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# Seedling Appearance, Survival and Flowering of *Trifolium pratense* in a Cutting Meadow

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## Abstract

Seedling appearance, survival and flowering of *Trifolium pratense* from naturally deposited seeds in meadow soil layers were monitored over a period of 8 years in a meadow for herbage cutting (hereafter referred to as "cutting meadow") in the central mountainous district of Japan. In the meadow, *Phleum pratense* predominated and herbage had been harvested for making hay or silage in early June and late August of a year since the establishment of the meadow in 1966. *T. pratense* seedlings appeared from March to May (namely spring cohort) and from September to November (namely autumn cohort) every year. Estimated seedling density ranged yearly from 18 to 1,215 seedlings/$m^2$ in the spring cohorts and from 1 to 245 seedlings/$m^2$ in the autumn cohorts, with an average of 199 and 86 seedlings/$m^2$, respectively. Half-life ranged yearly from 4 to 63 days for the spring cohorts and from 33 to 248 days for the autumn cohorts, with an average of 42 and 135 days, respectively. Of the 13,485 seedlings marked, no individuals survived for more than 4 years. Most of the individuals undergoing flowering (hereafter referred to as "flowering individuals") originated from the autumn cohorts. Thirty-two percent of the survivors began to flower in the first summer after their appearance. Ratio of flowering individuals to survivors rose to 81% in the second summer and fell to 50% in the third summer. It was suggested that during mechanical operations for harvesting in late August, *T. pratense* seeds were dropped and that the lack of third harvesting prolonged the half-life of the autumn cohorts which had succeeded in reproducing in this meadow.

**Discipline:** Grassland
**Additional key words:** demography, natural reseeding, population, red clover, reproduction

## Introduction

*Trifolium pratense* L. is an important forage legume in many countries, as it produces large quantities of high-quality forage for grazing animals[2,4,16,17]. In Japan, when sown with forage grasses, it is used for hay and pasture. Nationwide, clover and grass mixtures have been cultivated over an area of approximately 0.6 million ha for the past 10 years[14,15]. Most of the clover seeds were imported, with *T. pratense* seeds accounting for approximately 20% of the total[19].

It has long been recognized that the usefulness of *T. pratense* may be reduced because of the lack of persistence. Though the species is physiologically a perennial, it usually behaves as a short-lived perennial, a biennial, or an annual depending on the field conditions[4]. Hence its seeds must be sown every several years for permanent use. A large number of investigations have been conducted on the process and cause of internal breakdown of the crown of *T. pratense*[1,3,5,8,9,11–13,18]. Plant breeding has improved the persistence of *T. pratense* through the selection of cultivars with disease, nematode and insect resistance[4,17].

However, there are few studies on the demography of *T. pratense* in permanent grasslands, including the importance of reproduction and seedling regeneration in its persistence. Fergus and Hollowell[4] observed a 9-year-old *T. pratense* stand in an isolated irrigated area near Fowler, Colorado. Rabotnov[10] reported that the longevity of *T. pratense* individuals reached 20 years on subalpine meadows near Kislovodsk, North Caucasus, based on the average annual mortality of mature individuals during a 20-year observation period. In a cutting meadow man-

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aged by the National Institute of Livestock and Grassland Science, Japan (NILGS), a *T. pratense* stand has persisted for 20 years since its establishment under regular cutting. These observations suggest that *T. pratense* populations have reproduced effectively through seeds in the fields, and that a sustainable amount of the legume can be harvested once the population is established under certain circumstances.

The objective of this study was to describe the demography of *T. pratense* and to assess the effect of grassland management on it in cutting meadows. In the present paper, seedling appearance, survival and flowering of *T. pratense* and the effect of hay-making on them in a cutting meadow were described. *T. pratense* seedlings from naturally deposited seeds were marked individually and the fate of the seedlings was monitored regularly. This procedure was necessary to consider the possibility of persistence of the *T. pratense* population.

## Methods

### 1. Study site

The study was conducted from 1987 to 1995 in a meadow managed by the Department of Mountainous Grassland Farming of NILGS (36º21'N, 138º30'E) which was formerly the Mountainous Region Branch of the National Grassland Research Institute. The meadow studied was located on a 5º to 16º slope of Mt. Asama facing south at an altitude of approximately 1,200 m and it covered an area of about 1.2 ha.

Mean annual temperature was 8.1ºC with a monthly mean maximum of 20.5ºC in August and a monthly mean minimum of –3.5ºC in January. Mean annual rainfall was 1,013 mm with a wet summer season (633 mm precipitation from June to September). Although there were several occurrences of snowfall with a depth of more than 15 cm, snow seldom remained during the winter. Soil freezing in winter might reach a depth of 30 cm underground.

**Table 1. Cutting and harvesting dates for hay-making in the meadow studied**

| Year | Date of first hay-making | | Date of second hay-making | |
|---|---|---|---|---|
| | Cutting | Harvesting | Cutting | Harvesting |
| 1987 | June 1 | June 4 | August 17 | August 21 |
| 1988 | June 6 | June 8 | August 22 | August 27 |
| 1989 | June 2 | June 5 | August 21 | August 23 |
| 1990 | June 8 | June 13 | August 27 | August 29 |
| 1991 | June 21 | June 27 | August 19 | August 22 |
| 1992 | June 19 | June 22 | August 31 | September 2 |
| 1993 | July 28 | August 2 | September 25 | September 29 |
| 1994 | June 6 | June 10 | August 23 | August 26 |

The meadow was predominantly covered with *Phleum pratense* L. and secondarily with *Trifolium pratense* L., with limited amounts of *Agrostis alba* L., *Dactylis glomerata* L., *Festuca arundenacea* Schreb, *Poa pratensis* L., *Trifolium repens* L. and several dicotyledonous species.

Fertilizer was applied every year at a rate of 160, 200, and 160 kg/ha for N, $P_2O_5$ and $K_2O$, respectively during the study period. Cutting was being conducted twice a year (usually in early June and late August) for making hay or silage since the establishment of the meadow. Table 1 lists the cutting and harvesting dates in the meadow. Time for first hay-making was delayed in 1991, 1992 and 1993.

### 2. Observations

Ten permanent 1 m × 1 m quadrats were set in the meadow. Positions of the *T. pratense* seedlings were recorded with mapping on papers on a scale of one fifth of coordinate from 5 mm × 5 mm grid in the quadrats. Consecutive maps were superimposed to identify new recruits and dead individuals between the observations. Each individual was assigned a number and a card on which the dates of its first record, flowering and death were listed. Observations were usually made every 2 weeks from March to October and monthly from December to February. It was impossible to map all the seedlings in the quadrats in the spring of 1991 because of the large number of seedlings which appeared. Therefore, an area corresponding to one fifth in each quadrat was taken for the census for mapping the seedlings in this season. The total number of seedlings marked was 13,485 individuals for 8 years.

Ten mature individuals of *T. pratense* were transplanted from the meadow to Wagner pots (1/5000 a) in March 1989 in single planting and grown outdoors. Cutting was performed on June 2 and then the flowers were counted every 1–4 days until August 31 to determine the flowering rate after the first hay-making.

### 3. Estimation of the number of seedlings

Although the survival or death rates of a cohort could be calculated based on the data observed in a given interval, the number of seedlings was estimated because the seedlings which survived during periods shorter than that between censuses could not be recorded. An equation was obtained for estimating the number of seedlings appearing between 2 censuses:

$$E = \frac{L \ln(F/S)}{1 - S/F},$$

where $E$ represents the estimated number of seedlings in

2 censuses, *F* is the number of seedlings observed in the former census, *L* is the number of seedlings observed in the latter census and *S* is the number of observed survivors of *F* in the latter census (refer to appendix for the equation). The estimates were used for calculating the number and rate of *T. pratense* seedlings appearing in every cohort.

## Results

Fig. 1 shows the seasonal changes in the appearance rate of *T. pratense* seedlings in the meadow studied, including the estimates for 8 years. The seedlings appeared in spring and autumn every year. In spring, the seedlings appeared from March to June and the peak of the appearance rate occurred at the end of April. In autumn, the period of seedling appearance extended from the end of August, which immediately followed the second hay-making, to November and the peak of appearance rate occurred in mid-September. Hereafter, a seedling group which appeared in spring and autumn will be referred to as a spring cohort and an autumn cohort, respectively.

The density of *T. pratense* seedlings varied yearly in both kinds of cohorts (Fig. 2). In the spring cohorts, it decreased gradually from approximately 100 seedlings/m$^2$ in 1987 and 1988 to 47 seedlings/m$^2$ in 1990. Although 1,215 seedlings/m$^2$ appeared in 1991, the density decreased again to 18 seedlings/m$^2$ in 1994. In 1995, it rose to 81 seedlings/m$^2$. The density averaged 199 seed-

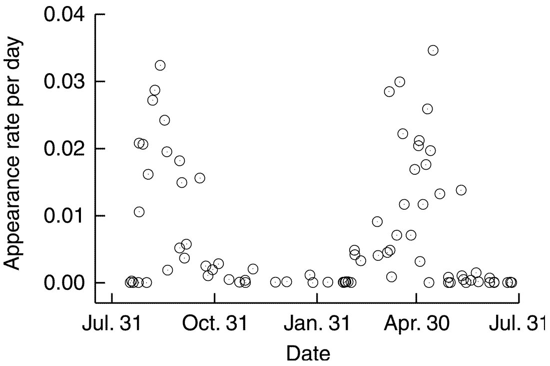


**Fig. 1. Seasonal changes in the appearance rate of *T. pratense* seedlings in the meadow studied**
The appearance rate is expressed as the daily averaged rate of the number of seedlings in a census period to the total number of seedlings in a season (spring and autumn).

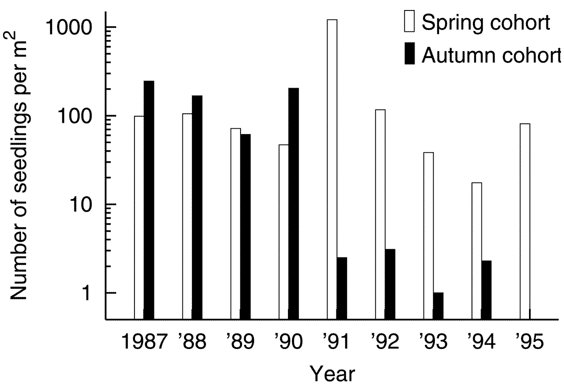


**Fig. 2. Annual changes in the density of *T. pratense* seedlings which appeared in a season in the meadow studied**

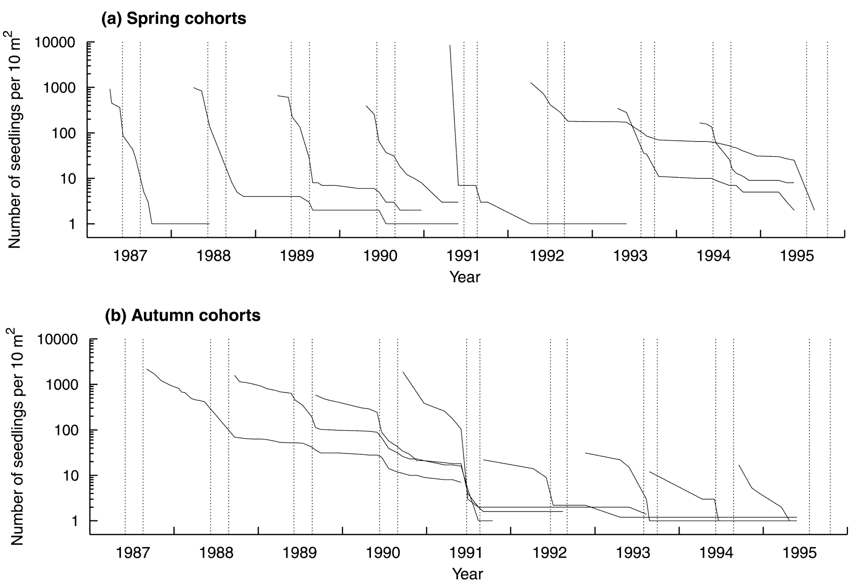


**Fig. 3. Survivorship curves of (a) spring cohorts and (b) autumn cohorts of *T. pratense* in the meadow studied**
Vertical dotted lines denote the time of hay-making.

lings/m$^2$ in the spring cohorts from 1987 to 1995. In the autumn cohorts, the density decreased gradually from 245 seedlings/m$^2$ in 1987 to 62 seedlings/m$^2$ in 1989. Although 204 seedlings/m$^2$ appeared in 1990, the density decreased drastically to a few seedlings/m$^2$ every year from 1991 to 1994, averaging 86 seedlings/m$^2$ in the autumn cohorts from 1987 to 1994.

Fig. 3 shows the survival rate of *T. pratense* in the meadow studied. In both kinds of cohorts, the survival rate of the juveniles was low. Many individuals died at the time of hay-making. The survival rate of the autumn cohorts was higher than that of the spring cohorts from 1987 to 1991. The differences in the survival rates between the spring and autumn cohorts were not clear after 1991. Half-life of *T. pratense* in the spring and autumn cohorts averaged 42 and 135 days, respectively for 8 years (Table 2). It also varied yearly in both kinds of cohorts. In the spring cohorts, the half-life in 1987 and 1991 was considerably shortened compared with other years, being 6.9 days and 3.6 days, respectively. In the autumn cohorts, the half-life in 1990 and 1994 was shorter than that in other years, 39.9 days and 33.4 days, respectively. Of the 13,485 seedlings marked, no individuals survived for more than 4 years.

Fig. 4 shows the flowering rate of *T. pratense* following the first hay-making. The pattern of the flowering rate was unimodal. Flowering began on June 27 and ended on August 22 with a peak in late July. Table 3 shows the number and ratio of the flowering individuals. Because most of the reproducing individuals were observed during the period from the first hay-making to the second one, the measurements were conducted immediately before the second hay-making. Although the spring and autumn cohorts were able to flower in the first

**Table 2. Half-life of *T. pratense* cohorts in the meadow studied**

| Year | Half-life (days) | |
|---|---|---|
| | Spring cohort | Autumn cohort |
| 1987 | 6.9 | 81.0 |
| 1988 | 45.0 | 148.8 |
| 1989 | 57.4 | 227.7 |
| 1990 | 38.7 | 39.9 |
| 1991 | 3.6 | 247.6 |
| 1992 | 62.6 | 186.7 |
| 1993 | 56.6 | 115.0 |
| 1994 | 63.0 | 33.4 |
| Average | 41.7 | 135.0 |

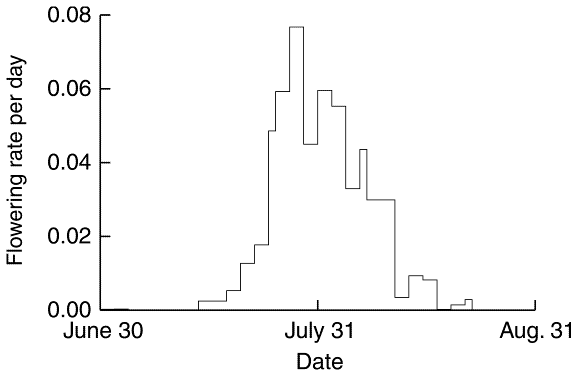


**Fig. 4. Flowering rate of *T. pratense* following the first hay-making**

The flowering rate is expressed as the daily averaged rate of the number of flowers to the total number of flowers. The total number of flowers was 653.8 flowers per individual.

**Table 3. Sequential changes in the number (per 10 m$^2$) and averaged ratio of flowering individuals in *T. pratense* cohorts in the meadow studied**

| Year | Flowering individuals in spring cohorts | | | | Flowering individuals in autumn cohorts | | |
|---|---|---|---|---|---|---|---|
| | Present year | 1 year later | 2 years later | 3 years later | 1 year later | 2 years later | 3 years later |
| 1987 | 0 ( 27) | 0 ( 0) | 0 ( 0) | 0 ( 0) | * | 33 ( 36) | 8 ( 11) |
| 1988 | * | 0 ( 3) | 0 ( 1) | 0 ( 0) | 68 (193) | 24 ( 32) | 0 ( 1) |
| 1989 | 2 ( 28) | 0 ( 3) | 0 ( 0) | 0 ( 0) | 9 ( 44) | 1 ( 2) | 0 ( 1) |
| 1990 | 0 ( 31) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 2) | 2 ( 2) | 0 ( 1) |
| 1991 | 0 ( 7) | 0 ( 1) | 0 ( 0) | 0 ( 0) | 0 ( 2) | 0 ( 1) | 0 ( 1) |
| 1992 | 7 (284) | 1 ( 85) | 5 ( 52) | 0 ( 2) | 0 ( 1) | 0 ( 1) | 0 ( 1) |
| 1993 | 0 ( 34) | 0 ( 7) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 0) |
| 1994 | 1 ( 26) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 0) | 0 ( 0) |
| Total | 10 (437) | 1 ( 99) | 5 ( 53) | 0 ( 2) | 77 (242) | 60 ( 74) | 8 ( 16) |
| Ratio (%) | 2.3 | 1.0 | 9.4 | 0.0 | 31.8 | 81.1 | 50.0 |

Values in parentheses denote the number of survivors. * Not determined.

summer after their appearance, most of the flowering individuals originated from the autumn cohorts. Thirty-two percent of the survivors began to flower in the first summer after their appearance. The ratio of flowering individuals to survivors rose to 81% in the second summer and fell to 50% in the third summer. Although the number of flowering individuals decreased yearly, the ratio peaked 2 years after seedling appearance in both kinds of cohorts.

## Discussion

In the central mountainous district of Japan, it is usual to harvest forage 3 times a year for making hay or silage. In fact, in other meadows managed by the Department of Mountainous Grassland Farming of NILGS, forage had been harvested 3 times a year. In these meadows, the first cutting occurred at the end of May to early June, the second cutting in mid- to late July, and the third cutting in early to mid-October. However, in the meadow studied, forage had been harvested twice a year. Although the time of the first cutting was the same as that in the other meadows, the second cutting occurred in mid- to late August. The regrowth of *P. pratense*, which is the dominant species in the meadow, is slow compared with that of *D. glomerata* or *F. arundenacea*[6], which are the predominant species in other meadows. Therefore, in the meadow studied, second harvesting could not take place until mid- to late August to yield a sufficient amount of forage.

Time and frequency of harvesting considerably influence the reproduction of *T. pratense*. The legume sets seeds in the meadow in summer to autumn. When the second harvest took place in mid- to late August, *T. pratense* had already set many seeds. Delay in the time of the first hay-making may prevent seed-setting of *T. pratense* because of the lack of sufficient time for reproduction, which was observed in the latter half of the study period (1991–93). A significant negative correlation was found between the time of the first hay-making and the density of *T. pratense* seedlings which appeared in the autumn of the same year (Fig. 5). It was suggested that the seedling density in autumn decreased when the time of the first hay-making was delayed. Let $y$ represent the seedling density, and $x$ represent the days from May 31 to the date of the first hay-making. A power regression was applied to the relationship: $y = 984x^{-1.71}$. On the other hand, no significant correlation was found between the time of the first hay-making and the density of the seedlings which appeared in spring of the next year, although another power regression was applied to the relationship: $y = 187x^{-0.331}$. Seedling density decreased drastically in both kinds of cohorts after 1991, resulting in population decrease. The differences in the survival rate between the spring and autumn cohorts after 1991 were not clear.

Products from the meadow might considerably affect seed fall of *T. pratense*. In the meadow, hay has been made chiefly. The sequential operations for hay-making include cutting, tedding and harvesting. A special machine is used for each operation. If a series consisted only of cutting and harvesting as for silage, the influence would be different from that for hay-making.

Most of the seeds of *T. pratense* have a hard coat[7]. Since time is required for seeds to become permeable and germinate, most of the seeds do not germinate immediately after falling to the ground. Most of them can germinate in the following spring. Therefore, the seedlings were usually considered to appear in spring. In fact, many *T. pratense* seedlings were observed in spring in this meadow, although some also appeared in autumn. The autumn cohorts appeared for 3 reasons: firstly, many seeds were produced during the period from the first to second harvestings; secondly, there was some seed permeation due to the somewhat insufficient seed set; and thirdly, mechanical operations for hay-making led to the fall of the seeds on the ground. It is considered that the time of the second hay-making and mechanical operations are effective in causing seed fall.

Mechanical operations for hay-making adversely affected the survival of *T. pratense*. Many individuals from the spring and autumn cohorts died when forage was harvested. On the other hand, harvesting removed the aboveground part of the dominant plants which grew profusely. These conditions were favorable for the

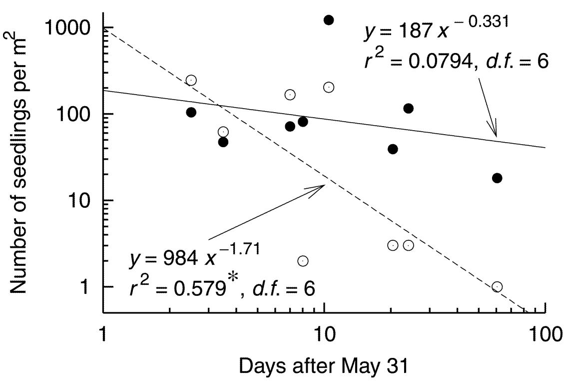


**Fig. 5. Relationship between the time of first hay-making and the number of *T. pratense* seedlings in the meadow studied**

● : Relationship in spring.
○ : Relationship in autumn.
— : Regression line of the relationship in spring.
--- : Regression line of the relationship in autumn.
* : Significant correlation at 5% level.

regrowth of *T. pratense* after hay-making.

The survival rate of the autumn cohorts was higher than that of the spring cohorts. It is possible that the seedlings of the autumn cohorts did not compete strongly with existing plants whose growth rates decreased from autumn to winter. In addition, the autumn cohorts did not sustain mechanical damage through forage harvesting until the year following seedling appearance. Autumn cohorts were the major constituents of *T. pratense* population in this meadow.

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## Appendix

Let us obtain an equation for estimating the number of seedlings appearing at an interval of $t$ days between 2 censuses. Let $E$ denote the estimated number of seedlings in the interval. Moreover, let $a$ denote the appearance rate per day of the seedlings in the interval. Assuming that the appearance rate is constant, we express the estimation as a function of $t$ by the following equation:

$$E = at. \qquad (1)$$

It is difficult to determine the appearance rate without continuous observations. We should substitute it for the number which we can determine with intermittent observations. Let us calculate approximately the appearance rate by using the mortality of the seedlings in the interval. Let $m$ denote the mortality per day for the seedlings. Suppose that the mortality is constant. Of the seedlings appearing at a given time d$x$, the number of survivors in the latter census can be determined by the following equation:

$$a\mathrm{e}^{-m(t-x)}\mathrm{d}x, \qquad (2)$$

where e and $x$ denote Napier's number (= 2.71828···) and a given time, respectively. Integrating Eq. (2) for $x$ from zero to $t$ days, we obtain the number of seedlings surviving in the latter census by the following equation:

$$L = \int_0^t a\mathrm{e}^{-m(t-x)}\mathrm{d}x = \frac{a}{m}(1-\mathrm{e}^{-mt}), \qquad (3)$$

where $L$ denotes the number of seedlings in the latter census. Substituting *a* in Eq. (1) for Eq. (3), we obtain an equation for estimating the number of seedlings appearing between 2 censuses:

$$E = \frac{Lmt}{1 - e^{-mt}}. \tag{4}$$

We approximate the mortality with 2 numbers that can be determined by the following equation:

$$mt = \ln\frac{F}{S}, \tag{5}$$

where *F* denotes the number of seedlings observed in the former census and *S* is the number of survivors of *F* observed in the latter census. Substituting *mt* in Eq. (4) for Eq. (5), we obtain another equation for estimating the number of seedlings appearing between the 2 censuses:

$$E = \frac{L\ln(F/S)}{1 - S/F}.$$