0.00	0.07	0.58	0.24	0.11	0.32
L3	0.00	0.00	0.13	0.65	0.55	0.21		0.00	0.00	0.13	0.60	0.70	0.20		0.00	0.01	0.14	0.56	0.17	0.07
G	0.00	0.00	0.01	0.12	0.24	0.06		0.00	0.00	0.01	0.08	0.04	0.02		0.00	0.00	0.01	0.07	0.05	0.00
D	0.00	0.01	0.04	0.04	0.03	0.33		0.00	0.00	0.04	0.02	0.01	0.00		0.00	0.02	0.05	0.04	0.02	0.00
Treatment EM																			
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	23.83	0.00		0.00	0.00	0.00	0.00	2.89	0.00		0.00	0.00	0.00	0.00	0.00	0.00
L1	0.36	0.34	0.03	0.00	0.00	0.10		0.31	0.53	0.15	0.02	0.01	0.33		0.22	0.38	0.10	0.02	0.00	0.33
L2	0.00	0.16	0.68	0.13	0.13	0.48		0.00	0.01	0.63	0.53	0.35	0.39		0.00	0.04	0.59	0.41	0.00	0.41
L3	0.00	0.00	0.07	0.60	0.53	0.18		0.00	0.00	0.02	0.27	0.38	0.15		0.00	0.00	0.04	0.40	0.00	0.16
G	0.00	0.00	0.01	0.14	0.18	0.06		0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.01	0.00	0.06
D	0.00	0.00	0.06	0.04	0.08	0.18		0.00	0.00	0.04	0.05	0.04	0.13		0.00	0.04	0.06	0.03	0.00	0.04
Treatment RM																			
	S	L1	L2	L3	G	D		S	L1	L2	L3	G	D		S	L1	L2	L3	G	D
S	0.00	0.00	0.00	0.00	90.83	0.00		0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
L1	0.16	0.62	0.19	0.02	0.00	0.00		0.03	0.39	0.17	0.02	0.00	0.33		0.07	0.57	0.21	0.00	0.00	0.36
L2	0.00	0.00	0.61	0.68	0.39	0.67		0.00	0.00	0.47	0.72	0.00	0.17		0.00	0.04	0.60	0.33	0.00	0.52
L3	0.00	0.00	0.00	0.22	0.61	0.00		0.00	0.00	0.00	0.04	0.00	0.00		0.00	0.00	0.01	0.33	0.00	0.00
G	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.02	0.01	0.00	0.00		0.00	0.00	0.08	0.02	0.00	0.17		0.00	0.00	0.03	0.00	0.00	0.11

Table S3. Vital rate values for the averaged matrices of the Austrian treatments (C = control, F = flower removal, EM = early May cut, LM = late May cut).

		2008-09				2009-10				2010-11			
		EM	**LM**	**F**	**C**	**EM**	**LM**	**F**	**C**	**EM**	**LM**	**F**	**C**
Survival	σ 1	0.921	0.798	0.659	0.902	0.000	0.441	0.451	0.421	0.000	0.323	0.506	0.647
	σ 2	0.923	0.878	0.814	0.786	0.791	0.784	0.821	0.847	0.601	0.629	0.661	0.786
	σ 3	0.847	0.921	0.925	0.919	0.882	0.899	0.902	0.899	0.779	0.804	0.863	0.911
	σ 4	0.948	0.949	0.953	0.978	0.884	0.901	0.969	0.954	0.761	0.889	0.960	0.946
	σ 5	0.768	0.973	0.973	0.974	0.268	0.971	1.000	0.928	0.143	0.513	0.947	0.912
	σ 6	0.857	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.571	1.000	1.000	1.000
Stasis	γ22	0.963	0.824	0.782	0.698	0.957	0.847	0.761	0.853	0.971	0.978	0.903	0.920
	γ33	0.694	0.765	0.656	0.769	0.796	0.829	0.668	0.679	0.550	0.745	0.681	0.765
	γ44	0.342	0.513	0.713	0.586	0.439	0.628	0.754	0.581	0.124	0.239	0.794	0.725
	γ55	0.024	0.173	0.183	0.424	0.000	0.096	0.395	0.642	0.000	0.000	0.117	0.193
	γ66	0.117	0.188	0.370	0.349	0.011	0.259	0.292	0.420	0.018	0.180	0.123	0.190
Growth	γ21	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000
	γ32	0.034	0.171	0.204	0.299	0.043	0.151	0.212	0.145	0.026	0.016	0.069	0.078
	γ42	0.000	0.000	0.000	0.003	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
	γ52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	γ43	0.007	0.029	0.154	0.080	0.021	0.073	0.249	0.227	0.010	0.010	0.138	0.066
	γ53	0.000	0.000	0.009	0.013	0.000	0.006	0.014	0.032	0.000	0.000	0.012	0.002
	γ54	0.004	0.067	0.087	0.244	0.006	0.049	0.185	0.365	0.000	0.014	0.064	0.079
	γ26	0.219	0.229	0.149	0.084	0.461	0.401	0.173	0.133	0.366	0.449	0.462	0.287
	γ36	0.581	0.490	0.327	0.466	0.528	0.286	0.343	0.339	0.616	0.318	0.348	0.295
	γ46	0.083	0.093	0.147	0.089	0.000	0.044	0.168	0.108	0.000	0.053	0.067	0.228
	γ56	0.000	0.000	0.007	0.012	0.000	0.010	0.024	0.000	0.000	0.000	0.000	0.000
Retrogression	ρ62	0.003	0.004	0.010	0.000	0.000	0.002	0.017	0.001	0.001	0.002	0.012	0.001
	ρ23	0.284	0.184	0.108	0.105	0.170	0.086	0.048	0.051	0.427	0.232	0.118	0.146
	ρ63	0.015	0.022	0.072	0.034	0.013	0.007	0.021	0.012	0.014	0.014	0.052	0.020
	ρ24	0.015	0.002	0.005	0.003	0.005	0.006	0.002	0.000	0.031	0.014	0.001	0.007
	ρ34	0.620	0.401	0.166	0.142	0.550	0.303	0.044	0.039	0.837	0.716	0.123	0.172
	ρ64	0.019	0.018	0.030	0.025	0.000	0.014	0.015	0.015	0.009	0.017	0.018	0.018
	ρ25	0.011	0.010	0.005	0.002	0.000	0.000	0.000	0.000	0.000	0.015	0.006	0.003
	ρ35	0.330	0.343	0.106	0.106	0.133	0.300	0.040	0.049	1.000	0.656	0.211	0.174
	ρ45	0.621	0.458	0.660	0.465	0.867	0.597	0.552	0.301	0.000	0.329	0.648	0.626
	ρ65	0.014	0.015	0.047	0.003	0.000	0.006	0.012	0.008	0.000	0.000	0.017	0.004
Generative reproduction	Φ5	0.000	2.819	2.043	1.234	0.000	9.252	13.64	3.723	0.000	1.101	2.801	3.198

to be continued…

Table S3. – Continued.

Vegetative Reproduction													
		2008-09				2009-10				2010-11			
		EM	LM	F	C	EM	LM	F	C	EM	LM	F	C
Survival	σ 2	0.002	0.000	0.001	0.000	0.002	0.001	0.006	0.000	0.000	0.000	0.000	0.000
	σ 3	0.008	0.014	0.004	0.012	0.003	0.000	0.009	0.004	0.002	0.000	0.001	0.001
	σ 4	0.011	0.006	0.056	0.039	0.000	0.003	0.052	0.042	0.000	0.000	0.021	0.010
	σ 5	0.007	0.015	0.016	0.058	0.000	0.000	0.043	0.033	0.000	0.000	0.036	0.016
	σ 6	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.011	0.000
Vegetative reproduction	K22	0.002	0.000	0.001	0.000	0.002	0.001	0.006	0.000	0.000	0.000	0.597	0.724
	K32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.062
	K42	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K23	0.000	0.013	0.001	0.000	0.001	0.000	0.000	0.002	0.002	0.000	0.102	0.133
	K33	0.008	0.001	0.003	0.012	0.002	0.000	0.009	0.002	0.000	0.000	0.587	0.697
	K43	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.119	0.060
	K53	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.002
	K24	0.003	0.000	0.004	0.007	0.000	0.000	0.006	0.003	0.000	0.000	0.001	0.006
	K34	0.006	0.006	0.020	0.005	0.000	0.003	0.016	0.014	0.000	0.000	0.118	0.162
	K44	0.002	0.000	0.024	0.022	0.000	0.000	0.030	0.024	0.000	0.000	0.762	0.685
	K54	0.000	0.000	0.007	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.075
	K25	0.003	0.006	0.004	0.012	0.000	0.000	0.009	0.004	0.000	0.000	0.006	0.003
	K35	0.004	0.008	0.006	0.020	0.000	0.000	0.025	0.008	0.000	0.000	0.200	0.159
	K45	0.000	0.000	0.005	0.009	0.000	0.000	0.009	0.007	0.000	0.000	0.614	0.571
	K55	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.015	0.000	0.000	0.111	0.176
	K26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.462	0.287
	K36	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.348	0.295
	K46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.228
	K56	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table S4. Vital rate values for the averaged matrices of the German treatments (C = control, F = flower removal, EM = early May cut, LM = late May cut). C* = Control of region G2 only.

		2008-09					2009-10					2010-11				
		RM	EM	LM	C	C*	RM	EM	LM	C	C*	RM	EM	LM	C	C*
Survival	σ 1	0.163	0.357	0.402	0.357	0.507	0.033	0.315	0.326	0.355	0.209	0.067	0.220	0.268	0.319	0.368
	σ 2	0.619	0.507	0.734	0.705	0.667	0.394	0.536	0.733	0.687	0.716	0.609	0.466	0.508	0.560	0.599
	σ 3	0.828	0.860	0.909	0.893	0.967	0.721	0.845	0.877	0.893	0.973	0.850	0.790	0.854	0.771	0.921
	σ 4	0.937	0.914	0.939	0.933	0.980	0.797	0.857	0.919	0.904	0.948	0.667	0.865	0.909	0.884	0.939
	σ 5	1.000	0.929	0.928	0.944	0.952	0.000	0.760	0.981	0.827	0.978	0.000	0.000	0.360	0.533	0.856
	σ 6	0.667	1.000	1.000	1.000	1.000	0.667	1.000	1.000	1.000	1.000	1.000	1.000	0.444	0.889	1.000
Stasis	γ22	1.000	0.679	0.745	0.643	0.311	0.992	0.989	0.951	0.798	0.759	0.939	0.812	0.815	0.801	0.670
	γ33	0.740	0.797	0.757	0.745	0.874	0.658	0.748	0.723	0.624	0.586	0.710	0.752	0.679	0.646	0.691
	γ44	0.236	0.655	0.687	0.506	0.621	0.048	0.313	0.648	0.570	0.540	0.500	0.461	0.615	0.515	0.449
	γ55	0.000	0.196	0.262	0.365	0.253	0.000	0.000	0.043	0.288	0.420	0.000	0.000	0.150	0.311	0.413
	γ66	0.000	0.183	0.325	0.381	0.355	0.250	0.126	0.000	0.059	0.079	0.111	0.045	0.000	0.046	0.070
Growth	γ21	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	γ32	0.000	0.314	0.248	0.310	0.589	0.008	0.008	0.047	0.175	0.241	0.061	0.094	0.143	0.143	0.213
	γ42	0.000	0.002	0.000	0.000	0.000	0.000	0.001	0.002	0.009	0.000	0.000	0.000	0.012	0.022	0.062
	γ52	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	γ43	0.000	0.083	0.141	0.149	0.069	0.000	0.024	0.147	0.194	0.154	0.009	0.045	0.163	0.231	0.206
	γ53	0.000	0.016	0.016	0.016	0.000	0.000	0.000	0.009	0.028	0.018	0.000	0.000	0.009	0.049	0.050
	γ54	0.000	0.152	0.131	0.377	0.353	0.000	0.000	0.086	0.276	0.367	0.000	0.012	0.073	0.340	0.492
	γ26	0.000	0.097	0.053	0.084	0.067	0.500	0.331	0.071	0.151	0.199	0.364	0.329	0.118	0.210	0.059
	γ36	1.000	0.482	0.353	0.413	0.492	0.250	0.391	0.706	0.606	0.606	0.525	0.411	0.729	0.329	0.452
	γ46	0.000	0.181	0.213	0.084	0.062	0.000	0.152	0.204	0.144	0.079	0.000	0.160	0.153	0.269	0.319
	γ56	0.000	0.058	0.056	0.037	0.025	0.000	0.000	0.020	0.039	0.037	0.000	0.056	0.000	0.146	0.100
Retrogression	ρ62	0.000	0.001	0.004	0.018	0.044	0.000	0.000	0.000	0.008	0.000	0.000	0.020	0.008	0.011	0.020
	ρ23	0.233	0.036	0.037	0.036	0.011	0.232	0.178	0.074	0.040	0.032	0.250	0.123	0.092	0.021	0.029
	ρ63	0.028	0.068	0.049	0.054	0.046	0.110	0.049	0.048	0.114	0.210	0.031	0.080	0.057	0.053	0.024
	ρ24	0.025	0.005	0.003	0.004	0.000	0.020	0.023	0.017	0.010	0.000	0.000	0.019	0.010	0.009	0.013
	ρ34	0.727	0.140	0.138	0.074	0.021	0.909	0.606	0.231	0.106	0.056	0.500	0.468	0.257	0.104	0.034
	ρ64	0.013	0.048	0.040	0.038	0.005	0.024	0.058	0.017	0.039	0.037	0.000	0.040	0.045	0.031	0.012
	ρ25	0.000	0.000	0.012	0.002	0.000	0.000	0.009	0.011	0.005	0.009	0.000	0.000	0.000	0.004	0.000
	ρ35	0.389	0.145	0.101	0.074	0.088	0.000	0.443	0.225	0.111	0.045	0.000	0.000	0.302	0.098	0.058
	ρ45	0.611	0.572	0.592	0.532	0.616	0.000	0.499	0.710	0.555	0.486	0.000	0.000	0.481	0.548	0.480
	ρ65	0.000	0.087	0.033	0.027	0.044	0.000	0.049	0.011	0.041	0.040	0.000	0.000	0.066	0.039	0.050
Generative reproduction	Φ5	90.83	23.83	10.70	7.409	9.548	0.000	2.890	3.169	2.491	4.102	0.000	0.000	0.576	2.797	3.783

to be continued…

Table S4. – Continued.

Vegetative Reproduction																
		2008-09					2009-10					2010-11				
		RM	EM	LM	C	C*	RM	EM	LM	C	C*	RM	EM	LM	C	C*
Survival	σ 2	0.000	0.000	0.000	0.012	0.037	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.037
	σ 3	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.002	0.011	0.000	0.000	0.000	0.000	0.025	0.000
	σ 4	0.000	0.002	0.010	0.013	0.000	0.000	0.001	0.000	0.019	0.028	0.000	0.005	0.002	0.027	0.000
	σ 5	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.020	0.056	0.000	0.000	0.000	0.000	0.000
	σ 6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Vegetative reproduction	K22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.572	0.000	0.415	0.449	0.401
	K32	0.000	0.000	0.000	0.012	0.037	0.000	0.002	0.000	0.000	0.000	0.037	0.000	0.073	0.080	0.127
	K42	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.012	0.037
	K52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K23	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.011	0.000	0.213	0.000	0.078	0.017	0.027
	K33	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.002	0.000	0.000	0.604	0.000	0.580	0.499	0.636
	K43	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.139	0.178	0.190
	K53	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.038	0.046
	K24	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.004	0.011	0.000	0.005	0.009	0.008	0.012
	K34	0.000	0.001	0.006	0.010	0.000	0.000	0.001	0.000	0.009	0.000	0.333	0.000	0.233	0.092	0.032
	K44	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.006	0.017	0.333	0.000	0.558	0.456	0.422
	K54	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.301	0.462
	K25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.022	0.000	0.000	0.000	0.002	0.000
	K35	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.007	0.017	0.000	0.000	0.109	0.052	0.049
	K45	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.017	0.000	0.000	0.173	0.292	0.410
	K55	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.166	0.353
	K26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.364	0.000	0.052	0.187	0.059
	K36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.525	0.000	0.324	0.293	0.452
	K46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.239	0.319
	K56	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.100

Table S5. Effects of between-subject (BS) factors (R = three regions per country; T = four (Austria) or three (Germany) treatments, see Table 1; St = two life cycle stages, L2, L3, see Figure 1) and within-subject (WS) factors (Y, year and its interactions) on transition probability of life stages to the generative stage (G) in the next year. Between-subject effects were determined by ANOVA, whereas within-subject effects were determined by MANOVA. DF = degrees of freedom, MQ = mean sum of squares.

Austria

BS	Source of variation	DF	MQ	*F*	*P*
	R (region)	2	0.03	1.91	0.16
	T (treatment)	3	0.53	29.47	<0.0001
	St (stage)	1	1.46	81.43	<0.0001
	R x T	6	0.01	0.32	0.92
	R x St	2	0.02	0.84	0.44
	T x St	3	0.24	13.46	<0.0001
	R x T x St	6	0.003	0.19	0.98
	Error	32	0.02		

WS	Source of variation	Wilks' Lambda	$DF_{Hypothesis}$	DF_{Error}	*F*	*P*
	Y (year)	0.46	2	31	18.11	<0.0001
	Y x R	0.88	4	62	1.07	0.38
	Y x T	0.53	6	62	3.80	0.003
	Y x St	0.67	2	31	7.69	0.002
	Y x R x T	0.71	12	62	0.98	0.48
	Y x R x St	0.95	4	62	0.40	0.81
	Y x T x St	0.76	6	62	1.52	0.19
	Y x R x T x St	0.60	12	62	1.52	0.14

Germany

BS	Source of variation	DF	MQ	*F*	*P*
	R (region)	2	0.02	0.34	0.71
	T (treatment)	2	0.72	15.49	<0.0001
	St (stage)	1	2.32	49.57	<0.0001
	R x T	4	0.04	0.93	0.46
	R x St	2	0.02	0.48	0.62
	T x St	2	0.42	8.90	<0.001
	R x T x St	4	0.05	1.03	0.40
	Error	36	0.05		

WS	Source of variation	Wilks' Lambda	$DF_{Hypothesis}$	DF_{Error}	*F*	*P*
	Y (year)	0.76	2	35	5.40	0.009
	Y x R	0.86	4	70	1.36	0.26
	Y x T	0.84	4	70	1.56	0.20
	Y x St	0.85	2	35	3.19	0.053
	Y x R x T	0.75	8	70	1.35	0.24
	Y x R x St	0.89	4	70	1.00	0.41
	Y x T x St	0.96	4	70	0.36	0.84
	Y x R x T x St	0.88	8	70	0.55	0.81

Table S6. Population growth rate for each population and treatment in Austria and Germany. Treatments: C = control, F = flower removal, EM = early May cut, LM = late May cut, RM = repeated May cut. For details on populations see Table S1.

Transition period	Treatment	Populations in Austria							Populations in Germany								
		A1-1	A1-2	A2-1	A2-2	A3-1	A3-2	A3-3	G1-1	G1-2	G1-3	G2-1	G2-2	G2-3	G3-1	G3-2	G3-3
	C	1.12	0.92	1.02	1.15	0.91	0.89	1.06	1.07	0.60	1.68	1.50	1.00	0.95	0.99	1.00	1.07
	F	1.07	0.92	0.92	1.15	0.89	0.96	0.99									
2008/09	LM	1.06	0.74	0.94	1.04	0.99	0.85	0.96	0.86	0.77	0.96	0.96	1.00	1.00	0.95	1.18	1.03
	EM	1	1	0.92	0.97	0.91	0.71	0.93	0.89	0.56	0.83	0.91	1.00	0.92	0.88	0.99	1.00
	RM											0.86	1.00	1.00			
	C	1.29	0.84	1.12	1.13	1.09	0.96	1.01	1.07	0.76	0.48	1.00	1.00	1.01	0.96	0.98	1.04
	F	1.29	0.94	1.22	1.20	1.20	0.96	1.12									
2009/10	LM	0.97	0.71	0.92	1.02	0.98	0.75	0.94	0.91	0.73	0.66	0.75	0.86	0.99	0.95	0.97	1.00
	EM	0.91	0.63	0.86	0.90	0.95	0.71	0.89	0.73	0.59	0.50	0.71	0.98	1.00	0.57	0.89	1.00
	RM											0.62	0.43	0.76			
	C	0.93	0.83	0.90	0.92	0.94	0.86	0.97	0.88	0.50	0.50	0.98	1.08	0.99	0.77	0.96	0.80
	F	0.90	0.98	0.93	0.94	0.91	0.85	0.93									
2010/11	LM	0.74	0.48	0.67	0.77	0.79	0.61	0.83	0.56	0.71	0.79	0.87	0.88	0.96	0.93	0.93	0.81
	EM	0.70	0.53	0.64	0.52	0.64	0.53	0.77	0.70	0.51	0.36	0.72	0.83	0.82	0.79	0.82	0.92
	RM											0.53	1.00	0.71			

Table S7. LTRE-contributions for the vital rates of the EM, LM and F treatments in comparison to the control treatment in Austria. Treatments: F = flower removal, EM = early May cut, LM = late May cut. Absolute LTRE-values ≥ 0.01 are given in bold.

		2008-09			2009-10			2010-11		
		EM	**LM**	**F**	**EM**	**LM**	**F**	**EM**	**LM**	**F**
Survival	σ 1	-0.002	-0.002	-0.003	**-0.031**	0.003	0.006	-0.002	-0.002	-0.003
	σ 2	**-0.108**	**-0.057**	**-0.017**	**-0.019**	**-0.017**	-0.007	**-0.108**	**-0.057**	**-0.017**
	σ 3	**-0.043**	**-0.051**	**-0.014**	-0.007	0.000	0.001	**-0.043**	**-0.051**	**-0.014**
	σ 4	-0.009	-0.005	0.007	**-0.012**	**-0.015**	0.005	-0.009	-0.005	0.007
	σ 5	-0.003	-0.003	0.002	**-0.042**	0.005	**0.013**	-0.003	-0.003	0.002
	σ 6	-0.004	0.000	0.000	0.000	0.000	0.000	-0.004	0.000	0.000
Stasis	γ22	**0.019**	**0.014**	-0.001	**0.028**	-0.001	**-0.015**	**0.019**	**0.014**	-0.001
	γ33	**-0.069**	-0.009	**-0.021**	**0.043**	**0.041**	-0.002	**-0.069**	-0.009	**-0.021**
	γ44	**-0.030**	**-0.047**	**0.033**	**-0.023**	**0.011**	**0.048**	**-0.030**	**-0.047**	**0.033**
	γ55	0.000	-0.001	-0.005	**-0.024**	**-0.083**	**-0.055**	0.000	-0.001	-0.005
	γ66	-0.001	0.000	-0.002	-0.004	-0.001	-0.002	-0.001	0.000	-0.002
Growth	γ21	-0.001	0.000	0.000	**-0.016**	0.000	0.000	-0.001	0.000	0.000
	γ32	**-0.041**	**-0.041**	-0.003	**-0.044**	0.002	**0.031**	**-0.041**	**-0.041**	-0.003
	γ42	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000
	γ52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	γ43	**-0.024**	**-0.039**	**0.034**	**-0.087**	**-0.075**	0.007	**-0.024**	**-0.039**	**0.034**
	γ53	-0.001	-0.002	0.006	**-0.013**	**-0.023**	**-0.010**	-0.001	-0.002	0.006
	γ54	-0.004	-0.008	-0.009	**-0.054**	**-0.133**	**-0.086**	-0.004	-0.008	-0.009
	γ26	0.000	0.001	0.001	0.002	0.001	0.000	0.000	0.001	0.001
	γ36	0.003	0.000	0.001	0.002	0.000	0.000	0.003	0.000	0.001
	γ46	-0.003	-0.005	-0.007	-0.001	-0.001	0.002	-0.003	-0.005	-0.007
	γ56	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000
Retrogression	ρ62	0.000	0.001	0.003	-0.001	0.000	0.007	0.000	0.001	0.003
	ρ23	**0.043**	**0.014**	-0.002	**0.027**	0.004	0.000	**0.043**	**0.014**	-0.002
	ρ63	-0.002	-0.003	0.008	0.001	-0.001	0.001	-0.002	-0.003	0.008
	ρ24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	ρ34	**0.025**	**0.034**	**-0.012**	**0.072**	**0.036**	0.001	**0.025**	**0.034**	**-0.012**
	ρ64	0.000	0.000	0.000	-0.002	0.000	0.000	0.000	0.000	0.000
	ρ25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	ρ35	0.001	0.002	0.001	0.003	**0.012**	-0.001	0.001	0.002	0.001
	ρ45	-0.001	-0.002	0.001	**0.022**	**0.025**	**0.033**	-0.001	-0.002	0.001
	ρ65	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Generative reproduction	Φ5	-0.002	-0.002	-0.002	-0.031	0.049	0.097	-0.002	-0.002	-0.002
Net effect		-0.061	-0.011	-0.049	-0.181	-0.209	-0.020	-0.256	-0.209	0.002

to be continued …

Table S7. – Continued.

Vegetative reproduction										
		2008-09			2009-10			2010-11		
		EM	LM	F	EM	LM	F	EM	LM	F
Survival	σ 2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	σ 3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	σ 4	**-0.011**	**-0.012**	0.006	**-0.024**	**-0.021**	0.006	-0.003	-0.003	0.004
	σ 5	-0.007	-0.006	-0.007	0.000	0.000	0.000	0.000	0.000	0.001
	σ 6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Vegetative reproduction	K22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K42	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K43	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K53	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K44	-0.007	-0.008	-0.004	-0.012	-0.012	0.001	-0.001	-0.001	0.001
	K54	-0.002	-0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001
	K25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K35	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K45	-0.002	-0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.001
	K55	-0.003	-0.004	-0.004	0.000	0.000	0.000	0.000	0.000	0.000
	K26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K36	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K56	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Net effect		-0.031	-0.034	-0.005	-0.035	-0.033	0.007	-0.004	-0.004	0.007

Table S8. LTRE-contributions for the vital rates of the RM, EM and LM treatments in comparison to the control treatment in Germany. Treatments: EM = early May cut, LM = late May cut, RM = repeated May cut. Absolute LTRE-values ≥ 0.01 are given in bold.

		2008-09			2009-10			2010-11		
		RM	**EM**	**LM**	**RM**	**EM**	**LM**	**RM**	**EM**	**LM**
Survival	σ 1	**-0.065**	0.000	0.009	-0.008	-0.001	-0.002	**-0.028**	-0.005	-0.003
	σ 2	**-0.010**	**-0.045**	0.006	**-0.051**	**-0.020**	0.006	0.002	**-0.010**	-0.005
	σ 3	**-0.068**	**-0.012**	0.005	**-0.132**	**-0.024**	-0.006	**-0.034**	0.009	**0.031**
	σ 4	-0.008	-0.005	0.002	**-0.019**	**-0.010**	0.005	**-0.048**	-0.005	0.009
	σ 5	0.002	-0.002	-0.002	**-0.049**	-0.003	**0.014**	**-0.070**	**-0.041**	**-0.019**
	σ 6	**-0.015**	0.000	0.000	**-0.034**	0.000	0.000	0.000	0.005	**-0.018**
Stasis	γ22	**0.068**	0.003	**0.011**	**0.015**	**0.012**	**0.012**	**0.026**	0.000	0.001
	γ33	**-0.055**	**0.013**	0.003	**0.035**	**0.057**	**0.030**	0.009	**0.042**	**0.010**
	γ44	**-0.066**	**0.036**	**0.047**	**-0.061**	**-0.051**	**0.026**	0.007	**-0.014**	**0.035**
	γ55	**-0.026**	**-0.029**	**-0.018**	-0.008	**-0.014**	**-0.025**	**-0.012**	-0.004	-0.006
	γ66	**-0.012**	-0.009	-0.002	**0.016**	0.005	-0.003	0.001	0.000	-0.001
Growth	γ21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	γ32	**-0.136**	0.001	**-0.016**	**-0.050**	**-0.040**	**-0.031**	**-0.038**	-0.007	0.000
	γ42	0.000	0.001	0.000	0.000	-0.002	-0.002	**-0.013**	-0.003	-0.002
	γ52	0.000	**-0.010**	-0.009	0.000	0.000	0.000	0.000	0.000	0.000
	γ43	**-0.129**	**-0.038**	-0.004	**-0.085**	**-0.095**	**-0.018**	**-0.079**	**-0.085**	**-0.025**
	γ53	0.000	0.000	-0.001	-0.006	**-0.019**	-0.009	**-0.016**	**-0.010**	**-0.010**
	γ54	**-0.164**	**-0.097**	**-0.110**	**-0.030**	**-0.065**	**-0.080**	**-0.057**	**-0.038**	**-0.064**
	γ26	-0.001	0.000	0.000	**0.010**	0.003	-0.001	0.004	0.002	-0.001
	γ36	**0.015**	0.003	-0.002	**-0.041**	**-0.014**	0.004	0.002	0.005	**0.012**
	γ46	-0.008	0.008	0.009	**-0.010**	0.001	0.003	-0.009	-0.007	-0.004
	γ56	-0.009	0.003	0.002	-0.003	-0.004	-0.001	-0.002	-0.003	-0.004
Retrogression	ρ62	**-0.012**	-0.005	-0.004	0.000	-0.002	-0.002	-0.005	0.001	0.000
	ρ23	**0.039**	0.000	0.000	0.030	**0.016**	0.003	**0.040**	**0.012**	0.006
	ρ63	-0.009	0.004	-0.001	**-0.041**	**-0.031**	**-0.022**	0.004	**0.011**	0.001
	ρ24	0.000	0.000	0.000	0.001	0.001	0.001	-0.001	0.001	0.000
	ρ34	**0.026**	0.007	0.009	**0.095**	**0.081**	**0.032**	**0.079**	**0.080**	**0.046**
	ρ64	0.000	0.001	0.000	-0.001	0.003	-0.006	-0.002	0.002	0.003
	ρ25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	ρ35	0.003	0.003	0.001	-0.001	**0.011**	0.007	-0.003	-0.002	**0.010**
	ρ45	0.000	0.004	0.006	**-0.014**	-0.002	**0.012**	**-0.018**	**-0.014**	-0.004
	ρ65	0.000	0.003	0.000	-0.001	0.000	-0.002	-0.002	-0.001	0.001
Generative reproduction	Φ5	**0.103**	0.088	**0.028**	**-0.011**	0.002	0.005	**-0.040**	**-0.025**	**-0.025**
Net effect		-0.641	-0.163	-0.059	-0.442	-0.210	-0.055	-0.262	-0.077	-0.002

to be continued…

Table S8. – Continued.

Vegetative reproduction										
Survival	σ 2	0.000	-0.006	-0.006	0.000	0.000	0.000	0.000	0.000	0.000
	σ 3	0.000	**-0.014**	**-0.014**	0.000	0.000	0.000	0.000	-0.007	-0.008
	σ 4	0.000	0.000	0.000	**-0.017**	-0.005	-0.006	0.000	**-0.011**	**-0.014**
	σ 5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	σ 6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Vegetative reproduction	K22	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.001
	K32	0.000	-0.003	-0.003	0.000	0.000	0.000	-0.038	-0.007	0.000
	K42	0.000	0.000	0.000	0.000	0.000	0.000	-0.013	-0.003	-0.002
	K52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K23	0.000	-0.003	-0.003	0.000	0.000	0.000	0.040	0.012	0.006
	K33	0.000	-0.004	-0.004	0.000	0.000	0.000	0.009	0.042	0.010
	K43	0.000	0.000	0.000	0.000	0.000	0.000	-0.079	-0.085	-0.025
	K53	0.000	0.000	0.000	0.000	0.000	0.000	-0.016	-0.010	-0.010
	K24	0.000	0.000	0.000	0.000	0.000	0.000	-0.001	0.001	0.000
	K34	0.000	0.000	0.000	0.000	0.000	0.000	0.079	0.080	0.046
	K44	0.000	0.000	0.000	-0.009	-0.003	-0.003	0.007	-0.014	0.035
	K54	0.000	0.000	0.000	0.000	0.000	0.000	-0.057	-0.038	-0.064
	K25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	K35	0.000	0.000	0.000	0.000	0.000	0.000	-0.003	-0.002	0.010
	K45	0.000	0.000	0.000	0.000	0.000	0.000	-0.018	-0.014	-0.004
	K55	0.000	0.000	0.000	0.000	0.000	0.000	-0.012	-0.004	-0.006
	K26	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.002	-0.001
	K36	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.012
	K46	0.000	0.000	0.000	0.000	0.000	0.000	-0.009	-0.007	-0.004
	K56	0.000	0.000	0.000	0.000	0.000	0.000	-0.002	-0.003	-0.004
Net effect		0.000	-0.029	-0.029	-0.026	-0.008	-0.009	0.000	-0.031	-0.025

Table S9. Effects of between-subject (BS) factors (R = regions; T = treatments, see Table 1) and within-subject (WS) factors (Y, year and its interactions) on Shannon Index. Between-subject effects were determined by ANOVA, whereas within-subject effects were determined by MANOVA. DF = degrees of freedom, MQ = mean sum of squares.

BS	Source of variation	DF	MQ	*F*	*P*
	R (region)	2	6.29	11.50	<0.0001
	T (treatment)	2	0.29	0.52	0.60
	R x T	4	0.14	0.25	0.91
	Error	63	0.55		

WS	Source of variation	Wilks' Lambda	$DF_{Hypothesis}$	DF_{Error}	*F*	*P*
	Y (year)	0.66	3	61	10.38	<0.0001
	Y x R	0.63	6	122	5.38	<0.0001
	Y x T	0.82	6	122	2.18	0.0497
	Y x R x T	0.83	12	161.68	1.00	0.45

Versicherung

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

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