

University of South Bohemia in České Budějovice  
Faculty of Science

**Victims of agricultural intensification**  
**Mowing date affects *Rhinanthus* spp.**  
**regeneration and fruit ripening.**

RNDr. Thesis

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## **Annotation**

The recent population declines of annual hemiparasitic *Rhinanthus* species may be a result of changes in mowing dates associated with the intensification of grassland management, but the causal mechanisms are not well understood. This thesis describes the dynamics of *Rhinanthus* spp. regeneration after cutting and of fruit ripening under silage or hay making, and suggest recommendations for conservation of the target species.

## **Authors' contribution**

The thesis is based on an original research article:

**Blažek P. & Lepš. J.** (2015) Victims of agricultural intensification: Mowing date affects *Rhinanthus* spp. regeneration and fruit ripening. *Agriculture, Ecosystems and Environment* 211: 10–16 (IF = 3.4). <http://dx.doi.org/10.1016/j.agee.2015.04.022>

Petr Blažek performed all steps of the work himself with the supervision of Jan Lepš.

## **Declaration [in Czech]**

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Petr Blažek

# Victims of agricultural intensification: Mowing date affects *Rhinanthus* spp. regeneration and fruit ripening.

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## Abstract

The recent population declines of annual hemiparasitic *Rhinanthus* species may be a result of changes in mowing dates associated with the intensification of grassland management, but the causal mechanisms are not well understood. We aimed to determine the dynamics of *Rhinanthus* regeneration after cutting and of fruit ripening under silage or hay making. Mowing was simulated on several dates from mid-May to mid-July in populations of a vernal ecotype of *Rhinanthus minor* and an aestival ecotype of *Rh. alectorolophus*. Survival and regeneration of clipped plants, as well as fruit ripening were monitored in the experiments. We showed that *Rhinanthus* species were capable of resprouting, albeit with high mortality, but only in early spring before the lower leaves were shed. The time of fruit ripening differed among phenological types by over a month and a considerable number of fruits ripened during hay making. If meadows are mown in the period when plants are not able to regenerate and not enough fruits have ripened, *Rhinanthus* populations could undergo a massive decline. Postponing the first cut until fruits start to ripen is necessary for the protection of these species. Current Czech agri-environmental measures (AEMs) subsidize postponing the first cut only in protected areas, which limits the distribution of *Rhinanthus*. Moreover, the earliest date for a postponed cut is mid-July, which is too late considering forage quality. We suggest implementing a late-June mowing, which would diversify the mosaic of various mowing dates within protected areas, and could be widely acceptable for farmers in nonprotected landscapes.

## Keywords

Yellow rattle, Hemiparasite, Agri-environmental schemes, Agricultural intensification, Delayed mowing.

## Abbreviation

AEMs: agri-environmental measures.

## Introduction

The agricultural amelioration of grasslands (i.e. fertilizer application, more frequent mowing, silage making etc.) increases the yield and quality of fodder (Zechmeister et al. 2003), but its detrimental effects on biodiversity are numerous documented (Zechmeister et al. 2003; Kleijn et al. 2009; Čížek et al. 2012). At the same time, as the demand for grassland fodder decreases (Hodgson et al. 2005), some grasslands are threatened by management abandonment (Isselstein et al. 2005; Poptcheva et al. 2009). A system of subsidies was established first to allow farmers to keep farming. Additionally, agri-environmental measures (AEMs) were introduced to motivate farmers to give up intensive farming practices by compensating for the loss in profitability. These AEMs could be a strong tool for nature conservation, but they have not been as effective in the protection of biodiversity as they could be (Coulson et al. 2001; Zechmeister et al. 2003; Hodgson et al. 2005). If AEMs are to effectively protect biodiversity and promote the survival of endangered species, they must be based on a more intimate knowledge of the needs of these species, based on real field data.

*Rhinanthus* species (Orobanchaceae) are annual hemiparasitic herbs growing in various grasslands in Europe, Asia and North America (Skála & Štech 2000; Těšitel et al. 2010). While these species can be harmful weeds in grasslands and corn fields (Carruthers 1899, 1903; Bastin 1915; Rabotnov 1956; Mizianty 1975), with *Rh. minor* even invading North America (van Hulst et al. 1987; Smith & Cox 2014), they are nowadays declining throughout Europe (Linusson et al. 1998; Westbury 2004; Ameloot 2007). The sites where they still grow are usually in areas without intensive management due to unsuitable environmental conditions or nature protection limits. They are missing in productive grasslands (ter Borg 1972; Westbury 2004), as well as in abandoned grasslands (ter Borg 1972; Lindborg et al. 2005; Ameloot et al. 2006). This distribution suggests that *Rhinanthus* species are harmed by changes in grassland management, but the reasons remain unclear.

The response of *Rhinanthus* to increased grassland productivity is rather complex due to its hemiparasitic life strategy. The plants are outcompeted when biomass is too high, but utilizing host resources, they can tolerate an increase in biomass up to about 500 g·m<sup>-2</sup> (van Hulst et al. 1987; Hwangbo & Seel 2002; Westbury et al. 2006; Hejcman et al. 2011) and even largely reduce host biomass (Ameloot et al. 2005; Westbury & Dunnett 2007; Mudrák & Lepš 2010).

The response of *Rhinanthus* to increased mowing frequency is less understood. If mowing starts to interfere with *Rhinanthus* flowering, which can happen when the first cut is shifted to an earlier date, *Rhinanthus* seed production could be decreased. As an annual with poor dispersal (Bullock et al. 2003) and an only shortterm persistent seed bank (ter Borg 1985; van Hulst et al. 1987; Mudrák et al. 2014), *Rhinanthus* is expected to respond rapidly to a decrease in seed production (Smith et al. 2000; Westbury 2004; Bullock & Pywell 2005; Bullock et al. 2008). The impact of seed loss caused by early mowing on *Rhinanthus* population persistence has been documented (Smith et al. 2000; Magda et al. 2004), but neither of these studies described the seasonal dynamics in detail. Seed loss can be amplified

by making silage instead of hay, which prevents some fruits from ripening after the cut (Smith et al. 1996; Svensson & Carlsson 2005), but the effect of this difference on seed production has not been quantified.

If *Rhinanthus* fails to produce seeds due to early mowing, it must be able to resprout and flower once more to keep its population stable. Despite being annuals without storage organs, *Rhinanthus* species have a limited regeneration capability (ter Borg 1972; Huhta et al. 2000). However, no study has investigated how the combination of both regeneration and the timing of fruit ripening could affect the persistence of *Rhinanthus* populations.

In this study we simulated mowing of meadows with *Rhinanthus* in order to ascertain the response of *Rhinanthus* to various mowing dates. We tried to answer two principal questions: (1) What is the latest mowing date for *Rhinanthus* to still be capable of regeneration, and which factors affect the regeneration rate? (2) What is the earliest mowing date for *Rhinanthus* to still produce ripe fruits, and is there any difference between hay and silage making?

## Materials and methods

### Study species and study sites

*Rhinanthus* species are known for seasonal polymorphism. A number of forms varying in plant architecture and phenology can be found within each species, ranging from small unbranched types which flower from mid-May (vernal ecotypes), through intermediate types (aestival ecotypes), to big branched types which flower from July (autumnal ecotypes; terminology according to Zopfi, 1993, 2011; ter Borg 1972, 1985; Skála & Štech 2000; Westbury 2004). We deliberately put higher importance on the ecotypes than on the actual species identity. To underlie this variability, we have chosen two natural populations whose onset of fruit ripening is shifted by over a month.

The first experimental population represents the vernal ecotype of *Rhinanthus minor* L. characterized by very small and early flowering individuals (Table 1). This species is still relatively common in low-productivity meadows and pastures, and it also inhabits disturbed places such as road and path verges (Skála & Štech 2000; Westbury 2004). The experimental site was situated in a meadow near Hejdlov (Blanský les protected landscape area, South Bohemia, Czech Republic, 48°52'3.8"N, 14°14'46.4"E) at an altitude of 740 m a.s.l. on a south-east facing slope of 13°, in a low productive species rich mesic meadow (*Arrhenatherion* alliance).

The other population is the aestival ecotype of *Rhinanthus alectorolophus* (Scop.) Pollich, representing big branched, and phenologically intermediate forms (Table 1). This species was once thought of as a corn-field weed (Skála & Štech 2000) but is now considered vulnerable according to the Czech Red List (Grulich 2012) and is also declining in Germany (Zopfi 2011). This is one of the tallest *Rhinanthus* species, which can grow also in slightly more productive meadows, fallow land, or road verges (Skála & Štech 2000). The

experimental site was situated in a meadow near Hrabětice (Jizerské hory protected landscape area, north-east Bohemia, Czech Republic, 50°46'48.3"N, 15°11'26.2"E) at an altitude of 770 m a.s.l. on a west facing slope of 6°, in a medium-productive species-rich mesic meadow (Polygono–Trisetion alliance).

## Regeneration

The first experiment tested the response of *Rhinanthus* individuals to being clipped at various dates and heights. The experiment for *Rh. minor* was carried out in 2011. We marked out 6 points in a rectangular grid of 2 × 3 points in a place with visually homogeneous distribution of *Rh. minor*. The distance of neighbouring points was 2.5 m. We simulated mowing on 3 dates from mid to the end of May (14, 22, 28 May) around two randomly selected points (out of the six) on each of the three dates. The area around each point was split into three sectors with different clipping heights (3, 6 and 9 cm) assigned randomly to the sectors. In each sector, we labelled 10 *Rh. minor* plants, nearest to the point, with small plastic tags. In total, we labelled 180 plants: 3 dates × 2 replications (points) × 3 heights (sectors) × 10 plants. We clipped each plant to a given height and recorded its original height, the number of leaves remaining after clipping, and its phenological stage (no flower buds present, only flower buds present, flowers or fruits also present). There were no lateral shoots on the plants, only the buds. We clipped also the surrounding vegetation at the same height to the distance of about 15 cm further from the point than the furthest labelled plant occurred. Three weeks after clipping, we checked each labelled plant and counted the lateral shoots shorter and longer than 1 cm separately. At the end of June (at one date for all clipping dates), we checked the labelled plants once more and again counted the lateral shoots and flowers or flower buds on each shoot.

Data collection for the experiment with *Rh. alectorolophus* was carried out in 2013 using an analogous methodology adjusted for the different phenology of this species. We marked out 4 points and simulated mowing in two dates (18 May, 8 June) around two randomly selected points (out of the four) on each of the two dates resulting in 120 plants clipped in total.

## Fruit ripening

The second experiment focussed on the dynamics of fruit ripening in response to making hay or silage. Data collection took place in the same sites and in the same years as the above described experiment. For *Rh. minor*, in a place with visually homogeneous distribution of its individuals, we marked out 9 points in a rectangular grid of 3 × 3 points. The distance of neighbouring points was 2.5 m. We simulated mowing in 3 dates from the end of May to mid-June (28 May, 5, 13 June 2011) around 3 randomly chosen points in each date. We clipped 20 *Rh. minor* plants, nearest to the point on the given date (3 replications (points) × 3 dates × 20 plants = 180 plants in total). On each plant, we assigned each fruit to a ripeness category (Appendix 1), and put the plant into a labelled paper bag. Then we simulated hay

making by drying the bags in about 3 layers at room temperature. One week after clipping, we assigned each fruit to a ripeness category again and counted the seeds that had fallen out spontaneously of the ripened fruits.

Data collection for the experiment with *Rh. alectorolophus* followed an analogous methodology adjusted for the different phenology of this species. We marked out 6 points and collected plants on 2 dates (26 June, 14 July 2013) around 3 randomly chosen points in each date, resulting in 120 plants in total. The site was mown just after 15 July.

## Data analysis

As the vast majority (88%) of *Rh. minor* shoots that were shorter than 1 cm 3 weeks after clipping died or did not overgrow the 1 cm limit, they were considered unviable and omitted from further analyses. A multiple logistic regression (i.e. a generalized linear model with binomial distribution of response variable) was used to test the relationship between the percentage of regenerated plants in the sector and the clipping date and height. We also tested the correlations between the plant characteristics at the time of clipping (i.e. plant height, number of leaves remaining after clipping, phenological stage – the three stages transformed to numerical values 1, 2 and 3) with treatments, and intercorrelations between each pair of these characteristics. Eventually, simple logistic regressions were used to test how regeneration of individual plants depends on their characteristics, and the threshold values of these characteristics were defined for potentially successful regeneration. We considered individual plants to be independent observations in all analyses where plant characteristics were involved. As *Rh. alectorolophus* did not regenerate at all, we could only calculate the correlations of plant characteristics and treatments for this species.

Summary statistics were computed for the number of flowers per plant and seeds per fruit. The dynamics in fruit ripening were simply plotted together with the overall results of the regeneration experiment. Since the number of fruits does not change linearly with clipping date, the differences among individual date were demonstrated using analysis of variance followed by the Tukey HSD test. To evaluate which fruits were able to ripen during haymaking, a transitional matrix between fruit ripeness categories before and after haymaking was created.

Table 1: An overview of the growth habit and phenology of the studied *Rhinanthus* types. See methods for details about data collection. Data from the first clipping date in the regeneration experiment (for plant height) and the first clipping date in the fruit ripening experiment (for flowers per plant) were excluded, because the parameters did not reach final values. Note that the number of seeds per fruits refers only to seeds that fell out of fruits during haymaking simulation.

|                                  | <i>Rh. minor</i>      | <i>Rh. alectorolophus</i> |
|----------------------------------|-----------------------|---------------------------|
| Ecotype                          | Vernal                | Late aestival             |
| Onset of flowering               | 20 May                | 20 June                   |
| First fruits open                | 5 June                | 15 July                   |
| Branches                         | 0                     | 2 – 10                    |
| Internodes                       | 5 – 8 short           | 6 – 10 long               |
| Plant height [cm] $\pm$ s.d. (n) | 16.3 $\pm$ 4.6 (120)  | 25.7 $\pm$ 5.9 (60)       |
| Flowers per plant $\pm$ s.d. (n) | 2.63 $\pm$ 1.64 (120) | 27.5 $\pm$ 22.9 (60)      |
| Seeds per fruit $\pm$ s.d. (n)   | 8.68 $\pm$ 2.80 (79)  | 3.17 $\pm$ 1.54 (30)      |

## Results

### Regeneration

In total, 11% of *Rh. minor* plants regenerated (i.e. produced at least one shoot longer than 1 cm three weeks after clipping). Regeneration success increased with clipping height and decreased with clipping date (Table 2, Fig. 1). The highest regeneration (up to 80%) was found in mid-May in the highest clipping height. On the contrary, no plants regenerated at all when clipped to 3 cm. Regeneration dropped below a level which would be sufficient for keeping a population stable (i.e. over 22%; Appendix 2) around mid-May (Fig. 2).

The plant characteristics were correlated with treatments. Plant height and phenological stage increased and the number of remaining leaves decreased in time (plant height:  $r = 0.51$ ,  $p < 0.001$ ; phenological stage:  $r = 0.50$ ,  $p < 0.001$ ; remaining leaves:  $r = -0.49$ ,  $p < 0.001$ ; Table 3, Appendix 3). Only the number of remaining leaves increased with clipping height ( $r = 0.51$ ,  $p < 0.001$ ; Table 3). These characteristics were also correlated with each other (plant height vs. phenology:  $r = 0.73$ ,  $p < 0.001$ ; plant height vs. remaining leaves:  $r = -0.47$ ,  $p < 0.001$ ; remaining leaves vs. phenology:  $r = -0.41$ ,  $p < 0.001$ ).

Logistic regressions demonstrated that regeneration of individual *Rh. minor* plants significantly decreased with plant height ( $\chi^2_1 = 9.6$ ,  $p = 0.002$ ), and phenological stage ( $\chi^2_1 = 8.8$ ,  $p = 0.003$ ), and increased with the number of remaining leaves ( $\chi^2_1 = 64.9$ ,  $p < 0.001$ ). At the time of clipping, all plants that later regenerated were shorter than 20 cm, a minimum of 4 leaves remained and no flowers were present but only 39% of plants that fulfilled all of these criteria regenerated.

There were 7% *Rh. alectorolophus* plants (all 18 May, 9 cm) that fulfilled the criteria for potentially successful regeneration of *Rh. minor*, but none of them eventually regenerated. See Appendix 3 for correlations of plant characteristics and treatments.



|                               | Deviance<br>( $\chi^2$ ) | Df | P       |
|-------------------------------|--------------------------|----|---------|
| Total                         | 59.08                    | 17 |         |
| Date                          | 21.95                    | 1  | < 0.001 |
| Height                        | 18.53                    | 1  | < 0.001 |
| Date   Height                 | 24.84                    | 1  | < 0.001 |
| Height   Date                 | 21.42                    | 1  | < 0.001 |
| Date + Height                 | 43.37                    | 2  | < 0.001 |
| Date * Height   Date + Height | 1.86                     | 1  | n.s.    |

Table 2: Analysis of variance table of the generalized linear model with binomial distribution of response variable. Response variable: percentage of regenerated plants in the sector. Date and Height refer to clipping date and clipping height (both expressed as quantitative variables). | separates the tested effect (left) from the covariates (right), i.e. the variability explained by the first variable was tested when controlled for the second one.

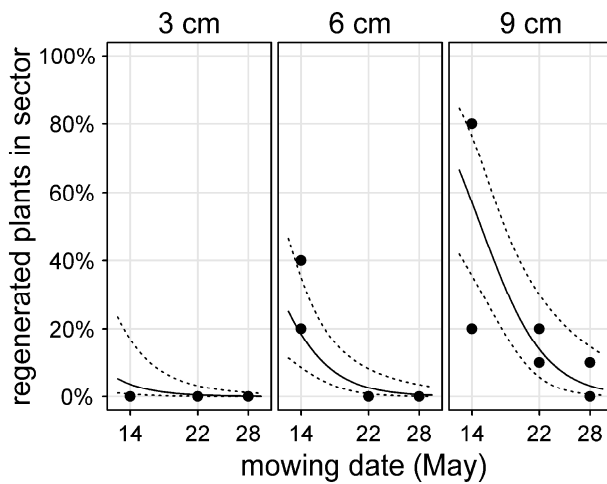


Figure 1: Dependence of percentage of regenerated *Rhinanthus minor* plants on mowing date, separate for each clipping height. Each point represents 10 plants in a sector. The result of multiple logistic regression with 95% confidence band is shown.

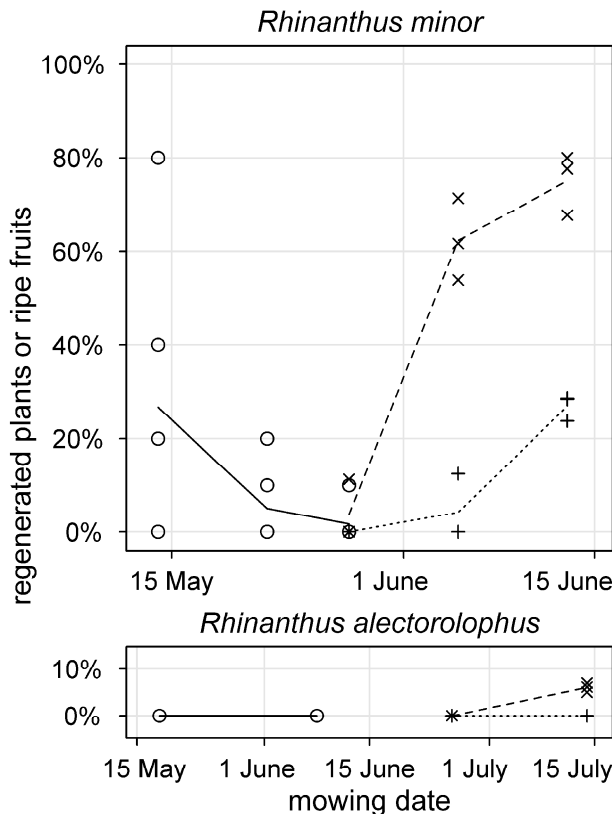


Figure 2: Dynamics in percentage of regenerated plants in each section and percentage of ripe fruits around each point.  $\circ$ , solid line: percentage of regenerated plants; +, dotted line: percentage of fruits ripe at the time of clipping;  $\times$ , dashed line: percentage of fruits ripe after drying.

Table 3: Plant characteristics at individual dates: plant height, percentage of plants in individual flowering stages, and mean number of remaining leaves in each clipping height. See Appendix 3 for the regression results.

| Species (year)                        |                   | <i>Rh. minor</i> (2011) |                |                | <i>Rh. alectorolophus</i> (2013) |                |
|---------------------------------------|-------------------|-------------------------|----------------|----------------|----------------------------------|----------------|
|                                       |                   | 14 May                  | 22 May         | 28 May         | 18 May                           | 8 June         |
| Clipping date                         |                   |                         |                |                |                                  |                |
| Plant height $\pm$ s.d. [cm]          |                   | 11.5 $\pm$ 2.9          | 15.2 $\pm$ 4.5 | 17.3 $\pm$ 4.5 | 10.9 $\pm$ 2.7                   | 25.7 $\pm$ 5.9 |
| Percentage of plants with [%]         | no flower buds    | 58                      | 33             | 18             | 100                              | 100            |
|                                       | flower buds only  | 42                      | 33             | 22             | 0                                | 0              |
|                                       | flowers or fruits | 0                       | 33             | 60             | 0                                | 0              |
| Number of remaining leaves $\pm$ s.d. | clipped in 3 cm   | 1.1 $\pm$ 1.2           | 0.1 $\pm$ 0.3  | 0.1 $\pm$ 0.4  | 0.0 $\pm$ 0.0                    | 0.0 $\pm$ 0.0  |
|                                       | clipped in 6 cm   | 4.2 $\pm$ 1.9           | 1.8 $\pm$ 1.9  | 0.1 $\pm$ 0.4  | 0.7 $\pm$ 1.0                    | 0.0 $\pm$ 0.0  |
|                                       | clipped in 9 cm   | 4.7 $\pm$ 2.4           | 3.8 $\pm$ 1.7  | 1.4 $\pm$ 1.7  | 2.4 $\pm$ 1.7                    | 0.0 $\pm$ 0.0  |

## Fruit ripening

*Rh. alectorolophus* produced about ten times more flowers per plant than *Rh. minor* and less than half the number of seeds per fruit (but not all seeds fell out of some capsules in both species), and flowering started about one month later (Table 1). A sufficient percentage of ripe fruits for keeping the population stable (i.e. over 22% and 6% for *Rh. minor* and *Rh. alectorolophus*, respectively; Appendix 2) was reached before mid-June in *Rh. minor*, and this percentage was able to ripen during haymaking around the end of May. No fruits of *Rh. alectorolophus* were ripe by mid-July, but some ripened during drying, roughly in amount needed for keeping the population stable (Table 4, Fig. 2).

The majority of large green fruits but only a small percentage of medium fruits ripened in *Rh. minor*. Ripening was lower in *Rh. alectorolophus*, but large green fruits were able to ripen at a high rate, whereas medium fruits ripened at a low rate (Table 5).

Table 4: Flower and fruit counts per plant ( $\pm$ s.d.) and ripe fruit percentage of total flower count. Values for each point were calculated first and these were averaged afterwards. Not all flower buds were detectable on the plant apices on the first date for both species, so that the non-zero percentage of ripe fruits could be slightly overestimated on these dates. Groups indicated with different letters differ significantly (analysis of variance,  $p < 0.05$ ).

| Species                  | <i>Rh. minor</i> (2011)                  |  |  | <i>Rh. alectorolophus</i> (2013)        |   |
|--------------------------|--|--|--|---|---|
|                          | 28 May                                   | 5 June                                   | 13 June                                  | 26 June                                 | 14 July                                 |
| Total flowers and fruits | 2.13 $\pm$ 0.84                          | 2.58 $\pm$ 0.23                          | 2.68 $\pm$ 0.83                          | 7.47 $\pm$ 2.69 <sup>a</sup>            | 27.47 $\pm$ 6.86 <sup>b</sup>           |
| Fruits ripe at clipping  | 0.00 $\pm$ 0.00 <sup>a</sup><br>( 0.0 %) | 0.12 $\pm$ 0.20 <sup>a</sup><br>( 4.2 %) | 0.72 $\pm$ 0.23 <sup>b</sup><br>(26.9 %) | 0.00 $\pm$ 0.00<br>(0.0 %)              | 0.00 $\pm$ 0.00<br>(0.0 %)              |
| Fruits ripe after drying | 0.12 $\pm$ 0.20 <sup>a</sup><br>( 3.8 %) | 1.62 $\pm$ 0.33 <sup>b</sup><br>(62.3 %) | 2.00 $\pm$ 0.60 <sup>b</sup><br>(75.1 %) | 0.00 $\pm$ 0.00 <sup>a</sup><br>(0.0 %) | 1.70 $\pm$ 0.69 <sup>b</sup><br>(6.0 %) |

Table 5: The course of fruit ripening. Left – sum of fruits in the ripeness categories. Middle – a matrix summarising the transition of fruits between ripeness categories during haymaking simulation. Some categories are merged. Right – percentage of fruits in the ripeness categories within a date. See Appendix 1 for description of the ripeness categories.

| <i>Rh. minor</i>           |       |                             |                  |         |                   |         |         |
|----------------------------|-------|-----------------------------|------------------|---------|-------------------|---------|---------|
| Starting ripeness category | Count | Final ripeness category [%] |                  |         | Clipping date [%] |         |         |
|                            |       | Unopened                    | Opened unspilled | Spilled | 28 May            | 5 June  | 13 June |
| Flower buds, flowers       | 87    |                             |                  |         | 53                | 6.5     | 5.6     |
| Small fruit                | 49    | 100                         | 0                | 0       | 25                | 6.5     | 4.3     |
| Medium fruit               | 49    | 53                          | 43               | 4.1     | 13                | 15      | 5.6     |
| Large green fruit          | 112   | 4.5                         | 24               | 71      | 9.4               | 45      | 19      |
| Yellow unopened fruit      | 70    | 0                           | 5.7              | 94      | 0                 | 20      | 24      |
| Opened unspilled fruit     | 27    |                             | 3.7              | 96      | 0                 | 1.9     | 15      |
| Opened spilled fruit       | 50    |                             |                  | 100     | 0                 | 4.5     | 27      |
| <i>Rh. alectorolophus</i>  |       |                             |                  |         |                   |         |         |
| Starting ripeness category | Count | Final ripeness category [%] |                  |         | Clipping date [%] |         |         |
|                            |       | Unopened                    | Opened unspilled | Spilled | 26 June           | 14 July |         |
| Flower buds, flowers       | 1323  |                             |                  |         | 94                | 54      |         |
| Small fruit                | 204   | 100                         | 0                | 0       | 3.8               | 11      |         |
| Medium fruit               | 345   | 91                          | 7.2              | 1.7     | 1.8               | 20      |         |
| Large green fruit          | 226   | 30                          | 27               | 42      | 0                 | 14      |         |
| Other                      | 0     |                             |                  |         | 0                 | 0       |         |

## Discussion

We found that there is a period in which *Rhinanthus* plants neither produce enough ripe fruits, nor resprout sufficiently. Mowing within this critical period could have a detrimental effect on the persistence of *Rhinanthus* populations. The length of this gap varies largely among species and ecotypes. It was about one month long in our early flowering *Rh. minor* population, and haymaking shortened the gap by about one or two weeks. In contrast, this gap in our late flowering *Rh. alectorolophus* population was longer than the duration of our experiment. We found no regeneration even in the first experimental date and some fruits could ripen only in hay at the end of the experiment (Fig. 2). In both sites, the usual mowing date would interfere with the critical period, but as mowing is postponed by agrienvirommental measures (AEMs) to after mid-July, both populations are persisting. Whereas the timing is very tight for *Rh. alectorolophus*, the site with *Rh. minor* could be mown one month earlier without a negative impact on its population.

## Regeneration

Despite being annuals without storage organs, regeneration of *Rhinanthus* plants after mowing was observed in this study, as well as in other studies (ter Borg 1972; Huhta et al. 2000). *Rh. minor* was able to resprout and even individuals from a population of normally unbranched plants could produce lateral shoots. Regeneration decreased with clipping date and increased with clipping height. While regeneration could be sufficient to keep the population stable (Appendix 2) until mid-May in *Rh. minor*, *Rh. alectorolophus* did not regenerate at all (Fig. 2). We cannot rule out that the *Rh. alectorolophus* would regenerate after earlier mowing, but this date would be too early for farmers. We expect that in the case of real mowing, individual plants will differ in the height where they are cut (e.g. due to soil surface relief) and this will also increase the variability in the number of remaining leaves within a single clipping date.

The effects of clipping date and height are directly interpretable in terms of agricultural practices, but the effects of these treatments are probably mediated by plant characteristics at the time of clipping. The characteristics we measured (i.e. plant height, number of leaves remaining after clipping, phenological stage) were to a large extent determined by the treatments, but they were also highly correlated with each other, so it is difficult to determine statistically which ones directly affected survival. Physiological considerations suggest that the number of remaining leaves probably plays a key role. Leaves can act as a source of energy, thereby compensating for the absence of storage organs. Huhta et al. (2000) has shown that plants that regenerated had at least one or two nodes with leaves. Our data similarly demonstrate that plants with less than four remaining leaves (i.e. 2 nodes) never regenerated. No plants with flowers at the time of clipping regenerated in our experiment (nevertheless, there were only few that had both flowers and at least four leaves remaining after clipping). The plants that have already invested in flower production have very likely no energy to invest into regeneration. The effect of plant height is probably indirect, mediated by the number of remaining leaves, because lower leaves are shed as the plants grow. ter Borg (1972) explained the differences in regeneration success by growth habit, with the focus on the length of lower internodes and branching. She found, for instance, a population of autumnal *Rh. major* (= *Rh. angustifolius* or *Rh. serotinus*) whose lower internodes were short and branched when young, making a rosette-like habit. Large parts of these plants remained after mowing and the capability of resprouting was very high. We have also found similar populations in Czechia, which were branched in lower nodes and regenerate with flowers after mowing (around Horní Planá in South Bohemia, late June 2014). We attribute the failure of our *Rh. alectorolophus* to regenerate to its long basal internodes and hence lack of remaining leaves after clipping even in the earliest date. As plant characteristics (Table 3) are more relevant for determining of the date, before which *Rhinanthus* plants resprout sufficiently, we suggest estimating whether there is still a sufficient percentage of plants (Appendix 2) which have at least four leaves remaining below the height of cutting.

## Fruit ripening

In our fruit ripening experiment, *Rhinanthus* seed production was decreased by cutting on all dates (as compared to full production without cutting), but this decrease can be to some extent compensated for by lower density-dependent mortality and higher fecundity of the next generation who will be free from intraspecific competition (Westbury & Dunnett 2007; Mudrak & Lepš 2010). The estimation of minimum seed production which will not lead to a population decline based on one-year data is provided in Appendix 2. A sufficient percentage of ripe fruits in *Rh. minor* population was attained in about mid-June if silage was made, but already by early June if hay was made. While we consider mid-June mowing to be already safe for our *Rh. minor* population, in agreement with Bullock et al. (2003), Smith et al. (2000) found it detrimental for their *Rh. minor* ecotype. *Rh. alectorolophus* had no ripe fruits even in mid-July (the cutting date postponed for the sake of subsidies), but as some fruits were already able to ripen during hay drying (about the minimum sufficient percentage), we expect fast onset of fruit production after this date.

The dynamics of fruit ripening vary not only among species and ecotypes, but also among years and regions with different climates, so we propose using plant characteristics instead of calendar dates to define cutting dates (Table 5). The date before which a population would undergo a decline must be determined individually by simply estimating if plants have already produced enough ripe fruits (Appendix 2). Where hay is made on the meadow, it is possible to count also fruits that are not yet ripe (are unopened and green), but are full size.

## Practical applications

*Rhinanthus* species have long been recognised as weeds that can reduce grassland productivity and forage quality (Carruthers 1899; Bastin 1915; Rabotnov 1959; Mizianty 1975) or even destroy cereal crops (Carruthers 1903; Mizianty 1975). It was recommended to pull out individual plants by hand or to cut them off before seeding, but not too early because of the risk of resprouting (Bastin 1915; Rabotnov 1959). The approach to grasslands has changed over the century (Isselstein et al. 2005), but the biological processes remain the same. Our results imply that intensive farming methods caused *Rhinanthus* to disappear from productive grasslands, which are mown before *Rhinanthus* fruiting. The uniform early mowing, usually done in late May or at the beginning of June, had a negative impact also on other plants flowering at the same time (Kirkham & Tallowin 1995; Zechmeister et al. 2003; Humbert et al. 2012), as well as birds during nesting (Perkins et al. 2013) and arthropods, who are dependent on various specific plant resources in all of their lifecycle stages (Konvicka et al. 2008; Cizek et al. 2012; Buri et al. 2013).

Postponing the first cut until *Rhinanthus* fruits start to ripen is therefore necessary for its protection. The most important tool for regulating grassland management in Europe is agri-environmental measures (AEMs). The design of Czech AEMs for mesic meadow management, the most common habitat of *Rhinanthus* species, depends on the conservational

status of a site (Ministry of Agriculture of the Czech Republic 2013). Outside protected areas, only the amount of fertilizers is regulated effectively, only indirectly affecting mowing frequency. Inside protected areas, the nature protection administration has assigned postponed mowing to individual meadows, and it is subsidized after 15 July or after 15 August.

These dates for the postponed cut are suitable for both *Rhinanthus* types in this study, which should produce enough ripe fruits by that time, but they are rather problematic for farmers. The forage quality decreases throughout season, and it is already quite low in mid-July (Kirkham & Tallowin 1995; Isselstein et al. 2005), making this measure barely acceptable at the cost of rather high financial compensation (Zechmeister et al. 2003). *Rhinanthus* suppresses nutritionally valuable grasses, and, from *Rhinanthus* plants, only the lignified leafless shoots remain in late-harvest hay, considerably decreasing its quality (Mizianty 1975; Ameloot et al. 2005). We thus suggest introducing a late-June mowing, which is a month earlier than the current date for a postponed cut, but still a month later than the usual mowing date in productive sites. This would allow for occasional control of *Rhinanthus* in case it gets overpopulated (Bullock & Pywell 2005) and it would also increase the fodder quality, so lower financial compensation should suffice. The suggested late-June cut would also be an interesting alternative for nature conservation and it could supplement current dates. The aim of nature conservation is not only to postpone, but also to differentiate the first mowing in a landscape mosaic, because there is no universal mowing date that would suit all organisms (Čížek et al. 2012; Humbert et al. 2012).

It is clear from our results that some *Rhinanthus* types would not survive in late-June mown plots, but many of them would. A thriving *Rh. minor* population in a scout campground near Frantoly, South Bohemia (pers. obs.), nicely illustrates that this date can be really favourable. The site is mown shortly before the Czech school holidays, which start at the beginning of July. Our experimental plot in Benešov (reported in Mudrak et al. 2014) is also usually mown before the end of June, and the population of *Rh. minor*, spreading vigorously throughout the locality after artificial introduction, survives more than a decade on. Not only seed production, but also seed dispersal was shown to be very limiting for *Rhinanthus* survival in the landscape (Bullock et al. 2008). Its main vector of seed transport between sites is mowing machinery, but it works only when there are still some seeds in the capsules, so the efficiency decreases over time (Smith et al. 1996; Strykstra et al. 1996, 1997; Coulson et al. 2001; Bullock et al. 2003). For instance, Coulson et al. (2001) consider mid-July to be ideal for *Rhinanthus* seed dispersal, but Bullock et al. (2003) found mid-June to be even better. Not all fruits may be ripe at the time of mowing, but a reasonable seed loss is an acceptable price for better dispersal, which is a vital process in the life of an annual (Bullock et al. 2008).

Whereas mowing can be postponed in protected areas (though the current options are not ideal), it is not regulated at all outside protected areas, which is detrimental for the whole *Rhinanthus* metapopulation. Postponing the first cut by at least a couple of weeks is essential for protection of *Rhinanthus* and so AEMs support of late-June mowing should be introduced also outside protected areas. This date should be widely acceptable for farmers, even with lower financial support. Making hay instead of silage would even increase the positive effect (Smith et al. 1996; Svensson & Carlsson 2005). We have shown that it would not suit all *Rhinanthus* types, but at least its common vernal types would be supported by mowing on this compromise date on a large scale.

Except for natural sites, *Rhinanthus* also occurs in places where species rich grasslands are being restored on formerly intensive grasslands or arable fields. *Rhinanthus* is included in sown seed mixtures as one of the desired indigenous species (Smith et al. 2000), or even as a treatment that should help with lowering biomass thanks to its parasitism (Bullock & Pywell 2005; Westbury et al. 2006; Westbury & Dunnett 2007; Mudrak et al. 2014). To keep permanent *Rhinanthus* populations in such sites, the proper timing of mowing must be applied and our results can be used as a guideline.

## Conclusions

This study not only showed that *Rhinanthus* species are seriously harmed by early mowing, but also provided details about the dynamics in crucial processes in their life cycle, highlighting the differences between phenological types. Based on this information, we conclude that the absence of *Rhinanthus* species in intensively managed grasslands and their general decline is tightly connected to changes in mowing dates and to more common making of silage. A postponed cut, which should promote plant diversity in general, is beneficial for *Rhinanthus* survival, and the grasslands where it is applied are one of its most common habitats nowadays. However, current Czech AEMs, which limit support for a postponed cut to only protected areas and support only late mowing there, can never combine both conservational effectiveness and agricultural acceptability. We suggest a compromise mowing date which should still support *Rhinanthus* survival and, at the same time, should be widely acceptable even outside protected areas, where no regulation is applied. In our opinion, AEMs are a method of cooperation with farmers, and so they should accommodate their needs in cases where it is not to the detriment of nature conservation. Making AEMs more flexible is important for diversifying the landscape mosaic and protecting plant and animal diversity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2015.04.022>.

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