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Original article

Impact of late frost on height growth in young sessile oak regenerations

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Abstract – The damage due to late frost during the 1995 and 1996 growing seasons was analysed in sessile oak seedlings in a natural regeneration. The seedlings were 5 years old at the beginning of the 1995 growing season. In 1995, frost occurred after the complete elongation of the growth unit (GU) of the stem which was produced during the first period of elongation. In 1996, frost occurred during the elongation period of the first GU. Damage intensity was observed visually and ranged from simple necroses of the leaf or the terminal bud of the new GU to the total loss of the GU. Damage intensity appeared to depend on the growing season and the bud phenological stage when frost occurred but not on the initial dimensions (height and collar diameter) of the seedling. Damage was higher when the seedling was at the shoot elongation stage (1996 growing season) and lower when the seedling was in apparent rest (1995 growing season). The consequences of frost damage on growth were greater in the 1996 growing season. The bud resting period after the first period of elongation and before regrowth was shorter in 1996. Regrowth occurred from either a bud from the damaged GU or a bud from the GU grown during the previous growing season. In the latter case, the GU was sometimes longer than the GU from an axillary bud of the damaged GU. The second elongation period was shorter in 1996 than in 1995 and led to a relatively small GU. The intensity of frost damage, which had an effect on the length of the GUs produced during the second period of elongation, had no subsequent effect on the length of those produced during the third elongation period. The number of GUs finally contained in the stem and the annual shoot length of the stem were negatively affected by frost since the GU established during the first elongation period did not often belong to this stem. Monitoring growth allowed us to count the number of elongation periods and GUs established and to differentiate the GUs produced during different elongation periods. (© Inra/Elsevier, Paris.)

late frost / rhythmic growth / *Quercus petraea* seedlings

Résumé – Impact des gelées tardives sur la croissance en hauteur des jeunes régénérations de chêne sessile. Des dégâts occasionnés par des gelées tardives, intervenues au cours de deux saisons de croissance 1995 et 1996, sur des plants de Chêne sessile d'une régénération naturelle ont été analysés. Les plants étaient âgés de 5 ans au début de la saison 1995. En 1995, la gelée est survenue après élongation complète de l'unité de croissance (UC) de la tige mise en place au cours de la première vague. En revanche, en 1996, la gelée est intervenue en cours d'élongation de cette UC. L'intensité des dégâts a été observée visuellement, allant de simples nécroses des feuilles ou du bourgeon terminal de l'UC nouvellement mise en place, à la destruction totale de cette UC. Cette intensité des dégâts est apparue dépendante de l'année et du stade de développement du plant lorsque la gelée est survenue, mais indépendante de leurs dimensions initiales (hauteur et diamètre au collet). Les dégâts étaient plus importants lorsque le plant se trouvait au stade élongation de la pousse (saison 1996) que lorsque le plant était en repos apparent (saison 1995).
Les conséquences de ces dégâts de gel sur la croissance étaient plus importantes au cours de la saison 1996. La durée du repos entre la première et la deuxième vagues de croissance a été relativement plus courte en 1996. La reprise de croissance a été assurée soit à partir d'un bourgeon issu de l'UC endommagée, soit à partir d'un bourgeon issu de l'UC formée la saison précédente. Dans ce dernier cas l'UC formée était parfois plus longue que celle issue d'un bourgeon axillaire de la pousse endommagée. La durée de la

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période d'élongation de la deuxième vague était plus courte en 1996 qu'en 1995, et s'est traduite par la mise en place d'une UC de faible longueur. L'intensité des dégâts de gel n'a pas eu de conséquences sur la longueur de l'UC mise en place au cours de la troisième vague de croissance. Le nombre d'unités de croissance qui forment finalement la tige et la longueur de la pousse annuelle ont été négativement affectés par le gel, puisque l'UC établie au cours de la première vague ne faisait souvent pas partie de la tige. Le suivi de croissance nous a permis de comptabiliser les nombres de vagues et d'unités de croissance établies et de distinguer les unités de croissance allongées durant des vagues de croissance différentes. (© Inra/Elsevier, Paris.)

gelées tardives / croissance rythmique / *Quercus petraea*

1. Introduction

Frost damage can be observed in spring (late frost), autumn (early frost) and winter (winter frost). In sessile and common oaks, damage due to late frost is more frequent than damage due to winter frost [27]. In northeastern France, early frost has less impact than late frost since most species start their dormancy period before the frost risk period [5]. Late frost in France represents a major constraint for starting natural or artificial regenerations [3].

Frost damage seems to be more frequent when climatic conditions vary towards higher temperatures and increasing weather instability [6].

The extent of damage due to late frost depends on the species, the provenance, the lowest temperature reached and the phenological stage of the plant when frost occurred [4, 29]. Day and Pearce [14] reported that oak is more sensitive to late frost than ash and beech. In a recent study, it has been shown that the *Betula alleghaniensis* Britton was less sensitive to frost than sugar maple (*Acer saccharum* Marsh.) and red oak (*Quercus rubra* L.) [8]. Major variations due to the latitude and altitude have been shown to occur in the sensitivity of sessile oak provenances to late frost [16, 17, 21, 29, 36]. In spring, provenances from southern and southeastern Europe burst bud earlier and are more sensitive to late frost than provenances from northern Europe or at high altitudes which burst bud later. Sessile oak seedlings presenting swollen buds, non-spreading young leaves or elongated young leaves are sensitive to frost from temperatures ranging between –4 and –8 °C [29].

Frost damage is more serious than other physical damages (insect defoliation, browsing by herbivores, etc.) since it affects very sensitive parts of the plant, such as young growth units (GUs) or flowers during the growing period. During this period, plant cells contain much water, whose transformation into ice leads to the destruction of the cell components. The water content of stems varies according to the phenological stage of the plant, and frost damage consequently depends on this stage [2, 3, 16]. Damage is very serious in oak seedlings when they are at the stage of shoot elongation [29].

Oak is characterised by a rhythmic growth which consists of several successive growth flushes (short elongation periods) [7, 28, 33]. The stem can show up to four growth flushes per year [10, 30] separated by apparent rest periods. The duration of these periods depends on several factors, in particular climatic conditions, site characteristics and insect attacks [26]. The shoots of the second growth flush, also called 'mid-summer growth units' [12] or lammas shoots, depending on the period of elongation, are more frequent after insect attacks [26].

Late frost causes damage to the spring GU (produced during the first growth flush) but, according to our knowledge, there are no quantified data on this damage and on the variation in the sensitivity of plants within the same provenance. Moreover, the consequences of this damage for the subsequent height growth of the plants, in particular on the establishment of GUs during the second growth flush, have not been analysed.

The objectives of this study were 1) to determine whether some seedlings were particularly sensitive to late frost; 2) to determine the external characteristics of the seedlings that would make it possible to predict this sensitivity; 3) to assess the consequences of the damage due to late frost on the subsequent height growth. The intensity of frost damage was not manipulated and, consequently, this experiment did not aim to study the effects of late frost on oak growth; however, it aimed to study the subsequent growth of oak seedlings subjected to varying degrees of damage which were identified a posteriori.

2. Materials and methods

2.1. Materials

Observations were made in a natural regeneration of the Champenoux forest (48°44' N, 6°14' E, altitude 237 m) east of Nancy (France). The soil is a leached brown soil developed on a pseudogley horizon. It has a silty clay texture. Rainfall was well distributed with an annual average of 769 mm (over the 1975–1994 period); a peak of 82 mm was observed in June and a minimum of 49 mm in April. Rainfall was 746, 802 and 605 mm in

1994, 1995 and 1996, respectively. The mean annual temperature was 9.6 °C. The maximum monthly mean was 18.4 °C in July and the minimum monthly mean was 1.5 °C in January. The probability of spring frost (corresponding to a minimum temperature < 0 °C under shelter) after 21 April was 72 % as calculated over the 1971–1995 period.

Late spring frosts occurred during the 1995 and 1996 growing seasons. The two frosts did not occur when the oak was at the same phenological stage. In 1995, the frost occurred during the night of 14–15 May during the apparent rest period of the sessile oak seedlings. This was after the total elongation of the first growth flush which was completed on 11 May. The minimum temperature recorded under shelter was –0.9 °C: no data on air temperature were available. A temperature of 0 °C under shelter would correspond to an air temperature close to –3.0 °C at a height of 40 cm above the soil [4]. In 1996, the frost occurred during the night of 4–5 May during the elongation period of the first growth flush. The minimum temperature recorded under shelter reached –0.3 °C.

The density of the oak seedlings in the regeneration site studied was about 80 seedlings/m^2. A total of 126 6-year-old seedlings were randomly sampled at the beginning of April 1995 in an enclosed plot (35 × 15 m^2). The mean heights and diameters (± standard deviation) of the seedlings were 88.6 cm (± 17.1 cm) and 8.4 mm (± 1.2 mm), respectively, at the beginning of the 1995 growing season and 111.9 (± 20.9 cm) and 10.9 (± 2.5 mm), respectively, at the beginning of the 1996 growing season.

2.2. Description methods

The intensity of frost damage was characterised visually on the stem of each seedling. The stem was defined as the main shoot which had the strongest elongation during the previous growing season. After frost, the seedlings were classified into four categories: F1, intact seedling, no damage; F2, necrosis of the terminal bud of the terminal shoot; F3, necrosis of the apical tip of the shoot (in 1996) and the whole rosette of subterminal buds (in 1995); and F4, necrosis of the whole shoot. For intensities F2, F3 and F4 leaves were necrotic.

In order to characterise the vegetative phenology and determine the bud burst dates of seedlings at the beginning of each growth flush, growth was monitored weekly or twice weekly during the 1995 and 1996 growing seasons on two or three buds likely to produce extension growth of the stem. The phenological stage of the buds was noted according to the following classification system: stage 1, dormant bud; stage 2, start of bud development (swollen bud); stage 3, green tip with appearance of the primordium of the first leaf; stage 4, elongation of the primordia contained in the bud; stage 5, at least one leaf has completely emerged; stage 6, intense elongation of internodes; and stage 7, elongation finished. This last stage was assessed by measuring the length of the shoot. Monitoring growth made it possible to determine the periods of elongation and apparent rest in each flush and thus to determine the number of growth flushes which appeared during each growing season (*Nflush*). The bud burst of each flush was chosen as being stage 2 and elongation cessation as being stage 7.

The length (*Length*) and the number of visible internodes (*Ni*) (length > 2 mm) of all growth units (GUi) (i.e. the portion of axis elongated during a growth flush or elongation period) were measured on each seedling during the 1995 and 1996 growing seasons.

The origin or the position of the bud, which was responsible for regrowth and the continuity of the stem during the second growth flush after frost damage, was determined. Five different origins were distinguished either on the GU produced during the first growth flush of the current growing season (A) or on the GU established at the end of the last growing season (B) (*figure 1*):

A) On the GU damaged, established during the first flush of the current growing season: the terminal bud (TB-GUl) (1); a subterminal bud (STB-GUl) (2) and a lateral bud (LB-GUl) (3).

B) On the GU established at the end of the previous growing season: a subterminal bud (STB-pGU) (4) and a lateral bud (LB-pGU) (5).

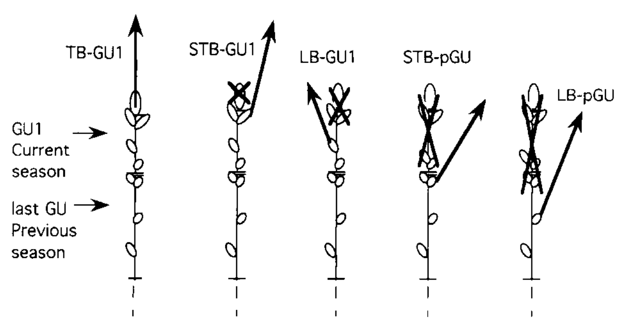

Figure 1. The position of the bud, which was responsible for regrowth and the continuity of the stem during the second growth flush after frost damage. A: The terminal bud (TB-GUl) (1); a subterminal bud (STB-GUl) (2); a lateral bud (LB-GUl) (3) of the GU produced during the first growth flush of the current growing season. B: A subterminal bud (STB-pGU) (4) and a lateral bud (LB-pGU) (5) of the growth unit established during the last growth flush of the previous growing season.

In cases (4) and (5), the GU established during the first flush did not belong to the stem at the end of the current growing season. The state of the terminal bud (dead or alive) of the GU of the other flushes was also determined. We also noted the position of the bud responsible for the elongation of the stem during these flushes when the terminal bud died.

At the end of each of the 1995 and 1996 growing seasons, we measured the number of growth units (*Ngu*) contained in the annual shoot, the length of the annual shoot (*Alength*) and the number of visible nodes per GU (*Ni*).

2.3. Statistical analysis

Two chi-square tests were used to study the relationships between the different frost damage categories and the bud burst date (*Beginning*) or initial dimensions (*Diam*: collar diameter and *Height*: height) of the seedlings at the beginning of the two 1995 and 1996 growing seasons, or the length of the GU produced during the first growth flush (*Length1*) before frost. For this, the *Diam*, *Height* and *Length1* variables were transformed into class variables. A chi-square test was also applied to study the relationship between the categories of frost damage in the 1995 growing season and those in the 1996 growing season.

Statistical models chosen according to the type of variables were used to quantify and test the effects of the intensity of frost damage on the variables measured as well as the relationships between these variables. Chi-square tests were thus used to analyse the relationship between the number of growth flushes established during each growing season (*Nflush*) or the number of GU on the stem (*Ngu*) and the intensity of frost damage (F1 to F4). A logistic model using cumulative logits [1] was used to take into account the ordinal nature of the dependent variables *Nflush* and *Ngu* and to quantify the differences between categories of frost damage.

For each growing season, an analysis of variance (ANOVA) was applied to compare the GU lengths (which are continuous quantitative variables) during each flush *i* (*Length i*) between the different categories of frost damage and between the different origins or positions of the GU. Moreover, a covariance analysis was applied to compare the annual shoot length (*Alength*) according to the different categories of frost damage, taking the number of GU established (*Ngu*) as a covariable.

The number of nodes is a discrete quantitative variable. A deviance analysis using the Poisson linear model [31] was thus applied in order to compare the number of nodes per GU (*Nn*) depending on the intensity of frost damage. A Poisson regression was adjusted between *Nn* and the log transformation of the *Length* of each flush.

The proportions of dead terminal bud at the end of each growth flush (ranging between 0 and 1) were compared for each growing season using a logistic model [13].

In order to analyse the relationships between the categories of frost damage with other qualitative variables (such as bud burst dates, dates of interruption of growth of the second and third flushes and number of growth flushes [*Nflush*]), chi-square tests were applied.

Calculations were made with SAS, version 6.09 [34, 35]. The procedures used were Proc Genmod, Proc Catmod, Proc Logistic and Proc Freq.

3. Results

3.1. Sensitivity of the seedlings to frost

Bud burst of the seedlings occurred between 21 April and 2 May for the 1995 growing season and between 17 April and 25 April for the 1996 growing season.

In 1995, the proportion of intact seedlings (F1) was the highest whilst that of the most damaged seedlings (F4) was the lowest. The reverse trend was observed in 1996: the proportion of F4 seedlings was the highest whilst that of F1 seedlings was the lowest (*figure 2*).

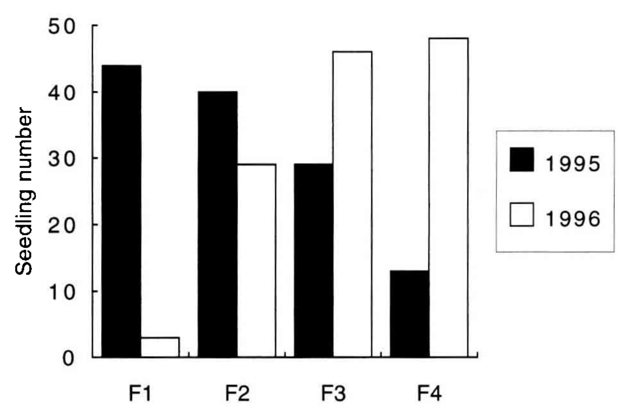

Figure 2. Number of seedlings as a function of the intensity of frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot) to which the seedlings were subjected during the 1995 and 1996 growing seasons.

Seedlings were consequently less damaged by frost during the 1995 season than during the 1996 season.

For both the 1995 and 1996 growing seasons, no relationship between the intensity of frost damage (F1 to F4) and the initial height ($P = 0.218$ and $P = 0.821$ in 1995 and 1996, respectively) or the initial collar diameter ($P = 0.271$ and $P = 0.743$ in 1995 and 1996, respectively) of the seedlings at the beginning of each season was observed. The length of the GU completely or partially established during the first growth flush before frost had no effect on the intensity of frost damage for the two growing seasons ($P = 0.490$ and $P = 0.135$ in 1995 and 1996, respectively).

For both growing seasons, a relationship between the bud burst date and the intensity of frost damage was observed. In 1995, the seedlings which had an early bud burst, i.e. before 21 April, tended to be less damaged by frost than the seedlings which burst bud after this date, around 2 May (*figure 3, top panel*). In 1996, we observed the reverse trend, i.e. the seedlings which burst bud early (around 17 April) tended to be more affected by frost (categories F3 and F4) than the seedlings which had a late burst bud (categories F1 and F2) (*figure 3, bottom panel*). The seedlings of the latter categories were at less advanced growth stages than the seedlings of categories F3 and F4 when frost occurred (stage observed on 3 May) (*figure 4*).

The date of bud burst appeared to be independent of the initial height ($P = 0.263$ and $P = 0.694$ in 1995 and 1996, respectively) or the initial collar diameter ($P = 0.168$ and $P = 0.927$ in 1995 and 1996, respectively) of the seedlings. There was no relationship between the categories of frost damage of the 1995 growing season and those of the 1996 season ($P = 0.716$).

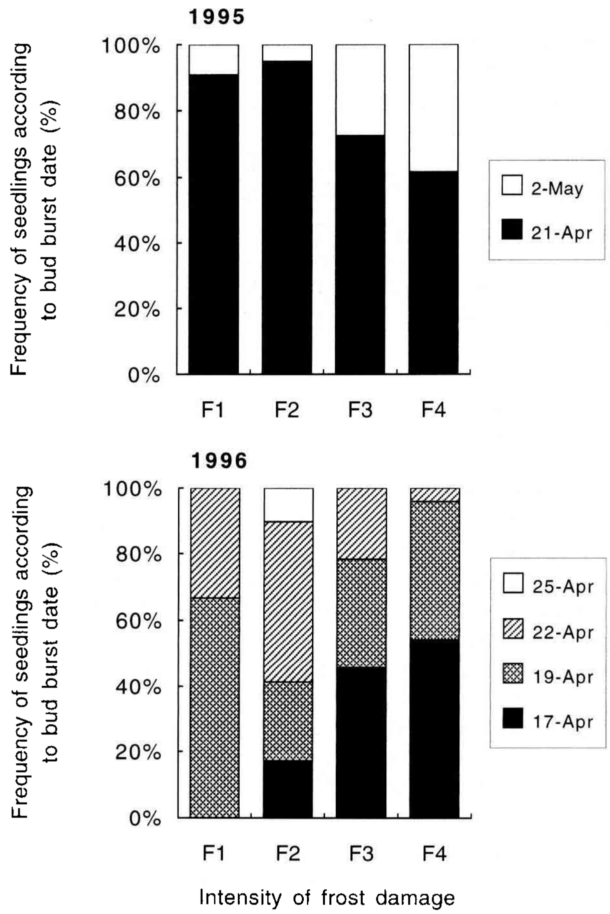

Figure 3. Distribution of the seedlings as a function of the bud burst date (stage 2, beginning of the bud development) for each intensity of the frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot) to which the seedlings were subjected during the 1995 and 1996 growing seasons.

3.2. Regrowth of the second flush

After frost damage, the position of the bud which developed during the second flush and which ensured the continuity of the stem was influenced by the intensity of frost damage in both the 1995 and 1996 growing seasons ($P < 0.001$) (*figure 5*). The bud located immediately below the wound (and only rarely further) usually ensured the continuity of the stem. In 1995, this bud was located close to the terminal bud of GU1 when damage intensity was low. When frost damage was small (categories F1 and F2), a bud of the GU of the first flush (GU1) often ensured the continuity of the stem. In contrast, when damage was higher (categories F3 and F4), an axillary bud on the GU developed during the previous season (pGU) usually ensured the continuity of the stem at the end of the growing season. This is evident for the damage category F4 for which the GU1 was lost, and also for category F3 because small buds at the base of the GU1 cannot develop to ensure the continuity of the stem. In these cases, the GU established during the first flush did not belong to the stem. In 1995, whatever the damage intensity, only 32 % of the buds which developed in the relay shoot were located on the GU developed at the end of the previous season. During the 1996 season, the reverse trend was observed: 84 % of the buds were located on the GU developed at the end of the previous season, whatever the damage intensity. For similar frost damage intensities, the position of the buds which

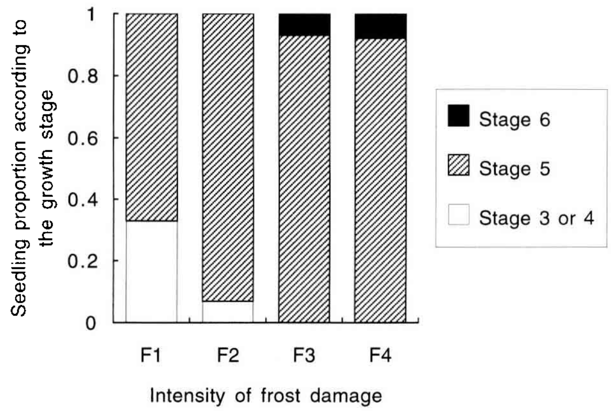

Figure 4. Proportions of seedlings according to the bud phenological stage as recorded on 3 May 1996 for each intensity of frost damage which occurred on 4–5 May 1996 (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot). Phenological stages: stage 3, green tip with appearance of the primordium of the first leaf; stage 4, elongation of the primordia contained in the bud; stage 5, at least one leaf has completely emerged; stage 6, intense elongation of internodes; and stage 7, elongation finished.

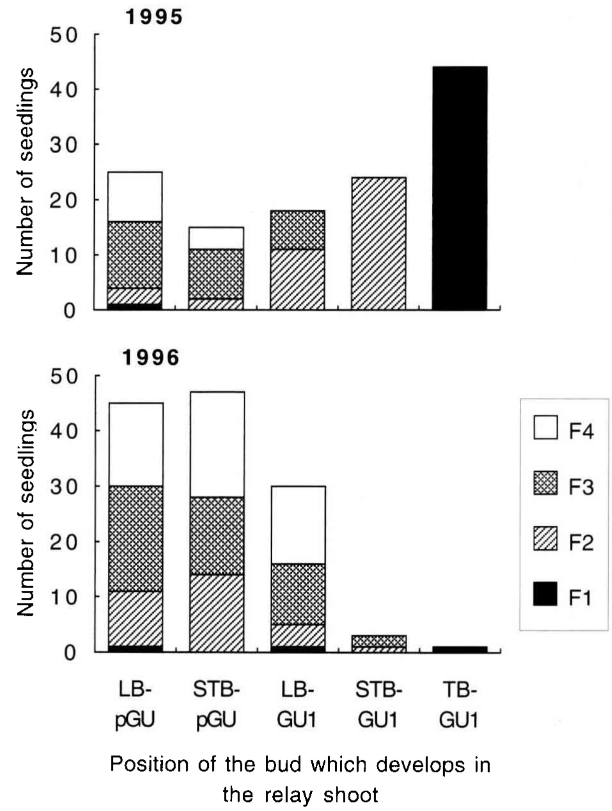

Figure 5. Number of seedlings as a function of the origin of the bud which ensured the continuity of the stem. LB-pGU: lateral bud of the GU established at the end of the previous season; STB-pGU: subterminal bud of the GU established at the end of the previous season; LB-GU1: lateral bud of the damaged GU of the first flush of the season; STB-GU1: subterminal bud of the GU of the first flush of the season; TB-GU1: terminal bud of the GU of the first flush of the season and according to the intensity of frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot) for each 1995 and 1996 season.

developed into relay shoots was different for the 1995 and 1996 growing seasons. The position was more apical in the 1995 growing season.

3.3. Number of growth flushes

Most seedlings produced three growth flushes in 1995 (85.7 %) and 1996 (84.1 %). The number of flushes, including the first flush and those formed after frost damage, appeared to depend on the growing season ($P = 0.016$) but not on the intensity of frost damage ($P = 0.770$ in 1995 and $P = 0.970$ in 1996).

3.4. Elongation and rest periods of each flush

Bud burst dates of the second flush occurred between 6 June and 27 July in 1995 and between 11 and 31 May in 1996. The apparent rest period after the first flush thus varied between 26 and 49 d in 1995, which was much longer on average than in 1996 (7 to 27 d). This rest period was apparently not related to the intensity of frost damage in 1995 (*table I*). The most damaged seedlings (F3 and F4) had a longer rest period than the less damaged seedlings (F1 and F2).

For the shoots with more than two GUs per year, the resting period after the second flush started between 22 June and 9 August in 1995 and between 28 May and 11 July in 1996. The elongation period of the second flush was 7 to 64 d long in 1995 and 3 to 31 d long in 1996. This elongation period was, on average, longer in 1995 than in 1996 and was only influenced by the intensity of frost damage in 1995. The less damaged seedlings (F1 and F2) had a longer elongation period than the most damaged seedlings (F3 and F4).

Table I. Durations of the elongation and apparent rest periods of each growth flush expressed in days for each intensity of frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot) for the two 1995 and 1996 growing seasons (mean ± standard deviation).

Intensity of frost damage	First flush Elongation	First flush Rest	Second flush Elongation	Second flush Rest	Third flush Elongation	Third flush Rest
F1						
1995	19.0 ± 3.2*a*	29.1 ± 5.4*a*	33.7 ± 7.7*a*	15.6 ± 8.7*a*	21.8 ± 7.1*a*	15.0
1996	14.0 ± 1.7*xy*	8.3 ± 2.3*x*	18.7 ± 2.3*x*	16.0 ± 5.0*y*	28.3 ± 0.6*x*	—
F2						
1995	19.4 ± 2.4*a*	30.3 ± 6.3*a*	31.8 ± 10.4*a*	15.3 ± 9.9*a*	22.1 ± 7.9*a*	—
1996	13.2 ± 2.6*y*	10.7 ± 5.0*x*	18.0 ± 5.6*x*	32.3 ± 7.5*x*	17.4 ± 7.6*y*	—
F3						
1995	16.9 ± 5.0*ab*	33.5 ± 10.9*a*	27.4 ± 11.6*ab*	12.2 ± 8.5*a*	23.9 ± 8.5*a*	—
1996	15.1 ± 2.0*xy*	9.9 ± 2.3*x*	19.4 ± 3.3*x*	28.4 ± 7.9*x*	20.9 ± 7.6*xy*	—
F4						
1995	15.8 ± 5.6*b*	33.5 ± 6.6*a*	23.8 ± 7.8*b*	13.3 ± 5.1*a*	22.9 ± 7.0*a*	—
1996	15.9 ± 1.3*x*	9.7 ± 2.9*x*	19.3 ± 3.8*x*	28.3 ± 9.4*x*	21.1 ± 8.3*xy*	—
All intensities						
1995	18.3 ± 3.9	30.9 ± 7.5	30.7 ± 10.1	14.9 ± 8.9	22.52 ± 7.6	15.0
1996	14.9 ± 2.2	9.9 ± 3.3	19.0 ± 4.1	28.8 ± 8.7	20.4 ± 7.9	—
Significance of the effect ($P > F$)						
1995	0.003	0.048	0.005	0.738	0.760	—
1996	< 0.0001	0.546	0.554	0.014	0.078	—

The effect of the intensity of frost damage on the duration of these periods was tested using the Fisher *F*-test and whether this effect is significant or not is indicated. Within each season, the values with the same letter *a* or *b* in 1995, *x* or *y* in 1996 are not significantly different according to the Bonferoni *t*-test at the 5 % level.

The bud burst dates of the third flush ranged between 18 July and 17 August in 1995 and 2 June and 23 July in 1996, which corresponded to an apparent rest period after the second flush of 2 to 49 d in 1995 and 10 to 53 d in 1996.

For the shoots with more than three GUs per year, the rest period after the third flush started between 3 August and 6 September in 1995 and between 9 July and 13 August in 1996. This corresponds to an elongation period of the third flush of 6 to 37 d in 1995 and 3 to 50 d in 1996.

For the shoots with four GUs per year, in 1995, the bud burst dates of the fourth flush ranged between 9 and 24 August and the resting date was on 6 September. The elongation period of this flush varied from 13 to 28 d. In 1996, bud burst occurred on 19 July and elongation was interrupted on 13 August.

The rest period after the second flush, the elongation period of the third flush, the rest period after the third flush and the elongation period of the fourth flush were not influenced by the intensity of frost damage.

3.5. Date of the complete cessation of elongation

The date growth stopped varied between 29 June and 6 September in 1995 and between 31 May and 13 August in 1996 (*figure 6*). In both cases, the date did not seem to depend on the intensity of frost damage ($P = 0.514$ in 1995 and $P = 0.954$ in 1996) but rather on the number of flushes established during the season ($P < 0.001$ in 1995 and $P = 0.003$ in 1996). Seedlings having three flushes or more interrupted their elongation later (around 6 September in 1995 and 13 August in 1996) than those having only two flushes (around 9 August in 1995 and 4 June in 1996).

3.6. GU length

The length of the GU varied between 0.5 and 47.2 cm for the 1995 growing season and between 0.4 and 37.5 cm for the 1996 growing season. During both seasons, the length of the GU significantly depended on the

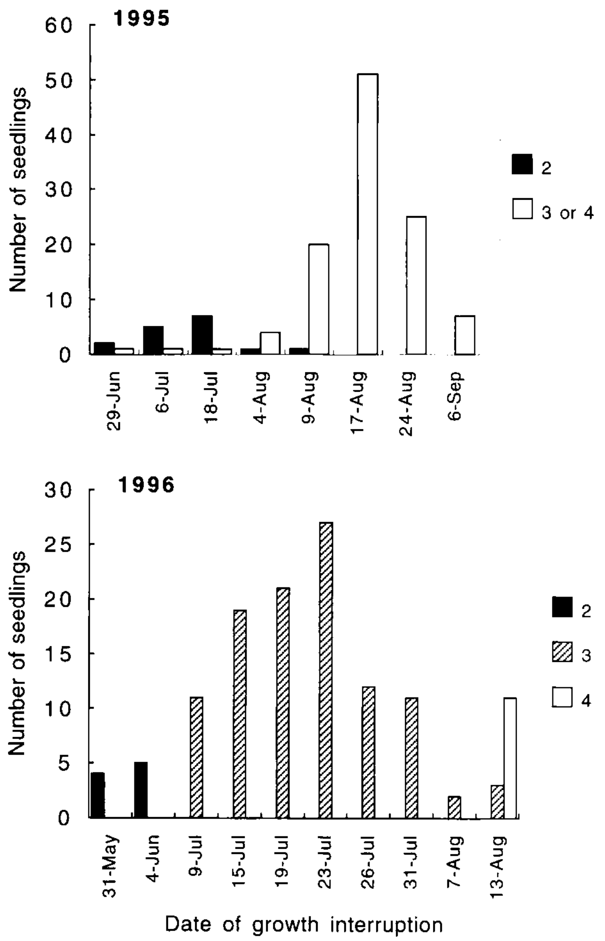

Figure 6. Number of seedlings which ceased growth at different dates according to the number of flushes (2, 3 or 4; see legend) established during the 1995 and 1996 growth seasons.

growth flush ($P < 0.001$). In 1995, the smallest mean length was that of the GU established during the first flush and the longest was that of the GU of the second flush. In 1996, the mean length of the GU produced during the second flush was smaller than that of the first flush and the longest mean length was that of the GU of the third flush.

The differences in the intensity of frost damage only had a significant effect on the GU length produced during the second flush in 1995 (*table II*). During the second flush of the 1995 season intact seedlings (category F1) produced the longest GU.

For the second flush, the origin of the GU, according to the classification provided in *figure 1*, had an effect on GU length only in 1995 ($P = 0.007$). In 1995, the GUs originating from the terminal bud (origin: TB-GU1) were the longest, followed by the GUs originating from the lateral bud of the GU produced during the previous season (origin: LB-pGU) (*table III*). During the 1996 season, due to the small number of GUs originating from the terminal bud or the subterminal bud of GU1 (origins: TB-GU1 and STB-GU1), these GUs were grouped together with the GUs originating from a lateral bud of the same GU1 (origin: LB-GU1) and then compared to the GUs originating from a bud (subterminal or lateral) of the GUs produced during the previous season (origins: STB-GUp and LB-GUp) (submeans, *table III*). After this, it appeared that the GUs originating from the bud of GU1 were, on average, significantly shorter (value 3.9 ± 3.4 cm) than those originating from the bud of the GU produced during the previous season (value 5.7 ± 4.3 cm; $P = 0.032$). The same was done for the 1995 growing season and it appeared that the mean values of the GUs originating from a bud of GU1 (15.2 ± 9.2 cm) were smaller than those originating from a bud of the GU produced during the previous season (16.1 ± 8.0 cm), but the difference was not significant.

For the two seasons, no significant effect of frost intensity damage was observed on the GU length produced during the third growth flush (*table II*). The origin of the GU (two possible origins: the terminal bud or the subterminal bud if the apex was dead) had also no significant effect on GU length ($P = 0.843$ in 1995 and $P = 0.247$ in 1996).

3.7. Number of nodes per GU

The number of nodes (*Ni*) per GU varied from 2 to 26 in 1995 and 2 to 24 in 1996, whatever the GU. On the one hand, the intensity of frost damage had no effect on *Ni* ($P = 0.415$ in 1995 and $P = 0155$ in 1996). On the other hand, the number of the growth flush (*Noflush*) had a highly significant effect on *Ni*. For both 1995 and 1996 growing seasons, the GU produced during the third flush contained the highest mean number of nodes (*table IV*). In 1995 the GU produced during the first flush contained the smallest mean number of nodes and in 1996 the fourth GU had the smallest mean number. Moreover, for each growth flush, the number of nodes appeared to be positively correlated with the length of the GU, but the relationship did not seem to be influenced by the intensity of frost damage.

3.8. Proportion of dead terminal buds

For both 1995 and 1996 growing seasons, the proportion of dead terminal buds on the GU of the stem

Table II. Mean length (± standard deviation) of the growth unit produced during each growth flush in 1995 and 1996 expressed in cm for each intensity of frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot).

Intensity of frost damage	Growth flush: First flush	Second flush	Third flush	Fourth flush
F1				
1995	6.3 ± 4.2*a*	18.3 ± 8.5*b*	14.0 ± 7.1*x*	—
1996	5.9 ± 7.2*x*	7.5 ± 2.2*x*	14.7 ± 9.1*a*	—
F2				
1995	7.8 ± 4.7*a*	15.6 ± 7.9*ab*	13.2 ± 6.5*x*	—
1996	7.9 ± 7.6*x*	5.5 ± 4.0*xy*	14.5 ± 8.4*a*	1.3 ± 0.9*x*
F3				
1995	6.3 ± 5.2*a*	11.7 ± 7.9*a*	17.9 ± 8.6*a*	—
1996	6.0 ± 3.8*x*	5.3 ± 4.4*xy*	15.4 ± 7.7*x*	1.7 ± 0.3*x*
F4				
1995	—	14.9 ± 8.6*ab*	14.4 ± 9.7*a*	—
1996	—	4.8 ± 4.0*y*	15.2 ± 8.5*x*	1.1 ± 0.1*x*
All intensities				
1995	6.9 ± 4.5*b*	15.5 ± 8.5*a*	14.2 ± 7.2*a*	—
1996	6.6 ± 5.3*y*	5.2 ± 4.1*yz*	15.1 ± 8.1*x*	1.4 ± 0.5*z*
Significance of the effect ($P > F$)				
1995	0.232	0.017	0.364	—
1996	0.683	0.686	0.980	0.336

The effect of the intensity of frost damage on the growth unit length produced during each flush is tested using the Fisher *F*-test and whether this effect is significant or not is indicated. For each season, the values with the same letter are not significantly different according to the Bonferoni *t*-test at the 5 % level.

Table III. Mean length (± standard deviation) of the growth unit (GU) expressed in cm during the second growth flush (GU2) of the two 1995 and 1996 seasons according to the origin of the bud which developed in the relay shoot: terminal bud (TB-GU1), subterminal bud (STB-GU1), lateral bud (LB-GU1) of the damaged GU of the first flush (GU1) or subterminal (STB-pGU) or lateral (LB-pGU) bud of the GU established during the previous season (pGU).

Origin of the bud which develops in the relay shoot	Growing season 1995	1996
GU1		
TB-GU1	18.9 ± 8.4*a*	5.3 ± 1.2*a*
STB-GU1	13.0 ± 5.9*b*	4.4 ± 2.6*a*
LB-GU1	14.0 ± 8.3*ab*	3.8 ± 3.6*a*
Submean	*15.2 ± 9.2 x*	*3.9 ± 3.4 x*
GUp		
STB-pGU	11.9 ± 8.3*b*	5.8 ± 4.7*a*
LB-pGU	16.7 ± 9.3*ab*	5.6 ± 3.9*a*
Submean	*16.1 ± 8.0 x*	*5.7 ± 4.3 y*

The values with the same letter (*a* or *b*) are not significantly different according to the Bonferoni *t*-test at the 5 % level. Submean: mean GU length (GU1 or pGU) whatever the origin of the bud which develops in the relay shoot. Submeans are compared by subscripts *x* and *y*.

Table IV. Average number of nodes (± standard deviation) carried by the growth unit during each growth flush of the two 1995 and 1996 seasons in oak seedlings.

Number of the flush	Growing season 1995	1996
First	8.5 ± 3.2*a*	6.8 ± 3.7*ab*
Second	10.7 ± 3.5*b*	8.6 ± 3.0*a*
Third	12.0 ± 3.9*c*	9.8 ± 3.2*a*
Fourth	—	3.5 ± 3.5*b*

The values with the same letter are not significantly different according to the chi-square test at the 5 % level.

depended greatly on the number of the growth flush ($P < 0.001$). The proportion of terminal bud death was the highest at the end of the first growth flush, and the lowest at the end of the second growth flush (*figure 7*).

3.9. Annual shoot length

The annual shoot length (*Alength*) varied between 1 and 73.2 cm for the 1995 season and between 2.6 and

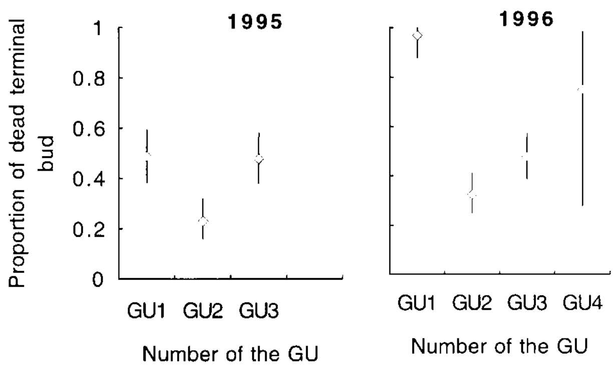

Figure 7. Proportion of dead terminal buds at the end of each growth flush during the 1995 and 1996 growing seasons. The vertical bars represent the limits of the confidence intervals at the 5 % level calculated using the maximum likelihood method around the value predicted by the logistic model (diamond symbol).

47.5 cm for the 1996 season. For both seasons, length did not depend on either the initial height of the seedling (*Hei*) at the beginning of the season ($P = 0.100$ in 1995 and $P = 0.342$ in 1996) or the collar diameter. In 1995, this annual shoot length was statistically dependent on the intensities of frost damage (*table V*). The least damaged seedlings (F1 and F2) had a higher mean annual shoot length than the most damaged seedlings (F3 and F4). This annual shoot length was also related to the number of GUs observed at the end of the growing season (*Ngu*), which depended on the intensity of frost damage (*figure 8*). For the same number of GU observed, there was no significant difference between the different intensities of frost damage (*table V*, see the adjusted annual shoot length GU). In 1996, both the intensity of frost damage and the number of GUs had no significant effect on the annual shoot length; its mean value was 22.4 cm (± 10.9 cm).

4. Discussion

Late frost in spring caused different types of damage in oak seedlings, i.e. from simple necroses of leaves to a complete necrosis of the GU [10]. The intensity of frost damage was higher in 1996 than in 1995. Damage intensity depends mostly on the phenological stage of the shoot when frost occurred and on the minimum temperature reached [2, 29]. In our experiment, the minimum temperatures reached in 1995 and 1996 when frost occurred were close and the differences in frost damage cannot consequently result from the differences in temperature during the two seasons. These differences in

Table V. Annual shoot length produced during the 1995 season by oak seedlings subjected to increasing intensities of frost damage (F1 to F4).

Intensity of frost damage	Annual shoot length (cm)	Adjusted annual shoot length GU (cm)
F1	36.6 ± 14.6*a*	32.5 ± 13.3*a*
F2	32.7 ± 12.7*a*	30.7 ± 12.0*ab*
F3	19.4 ± 13.8*b*	24.9 ± 13.5*b*
F4	18.3 ± 14.2*b*	26.1 ± 13.3*ab*
All intensities	35.0 ± 15.2	35.0 ± 15.2

Means (± standard deviations) and means adjusted to the number of growth units (GU) produced (± standard deviations) are expressed in cm. In each column, the values with the same letter are not significantly different according to the Bonferoni *t*-test at the 5 % level. F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot.

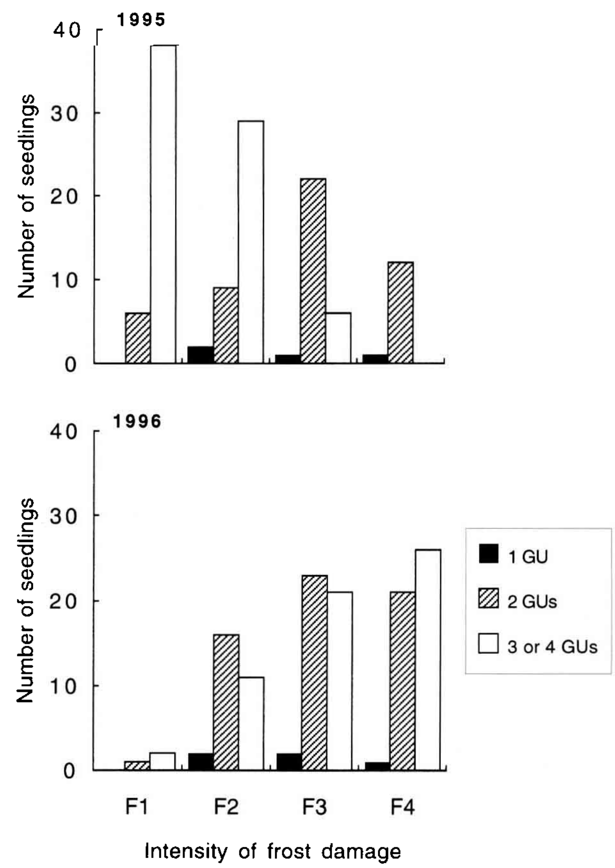

Figure 8. Number of seedlings as a function of the number of growth unit (GU) belonging to the stem for each intensity of frost damage (F1: intact seedling, no damage; F2: necrosis of the terminal bud of the terminal shoot; F3: necrosis of the apical tip of the shoot; F4: necrosis of the whole shoot) during the 1995 and 1996 seasons.

damage can be attributed to the phenological stage of the shoot since late frost did not occur at the same time or at the same phenological stage in 1995 and 1996. In 1996, late frost occurred when the new GU of oak seedlings was elongating and in 1995 frost occurred after the complete elongation of the GU, i.e. during the apparent rest period following the first flush. The elongating GU is more sensitive to frost than the GU after complete elongation, since it contains a lot of water, is not lignified and its terminal meristem is not yet protected by scaly leaves. A relationship between frost sensitivity and the water content of plant stems has been shown elsewhere [8, 9, 22].

Within the same season, differences in frost sensitivity were observed between seedlings. This sensitivity was statistically related to the bud burst date, directly linked to the phenological stage reached by the shoot when frost occurred. In 1995, the seedlings having early bud burst tended to be less affected by frost as they developed a more lignified shoot. The reverse trend was observed in 1996. The oak seedlings with early bud burst were at advanced elongation stages and were consequently more vulnerable to frost than the seedlings with late bud burst and which were at earlier phenological stages.

The initial dimensions of the seedlings, i.e. height and collar diameter, were not related to the intensity of frost damage. This result is in agreement with the observations of Day and Pearce [14] who reported that frost damage could be observed on all the dominant shoots of oak seedlings, whatever their dimensions. Frost damage sensitivity on young plants is rather influenced by the structure of the canopy and the proximity of damaged seedlings to each other (plants isolated or protected by neighbouring seedlings) with the presence of microvariations in the temperature around these seedlings (Aussenac, personal communication).

Moreover, the initial dimensions of the oak seedlings did not apparently influence the bud burst date. The absence of a relationship between the bud burst date and the initial dimensions of the seedlings explains the absence of a relationship between these parameters and the intensity of frost damage.

After frost damage, the height growth of seedlings sometimes started again from a bud of the GU on the shoot of the previous year. The first GU was lost and did not belong to the stem at the end of the season. The loss rate of the first GU was higher when frost occurred during the elongation of this GU (season 1996). Thus, the number of GU which finally belonged to the stem did not often correspond to the number of growth flushes expressed by stem. In addition, the late GU established at the end of the season, especially during the fourth flush, did not subsequently belong to the stem. To demonstrate this, monitoring growth provided much more information than retrospective growth analysis.

Regrowth occurred from a bud located below the injury caused by frost. The scaly buds did not participate in regrowth, probably because they were not well formed. The bud which developed in the relay shoot originated either from the damaged GU or from the GU produced at the end of the previous season (when the damaged GU did not belong to the stem). In the latter case, the GU was longer than the GU originating from an axillary bud of the new GU. This could be explained by the fact that an axillary bud originating from the GU established at the end of the previous season, which was previously inhibited by the elongating shoot of the first flush, can be better formed (i.e. contains more leaf organs) than a bud originating from the new GU. This is sustained by the fact that frost occurred during the elongation of the first GU which is composed of terminal or axillary meristems which are just beginning to be formed. A cytologic observation of buds of different origins is necessary to confirm this hypothesis.

The length of the GU of the third flush was not affected by the intensity of frost damage, which means that the effect of frost damage did not seem to persist beyond the following growth flush. Similar results were reported by Chaar et al. [11] who showed that the length of the GU of the third flush was not affected by controlled damage applied to the GU of the first flush, i.e. defoliation, decapitation of the terminal bud, a combination of the two previous treatments and removal of the apical tip of the GU.

During the 1995 season, the GU produced during the first growth flush were shorter than those produced during the second and third flushes. Similar results were reported by Chaar et al. [11], Harmer and Baker [25] and Harmer [24] in sessile oak seedlings. These differences can be partly attributed to changes in the nutritional status of the plant during the growing season [20, 23]. The length of the GU of the first flush depends mainly on the availability of the photosynthates stored in the plant during the previous season and the number of nodes, internodes and leaves preformed in the bud which pass winter. The GU of the second and third flushes are well supplied in photosynthates owing to the large leaf surface area already present at these development stages [23]. During the 1996 season, the GU produced during the second flush were shorter than those of the first and third flushes. This result may be partly explained by the fact that the GUs of the second flush were not well supplied with photosynthates by leaves which were partially damaged by late frost at the beginning of their expansion.

The intensity of frost damage had an effect neither on the positive relationship between annual shoot length and the number of GUs established during the growing season and belonging to the stem, nor on the relationship between the number of nodes and the length of the GU which seems to depend more on the growing conditions during the elongation period. The intensity of frost damage had an effect on the annual shoot length via the reduction of the number of GUs belonging to the stem (the GU produced during the first growth flush was often lost). The annual shoot length did not seem to depend on the initial dimensions of the seedlings. This result is not in agreement with that of Day and Pearce [14] who showed that after late frost the large young sessile oak trees (according to height and diameter values) had a better subsequent height growth increment than the small trees. This difference in the results could originate from the difference in the variability of tree dimensions, our plants being much more similar in dimensions than those of Day and Pearce [14].

The appearance of late frost increased the proportion of dead terminal buds at the end of the first flush. This proportion was higher when frost occurred during the first elongation period (1996 season). For the following growth flushes, the proportion of dead terminal buds was relatively lower. In the absence of late frost, the death of the terminal bud was rather low at the end of the first flush and increased for the subsequent growth flushes [23]. Terminal bud death could be due to numerous reasons: browsing by herbivores (this factor was controlled by the fence around the regeneration plot), attacks by insects or phytopathogenic fungi and low temperatures [24, 29]. The death of the bud might lead to the temporary loss of stem straightness [23] and might also lead to a forked appearance [19].

The apparent rest period that followed the first flush was shorter in 1996 than in 1995, which led to a time lapse between the subsequent elongation periods. The growing conditions of the first flush of the two 1995 and 1996 seasons were slightly different, but this did not explain the shorter apparent rest period in 1996. The apical tip of the elongating GU of the first flush often inhibits growth of the axillary buds of the previous GU. The frost which occurred during elongation damaged this GU with young leaves and suppressed this inhibition, which allowed the development of these axillary buds to ensure regrowth and the establishment of a new assimilating leaf surface area.

The frost that occurred after the complete elongation of the first GU (season 1995) also affected the apparent rest period of the seedlings. The rest period of the most damaged seedlings (categories F3 and F4) was the longest, probably because the subterminal and lateral buds of the GU established during the previous season and which often ensured regrowth required a longer period of reactivation than those of the first GU which often ensured the regrowth of the least damaged seedlings. These results are in agreement with those of Chaar et al. [11] who showed that controlled damage applied to the GU established during the first flush in sessile oak seedlings affected the rest period of this first flush. Leaf removal, decapitation of the terminal bud combined with leaf removal as well as the removal of the apical tip of the GU reduced the rest period of the first flush while decapitation of the terminal bud alone delayed the rest period. The elongation period of the GU of the second flush was longer in 1995 than in 1996, probably because damage was smaller and the leaves which ensured the carbon supply of this GU were less damaged in 1995.

This study focused on the effect of late frost on the height growth components of seedlings. Frost, in particular repeated late frost, is expected to have consequences on the shape and ramification of seedlings [29]. Defects in the shape of branching caused by frost or other factors, such as browsing by mammals and insect attacks, are often considered as being worse consequences than height growth losses [18, 32]. It would thus be interesting to complete the measurements of the height growth components by an assessment of the shape of the seedlings.

In this study, the intensity of frost damage for a given growing season did not vary greatly and we consequently did not have real control seedlings. Analysing the consequences of frost damage, after specifying the damage categories a fortiori, raises the problems of 1) the different numbers of seedlings in the different categories and 2) the absence of an actual control, not really damaged by frost. This introduces a bias in the statistical results. The extent of the damage caused by frost depends on the phenological stage of buds when frost occurs as well as on the low temperature the buds are subjected to [2, 29]. To better study the effects of frost on the subsequent growth of an oak seedling, it is necessary to be able to vary the low temperatures the buds are subjected to at different bud phenological stages under controlled conditions. Frost damage can be estimated visually by distinguishing damage categories, as in our study, or simply by noting the browning of the tissues (according to Liepe [29]), or using the relative conductivity method explained by Deans and Harvey [15], which seems to be a more objective method.

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