## White clover (*Trifolium repens*) population dynamics are partly dependent on timing of seminal taproot death

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#### **Abstract**

The expanded usage of white clover has increased the importance of understanding white clover dynamics. It is assumed that clover plants have a higher tolerance to moisture and nutrient deficiencies when the taproot is still present. Therefore, the survival of the seminal taproot can influence the dynamics of clover. There is no direct evidence whether increased survival of the taproot results in increased performance of white clover. In this study, we aimed to: (1) assess the relationship between taproot volume and taproot survival; and (2) whether the timing of death of the seminal taproot influences the population dynamics of white clover varieties. In a two-year field experiment with 18 white clover varieties grown in monoculture and in mixture with *Lolium perenne* L, the taproot characteristics and population dynamics were studied. It was shown that taproot volume was positively correlated to both leaf size and taproot presence during autumn 2017. The combination of the timing of death of the seminal tap root and the development of stolons seems to play a more important role in increasing the persistence of white clover than the survival of the seminal taproot. Future research should focus on understanding the transition from a taprooted white clover to a stolonous white clover plant.

Keywords: nodal roots, breeding, morphology, stolons, regenerative agriculture, organic agriculture

#### Introduction

An issue concerning the use of white clover is the persistence of white clover over years in grass-clover pastures, which influences the stability of dry matter production and feeding value of grass-clover. Reasons for reduced persistence of white clover include diseases, pests and drought stress, possibly related to the survival of the taproot (Nichols *et al.*, 2015). A possible change in the root morphology of white clover could be the increase in lifetime of the seminal taproot. A major decline in clover content has been observed after the death of the seminal taproot (Nichols *et al.*, 2015; Westbrooks and Tesar, 1955). Death of the taproot usually occurs between twelve and eighteen months after plant formation, but the variation is large, ranging from six months to over 24 months (Brock and Tilbrook, 2000; Nichols *et al.*, 2015). Therefore, in this study we investigated the relationship between taproot characteristics of white clover and the persistence of the taproot in 18 white clover varieties and related this to the population dynamics of white clover in the period during which the taproot disappears. We hypothesized that: (1) a higher taproot volume will increase the lifespan of the seminal taproot; and (2) the timing of death of the seminal taproot has an effect on the short-term population dynamics and performance of white clover varieties.

#### Materials and methods

In an experiment, which was part of the Dutch VCU (Value for Cultivation and Use) testing programme, 18 white clover varieties (*Trifolium repens* L.) were tested in a randomized block design with four replicates. The different clover varieties were sown both in monoculture and in mixture with perennial ryegrass (*Lolium perenne*, varieties Sputnik and Dromara (50/50)). In these plots, clover cover was determined according to VCU protocol, leaf size of white clover was measured, presence of taproot was determined at four different times and size of taproot was determined during first taproot presence measurement. On each monoculture plot, a clod of 20×20 cm square and 25 cm depth was harvested

in a place where white clover was abundantly present. Taproots were measured at base, 1 cm below base and 10 cm below base. Taproot volume was determined through these three measurements. Correlation coefficients between variables were based on Pearson's R test. Data were checked for normality and log-transformed if required. The effect of white clover variety and leaf size group was tested in separate Anovas. Post hoc analysis was done using Fishers test of least significant difference for varieties and Tukey's test for honest significant difference for differences between leaf size groups.

#### Results and discussion

Taproot survival showed a gradual decline from an average of 85% in spring 2017 to 31% in autumn 2018 (Figure 1). The survival of the taproot measured during autumn 2017 significantly positively correlated with taproot volume (P<0.05), average clover cover over all years (P<0.01, Table 1), leaf size (P<0.001), average clover cover in 2017 (P<0.01) and average clover cover in 2018 (P<0.05). A significant correlation was observed between the taproot volume and the survival of the taproot in autumn 2017 (P<0.05, Table 1) but not with the survival of the taproot in spring 2017, spring 2018 and autumn 2018. Previous research and breeding efforts have focussed on a longer lifespan of the seminal taproot (Caradus and Woodfield, 1998). It has, however, remained unclear to what extent the longer lifespan and timing of death of the seminal taproot has in terms of effect on white clover performance in pastures. The first hypothesis of this research was that an increase in taproot volume would increase the survival of the taproot in white clover. However, despite the large range of taproot sizes in our experiment, we only measured a significant positive correlation of taproot volume with taproot survival in the autumn of 2017, but not in the other periods. Therefore we cannot conclude that an increase in taproot volume increased the survival of the taproot over the measured years. The second hypothesis of this research was that the timing of death of the taproot would have an effect on the persistence of white clover. At the end of the experiment in September 2018 we did not find a relation between taproot survival and the persistence of white clover in the monoculture and mixture. However, we did find a positive correlation between the survival of the seminal taproot in autumn 2017 and the white clover cover in 2018. A cause that has been mentioned for the decline of white clover is its winter survival.

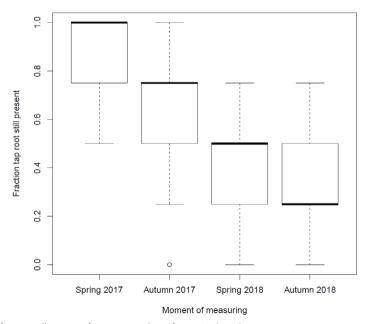


Figure 1. Fraction of taproot still present at four measuring dates after sowing (n=18).

Table 1. Correlation table of taproot volume, leaf size and taproot survival with taproot volume, leaf size and clover cover. 1

Correlation matrix	Taproot volume (cm <sup>3</sup> )	Leaf size (cm <sup>2</sup> )	Plant density	Average clover cover mono (%)	Average clover cover mono 2017 (%)	Average clover cover mono 2018 (%)
Taproot volume (cm <sup>3</sup> )		0.27*	-0.35**	0.22	0.19	0.17
Leaf size (cm <sup>2</sup> )	0.27*		-0.04	0.28*	0.35	0.08
Plant density (# plants m <sup>2</sup> )	-0.35**	-0.04		-0.13	-0.14	-0.06
TR survival spring 17	-0.02	0.11	0.04	0.10	0.11	0.05
TR survival autumn 17	0.29*	0.43***	-0.25*	0.38**	0.36**	0.26*
TR survival spring 18	-0.21	-0.21	-0.10	-0.17	-0.13	-0.16
TR survival autumn 18	-0.06	0.16	0.07	0.00	-0.08	0.09

<sup>1</sup>P-values: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. Taproot volume and plant density were measured in spring 2018, leaf size was measured autumn 2018. TR = Taproot, TR survival is fraction.

#### Conclusions

We found a positive correlation between leaf size and taproot volume. However, no correlations were found between taproot characteristics and taproot survival. We found indications that the timing of death of the seminal taproot can contribute to the winter hardiness of these varieties, possibly resulting in a higher persistence over the years. The combination of the timing of death of the seminal tap root and the development of stolons seems to play a more important role in increasing the persistence of white clover than the absolute survival of the seminal taproot. Future research should focus on understanding the transition from a taprooted white clover to a stoloniferous white clover plant in relation to specific weather events such as winter frost conditions.

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