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Weed Suppression Ability and Yield Impact of Living Mulch in Cereal Crops

Roland Gerhards

Department of Weed Science, University of Hohenheim, 70593 Stuttgart, Germany; gerhards@uni-hohenheim.de; Tel.: +49-711-45922399

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Abstract: Intercropping provides several benefits to the agro-ecosystem and plays an important role in Integrated Weed Management (IWM). In this study, we investigated the impact of living mulch in cereal crops on weed density and grain yield. Seven field experiments were conducted in Southwestern Germany. Perennial ryegrass and white clover were sown on the same day as the cereal crop (early) and when cereals had produced 3–5 leaves or the first tillers (late). Average weed density in the control plots without living mulch was 45 weeds m^{-2}. Perennial ryegrass and white clover significantly reduced weed density to 22 plants m^{-2} and 25 plants m^{-2}. Sowing date of living mulch had no effect on weed density. Grain yield was equal in all treatments. The results show that living mulch can suppress weeds without competing with the cereal crop.

Keywords: living mulch; undersown crop; Integrated Weed Management

1. Introduction

Shortstature cultivars of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) are common living mulch species in German cereal production systems. The term living mulch can be used synonymously with the undersown crop. Living mulches are usually sown between rows of cereals and maize. Alternatively, living mulch seed can be broadcast over the soil and incorporated with a harrow. The timing of living mulch sowing is critical. If sowing is too early or if tall-growing species and cultivars such as Italian ryegrass (*Lolium multiflorum* Lam.) and red clover (*Trifolium pratense* Lam.) are used, living mulches may compete with the crop, significantly reducing grain yield [1]. If sowing is too late, the crop may completely suppress the living mulch, thus negating its benefits.

Living mulches provide numerous benefits such as increasing microbial activity in the soil, preventing soil erosion, allowing symbiotic nitrogen fixation (only legumes), conserving nutrients in the top soil layer, increasing biodiversity, and suppressing weeds [2,3]. This approach can play a major role in IWM, because living mulches can suppress weeds over a long period starting in the vegetative growth stages of the cereal crop until sowing of the following crop. The suppressive ability of living mulches against weeds and their impact on cereal grain yield has been investigated in several studies [1,2,4,5]. Weed densities were equal to or up to 55% lower with than without living mulch. Grain yields with living mulch were in the range of 14% less to 22.0% greater compared to the treatments without living mulch [6,7]. Densities and biomass of perennial weed species such as *Elymus repens* (L.) Gould, *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop. were not affected by living mulch [8].

The objectives of this study were to investigate the impact of white clover and perennial ryegrass living mulches on weed density and yield in cereal crops. The following were tested: (1) if white clover and perennial ryegrass planted as living mulches can suppress weeds; (2) if these living mulches reduce crop yield when sown simultaneously with the cereal crop; and (3) if perennial ryegrass is more competitive than white clover.

2. Materials and Methods

Field trials were located in Southwestern Germany near Stuttgart on the Research Station of the University of Hohenheim. Seven experiments were set up in spring wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), spelt wheat (*Triticum spelta* L.) and spring barley (*Hordeum vulgare* L.) from 2009 until 2017. Spring cereals were sown in March. Spelt wheat was sown in the first week of October. The average annual temperature is 9.1 °C and the average annual precipitation 825 mm. Average temperatures were 1.1–1.9 °C greater than this average annual temperature during all six years of study. In 2009 and 2012, it was moist and warm in April and May with favorable growing conditions. However, in 2009 it was very dry in June and July. The growing seasons in 2010, 2011 and 2014 were very dry before and after living mulch sowing. The summers were moist and warm. In 2017, the weather was very cold at time of living mulch sowing. The soil type was a Haplic Luvisol and soil texture was clayey loam. Experimental design was a completely randomized block with four replicates. The size of a plot varied from 3–6 m × 12–15 m. This was due to the different dimensions of machinery used in the experiments. Sampling areas for weed density, living mulch dry biomass and yield were equal in all experiments. The first factor was the living mulch species including white clover (*Trifolium repens* L.) with a sowing density of 9.2 kg ha^{-1} and perennial ryegrass (*Lolium perenne* L.) with a sowing density of 24.4 kg ha^{-1}. In the "weedy" control treatment, no living mulch was sown. The second factor was the date of living mulch establishment. In the early treatments, living mulch and cereals were sown on the same date. In the late treatments, living mulch was sown 25–107 days after the crop had been sown (DAS) (Table 1). Living mulch was sown with a single disc drill at 2-cm depth between the cereal crop rows. Crop row distance was 15–17 cm. All crops were sown and fertilized as usual. No mechanical and chemical weed control methods were applied in the experiments. Throughout the study, only non-inversion tillage operations (typical for Southwestern Germany) not deeper than 0.12 m with a chisel plow followed by seed-bed preparation with a rotary tiller were performed. Grain yield was determined at time of harvest with a plot-harvester and grain weight was determined for 86% dry matter. The plot harvester collected the grains of a sub-plot of 2 m × 10 m in the center of each plot.

Table 1. Details of the field experiments with living mulch for weed suppression. White clover and perennial ryegrass were sown between the crop rows in all experiments on the same day with the crop (early) and during tillering of the cereal (late).

No.	Year	Cereal Crop	Living Mulch	Time of Living Mulch Establishment	Dominant Weed Species
1	2009	Spring wheat	*Trifolium repens*, *Lolium perenne*	Early [1], 31 DAS [2]	*Viola arvensis* Murray, *Lamium purpureum* L., *Galium aparine* L., *Veronica persica* Poir., *Poa annua* L.
2	2010	Spelt wheat	*T. repens*, *L. perenne*	Early, 107 DAS	*Stellaria media* (L.) Vill., *G. aparine*, *Matricaria recutita* L.
3	2011	Spring wheat	*T. repens*, *L. perenne*	Early, 27 DAS	*S. media*, *Chenopodium album* L., *Fumaria officinalis* L., *L. purpureum*
4	2012	Spring barley	*T. repens*, *L. perenne*	Early, 26 DAS	*S. media*, *L. purpureum*, *V. persica*, *Alopecurus myosuroides* Huds.
5	2012	Spring barley	*T. repens*, *L. perenne*	Early, 25 DAS	*Poa annua*, *L. purpureum*, *V. persica*, *A. myosuroides*
6	2014	Spring wheat	*T. repens*, *L. perenne*	Early, 33 DAS	*S. media*, *Vicia hirsuta* (L.) Gray, *G. aparine*, *C. album*
7	2017	Oats	*T. repens*, *L. perenne*	Early, 29 DAS	*C. album*, *G. aparine*, *Polygonum convolvulus* (L.) Á.Löve 1970, *Sinapis arvensis* L., *S. media*

[1] Living mulch was sown on the same day as the crop; [2] Days after sowing.

Weed density was measured between 48 and 54 DAS in spring cereals and 140 DAS in spelt wheat, when the crop had produced the first tillers and the living mulch had emerged in all plots. A frame of 0.1 m^{-2} was placed six times randomly in each plot. Weeds within the frame were counted separately for each species and averaged over the six observations. Experimental details and the weed species with highest densities are listed in Table 1. In three experiments (No. 2, 4, 5), emergence of white clover and perennial ryegrass was recorded daily until the maximum living mulch density was reached. In three experiments (No. 2, 4, 7), dry biomass of living mulch was measured in a frame of 0.25 m^{-2} per plot.

All data were analysed with the statistic program *R* version 3.0.2 (*R* Core Team 2016, Boston, MA, USA). Prior to analysis, data were tested for normality using the Shapiro-Wilk-test and for homogeneity using the Levene-test of variance. It was not necessary to transform the data for ANOVA. Weed density and Grain yield data were analyzed using the following mixed linear model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_l + e_{ijkl}, \quad ()$$

where Y_{ijkl} is the model's dependent variable, with subscript i indicating the living mulch species, j the sowing date, k the year, and l the cereal crop. Further, μ is the general mean, whereas α, β are the fixed effects of mulch species, and sowing date with the two-way interaction marked as $\alpha\beta$. γ_k is the random effect of year k and δ_l cereal crop. Multiple mean comparison tests were performed using the Tukey-test at a significance level of $\alpha \leq 0.05$.

3. Results

The dominant weed species for all experiments in this study are listed in Table 1. Almost no perennial weed species were observed in the trials. The species are typical for cereal production in Germany. *Stellaria media*, *Galium aparine*, *Lamium purpureum*, *Veronica persica* and *Poa annua* occur in winter- and spring cereals. *Chenopodium album*, *Polygonum convolvulus* and *Sinapis arvensis* mostly occur in spring cereals. *Alopecurus myosuroides* predominantly grows in winter annual crops. *Vicia hirsuta* indicates lower nitrogen contents in the soil. Therefore, it is more abundant in organic fields. *Galium aparine*, *C. album*, *P. convolvulus* and *V. hirsuta* were the most competitive species [3].

Living mulches emerged within 21 DAS. White clover emerged slightly faster than perennial ryegrass. The average time until 50% of the maximum living mulch plants emerged (T_{50}-value) [4] was 9.2 days for white clover and 11.0 days for perennial ryegrass.

Average weed density in the control plots ranged from 6 plants m^{-2} in spring wheat 2014 and oats 2017 to 93 plants m^{-2} in spring wheat 2009 (Tables 2 and 3). Living mulch significantly suppressed weed densities. Early and late sowing perennial ryegrass reduced weed density by 97% in spring barley 2012, probably due to the warm and moist weather in spring. Under similar growing conditions in spring wheat 2009, perennial ryegrass suppressed 40% of the weeds after early sowing and 86% after late sowing. White clover in the same experiment reduced weed density by 42% and 76% compared to the "weedy control". The least weed reduction was observed in spelt wheat with only 2–7% weed reduction. Possibly, the cold temperatures during winter reduced weed suppression ability of living mulch and favored weed development. The mean weed density over all experiments was 45.3 plants m^{-2} in the control plots, 21.7 plants m^{-2} in perennial ryegrass, and 25.4 plants m^{-2} in white clover (Table 4). Weed densities were similar in the early and late sowing treatments. Living mulch dry biomass was greater after early sowing than after late sowing. In the early sown plots, white clover produced up to 8 g m^{-2} aboveground dry biomass and perennial ryegrass 36 g m^{-2} until 95 DAS compared to only 2–3 g m^{-2} dry biomass in the late sowing treatments. Weed reduction in the early sown plots is probably affected by competition and shading, whereas weed reduction after late sowing of living mulch was rather caused by uprooting and covering of weeds with the pipes of the drilling machine.

Cereal grain yield was similar in all treatments with and without living mulch. Early and late sowing of living mulch resulted in equal grain yields of 6.7 and 6.8 t ha^{-1} (Table 5). Only spring wheat in 2009 and oats in 2017 had a lower yield probably due to the dry weather in June and July 2009 and the cold temperatures in spring 2017.

Table 2. Average weed densities (n m^{-2}) and average grain yields (t ha^{-1}) of the seven field experiments after early sowing of living mulch; Tukey-tests at a significance level of $\alpha \leq 0.05$ were performed for each single experiment; means with identical letters within each experiment do not differ significantly based on the Tukey-HSD test ($p < 0.05$).

No.	Crop	Average Weed Density (n m^{-2})			Average Grain Yield (t ha^{-1})		
		Control	Ryegrass	White Clover	Control	Ryegrass	White Clover
1	Spring wheat	93 ^{a}	37 ^{b}	35 ^{b}	4.6 ^{A}	4.7 ^{A}	4.8 ^{A}
2	Spelt wheat	80 ^{a}	74 ^{a}	79 ^{a}	7.0 ^{A}	7.0 ^{A}	6.8 ^{A}
3	Spring wheat	17 ^{a}	11 ^{b}	6 ^{b}	7.7 ^{A}	6.7 ^{A}	7.4 ^{A}
4	Spring barley	58 ^{a}	32 ^{b}	27 ^{b}	7.2 ^{A}	7.1 ^{A}	7.0 ^{A}
5	Spring barley	58 ^{a}	2 ^{c}	14 ^{b}	7.0 ^{A}	7.1 ^{A}	7.1 ^{A}
6	Spring wheat	6 ^{a}	3 ^{b}	4 ^{b}	8.3 ^{A}	7.9 ^{A}	7.5 ^{A}
7	Oats	6 ^{a}	3 ^{b}	4 ^{b}	6.2 ^{A}	5.8 ^{A}	6.4 ^{A}

Table 3. Average weed densities (n m^{-2}) and average grain yields (t ha^{-1}) of the seven field experiments after late sowing of living mulch; Tukey-tests at a significance level of $\alpha \leq 0.05$ were performed for each single experiment; means with identical letters within each experiment do not differ significantly based on the Tukey-HSD test ($p < 0.05$).

No.	Crop	Average Weed Density (n m^{-2})			Average Grain Yield (t ha^{-1})		
		Control	Ryegrass	White Clover	Control	Ryegrass	White Clover
1	Spring wheat	92 ^{a}	13 ^{b}	31 ^{b}	4.7 ^{A}	4.7 ^{A}	4.7 ^{A}
2	Spelt wheat	80 ^{a}	78 ^{a}	37 ^{b}	7.0 ^{A}	7.6 ^{A}	7.5 ^{A}
3	Spring wheat	18 ^{a}	13 ^{a}	17 ^{a}	7.6 ^{A}	7.3 ^{A}	7.5 ^{A}
4	Spring barley	54 ^{a}	30 ^{b}	32 ^{b}	7.1 ^{A}	6.9 ^{A}	7.1 ^{A}
5	Spring barley	58 ^{a}	2 ^{c}	24 ^{b}	7.1 ^{A}	7.1 ^{A}	7.0 ^{A}
6	Spring wheat	7 ^{a}	2 ^{b}	3 ^{b}	8.2 ^{A}	7.3 ^{A}	7.1 ^{A}
7	Oats	7 ^{a}	2 ^{b}	3 ^{b}	6.1 ^{A}	6.4 ^{A}	6.3 ^{A}

Table 4. Average weed density (n m^{-2}) and standard error in parenthesis averaged over the fixed factors living mulch species and time of living mulch sowing in all seven experiments; no interactions between living mulch species and sowing date were found; time of sowing was not significant; means with identical letters within each experiment do not differ significantly based on the Tukey-HSD test ($p < 0.05$).

Living Mulch Species	Early Sowing	Late Sowing
No living mulch	45.4 ^{a} (6.8)	45.1 ^{a} (6.7)
Perennial ryegrass	23.1 ^{b} (5.0)	20.3 ^{b} (5.2)
White clover	24.1 ^{b} (5.1)	26.7 ^{b} (4.8)

Table 5. Average grain yield (t ha^{-1}) and standard error in parenthesis averaged over the fixed factors living mulch species and time of living mulch sowing in all seven experiments; interactions between living mulch species and sowing date and both factors were not significantly different from each other.

Living Mulch Species	Early Sowing	Late Sowing
No living mulch	6.9 (0.2)	6.8 (0.2)
Perennial ryegrass	6.6 (0.2)	6.8 (0.2)
White clover	6.7 (0.2)	6.7 (0.2)

4. Discussion

In this study, white clover and perennial ryegrass as living mulch significantly reduced weed density in 6 out of seven experiments. This is in agreement with other studies on living mulch in cereal crops [2,5–7,9,10]. In spelt wheat, living mulches did not reduce weed densities. However, it was possible to establish living mulches in winter cereals almost three months after sowing (Figure 1). Living mulch had less effect on perennial weeds [11]. Weed suppressive ability of living mulch is thought to be due to competition for light, water und nutrient and inhibition of weed seed germination due to shading [9]. Dry biomass of living mulch and weed suppressive ability are often positively correlated [6]. Living mulch can be included into Integrated Weed Management (IWM) strategies in different ways. For spring cereals, weed suppressive ability of living mulch might be sufficient to prevent yield losses and no other weed control methods may be applied. In most other crops, it is necessary to combine different weed control methods [12,13]. Perennial ryegrass and white clover are tolerant to a few common herbicides in all major crops. However, most farmers would not see a necessity to grow living mulch if they use herbicides. Mechanical weed control, mainly pre-emergent and post-emergent harrowing is often combined with living mulch in an IWM system [1,3,10].

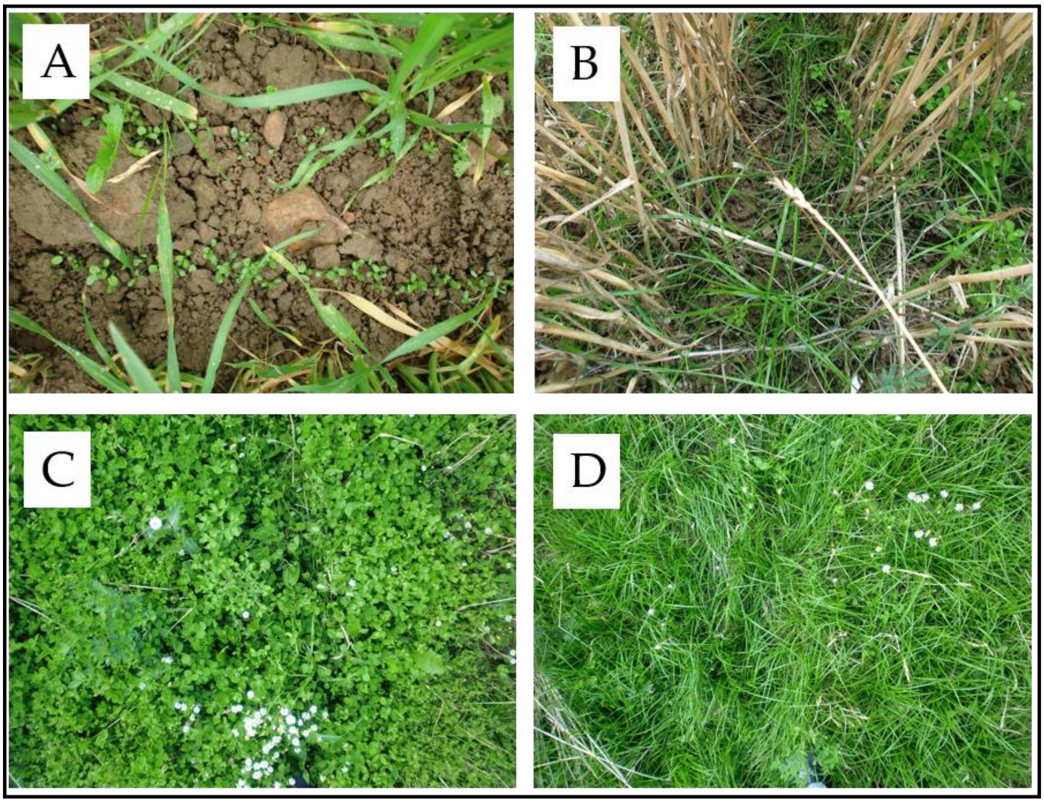

Figure 1. White clover (**A**,**C**) and perennial ryegrass (**B**,**D**) in spelt wheat and 21 days after spelt wheat harvest in the experiment No. 2 in 2010.

Treatments with living mulches provided similar yields to the "weedy" control plots regardless of sowing date for living mulch. Even when sown simultaneously with the cereal crop, living mulch did not reduce grain yield. This is in agreement with [1,2,4]. Only one study showed a 14% yield decrease of living mulch in cereals [7]. Living mulch in Norwegian spring cereal production significantly increased grain yield by 16–22% [11]. Clover living mulch in winter wheat increased the yield of subsequent spring barley due to nitrogen fixation in the soil [12]. In conclusion, short growing cultivars of living mulch seem to have a low risk of reducing cereal grain reduction.

In this study, perennial ryegrass and white clover had equal impact on weeds and cereal crops. Tall growing living mulch species such as red clover (*Trifolium pratense* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) exposed a stronger competition on cereals [2].

Approximately 14–21 days after harvest of the cereal, perennial ryegrass and white clover rapidly produced a dense canopy and covered the soil almost entirely (Figure 1). Living mulch can provide biomass for feed, energy use and green manure before and after winter [2]. In this study, it was cut once before winter and once in spring before sowing the next spring crop. Cutting and the dense cover guaranteed a good weed suppressing of weeds and volunteer crops in autumn and spring. The rapid development of living mulch after removing the cereal crops is one of the main advantages of living mulch compared to regular cover crops that are sown into the stubble of the cereal. Cover crops usually grow slowly and might completely fail under dry conditions. They often cannot compete with volunteer crops [4].

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