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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 in pastures. It is assumed that clover plants have a higher tolerance for moisture and nutrient deficiencies when the taproot is still present. Therefore, the survival of the seminal taproot can influence the dynamics of clover. Past breeding efforts in countries like New Zealand have focussed on increasing the taproot longevity through hybridisation with a close relative of white clover. However, there is no direct evidence whether increased survival of the taproot results in increased performance of white clover. In this study, we aimed to (i) assess the relationship between taproot volume and taproot survival, and (ii) 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, 1 year after sowing. 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 stolonous white clover plant in relation to specific weather events such as winter frost conditions.

KEYWORDS

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

1 | INTRODUCTION

Worldwide, the dairy sector is confronted with environmental challenges concerning water pollution (Leip et al., 2015), greenhouse gas emissions (Lesschen et al., 2011) and loss of biodiversity (Erisman et al., 2016). Furthermore, the use of artificial N fertilizers is linked with a high fossil energy usage and consequently a high level of greenhouse gas emissions (Wood & Cowie, 2004). As a result, forage legumes with their ability for symbiotic N_2 fixation play an increasingly important role in grassland forage production on conventional, nature inclusive, regenerative and organic dairy farms (Erisman et al., 2016; Gierus et al., 2012; Halling et al., 2004; Hejduk & Knot, 2010; Hoekstra et al., 2018; Lüscher et al., 2014; Mela, 2003). White clover (*Trifolium repens* L.) is an important legume on dairy farms because of its better resistance to grazing compared to other legumes such as red clover and alfalfa (Annicchiarico et al., 2015; Rochon et al., 2004; Weller & Bowling, 2007). Next to symbiotic nitrogen fixation, forage legumes stimulate aboveground and belowground biodiversity in grasslands (De Haas et al., 2019; Power & Stout, 2011; Van Eekeren et al., 2009).

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 (Loiseau et al., 2001; Schwinning & Parsons, 1996). Renewing grass-clover pastures when the clover content is low is a direct effect of poor persistence of white clover. This is unwanted because of costs for seeding, loss of soil organic matter, increase of greenhouse gas emissions and loss of soil biodiversity (Iepema et al., 2020; Van Eekeren et al., 2008). Re-introduction of white clover trough overseeding is a possible solution, but hard to achieve in practice (Wedderburn et al., 1996). Possible reasons for reduced persistence of white clover are diseases, pests and drought stress (Archer & Robinson, 1989; Gibson & Trautner, 1965; Woodfield & Caradus, 1987), possibly related to the survival of the taproot (Bonesmo & Bakken, 2005; Brock et al., 2000; Nichols et al., 2015). Woodfield et al. (1996) stated that breeding for white clover persistence should focus on developing varieties with resistance to pests like nematodes, and a root morphology which increases the persistence under moisture deficits.

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 & Tesar, 1955). Death of the taproot usually occurs between 12 and 18 months after plant formation, but the variation is large, ranging from 6 months to over 24 months (Nichols et al., 2015). The same author also stated that death of the taproot is pivotal in the decline of white clover. Death of the seminal taproot causes the plant to rely solely on the nodal roots. The smaller and more shallow nodal roots are less able to exploit the soil for water and nutrients, therefore they make white clover more vulnerable to moisture and nutrient stress and invasion by pathogens (Bonesmo & Bakken, 2005; Brock, 1988; Jones, 1980; Nichols et al., 2015; Timmermans & van Eekeren, 2016). The transition phase from a taprooted plant to a plant relying on nodal roots is crucial in the development of white clover (Brock et al., 2000; Brock & Tilbrook, 2000). It is generally acknowledged that the death of the seminal taproot is the start of the transition phase and is unavoidable. However, as variation in death of the seminal taproot is large, timing of the death can have a major influence on the survival of white clover varieties in this phase (Brock & Tilbrook, 2000). Over the past decades white clover varieties have shifted from smaller leaf varieties to larger leaf varieties, as larger leaved varieties are more erect and thus better at competing with ryegrass (Caradus et al., 1995). This could have increased the size of the taproot unintendedly, as leaf size and taproot size are strongly correlated (Caradus & Woodfield, 1998). This increase in taproot size could have positively affected the survival of the seminal taproot. However, there is no research on the relationship between taproot characteristics, timing of taproot death and population dynamics of white clover varieties.

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 (i) a higher taproot volume will increase the lifespan of the seminal taproot, and (ii) the timing of death of the seminal taproot has an effect on the short term population dynamics and performance of white clover varieties.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

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)). Of the 18 white clover varieties tested, four were previously tested white clover varieties and 14 were newly developed varieties. These varieties are part of breeding lines of breeders and are therefore analysed anonymously. The experiment was established on a sandy soil (Typic Haploquod, USDA) located on a dairy farm in Ysselsteyn, Limburg, The Netherlands (51°27'33 N, 5°53'47 E). The mean annual temperature at a nearby weather station in Volkel was 11.5°C, and the mean yearly precipitation was 676 mm between September 2016 and September 2018. Although the yearly precipitation of 2018 was around average, the growing season between April and September of 2018 was extremely dry (Figures 1 and 2). The cropping history of the experimental field was 10 years pasture (without clover), after which early potatoes were cultivated in 2016. After the potatoes were harvested the experimental field was established on August 5, 2016. The monocultures of white clover were sown in plots of 2 × 2 m at a seeding rate of 10 kg ha^{-1} and the mixtures of white clover with perennial ryegrass were sown in plots of 3 × 12 m at a seeding rate of 10 kg ha^{-1} for white clover and 27.5 kg ha^{-1} for perennial ryegrass. In 2017 and 2018, the field was fertilized with 25 m^3 cattle slurry and 80 kg N per hectare in the form of Calcium Ammonium Nitrate (CAN) before the first cut and 15 m^3 cattle slurry per hectare before the fourth cut. In 2017 and 2018, the first cut was mown, the second and third were grazed by dairy cows and the fourth was mown, in 2017 the fifth cut was also mown.

2.2 | Measurements

2.2.1 | Clover leaf size

A total of 10 leaf samples were collected in August 2018 in each monoculture plot and stored in air tight bags at 7 °C. The top fully developed

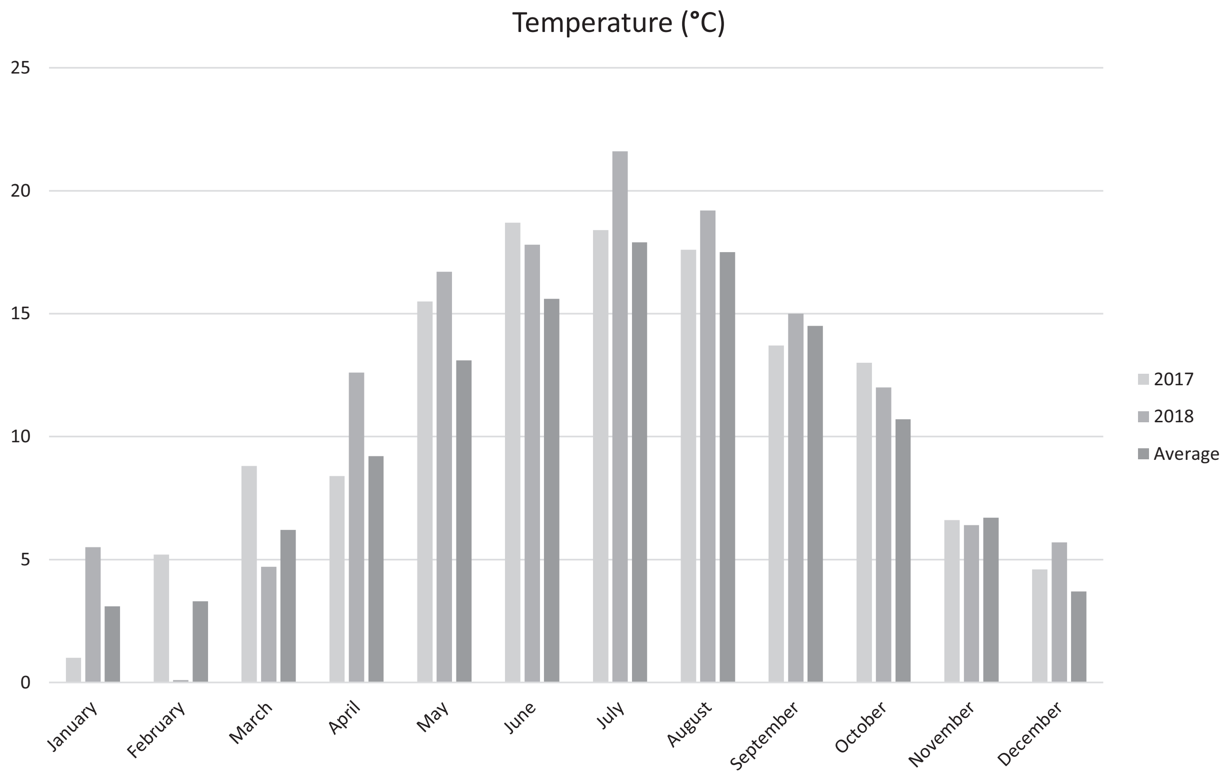

FIGURE 1 Average monthly temperature of years 2017 and 2018 displayed alongside long term average (1953–2020)

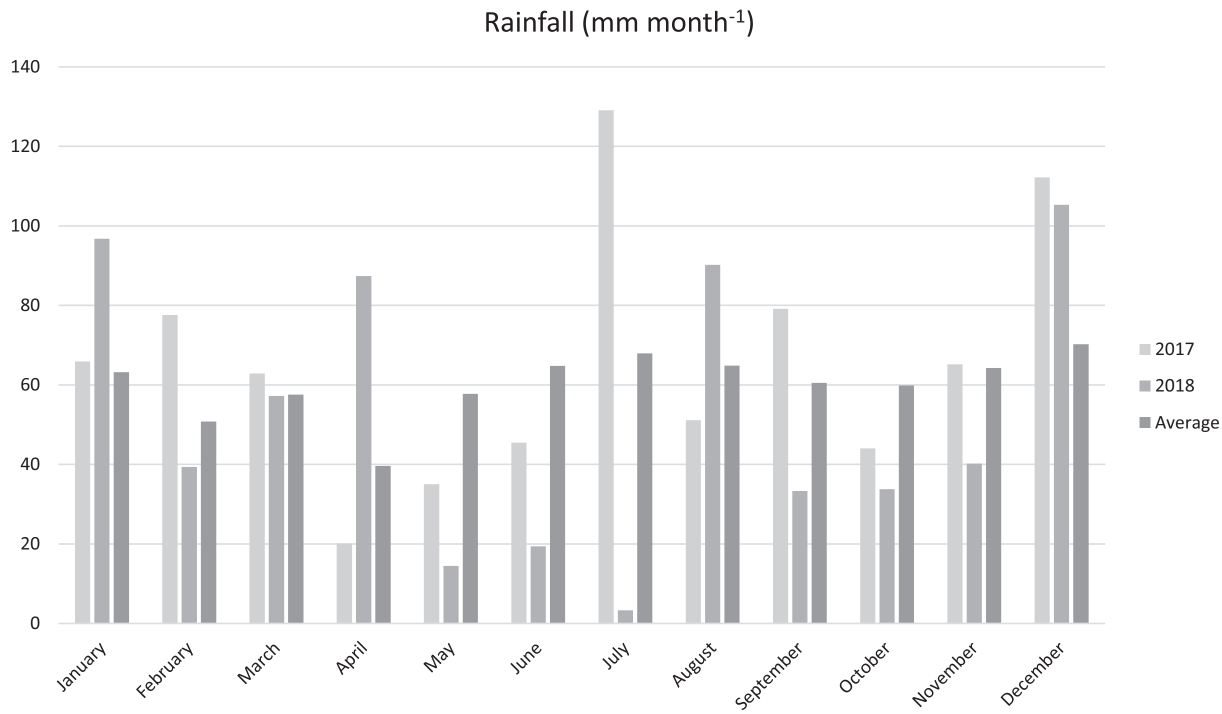

FIGURE 2 Average monthly rainfall of years 2017 and 2018 displayed alongside long term average (1974–2020)

leaf of 10 randomly selected plants was collected. The samples were subsequently scanned the day after collection for leaf size with the Petiole leaf size application (https://petioleapp.com). This application measures leaf size with an accuracy of 0.1 cm^2. Based on the measured leaf sizes, four leaf size groups were defined, small (<1.40 cm^2), medium (1.40–1.55 cm^2), large (1.55–1.70 cm^2) and very large (>1.70 cm^2).

2.2.2 | White clover cover and white clover content

The white clover cover in each monoculture plot was scored based on the percentage ground cover, while the white clover content in the mixture was done on basis of estimating the content in biomass. The measurements were executed by an expert based on the protocol of the Dutch VCU testing programme (VCU testing protocol forage grasses ("Gewasprotocollen CGO," 2021)). Each year clover cover and clover content of percentage was estimated three times during the growing season (April – October). Once in spring, once in summer and once in autumn. Yearly averages are the average of these three values.

2.2.3 | Dry matter production

Before the grass clover mixtures were mown or grazed by dairy cows, the dry matter production was measured with a Haldrup field harvesting machine. In 2017, five cuts were measured and in 2018, four cuts were measured. A surface of 1 × 4.5 m was mown at 6 cm height and fresh weight was determined. In a subsample, 1 kg fresh matter, the dry matter content was determined by drying the samples in an oven for 24 h at 104°C. Dry matter production was not determined in the clover monocultures.

2.2.4 | Taproot characteristics

Taproot survival was sampled at 9, 15, 21 and 27 months after seeding. On each monoculture plot, a clod of 20 × 20 cm square and 25 cm depth was harvested in a place were white clover was abundantly present. A scoring system was in place where 0 indicated the absence of a taproot and 1 indicated the presence of a taproot in each clod. Additionally, at the first sampling (9 months after sowing), the taproots were subjected to additional measurements. Per clod between three and nine taproots were collected. Taproot diameter was measured with a digital calliper (accuracy 0.01 mm) at the base of the taproot, 1 cm below the base and 10 cm below the base. Taproot length was measured with a thumb stick (accuracy 1 mm). Average values per clod were used. On basis of the above measured characteristics the volume of the taproot was calculated using the following equation (3 truncated cones):

$$\text{Volume} = \left(1/3\pi x\left(A^2+AB+B^2\right)\right)+\left(1/3\pi y\left(B^2+BC+C^2\right)\right)+\left(1/3\pi z\left(C^2+CD+D^2\right)\right)$$

In which: A = Diameter of the taproot at the base

B = Diameter of the taproot 1 cm below the base

C = Diameter of the taproot 10 cm below the base

D = Diameter of the taproot at the end of the taproot (0.1 mm)

x = Distance between base of the taproot and 1 cm below the base

y = Distance between 1 cm below the base of the taproot and 10 cm below the base

z = Distance between 10 cm below the base of the taproot and the end of the taproot

2.3 | Statistical analyses

All data were analysed using R version 3.5.2 (R Core Team 2017). 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

TABLE 1 Leaf size and clover cover (%) of the white clover varieties measured in the monocultures ($n = 4$)

		Clover cover (%)		
Variety	Leaf size (cm^2)	2017	2018	Average
1	1.69^{bcd}	43.8^{defg}	60.6^{abcd}	50.5^{bcde}
2	1.36^{cd}	39.6^{fg}	36.3^{ef}	38.3^{ef}
3	1.68^{bcd}	54.6^{bcd}	73.8^{a}	62.3^{ab}
4	1.25^{d}	52.5^{bcdef}	58.1^{abcd}	54.8^{bcd}
5	2.56^{a}	68.3^{a}	48.1^{de}	60.3^{abc}
6	1.83^{bc}	58.8^{abc}	70.0^{ab}	63.3^{ab}
7	1.51^{bcd}	36.3^{g}	23.1^{f}	31.0^{f}
8	1.45^{bcd}	46.3^{cdefg}	51.9^{bcde}	48.5^{cde}
9	1.69^{bcd}	57.1^{abcd}	58.8^{abcd}	57.8^{abc}
10	1.45^{bcd}	58.3^{abc}	61.9^{abcd}	59.8^{abc}
11	1.39^{bcd}	53.3^{bcde}	60.0^{abcd}	56.0^{abc}
12	1.87^{b}	57.9^{abc}	68.8^{abc}	62.3^{ab}
13	1.62^{bcd}	46.7^{cdefg}	50.6^{cde}	48.3^{cde}
14	1.61^{bcd}	70.4^{a}	47.5^{de}	61.3^{abc}
15	1.33^{d}	48.8^{cdefg}	46.9^{de}	48.0^{cde}
16	1.39^{bcd}	40.4^{efg}	45.0^{de}	42.3^{def}
17	1.65^{bcd}	65.4^{ab}	73.5^{a}	68.7^{a}
18	1.37^{cd}	70.4^{a}	25.6^{f}	52.5^{bcd}
Average	1.59	53.8	53.4	53.6
p	**	***	***	***
LSD	0.49	13.6	13.6	18.2
Leaf size group				
Small		50.8^{ab}	45.3^{b}	48.7^{bc}
Medium		46.9^{b}	45.6^{b}	46.4^{c}
Large		56.3^{ab}	60.8^{ab}	58.1^{ab}
Very large		61.7^{a}	62.3^{a}	61.9^{a}
p		*	**	***
CV		23.5	31.6	21.3

Note: Values within columns followed by the same letters are not significantly different ($p > 0.05$).
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

significant difference for differences between leaf size groups. Correlation coefficients between variables were based on Pearson's R test.

2.4 | Definitions

White clover population dynamics is defined as the dynamics between clover and bare soil in monoculture and the dynamics between white clover and perennial ryegrass in mixtures.

Persistence of white clover is defined as the ability of a variety to maintain an amount of clover in monocultures and in mixtures over time. White clover is known to decline over time, with its eventual disappearance as a result. A white clover variety that is persistent has the ability to regenerate itself in pastures and therefore does not disappear.

3 | RESULTS

3.1 | White clover leaf size, clover and dry matter production

The leaf size, cover and dry matter production of white clover were all tested to determine whether they were affected by variety. Significant differences in leaf size were observed between varieties ($p < .01$) and ranged from 1.25 to 2.56 cm^2 (Table 1).

The average clover cover in the white clover monocultures was 53.8% in 2017 and 53.4% in 2018 (Table 1). The clover cover was significantly affected by variety ($p < .001$) in both years. The average clover cover was also significantly affected by leaf size group ($p < .001$). The groups of large and very large had a significantly higher clover cover than the medium and small sized leaf groups. The clover cover was significantly affected by leaf size group in 2017 ($p < .05$) and in

TABLE 2 Clover content (%) in the mixtures of white clover and the dry matter production of the mixtures ($n = 4$)

	Clover content mixture			Dry matter production mixture (kg DM ha^{-1})	
Variety	2017	2018	Average	2017	2018
1	13.6^{abcd}	1.3	7.4^{bcdef}	9148	3276^{e}
2	6.6^{e}	0.8	3.7^{f}	10,215	3296^{e}
3	16.8^{ab}	2.5	9.6^{abc}	10,116	3844^{cde}
4	11.5^{bcde}	1.8	6.6^{bcdef}	9666	4626^{abcd}
5	18.8^{a}	6.8	12.8^{a}	10,312	4335^{abcd}
6	13.7^{abcd}	1.6	7.6^{bcdef}	9124	4108^{bcde}
7	7.3^{de}	0.6	3.9^{ef}	9297	4969^{ab}
8	11.3^{bcde}	0.4	5.9^{cdef}	9948	4400^{abcd}
9	14.8^{abc}	1.3	8.0^{bcde}	10,318	4070^{cde}
10	12.8^{abcde}	1.6	7.2^{bcdef}	9418	4710^{abc}
11	15.8^{ab}	2.4	9.1^{abcd}	9632	4968^{ab}
12	11.3^{bcde}	1.1	6.2^{cdef}	9459	4427^{abcd}
13	11.0^{bcde}	0.6	5.8^{cdef}	9134	4559^{abcd}
14	15.8^{ab}	1.9	8.9^{abcd}	10,001	5181^{a}
15	10.8^{bcde}	0.9	5.9^{cdef}	9617	4413^{abcd}
16	13.6^{abcd}	0.4	7.0^{bcdef}	10,015	3768^{de}
17	8.8^{cde}	1.1	5.0^{def}	9511	3880^{cde}
18	18.9^{a}	2.9	10.9^{ab}	9854	4115^{bcde}
Average	13.0	1.7	7.3	9710	4275
p	*		**		*
LSD	6.80		4.29		876
Leaf size group					
Small	12.9	1.5	7.1	9833	4198
Medium	10.4	0.9	5.6	9554	4693
Large	13.5	1.4	7.5	9705	4135
Very large	14.6	3.2	8.9	9631	4290
p	NS	NS	NS	NS	NS
CV	41.6	141.8	47.1	7.6	14.5

Note: Values within columns followed by the same letters are not significantly different ($p > 0.05$).
*$p < 0.05$. **$p < 0.01$.

2018 ($p < .01$). The very large leaf size group showed the highest clover cover in both years.

The average clover content in the mixtures was 13.0% in 2017 and 1.7% in 2018 (Table 2). White clover content was significantly affected by variety in 2017 ($p < .05$). In 2018 no significant effect of varieties on clover content was observed.

In 2017, the average dry matter production of the grass-clover mixtures was 9710 kg dry matter ha^{-1} (range 9124–10,318 kg ha^{-1}, Table 2) and was not significantly affected by variety or leaf size group. In 2018, the average dry matter production was 4275 kg ha^{-1} (range 3276–5181 kg ha^{-1}) and was significantly ($p < .05$) affected by variety but not by leaf size group.

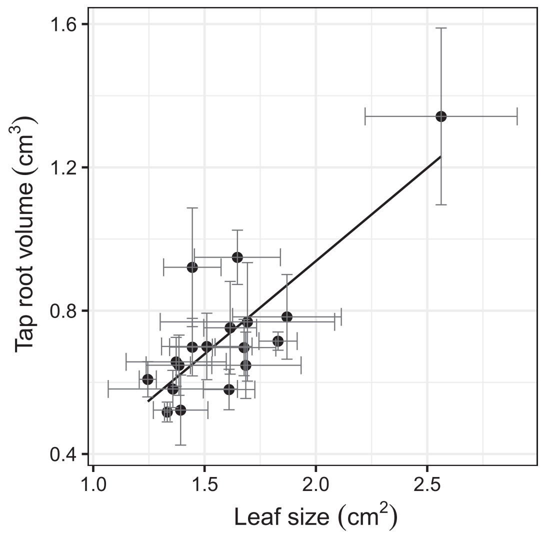

FIGURE 3 Correlation between leaf size and average volume of the taproot of the 18 white clover varieties (r 0.27; $p < .05$). Error bars = standard error, $n = 4$

3.2 | Taproot characteristics and plant density

The average diameter of the taproot at the base was 2.66 mm (Table 3). This characteristic was significantly affected by variety and by leaf size groups ($p < .001$).

TABLE 3 Taproot characteristics of different white clover varieties and leaf size groups in monoculture ($n = 4$)

Variety	Taproot diameter at base (mm)	Taproot diameter at 1 cm below base (mm)	Taproot diameter at 10 cm below base (mm)	Taproot length (cm)	Taproot volume (cm^3)	Plant density (plants m^{-2})
1	2.61	1.80	0.70	17.5	0.77	150
2	2.41	1.72	0.58	20.0	0.58	163
3	2.39	1.88	0.74	20.8	0.70	175
4	2.50	1.83	0.54	22.1	0.61	169
5	3.61	2.69	0.86	18.9	1.34	169
6	2.61	1.96	0.63	21.0	0.72	206
7	2.58	1.87	0.65	20.9	0.70	144
8	2.59	1.95	0.63	23.1	0.70	156
9	2.46	1.80	0.67	21.5	0.65	169
10	2.98	2.17	0.73	21.2	0.92	125
11	2.25	1.66	0.55	19.0	0.52	163
12	2.84	2.07	0.70	21.8	0.78	169
13	2.60	1.94	0.69	23.2	0.75	188
14	2.44	1.72	0.63	20.0	0.58	156
15	2.32	1.69	0.55	19.9	0.52	156
16	2.40	1.83	0.64	20.6	0.65	200
17	2.97	2.26	0.75	21.8	0.95	169
18	2.54	1.87	0.63	20.8	0.66	188
Average	2.66	1.94	0.66	20.7	0.73	167
p	*	NS	NS	NS	NS	NS
Leave size group						
Small	2.40^{b}	1.77^{b}	0.58^{b}	20.4	0.59^{b}	173
Medium	2.72^{ab}	1.99^{ab}	0.67^{ab}	21.5	0.77^{ab}	142
Large	2.58^{b}	1.93^{b}	0.70^{a}	20.7	0.73^{ab}	168
Very Large	3.02^{a}	2.24^{a}	0.73^{a}	20.4	0.95^{a}	181
p	***	**	***	NS	**	.
CV	14.9	16.6	15.7	13.2	33.6	24.0

Note: Values within columns followed by the same letters are not significantly different ($p > 0.05$).
*$p < 0.05$. $p < 0.1$. **$p < 0.01$. ***$p < 0.001$.

The average diameter at 1 cm below the base was 1.94 mm. The diameter at 1 cm below the base was not significantly affected by variety but there was a significant effect of leaf size group ($p < .01$). The group with a very large leaf size was significantly different from the groups with large and small leaf sizes.

An average diameter of 0.66 mm was measured at 10 cm below the base of the taproot. This diameter was not significantly affected by variety, but there was a significant effect of leaf size group. The group with a small leaf size was significantly lower compared to the three other groups ($p < .001$).

The taproot volume was not significantly affected by variety. When comparing leaf size groups for taproot volume a significant effect was found ($p < .01$). The small leaf size group had a smaller taproot volume compared to all other groups and the large leaf size group had a smaller taproot volume compared to the very large leaf size group. Taproot volume was significantly correlated with leaf size ($r = 0.27$; $p < .05$, Figure 3).

The plant density was not significantly affected by neither variety or leaf size group. It was however negatively correlated to ($p < .01$) taproot volume.

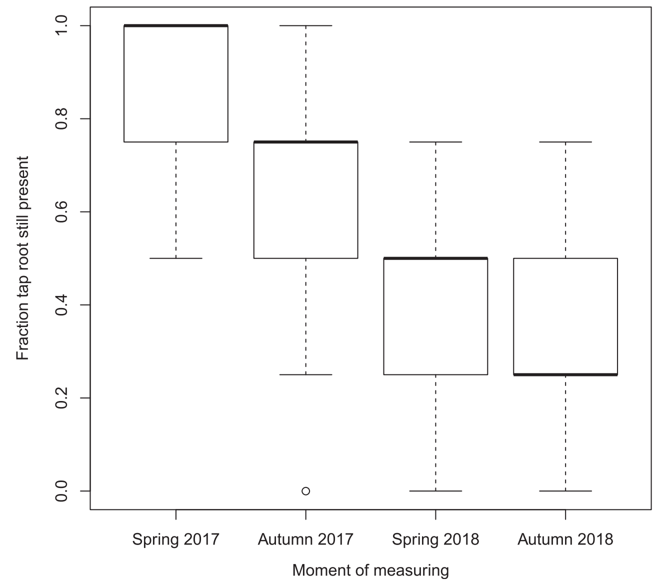

FIGURE 5 Fraction of taproot still present at four measuring dates after sowing. $n = 18$

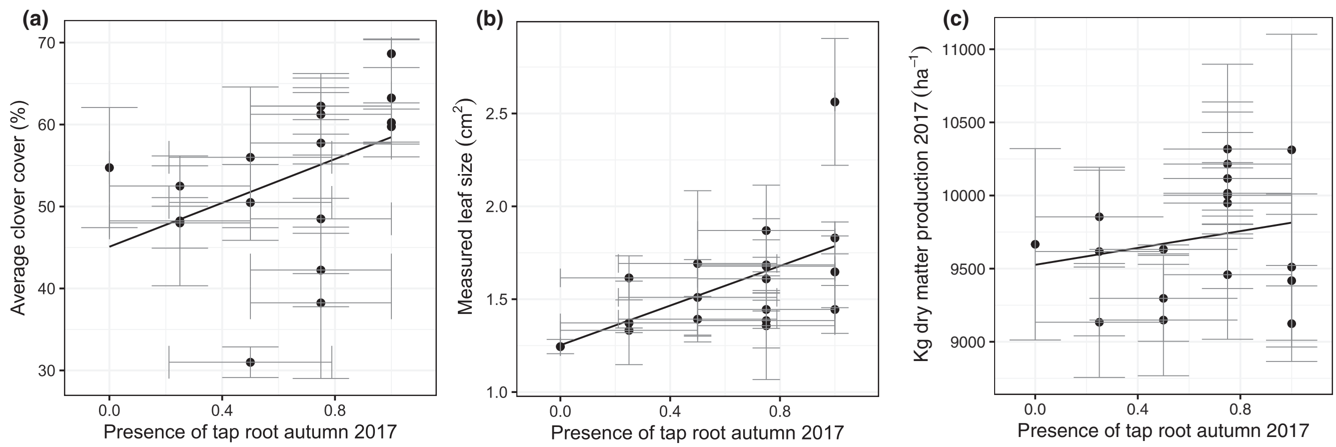

FIGURE 4 Correlation figures showing the effect of average presence of taproot per variety in the autumn of 2017 on (a) the average clover content (2017–2018) ($R = 0.38$, $p < .01$), (b) the leaf size ($R = 0.43$, $p < .001$) and (c) dry matter production in 2017 ($R = 0.26$, $p < .05$). (0: Taproot absent, 1: Taproot present) error bars = standard error, $n = 4$

TABLE 4 Correlation table of taproot volume, leaf size and taproot survival with taproot volume, leaf size and clover cover

Correlation matrix	Taproot volume	Leaf size	Plant density	Average clover cover mono	Average clover cover mono 2017	Average clover cover mono 2018
Taproot volume		**0.27***	**−0.35****	0.22	0.19	0.17
Leaf size	**0.27***		−0.04	**0.28***	0.35	0.08
Plant density	**−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

Note: Significant correlations written in bold. Taproot volume and plant density were measured in spring 2018, leaf size was measured autumn 2018.
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

3.3 | Taproot survival

Taproot survival showed a gradual decline from on average 85% in spring 2017 to 31% in autumn 2018 (Figure 5). The survival of the taproot measured during autumn 2017 significantly positively correlated with taproot volume ($p < .05$), average clover cover over all years ($p < .01$, Figure 4a), leaf size ($p < .001$, Figure 4b), average clover cover in 2017 ($p < .01$), average clover cover in 2018 ($p < .05$) and dry matter production 2017 ($p < .05$, Figure 4c) (Table 4). A significant correlation was observed between the taproot volume and the survival of the taproot in autumn 2017 ($p < .05$, Table 4) but not with the survival of the taproot in spring 2017, spring 2018 and autumn 2018.

4 | DISCUSSION

Understanding white clover dynamics is essential for maintaining a high production of permanent grass-white clover mixtures on dairy farms (Caradus & Williams, 1989). The disappearance of white clover after a certain period of time has been a key reason for dairy farmers to renew their pastures Previous research and breeding efforts have focussed on a longer lifespan of the seminal taproot (Caradus & Woodfield, 1998). It has however remained unclear to what extend the longer lifespan and timing of death of the seminal taproot has an effect on white clover performance in pastures. Through researching 18 varieties, the goal was to elucidate the role of the survival of the seminal taproot in the performance of white clover in pastures.

4.1 | Dry matter production

In 2017 the dry matter production of the grass clover mixture ranged between 9124 and 10,318 kg DM ha^{-1} . In general, dry matter production of grass in the Dutch VCU testing program was between 9000 and 15,000 kg DM ha^{-1} $year^{-1}$ (Schils et al., 2020). Therefore the yield in 2017 can be considered in line with average yields. In 2018 the DM productions in the mixture was very low (range 3276–5181 kg dry matter ha^{-1}) due to a severe drought during the summer of 2018 ("KNMI," 2018).

4.2 | Correlation between leaf size and root traits

We found a positive correlation between leave size and taproot volume. The volume of the taproot and the leaf size are known to be correlated (Caradus & Woodfield, 1998; Woodfield & Caradus, 1990). Leaf size also correlates with plant dry weight, but it is not known whether this is a result or a cause of a higher plant dry weight (Beinhart, 1963; Elgersma et al., 1998). The source of the higher plant weight could be found in genetics or in environmental factors. White clover plants are selected for large leaf size because it is known to be correlated with a high yield (Rhodes & Webb, 1993). Therefore, selection for higher plant yield could have had the unintended effect of selection for varieties with a larger taproot. Plant and root traits at the individual level are strongly correlated with stand density. However, due to the limitations of this study we were not able to untangle those effects. This would be an interesting aspect in future studies. A focus on individual root traits also might not be an effective breeding strategy as these are strongly entangled with plant density. Therefore, these could be considered at the stand level.

4.3 | Taproot volume and survival of taproot

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. In New Zealand, breeding efforts of hybridisation of white clover and *Trifolium uniflorum*, which has a thicker taproot, have focussed on increasing the taproot diameter and structure to elongate the lifespan of the seminal taproot (Hussain et al., 2017; Nichols et al., 2015; Nichols et al., 2016; Nichols, Hofmann, & Williams, 2014; Nichols, Hofmann, Williams, & Crush, 2014). In this work, a hybrid of white clover with *T. uniflorum* showed increased taproot survival but no significant difference between tap root sizes compared to white clover.

4.4 | Taproot survival and short term white clover population dynamics

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. This despite the severe drought in the summer of 2018, which could have exacerbated the potential benefit of taproot survival. 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 (Collins et al., 1991; Harris et al., 1983; Sanderson et al., 2003; Wachendorf et al., 2001). One important factor that determines the overwintering of white clover is the amount of carbohydrates in roots and stolons (Collins & Rhodes, 1995). This could be a possible explanation for the significant positive correlation between the taproot survival in the autumn of 2017 and the clover content in 2018. The winter of 2017–2018 had a short extremely cold spell during the ending of February and the beginning of March. This cold spell started at 16 months after the white clover was sown and during this period some varieties had already lost their taproot. However, a correlation between the presence of the taproot in autumn 2017 and the average clover cover of

2017 was also present. So the found correlation could be a leftover effect of white clover varieties which had a high clover content in 2017.

While carbohydrates are a major factor in the overwintering of white clover, there is only research about the role of stolons as a source of carbohydrates of white clover (Collins & Rhodes, 1995). In the situations where the stolons are not fully developed, the survival of the taproot might increase winter persistence of white clover varieties. This is in line with Westbrooks and Tesar (1955), who showed in their research that the supply of total available carbohydrates in the roots were relatively low during the winter of the second season and decreased until spring. However, they also stated that it is not clear whether this was the cause or the result of taproot starvation. Moreover, we have shown that one of the varieties (cv Alice) which does not have the highest winter hardiness but is known for its general persistence in practice (Elgersma & Schlepers, 1997), is one of the varieties which lost its taproot already after 9 months. These results may indicate that breeding programmes should not just focus on taproot persistence, as stated by Nichols et al. (2015), but should focus more on the transition of a taprooted plant to a stolonous rooted plant (Abberton & Marshall, 2005). Bonesmo and Bakken (2005) already identified the importance of the transition phase in the white clover life cycle. The current research shows that the timing of the death of the taproot could play a role in this transition. This is in line with Brock et al. (2000), who stated that breeding programmes should extend their focus from the tap-rooted phase to include the stolonous phase.

5 | 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 stolonous white clover plant in relation to specific weather events such as winter frost conditions.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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