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Article

Seasonal growth analysis of a white clover meadow (*Trifolium repens* L.)

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Abstract:

The objective of the present study was to assess a growth analysis of white clover (*Trifolium repens* L.) and determine the optimal harvest time per season. The experiment was carried out at the *Colegio de Postgraduados, Campus Montecillo*, *Texcoco*, Mexico. Twenty-four 3.7 X 1.7 m plots were used, distributed in a completely randomized design, with eight treatments and three replicates per station. The treatments consisted of successive weekly cuts, during a regrowth cycle of 8 wk, in each season of the year. At the beginning of the study, a uniform cut was made and the residual forage was determined. The evaluated variables were: accumulation of dry matter, botanical and morphological composition, and leaf area index of white clover. The highest forage accumulation (P<0.05) occurred in the eighth week in spring (2,688 kg DM ha^{-1}). Leaf production was higher (P<0.05) in spring, autumn and winter. The highest leaf area index was reached in the eighth week in spring (3.0; P<0.05). It is recommend exploiting the white clover meadow in the sixth week of the spring-summer period and in the seventh week of autumn-winter.

Key words: Growth analysis, *Trifolium repens* L., Dry matter accumulation, Botanical composition.

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Introduction

In the central zone of Mexico, white clover (*Trifolium repens* L.), perennial ryegrass (*Lolium perenne* L.), orchard grass (*Dactylis glomerata* L.), and alfalfa (*Medicago sativa* L.) planted on 171,520 hectares are the forage species that have exhibited the best performance under grazing conditions in pure or mixed pastures(1,2,3). However, due to its chemical composition, its persistence resulting from its creeping growth habit, and its adaptability to temperate zones, white clover is the species of greatest agronomic importance among the almost 300 species of the genus *Trifolium*(4). In addition, it can also improve soil fertility by supplying nitrogen in a proportion of up to 450 kg N ha^{-1} through symbiotic fixation(5,6,7).

Forage production patterns in Mexico are influenced by climate variations, with temperature and precipitation being the main factors(8); therefore, it is important to know the seasonal growth patterns of the most widely used forage species in each one of the ecological regions of the country(9). Previous works mention that the management strategies of a meadow, intensity and frequency through cutting or grazing can modify the botanical composition, yield, and nutritional quality(10,11). The severity in the use of the meadow can modify the carbohydrate reserves in the plant, which affects the growth pattern, reducing the number of stems, number of sprouts, and number of leaves in the plant(12).

Evaluating the seasonal growth of a pure or mixed meadow helps to understand the behavior of plants and the association of different species, since the balance between growth rate and tissue loss varies with the season throughout the year(9). Dry matter yield, crop growth rate, leaf area index, botanical and morphological composition, intercepted radiation, plant height, leaf: stem ratio, and leaf: non-leaf component are structural variables that help to understand the behavior of a meadow and must be considered to understand the response under a mowing or grazing system(13,14).

In a study conducted by Moreno *et al*(15), they reported that, when associated with irrigated grasses, white clover produced an average of 1,581 kg DM ha^{-1} (P<0.05) in its first year of evaluation, while Maldonado *et al*(3) reported an increase of 376 % (equivalent to 7,532 kg DM ha^{-1}) in their fourth year of evaluation under mixed pastures with irrigation due to their stoloniferous growth and persistence in the meadow. In another research(9), the highest leaf area index occurred at week five in summer (P<0.05), and the leaf was the largest component in spring. There is little research analyzing the growth of white clover in Mexico. The objective of the present study was to evaluate growth analysis of white clover (*Trifolium repens* L.) in order to determine the optimal physiological moment of grazing in each season of the year.

Materials and methods

The study was performed in a white clover meadow in the experimental field of the College of Postgraduates (Colegio de Postgraduados), in Montecillo, Texcoco, State of Mexico, at 19º 29' N and 98º 53' O, at 2,240 m asl. Broadcast sowing was carried out in February 2009 with a viable pure seed density of 6 kg ha^{-1}. The local climate is temperate sub-humid, with an average annual precipitation of 636.5 mm, and a rainfall regime in the summer (from June to October) and an average annual temperature of 15.2 ºC(16). The local soil is sandy loam, with a slightly alkaline (pH 7.8) and 2.4 % organic matter(17).

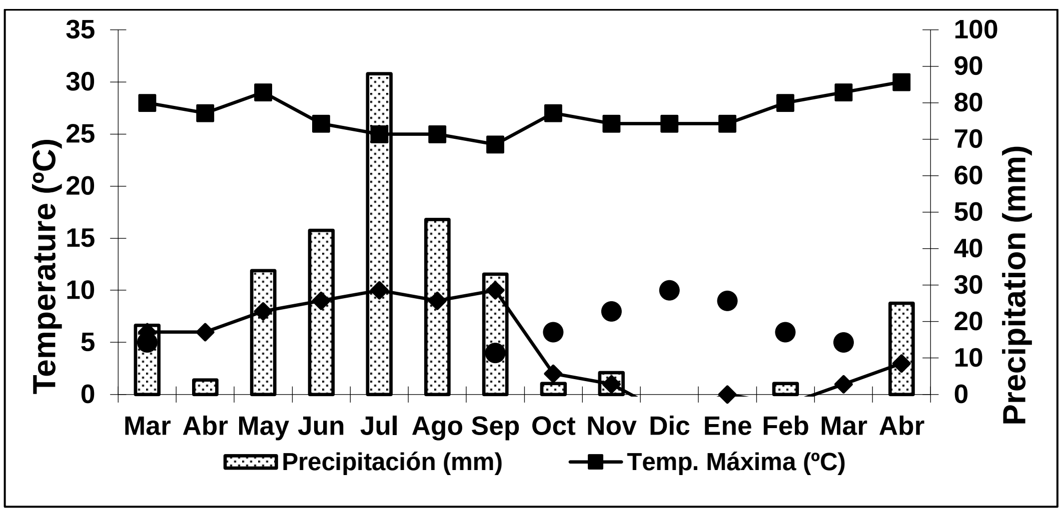
In the middle of each season of 2012, a uniform grazing was made and later a growth analysis was carried out; the treatments consisted of an eight-week growth analysis in spring-summer and a nine-week one in autumn-winter, since low temperatures promote a slower forage growth. Sheep were used as defoliators until the remaining forage was left at a height of approximately 5 cm above ground level, and, for better management, an electric fence was established in the experimental plots.

The plots were distributed in a completely randomized design with three replications. Twenty-four 3.7 x 1.7 m plots were drawn, among which the treatments were randomly distributed. During the dry season, the meadows were irrigated by gravity at field capacity; 16 irrigations were carried out every 2 wk with approximately 32 mm for each one, which gave a total of 512 mm of water, and the meadows were not fertilized.

Climate data

The monthly averages of outdoor temperature (maximum and minimum) and monthly rainfall during the study period were obtained from the agrometeorological station of the College of Postgraduates (Colegio de Postgraduados), located at a distance of 100 m from the experimental site (Figure 1). The maximum monthly temperature ranged from 22.1 to 30.2 °C, while the minimum temperature was from -2.6 to 11 °C. The highest temperature occurred in spring, registering a maximum of 30.2 °C in April, and the lowest temperature, of -2.6 °C was recorded in December. The accumulated precipitation from March 2012 to April 2013 was 312.3 mm, 70 % of which occurred in June, July, August, and September 2012, accumulating a precipitation of 220 mm.

Figure 1: Average maximum and minimum monthly temperature and accumulated precipitation per month during the study period (March 2012 - April 2013)



Dry matter accumulation

After uniform grazing, three 0.25 m^2 squares were cut at a height of 5 cm from the ground in each experimental plot for eight weeks. The forage harvested in each quadrant was washed and dried in labeled paper bags in a forced air oven at 55 °C during 72 h in order to estimate the amount of dry matter per hectare at the various regrowth ages.

Botanical and morphological composition

For the purpose of determining the botanical composition of the forage, a subsample of this was collected for a dry matter yield of approximately 20 %[11] and was separated into the following components: dead material, weeds, grasses and white clover. The morphological components of white clover (leaf, petiole, runner and flower) were separated. Each separate component was dried in a labeled paper bag and left in a forced air oven at 55 °C during 72 h in order to estimate its dry weight.

Leaf area index

The leaf area index was calculated by separating the leaves of five stolons were separated for each week's replicate and placing them in a leaf area integrator (LI 3100 LI-COR Inc.) from which the leaf area readings in cm^2 were obtained. These readings together with the number of stolons per square meter allowed estimating the leaf area index by means of the following formula:

LAI= LA * SD
Where: LAI= leaf area index; LA=leaf area per stem; and SD= stolon density (m^{-2}).

Statistical analysis

The data were analyzed by the GLM procedures of SAS[18], for a completely randomized experimental design, where the treatments were the weeks of evaluation with three repetitions per season and a regression analysis for each variable. The means were compared with the Tukey test (α= 0.05).

Results and discussion

Dry matter accumulation

Figure 2 shows the results of the accumulated dry matter yield, which increased with regrowth age, reaching its maximum in the eighth week of spring (2,688 kg DM ha^{-1}; P<0.05), the seventh week of summer (2,241 kg DM ha^{-1}; P<0.05), the eighth week of fall (1,781.3 kg; P<0.05), and the sixth week of winter (1,643 kg; P<0.05). The biomass accumulated in spring was higher by 20 % (447 kg DM ha^{-1}), 51 % (907 kg), and 64 % (1,045 kg), compared to summer, autumn and winter, respectively (P<0.05). The leaf for spring, autumn and winter increased as the regrowth weeks increased and it was greater than the petiole, however, in summer there was a higher petiole yield and less leaf. During the evaluation period in winter (March 3, 2013), an intense frost occurred; this coincided with the sixth week of regrowth, which limited the biomass yield for said sampling, drastically increasing the dead material.

For their part, in their assessment of white clover in the highlands of Mexico, Gutiérrez *et al*(9) mention an accumulation of forage as the age of the plant increased in all seasons, reaching the maximum yield for spring, autumn, and winter in the eighth week, with 2,953, 1,592, and 1,791 kg DM ha^{-1}, respectively, and in the seventh week for summer with 1,971 kg DM ha^{-1} (P<0.05).

When establishing associations of white clover with perennial ryegrass (*Lolium perenne*) and orchard grass (*Dactylis glomerata* L.), Moreno *et al*(15) found a maximum white clover yield of 513 kg DM ha^{-1} in their first sampling year. However, Maldonado *et al*(3) registered a significant increase in the yield of white clover in these same associations in their third and fourth year of production, adding up to an average of 7,220 kg DM $ha^{-1.}$ These same authors mention that white clover dominates over time in the meadow because it is a kind of stoloniferous growth habit that allows rapid growth compared to grasses, which are tufted. In another study(19), they reported that 65% of the annual yield occurred in spring and summer, 23 % in winter, and autumn was the season that exhibited the lowest value, of 12 % (P<0.05).

According to various authors(18,20), white clover requires temperatures of 18 to 30 °C, the optimal being 24 °C, and precipitations of 750 mm for best performance. These temperatures were reached in spring (Figure 1), favoring the growth of the meadow as a result of the increase in the leaf area per plant and probably due to the increase in leaf appearance and elongation rates(19). Conversely, the dry matter yield was low in winter; in this regard, various authors [3,12,21] argue that low temperatures limit growth and forage accumulation, due to their direct influence on a lower leaf appearance and foliar rate.

Botanical and morphological composition

Based on the morphological components and the estimated accumulated biomass yield, the plant morphology was variable (P<0.05). The highest leaf percentage, of 68 %, was produced in the third week in spring; likewise, in summer the highest percentage of leaf, of 46 to 40 %, was produced from the first to the third week, decreasing drastically in the fourth week, to 30 % (P<0.05). In autumn, the highest leaf percentage occurred in the first week (70 %) and remained until the seventh week (59 %), after which it decreased. Finally, in winter, the highest leaf percentage (60 %) occurred in the fifth week (Figure 3).

On the other hand, the greatest contribution of the petiole, of 38 %, occurred in summer (P<0.05), while the highest percentage of stolon was reported in spring, being greater only in the first two weeks of growth, when it amounted to 20 % in average (P<0.05). As for pastures, the largest percentage, an average of 15 %, occurred in the summer. The contribution of weeds and flowers was minimal in all seasons and weeks of regrowth, being 3 % in average.

Winter was the season that reported the largest amount of dead material and from the seventh week on there was a drastic increase, of 100 %, since there was a decrease in temperature (Figure 1) frost causing the death of white clover. In this regard, Brock and Tilrock[8] mention that all plants have an optimal growth temperature and when these surpass it or decrease drastically, there may be cell death, which causes a drastic increase in dead material. On the other hand, as the age of the plant increased, the dead material also built up (P<0.05), due to the maturation of the senescent leaves of the lower strata[9].

The large proportion of leaves with respect to the petiole and stolon indicates that it is a high-quality forage since this allows it to be more digestible. In addition to this, as observed in all the assessment stages, the content of flower was minimal (P>0.05), which indicates that this forage is not precocious and allows it to increase its nutritional value in the first weeks. As has been shown, the association of this legume with grasses confers it forage value, since it augments the total yield per surface unit to up to 14 t DM ha^{-1}; however, these qualities can also be affected by the season of the year[19,22].

In addition to this, white clover under grazing conditions is less susceptible to loss of apical meristems due to the horizontal arrangement of leaves and leaf primordia, which allow efficient capture of solar radiation compared to Gramineae[8,14].

Leaf area index

The leaf area index (LAI) allows to estimate the photosynthetic capacity of a plant per unit area and helps to understand its ability to assimilate solar energy and transform it into dry matter yield[23]. The highest LAI was reported during spring and autumn, with 3.0 and 2.6, respectively, in week eight, while in summer and winter the highest index was obtained in week five with 2.6 ($P<0.05$). On the other hand, in all seasons there was a close relationship between the LAI and the regrowth weeks, the highest r^2, of 0.97, occurring in spring, and the lowest, of 0.83, in summer (Figure 4).

The temperature range of 22 to 30 °C during the spring and summer and the higher precipitation in June, July, August, and September resulted in 70 % of the total accumulated precipitation in the experimental period, contributing to the greater growth of the plant, which profited from the biochemical and photosynthesis processes for its optimal development. However, the conditions were not favorable in the fall and winter, when the low temperatures ranged from -2 to 11 °C, causing a reduction of the tissue turnover in winter and thereby affecting the growth and development of the plant, which exhibited the lowest LAI and the lowest yield of accumulated biomass during this season.

In a trial to assess white clover[9], the highest leaf area index, of 3.0, was observed in the eighth week of spring; later, in the fifth week of summer, it was 1.7, and in the eighth week of autumn and of winter, it had a value of 1.4 and 1.6, respectively —results that agree with those of this research. In a study directed by Zaragoza *et al*[1], they reported the highest LAI ($P<0.05$) for the alfalfa crop in week five of spring (3.5), of summer (2.8), and of autumn (2.0), and in the sixth week of winter(1.9). However, the values were different when evaluating the orchard grass, since the highest LAI ($P<0.05$) occurred in week six of regrowth in spring(2.3), summer(1.4) and autumn (1.1), and in the seventh week of winter (1.0). Other researches on alfalfa[23,24] reported behaviors similar to those observed in this experiment, since the highest values of LAI ($P<0.05$), of 3.3 and 4.9, respectively, were recorded in spring-summer.

The LAI varies for each crop and depends on the environmental conditions present. Matthew *et al*[14] points out that the LAI is optimal when the net forage production is at a maximum point and the highest LAI is reached simultaneously; however, the LAI can be affected indirectly by low temperatures, according to the type of crop and the time of sampling.

Conclusions and implications

The accumulated biomass yield was higher in the spring and lower in autumn and winter. As the regrowth age increased, so did the dry matter. It is recommend profiting from the defoliation of the meadow in the sixth week of spring and summer and the seventh week of autumn and winter, based on the fact that an adequate dry matter yield, greater leaf component, and less dead material were obtained at those times, consequently optimizing the forage nutrients.

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Figure 2: White clover growth curves by season and by morphological component during a growth cycle of 8 and 9 weeks

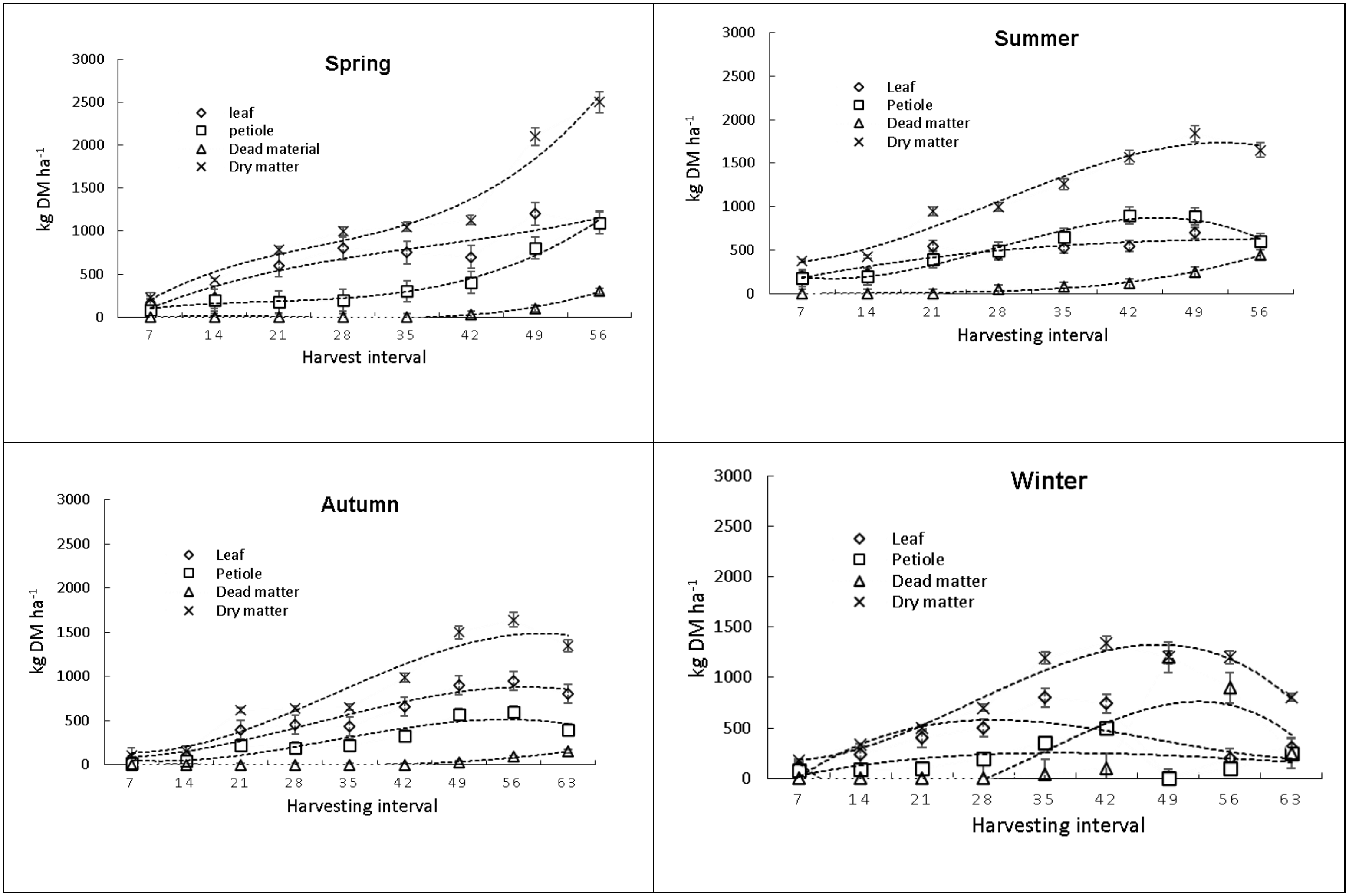


Figure 3: Botanical and morphological composition of white clover during an 8 and 9 week growth cycle. mm= dead material

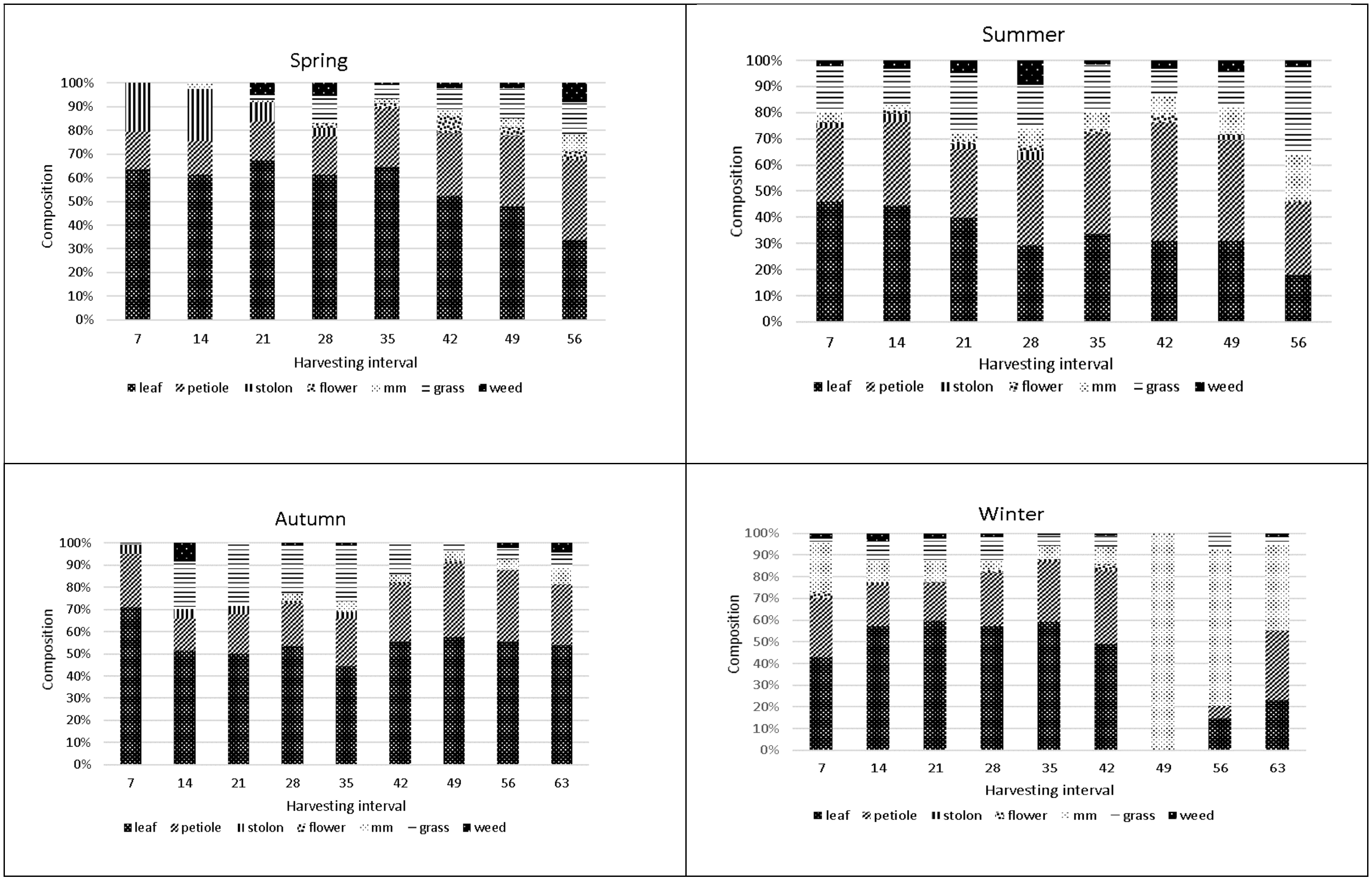
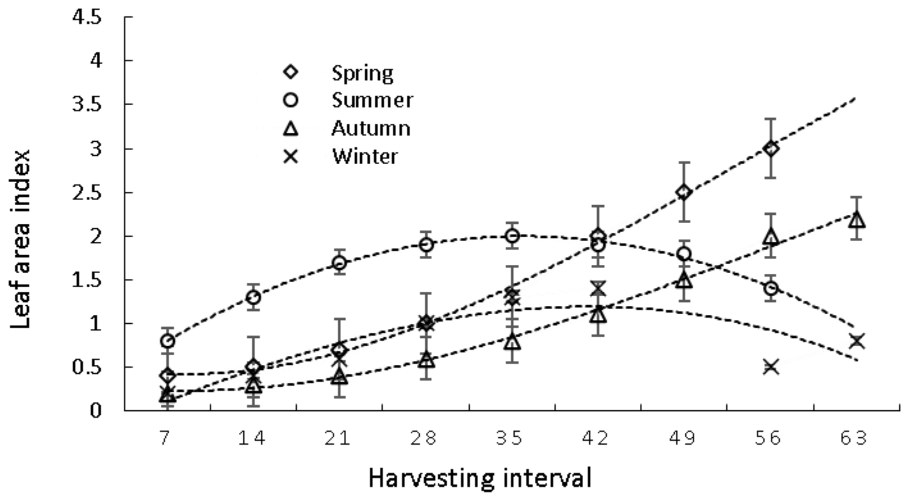


Figure 4: Leaf area index of white clover during a growing season



Spring= 4.67/(1+0.11*exp(-0.0619t))
r= 0.97
Summer= 0.03+0.11t-0.0015^2 r= 0.83

Autumn= 3.13/(1+26.85*exp(-0.0666t))
r= 0.98
Winter= 1.70/(1+18.91*exp(-0.1142t)) r= 0.96