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Approach to Demography of ALS-Resistant *Glebionis coronaria* as Influenced by Management Factors: Tillage, Allelopathic Crops and Herbicides

Zeineb Hada ^{1,2,*}, Messaad Khammassi ^{2,3}, Houda Jenfaoui ^{1,2}, Yosra Menchari ^{2,4}, Joel Torra ⁵ and Thouraya Souissi ^{1,2,*}

¹ Department of Plant Health and Environment, National Institute of Agronomy of Tunisia, University of Carthage, Tunis 1082, Tunisia; jenfhouda@gmail.com

² Laboratory of Bioaggressor and Integrated Management in Agriculture (LR14AGR02), National Institute of Agronomy of Tunisia, University of Carthage, Tunis 1082, Tunisia; kh_messad@yahoo.fr (M.K.); menchariyosra@yahoo.fr (Y.M.)

³ National Institute of Field Crops, Boussalem, Jendouba 8170, Tunisia

⁴ Higher Institute of Biotechnology of Beja, University of Jendouba, Jendouba 9000, Tunisia

⁵ Department of Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida, 25198 Lleida, Spain; joel.torra@udl.cat

* Correspondence: zeineb.hada@gmail.com (Z.H.); tsouissi@netcourrier.com (T.S.)



Citation: Hada, Z.; Khammassi, M.; Jenfaoui, H.; Menchari, Y.; Torra, J.; Souissi, T. Approach to Demography of ALS-Resistant *Glebionis coronaria* as Influenced by Management Factors: Tillage, Allelopathic Crops and Herbicides. *Agronomy* **2022**, *12*, 1083. <https://doi.org/10.3390/agronomy12051083>

Academic Editors: Jose L. Gonzalez-Andujar and Guillermo R. Chantre

Received: 30 March 2022

Accepted: 23 April 2022

Published: 29 April 2022

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Abstract: *Glebionis coronaria* (L.) Cass. ex Spach is one of the most serious weeds in cereal crops in Northern Tunisia. Our previous studies have confirmed the presence of resistance to ALS-inhibiting herbicides in *G. coronaria*, showing the evolution of cross-resistance through TS and NTS mechanisms. The purpose of this study was to evaluate the effects of (i) two tillage practices (conventional tillage and reduced tillage), (ii) three known allelopathic crops: *Hordeum vulgare* L. (Barley), *Brassica napus* L. (Rapeseed) and *Triticum durum* (Wheat) and (iii) herbicides belonging to different modes of action on the density, the cohort numbers and the growth of an ALS-inhibiting cross-resistant population of *G. coronaria*. Field experiments were conducted in two consecutive years (2017–2018 and 2018–2019) in the Fritissa-Mateur-Bizerte region. Our results revealed that barley considerably contributed to the decrease in *G. coronaria*'s density, cohort number, total biomass accumulation and plant height. The effect of rapeseed is likely dependent on the tillage practice and the timing of herbicide application, while *G. coronaria* could be successfully controlled in wheat using auxin herbicides. It is suggested that the management of resistant population of *G. coronaria* could be achieved by combining barely or rapeseed with right tillage practice and auxin herbicides' application.

Keywords: *Glebionis coronaria* (L.) Cass. ex Spach; tillage; allelopathic crops; *Hordeum vulgare* L.; *Brassica napus* L.; *Triticum durum* Desf.; ALS-inhibiting herbicides; auxin herbicides

1. Introduction

Glebionis coronaria (L.) Cass. ex Spach is a noxious weed in cereal cropping systems in Northern Tunisia, causing significant yield losses of up to 75% in wheat fields [1]. The ability of this weed to invade, grow and compete with the wheat crop has been attributed to several factors including rapid germination, extended emergence period and a vigorous growth providing strong competitive abilities. In the last few years, *G. coronaria* (*Glebionis coronaria* (L.) Cass. ex Spach) has become a growing threat in Tunisia in winter cereals because of the occurrence of herbicide-resistant (HR) populations, resistant to Acetolactate Synthase (ALS) inhibitors. Resistance to ALS inhibitors has been attributed to three-point mutations in the target ALS gene of some Tunisian populations. Evidence of enhanced metabolism developed by the weed, as non-target site resistance mechanism, has also been reported in these populations [2]. The co-existence of both mechanisms confer to the weed

high resistance levels to different ALS inhibitors and, it is likely, the ability to detoxify herbicides with different modes of action [3,4].

The genetic bases of herbicide resistance, as well as the biological and ecological traits of *G. coronaria*, mitigate the efficacy of the chemical approach to restrict the spread of resistant populations. Further research is required to explore more combinations of complementary selection pressures to be implemented in an integrated weed management (IWM) strategy. Particular attention needs to be focused on resistant population demographics [5], as it can be effectively used to improve crop management decisions [6,7]. Demography is the study of a population as it responds to intrinsic and extrinsic factors that act upon it [8,9]. Under the Mediterranean climate, weeds may occur in different cohorts that may differ in population dynamics and susceptibility to control measures [10]. Understanding the demographic traits of weeds, including emergence timing and survival of weed cohorts, together with the growth characteristics of individuals, are valuable to predict the competitive ability of the weed species within the cropping system [10,11].

In the Tunisian wheat cropping system, the control of ALS inhibitors cross-resistant populations of *G. coronaria* relies on synthetic auxins in herbicide rotation or mixed mainly with ALS inhibitors; however, this strategy is no longer effective enough with the occurrence of enhanced metabolism [2]. Among the safest tools to control resistant populations, crop rotation has been reported as reducing the selection intensity of relying solely on herbicides. It allows variation of the management of fields, creating an unstable and inhospitable environment which prevents weeds from proliferating as well as disrupting the weed population dynamics [12,13]. The introduction of allelopathic crops in rotation could be promising because of a large variety of secondary metabolites, commonly called allelochemicals, released into their environment [14]. In the field, allelochemicals have an inhibitory effect on seed germination and weed density [15]. However, the efficacy of allelopathy approaches is not enough unless combined within an IWM strategy [16]. Tillage is widely adopted as a major component of IWM programs. The main goal of tillage is to control the weed seedbank, to disrupt germination and to destroy emerged weeds to give the crop a competitive advantage in its first stages of growth [16]. However, tillage may increase the germination of some weeds, directly by increasing soil–seed contact or indirectly by increasing exposure to light, aeration of soil and soil temperature [17]. Individually, the effect of allelopathic crops and tillage has been well reported, however, their interactions are less studied [18].

The purpose of this field study was to test the hypothesis: if weed management practices such tillage, crops with allelopathic potential and herbicides with different modes of action could reduce the emergence of HR *G. coronaria* and suppress its growth, then the integration of these practices would improve the level of HR *G. coronaria* control under natural field conditions. Specific objectives are to determine in two successive growing seasons: (1) the evolution of weed density and the cohort's establishment, (2) the evolution of total dry matter along each growing season and (3) the weed height at flowering stage under different combinations of these practices.

2. Materials and Methods

2.1. Site Description

Field experiments were conducted during two consecutive growing seasons (2017–2018 and 2018–2019) at Fritissa, Mateur-Bizerte region (37°1'50.91" N, 9°42'26.92" E) under rainfed conditions. The soil was clay-loam soil, with 2.3% of organic matter and a pH of 8.5. Precipitation and temperature data were recorded daily by an automatic agrometeorological station placed in the experimental site. *G. coronaria* was the main weed species infesting the field and a few other species were present at low densities. The field had been under wheat mono-cropping system for the last ten years. The weed population infesting this field was confirmed as cross-resistant to ALS inhibitors, owing to both gene mutations and enhanced metabolism mechanisms [2].

2.2. Experimental Design, Crop Managements and Measurements

Field experiments were carried out in 2017–2018 to evaluate the effect of tillage systems, allelopathic crops and herbicide application on the demography of the above-mentioned herbicide-resistant (HR) population of *G. coronaria*. Two tillage practices were studied: conventional tillage (T1), with an autumn mouldboard ploughing (up to 40 cm) and then disk harrowing (up to 15 cm) for seedbed preparation the first season and only harrowing the second; and reduced tillage (T2), including only harrowing both seasons.

The two tillage practices were combined with three crop treatments: (1) barley (*Hordeum vulgare* L. var Rihane) for the first season in rotation with rapeseed (*Brassica napus* L. var Trapper) for the second; (2) rapeseed for the first season in rotation with barley for the second and 3) durum wheat (*Triticum durum* Desf. var Maali) for both seasons. For each crop × tillage combination, herbicides were applied in half of the cropped area (H) and the other half was left without herbicide treatments (NH). The same herbicide treatments and crop varieties were used in both growing seasons (Table 1).

Table 1. Management practices (crops and herbicides) during the two growing seasons.

Growing Seasons							
2017–2018				2018–2019			
Crop	Density Kg/Ha	Sowing Date	Harvest Date	Crop	Density Kg/Ha	Sowing Date	Harvest Date
Wheat	200	11–24	06–16	Wheat	200	11–22	06–20
Barley	140	11–24	05–18	Barley	140	11–22	06–04
Rapeseed	4.5	11–24	05–18	Rapeseed	4.5	11–09	06–04
Crop	Herbicide		Dates	Crop	Herbicide		Dates
Wheat	Prosulfocarb [†]		7 December 2017	Wheat	(2,4-D + Florasulam) ⁺⁺⁺		20 February 2019
	(2,4-D + Florasulam) ⁺⁺⁺		15 January 2018		+Clodinafop-propargyl ⁺⁺⁺		
	+Clodinafop-propargyl ⁺⁺⁺						
Barley	Dicamba + 2,4-D ⁺⁺⁺		16 March 2018	Barley	Dicamba + 2,4-D ⁺⁺⁺		18 March 2019
	(2,4-D + Florasulam) ⁺⁺⁺		15 January 2018		(2,4-D + Florasulam) ⁺⁺⁺		7 February 2019
	+Pinoxaden ⁺⁺⁺				+Pinoxaden ⁺⁺⁺		
Rapeseed	Dicamba + 2,4-D ⁺⁺⁺		16 March 2018	Rapeseed	Dicamba + 2,4-D ⁺⁺⁺		18 March 2019
	Trifluraline [†]		24 November 2017		Trifluraline [†]		8 November 2018
	Methazachlor ⁺⁺		7 December 2017		Methazachlor ⁺⁺		26 November 2018
	Clopyralid ⁺⁺⁺		15 January 2018		Clopyralid ⁺⁺⁺		18 January 2019

[†] PRE-emergence herbicide; ⁺⁺ Early-POST-emergence herbicide; ⁺⁺⁺ POST-emergence herbicide.

The treatments were arranged in a split–split–plot experimental design with tillage practices (T1 and T2) as the main-plot, the crops (barley, rapeseed and wheat) as the subplot and herbicide application (H and NH) as the sub-subplot factors. Each sub-subplot was 10 m × 50 m.

Three repetitions were used for each treatment (crop × tillage × herbicide) with a total of 36 sub-subplots in each growing season. Repetitions were separated by two corridors (of a width of 4 m) left as fallow, where *G. coronaria* grew in absence of crop competition (Figure 1).

Every year before sowing, the soil was amended by phosphorus fertilizers and the appropriate herbicides (Table 1). During the growing season, fungicides, early POST-herbicides and POST-herbicides were applied for each crop. Nitrogen was broadcasted as ammonium nitrate (33.5% N) in three fractions for wheat and barley (30% at 3-leaf stage, 40% at ear-1-cm and 30% at the stem elongation stage) and two fractions for rapeseed (50% at 4–6 leaf stage and 50% at stem elongation). PRE, early-POST and POST herbicides were applied at the labeled doses using a backpack sprayer calibrated to deliver 200 L ha^{−1} at a pressure of 3 KPa. The management program included the sowing densities and dates, the harvest dates and herbicide applications are represented in Tables 1 and 2.

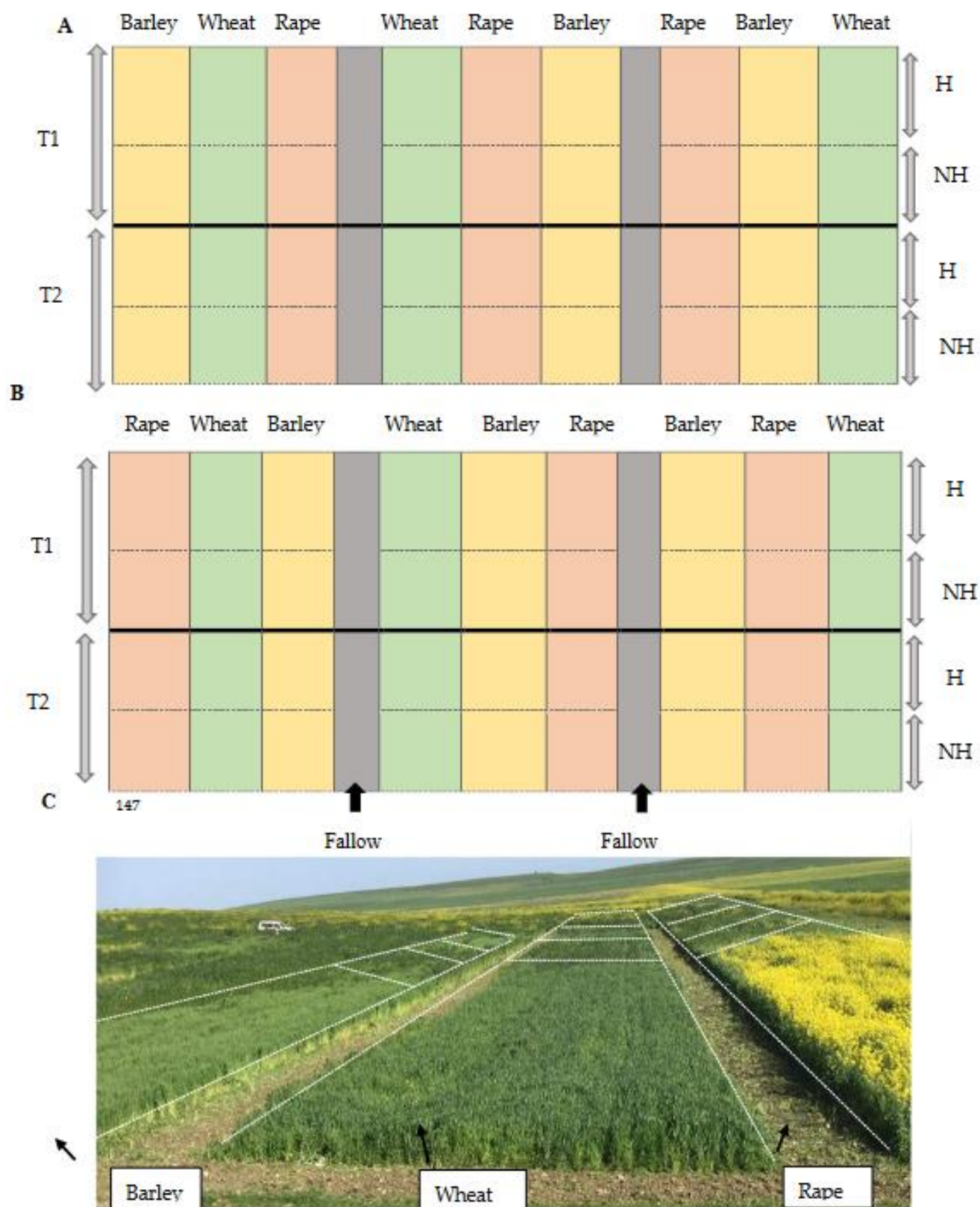


Figure 1. Schematic representation of the experimental design in (A) 2017–2018 and (B) 2018–2019 growing seasons; (C) Picture of the experimental unit (one repetition) within the field. T1 = Conventional tillage; T2, reduced tillage; H = with herbicide treatment; NH = without herbicide treatment.

Table 2. Herbicides applied during the two growing seasons.

Active Ingredients	Trade Name	HRAC Code	Applied Doses
Prosulfocarb	Minarix *	15	2.5 L ha ⁻¹
2,4-D + Florasulam	Nikos 306 SE	4 + 2	0.6 L ha ⁻¹
Clodinafop-propargyl	Topik 80 *	1	0.75 L ha ⁻¹
Pinoxaden	Axial 045 EC *	1	1 L ha ⁻¹
Dicamba + 2,4-D	Dialen super	4	0.8 L ha ⁻¹
Trifluraline	Trifluraline	3	2 L ha ⁻¹
Methazachlor	Twiga	15	2 L ha ⁻¹
Clopyralid	Lontrel	4	1 L ha ⁻¹

* These herbicides were applied to control grass weeds in the field.

During the two growing seasons, samplings were carried out monthly starting from the vegetative stage of the weed until crop harvest to determine the evolution of *G. coronaria* densities and the total dry biomass accumulated by the weed under different treatment combinations. Likewise, the main weed cohorts were recorded within each sub-subplot. In each sampling date, three quadrats (0.25 m²) per plot were sampled. At the flowering stage, weed heights (cm) were also recorded within cultivated plots and fallow (non-cultivated areas). Weed samples were oven-dried every time (for 48 h at 70 °C) before dry biomass measurements. The herbicide efficacies were evaluated based on the weed density using the following equation:

$$\text{Herbicide efficacy (\%)} = \frac{WDi - WDi + 1}{WDi} * 100$$

where *WDi* is the average density before herbicide treatment and *WDi + 1* is the average density measured 30 days after herbicide treatment.

2.3. Statistical Analysis

The effect of tillage, crops, herbicide applications and their combinations on *G. coronaria* densities, dry biomass and heights were subjected to a linear mixed model analysis (LMM). Three-way ANOVAs were performed for interactions and means were compared using the Duncan post hoc pairwise test (*p*-value = 0.05) using SPSS software (IBM SPSS statistics 20). All the effects are fixed, and every growing season was analyzed separately. Prior to each analysis, the normal distribution and homogeneity of the data were checked. The plots of density and biomass evolution were carried out using Sigma plot 11.0 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Climatic Data

The two growing seasons had the same pattern of temperature but differed in terms of the amount and distribution of rainfall (Figure 2). The average annual rainfall, from 2010 to 2019, for the Fritissa site was 493 mm and the total amount of rainfall varied considerably between the seasons. The 2011–2012 season was the wettest season (773 mm) and 2015–2016 was the driest season (315 mm). The total precipitation accumulated in the two growing seasons of the study were below the average. However, the 2017–2018 was considered relatively the wettest season (436 mm), characterized by wet winter (235 mm, December–February), considerable rainfall in spring (126 mm, March–May) and some rainfall in early summer (3 mm). The 2018–2019 growing season was drier compared to the previous season with a total precipitation of 395 mm but differed mostly in the distribution of rainfall along the season, having the highest precipitation amount in January (114 mm) and the lowest in April (6 mm).

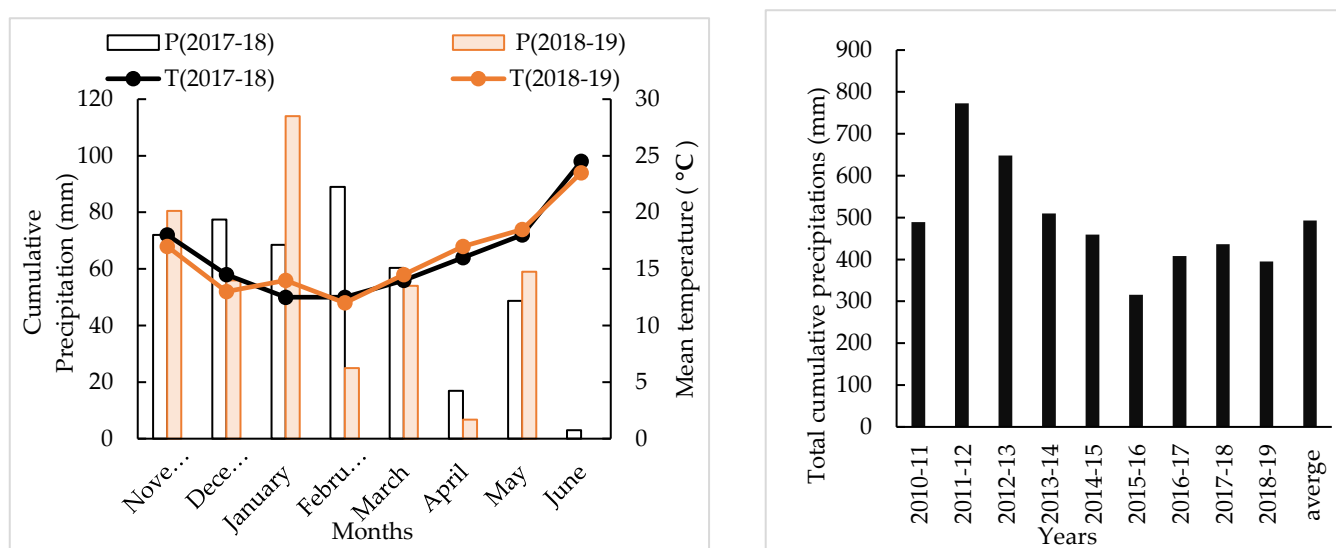


Figure 2. Left graph: Total monthly precipitations (mm) and mean temperatures (°C) during the growing seasons 2017–2018 and 2018–2019; right graph: Total cumulative precipitations of the two growing seasons 2017–2018 and 2018–2019 compared to the previous seasons (2010–2017) and their average (2010–2019) for the experimental site of Fritissa-Mateur-Bizerte.

3.2. Effects of Tillage, Crops, Herbicides and Their Interactions on the Evolution of the Densities of *G. coronaria*

Overall, our results showed that tillage, crops and herbicide applications have significant impact on the densities of the HR population of *G. coronaria*. Nevertheless, these effects were closely dependent on the growing seasons (Table 3, Figure 3).

Table 3. Results of main effects and interactions from three-way ANOVA of *G. coronaria* densities for 2017–2018 and 2018–2019.

Treatments	Main Effects				Interactions		
	Tillage	Crops	Herbicides	Tillage × Crop	Tillage × Herbicide	Crop × Herbicide	Tillage × Crop × Herbicide
2017–2018	**	***	***	**	NS	NS	NS
2018–2019	NS	***	NS	NS	NS	NS	NS

The symbols indicate the significance level at $p = 0.05$: *** < 0.001; ** < 0.01 and NS > 0.05.

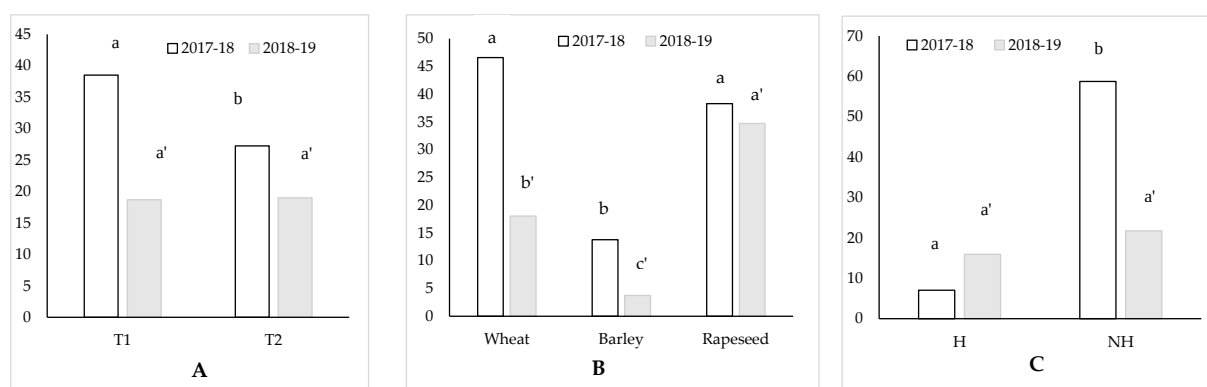


Figure 3. The effects of the management factors (A): tillage (T1 and T2); (B): Crops (wheat, Barley and Rapeseed); (C): herbicide applications (H and NH) on the density (plants/m²) of *G. coronaria* determined at crop harvest in the two growing seasons, Same superscript letters (a, b and for 2017–2018; a', b' and c' for 2018–2019) across treatments are statistically similar according to Duncan test ($p = 0.05$).

The analysis of variance showed significant effects of the crops ($p < 0.001$), herbicide application ($p < 0.001$) and tillage ($p < 0.01$) on the weed densities were observed during the first growing season 2017–2018. However, in 2018–2019, only the crops' effect was significant. The interactions between treatments were inconsistent between the two years of study. Only tillage by crop interaction was found to be significant ($p < 0.001$) in the first year and no other significant interactions were revealed.

At crop harvest, harrowing (T2) significantly reduced weed densities in 2017–2018, resulting in an average of 27 plants/m² compared to 39 plants/m² in plots with conventional tillage (T1). No effect of tillage was reported in 2018–2019 (Figure 3A). Similarly, the effect of herbicide application was only significant in the first season 2017–2018. An average density of 7 plants/m² was recorded in H-plots compared to 59 plants/m² in NH-plots (Figure 3C). The densities of *G. coronaria* were significantly different in the presence of different crops, with the lowest densities recorded in barley plots. Average densities ranged between 4 plants/m² to 47 plants/m² in barley (first season) and wheat (second season), respectively (Figure 3B).

Our results showed that the interaction between crops and tillage significantly impacted the densities of *G. coronaria* in 2017–2018 (Table 3). Along this growing season, the evolution of densities and the establishments of main cohorts were analyzed separately under H and NH treatments (Figure 4).

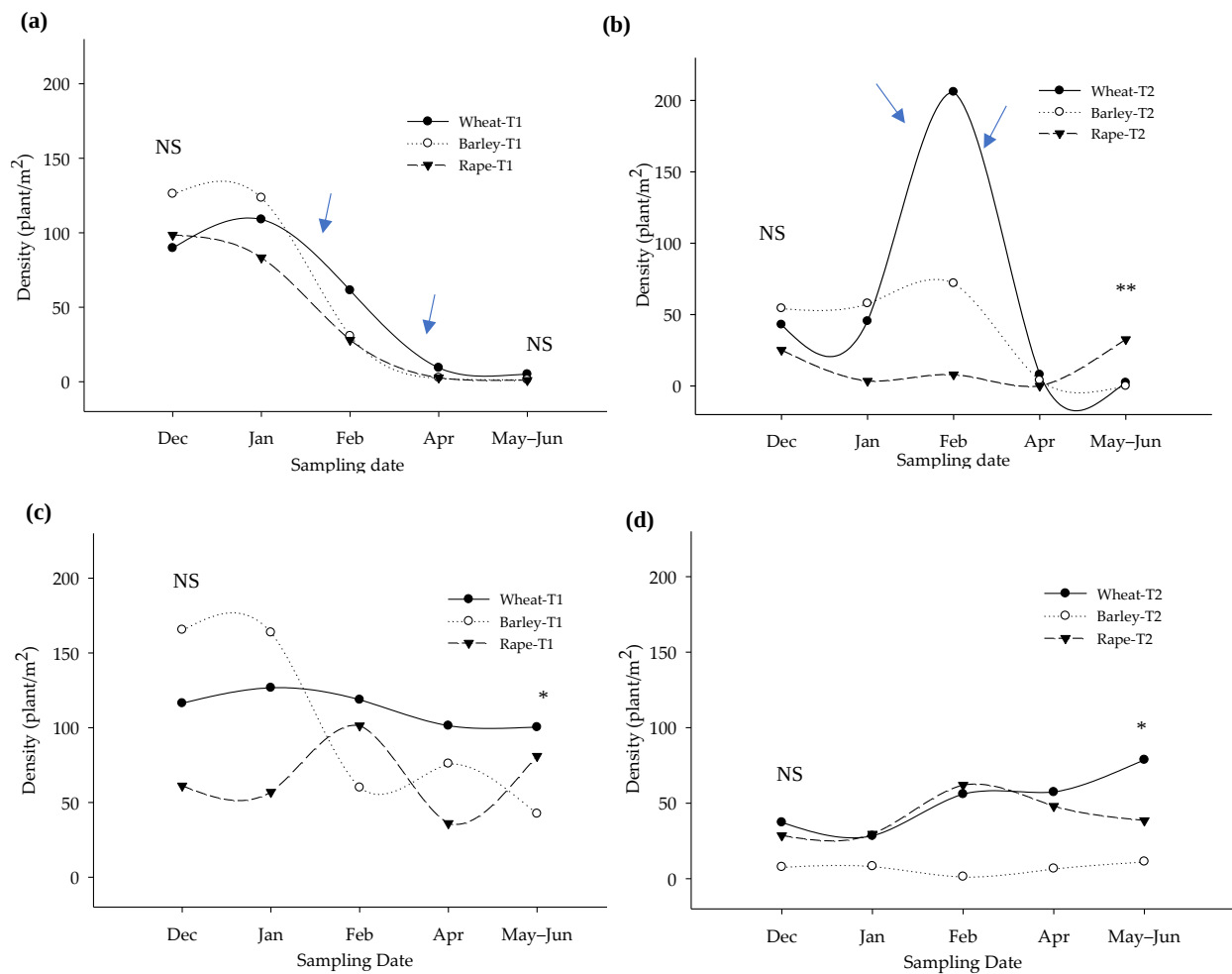


Figure 4. Evolution of *G. coronaria* densities (plants/m²) across 2017–2018 growing season, as influenced by tillage $\times$ crop interactions, (a,b): herbicide treated plots; (c,d): non-treated plots. The arrows indicate the timing of herbicide application. The symbols indicate the significance level at $p = 0.05$: * < 0.05 , ** < 0.01 and NS > 0.05 .

In herbicide-treated plots (H), the initial densities of *G. coronaria* were homogeneous and statistically similar among the plots with the same tillage practice. Early in the growing season 2017–2018 (30 days after sowing), the highest densities were recorded under T1 plots. The densities varied between 90 plants/m² and 126 plants/m² in wheat and barley plots, respectively. Lower densities were recorded under reduced tillage (T2), and varied between 25 plants/m² and 54 plants/m² in rapeseed and barley plots, respectively (Figure 4a,b).

Herbicide treatments resulted in significant reduction in the weed densities, as well as less dynamics in weed emergence along the growing season.

In T1 plots, the application of 2,4-D + florasulam on cereals and clopyralid on rapeseed reduced weed densities by 44%, 75% and 66% in wheat, barley and rapeseed, respectively. The Dicamba + 2,4-D applied in 20th of March, on cereals reduced the weed densities by 85% and 91% in wheat and barley, respectively. No more herbicide was applied on the rapeseed plots, however, our results showed that the weed density was reduced by up to 91% at the time of sampling (April). At crop harvest, the weed densities recorded in T1 plots decreased significantly, by up to 94% in wheat, 99% in barley and 99% in rapeseed plots, after all herbicide treatments (Figure 4a).

In T2 plots, low efficacy was observed with 2,4-D + florasulam application. The densities increased drastically in wheat to reach 200 plants/m² (Figure 4b). The application of Dicamba + 2,4-D caused a significant decrease in the densities in wheat and barley. After both treatments, densities were reduced up to 96% and 94% in wheat and barley, respectively (Figure 4b).

In the NH-plots, the initial densities of the weed were statistically similar between crops, with higher densities recorded under conventional tillage (T1) than reduced tillage (T2) (Figure 4c,d). In T1 plots, densities varied between 61 plants/m² and 165 plant/m² in rapeseed and barley, respectively. In T2 plots, recorded densities were about 8 plants/m² and 37 plants/m² in barley and rapeseed, respectively.

In the absence of a herbicide effect, the weed dynamics along the growing season depended mainly on the interactions between crops and tillage practices (Figure 4c,d).

Conventional tillage (T1) resulted in the highest constant emergence of *G. coronaria* in wheat plots with a density around 113 plants/m². In contrast, a more dynamic (with several cohort) emergence pattern was observed in barley and rapeseed plots. Interestingly, the weed had an opposite emergence timing in the presence of these two crops, with a first cohort in barley and lower delayed flushes in rapeseed (Figure 4c).

In barley, the highest density of the weed was observed at the early season (165 plants/m², December–January) then decreased considerably (60 plants/m², February) and again slightly increased (76 plants/m², April); by the end of the season 42 plants/m² were recorded in the barley plots. Conversely, a low density of *G. coronaria* in rapeseed was recorded in Dec-Jan (60 plants/m²) and a higher weed density in Feb (101 plants/m²), then the density decreased to 36 plants/m² in April; by the end of the season, another increase in the weed numbers was recorded (81 plants/m²) (Figure 4c).

Under reduced tillage (T2), the weed emergence appeared to be more constant compared to that in the T1 plots. In barley, the weed had the most constant emergence (7 plants/m², February) throughout the season. Simultaneously, an increase in densities was observed in both wheat (56 plants/m²) and rapeseed (62 plants/m²) in February 2018 (Figure 4c,d).

In 2018–2019, the statistical analysis revealed that the weed densities were only affected by the crops ($p < 0.001$) and no interactions were revealed at $p = 0.05$ (Table 3). At crop harvest, less contrast was observed between the H-plots and NH-plots under both tillage practices compared to the previous growing season, and the barley crop resulted in the lowest densities compared to rapeseed and wheat under different combinations of tillage and herbicides.

3.3. Effect of Treatments and Their Interactions on Dry Matter Evolution of HR *G. coronaria* during the Two Growing Seasons

Crops and herbicide applications had a significant impact on the dry matter of HR *G. coronaria* in both years of study. However, the effect of tillage ($p < 0.01$) was only observed in the 2017–2018 growing season (Table 4). Tillage $\times$ herbicide and crop $\times$ herbicide interactions were significant in 2017–2018, while only the tillage $\times$ herbicide interaction was found significant in 2018–2019 (Table 4).

Table 4. Results of main effect and interactions from three-way ANOVA of *G. coronaria* dry matter for 2017–2018 and 2018–2019.

Treatments	Main Effects				Interactions		
	Tillage	Crops	Herbicides	Tillage $\times$ Crop	Tillage $\times$ Herbicide	Crop $\times$ Herbicide	Tillage $\times$ Crop $\times$ Herbicide
2017–2018	**	***	***	NS	**	***	NS
2018–2019	NS	***	**	NS	**	NS	NS

The symbols indicate the significance level at $p = 0.05$: *** < 0.001 ; ** < 0.01 and NS > 0.05 .

At crop harvest, the statistical analysis showed that reduced tillage practice (T2) was effective in controlling *G. coronaria* biomass compared to conventional tillage (T1). In 2017–2018, weed biomass was around 96.3 g/m² within T2 plots and 155.2 g/m² within T1 plots (Figure 5A). Within the barley plots, *G. coronaria* accumulated less biomass than in the wheat and rapeseed plots. The average weed dry matters were 35.5 g/m² and 21.2 g/m² in 2017–2018 and 2018–2019, respectively (Figure 5B). Furthermore, herbicide application significantly decreased the biomass of *G. coronaria* in both growing seasons, resulting in 17.3 g/m² and 33.9 g/m², respectively (Figure 5C).

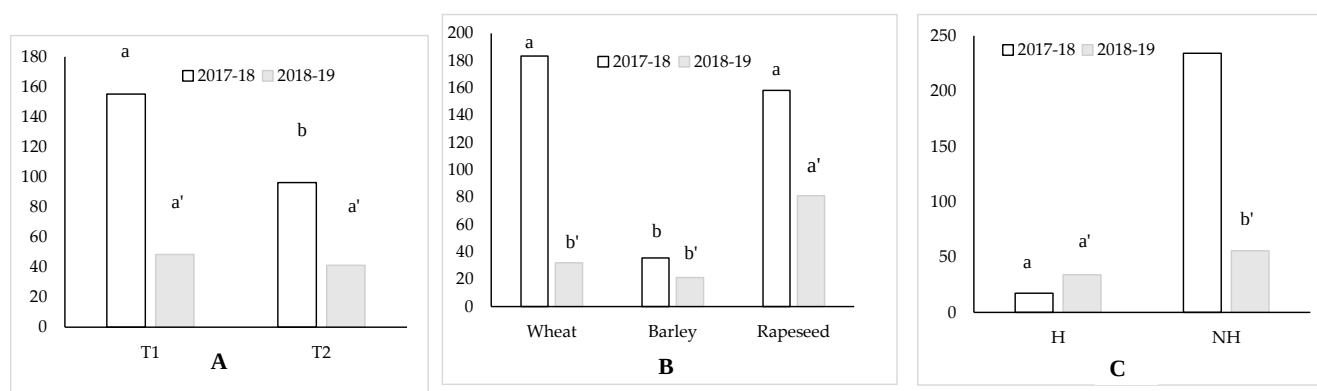


Figure 5. The effects of the management factors (A): tillage (T1 and T2); (B): crops (Wheat, Barley and Rapeseed); (C) herbicide applications (H and NH) and their interactions on the biomass (g/m²) of *G. coronaria* determined at crop harvest in the two growing seasons. Same superscript letters (a, b and for 2017–2018; a', b' and for 2018–2019) across treatments are statistically similar according to Duncan test ($p = 0.05$).

The two-way interaction between tillage and herbicide was significant on *G. coronaria* biomass in the two growing seasons. Therefore, the biomass evolution was presented and analyzed in each crop, separately (Table 4). Overall, the accumulation of the aboveground biomass of *G. coronaria* varied among the three crops used in our field experiments (Figure 6).

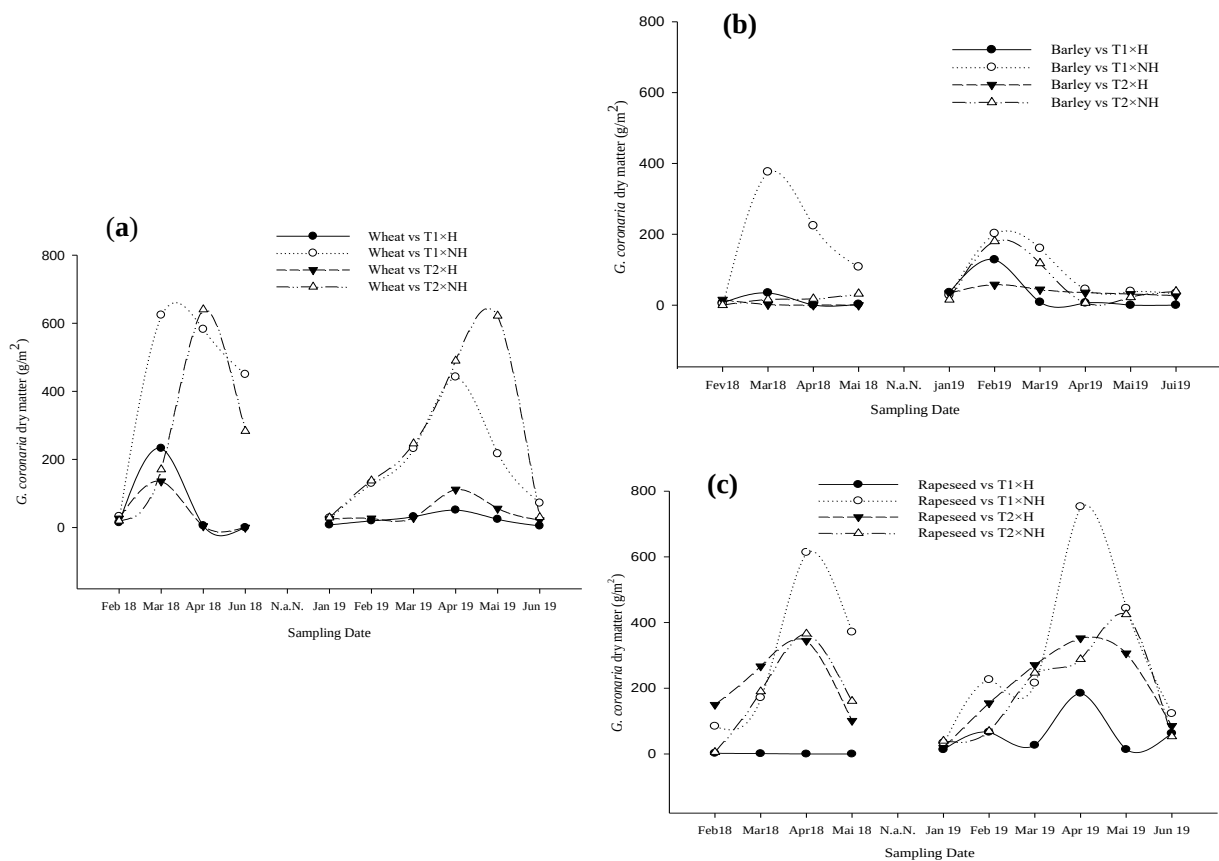


Figure 6. Effect of the interaction tillage $\times$ herbicides on the dry matter evolution of *G. coronaria* (g/m^2) throughout 2017–2018 and 2018–2019 growing seasons, (a): wheat; (b): barley; (c) rapeseed.

In the wheat plots, the biomass was significantly reduced by herbicide treatments for both seasons (Figure 6a).

During the growing season 2017–2018, a high biomass accumulation of *G. coronaria* until wheat harvest was observed in the NH plots. The highest biomass was recorded in spring (March–April 2018), being $624 \text{ g}/\text{m}^2$ and in conventional tillage (T1) and $640.3 \text{ g}/\text{m}^2$ in reduced tillage (T2). High biomasses of *G. coronaria* were also recorded at crop harvest with values ranging between $450 \text{ g}/\text{m}^2$ in T1 plots and $283 \text{ g}/\text{m}^2$ in the T2 plots. During 2018–2019, the highest weed biomasses were recorded in April–May 2019 with $442.3 \text{ g}/\text{m}^2$ and $621.7 \text{ g}/\text{m}^2$ in the T1 and T2 plots, respectively (Figure 6a).

For both growing seasons, less weed biomass in the treated plots (H), than in the non-treated plots (NH) was observed in wheat and the reductions were more important in 2018–2019. The maximum average biomass in 2017–2018 was $233 \text{ g}/\text{m}^2$ in the T1 plots, compared to $135.7 \text{ g}/\text{m}^2$ in the T2 plots. In 2018–2019, *G. coronaria* biomass was about $51 \text{ g}/\text{m}^2$ in the T1 plots compared to $111.6 \text{ g}/\text{m}^2$ in the T2 plots (Figure 6a).

In the barley plots, weed biomass was lower under the different tillage $\times$ herbicide treatments compared to the wheat plots (Figure 6b).

During 2017–2018, similar patterns of biomass accumulation were observed in the treated plots (T1 $\times$ H, T2 $\times$ H) and in the non-treated plots under reduced tillage (NH $\times$ T2). The average biomass ranged between $1.8 \text{ g}/\text{m}^2$ and $15.8 \text{ g}/\text{m}^2$ in T2 $\times$ H and T2 $\times$ NH plots, respectively. In contrast, increased biomasses of *G. coronaria* were recorded in the non-treated plots under conventional tillage (NH $\times$ T1), being $376 \text{ g}/\text{m}^2$ in March 2018 and $107.9 \text{ g}/\text{m}^2$ at harvest. In 2018–2019, higher biomasses were recorded under different treatments, except for T1 $\times$ NH treatment. Values ranged between $128.3 \text{ g}/\text{m}^2$ (T1) and $57.8 \text{ g}/\text{m}^2$ (T2) in the herbicide-treated plots and between $202 \text{ g}/\text{m}^2$ (T1) and $180.4 \text{ g}/\text{m}^2$ (T2) in non-treated plots (Figure 6b).

In the rapeseed plots, the herbicide application highly reduced the biomass of *G. coronaria* for both seasons (Figure 6c).

Under conventional tillage, *G. coronaria* was entirely controlled by herbicides in 2017–2018, and the average biomass was around 2 g/m². Under the same treatments (T1 × H), more weed biomass was recorded in 2018–2019 with maximum biomass averaged 185 g/m². In the absence of herbicide treatments (T1 × NH), weed biomass was higher during the two growing seasons and averaged 613.8 g/m² and 753 g/m², respectively.

Under reduced tillage (T2), less contrast was observed between the treated plots (H) and the non-treated plots (NH). In the T2 × H plots, the maximum weed biomass recorded were 345 g/m² and 353 g/m² in 2017–2018 and 2018–2019 growing seasons, respectively (Figure 6c).

3.4. Effect of Treatments and Their Interactions on *G. coronaria* Height in the Two Growing Seasons

The three-way ANOVA performed to investigate the effects of all treatments and their interactions on the height of *G. coronaria* at the flowering stage showed a significant effect of tillage ($p < 0.001$), crops ($p < 0.001$) and herbicide application ($p < 0.001$) in 2017–2018. However, in 2018–2019, the only significant effect was associated with herbicide application ($p < 0.001$), and neither crops nor tillage had significant effects on the height of *G. coronaria*. Significant interactions occurred only between herbicides and crops during 2017–2018 ($p < 0.001$), and no significant interactions were revealed in 2018–2019 (Table 5).

Table 5. Effect of tillage, crops and herbicide application on *G. coronaria* height (cm) at flowering stage in the two growing seasons. Results of main effect and interactions from a three-way ANOVA are also presented.

Treatments	Height (cm)	
	2017–2018	2018–2019
Main effects		
Tillage	***	NS
T1	78.3 a	71.6 a
T2	91.6 b	78.8 a
Crops	***	NS
Barley	64.2 a	66.4 a
Wheat	77.8 b	67.1 a
Rapeseed	96.2 c	76.4 a
Fallow	118.5 d	106.8 a
Herbicide	***	***
H	50.3 a	46.3 a
NH	108.4 b	93.6 b
Interactions		
Tillage × Crop	NS	NS
Tillage × Herbicide	NS	NS
Crop × Herbicide	***	NS
Barley × H	30.2 a	34.2 a
Wheat × H	38.7 a	46.3 a
Rapeseed × H	82.2 b	58.5 a
Barley × NH	98.2 c	98.7 a
Wheat × NH	116.8 d	87.8 a
Rapeseed × NH	110.2 cd	94.3 a
Tillage × Crop × Herbicide	NS	NS

Different letters indicate significant difference. Symbols indicate significance level at $p = 0.05$: *** < 0.001 and NS > 0.05 .

A comparison between the different treatments for both growing seasons showed that reduced heights were mainly associated with T1 tillage. The lowest average height of *G. coronaria* was recorded in the barley plots compared to the other crops and the non-cultivated plots (fallow).

For both growing seasons, the cumulative effect of the herbicides applied caused about 50% reduction in *G. coronaria* length compared to those in the NH plots. The average height of *G. coronaria*, was significantly the lowest in the treated plots of barley (30 cm) and wheat (39 cm), while the highest length was measured in the non-treated plots of wheat (Table 5, Figure 7). In the 2018–2019 season, even though no significant interaction was recorded, a similar trend of average weed lengths was recorded in crop $\times$ herbicide interactions. The height of *G. coronaria* was the lowest in the treated plots of barley (34 cm) followed by treated wheat (46 cm) and treated rapeseed (59 cm).



Figure 7. *G. coronaria* infestations under different crops compared to the non-cultivated plot at flowering stage, (A): herbicide-treated plot of barley; (B): herbicide-treated plot of rapeseed; (C): non-cultivated plot; (D): non-treated plot of wheat; Red arrow showed the height of wheat in non-treated plot.

4. Discussion

4.1. Effects of Tillage and Crops on the Emergence and Growth of HR *G. coronaria*

The aforementioned results suggest that the emergence patterns and the growth of *G. coronaria*, differed among the tillage practices and interspecific interactions. Higher initial *G. coronaria* densities were observed under conventional tillage (T1, 110 plants/m², on average) than reduced tillage (T2, 33 plants/m², on average) in 2017–2018, which emphasizes the

potential effect of tillage on weed emergence as well as on the weed seedbank dynamics. Indeed, it has been reported that different soil disturbance levels interact with the micro-environment of weed seeds and can influence the pattern of recruitment from the weed seed bank and the periodicity of weed emergence [17].

The soil disturbance that allows the seeds of *G. coronaria* to be more exposed to the fluctuations of temperature and humidity likely permits the weed to overcome its mechanical (physiological) dormancy imposed by its hard pericarp scaffold, resulting in a weak barrier to the water imbibition of the embryo. Thus, a higher number of fruit-coat rupture events might have happened under soil tillage leading to higher germination and emergence in T1 plots [19].

Under field conditions, *G. coronaria* emergence increases if tillage brings deep buried seeds to the surface, which is attributed to the small size of the embryo (1–2 mm), giving a small hypocotyl that cannot successfully support the emergence process if it is buried at more than 3 cm depth (data not published). According to our results, higher disturbance of the soil by deep tillage (40 cm) could bring deep buried seeds to the surface and stimulate high and simultaneous emergence. Deep tillage probably allows *G. coronaria* to form a persistent enough seed bank that each year is brought from the deep to surface layers. On the other hand, the lower initial densities and slower emergence occurred in T2 in early growing season, could be attributed to the stimulation of seeds only located at the top 5 cm layers of the soil (the layer of the “active” seed bank). Under reduced tillage, seeds are exposed to different factors (predation, less viability and higher germination) that, in the end, promotes the formation of less dense seed banks and therefore, less weed densities. In addition, the germination was progressive more likely because of the favorable conditions (mainly the available soil moisture) conserved by minimum disturbance of the soil and the high fluctuation of the temperature between night and day [20].

Among the crops, the lowest growth of the weed was associated with barley. At crop harvest, barley resulted in the lowest density (less than 20 plants/m²), minimum biomass and shorter plants of *G. coronaria*. These results suggest that the crop successfully overcame the HR plants of *G. coronaria*, by reducing its emergence and growth. On the contrary, high densities (more than 20 plants/m²) were recorded in rapeseed plots at crop harvest, likewise higher biomass and length of *G. coronaria* were associated with rapeseed. Previous studies reported differences in the patterns of the emergence and growth of weeds among phenotypically diverse crops, i.e., grass compared with broadleaf crops [21]. Each crop would result in different ecological conditions for weeds with regard to light interception, growth phenology [22] and allelopathic traits [23–25]. It has been reported that the competitiveness of barley exceeds that of wheat in controlling *Lolium rigidum* [26]. Moreover, barley has been reported as richer in allelopathic substances, such as phenolic acids, than wheat [27,28], which confer to barley an additional competitive trait to overcome weeds.

4.2. Effects of the Interactions between Treatments on Emergence Dynamics and Growth Evolution of HR *G. coronaria* under Field Conditions

The emergence of the weed and the establishment of main cohorts were variable depending on crops and tillage practices (crops × tillage). The first cohort coincided with late autumn and early winter (December–January) as a result of tillage disturbance, which occurred at the same time for all crop treatments. This cohort is likely to be the main one that may compete vigorously with the crop as previously reported in other weeds such as *Papaver rhoeas* [10], *Kochia scoparia* [29] and *Amaranthus palmeri* [30].

The magnitude, timing and the number of the next cohorts are likely influenced by the herbicide application.

In absence of any herbicide treatment, the interaction between barley and both tillage practices resulted in one main cohort, with less densities compared to rapeseed and wheat. The maximum growth of the weed was recorded early in the season and decreased over time in the barley plots. The differences in growth (density and biomass) and emergence patterns

(number of emerging cohorts) of HR *G. coronaria* among crops suggested differences in its adaptation to both physical competition (light quality and nutrient competition) and the allelopathic potential of each crop. The release of allelochemicals into the weed environment very early in the growth cycle [31], coupled with faster leaf area growth during vegetative stage [10,32], could explain low densities as well as less emergence of later cohorts within barley plots.

Overall, rapeseed had an opposite population dynamic compared to barley, in terms of highest density and timing of cohorts. Similarly, more cohorts and the highest biomass evolution of HR *G. coronaria* were recorded in rapeseed plots. In controlled conditions, rapeseed has been reported as producing allelochemicals that inhibit germination and growth of many weed species, with maximum allelochemicals released at flowering stage [25]. Our results showed that the peak of HR *G. coronaria* emergence was determined in February–March, coinciding with the flowering stage of rapeseed, which could indicate that *G. coronaria* overcame rapeseed in field conditions. The failure of rapeseed to control *G. coronaria*, observed in this study, could be explained by the poor establishment of the crop because of the high emergence rates of *G. coronaria* in the first cohort and poor competitive abilities compared to grass crops. However, the combination of rapeseed with herbicides (belonging to the groups 3, 4 and 15) controlled effectively the weed (>90%) if associated with deep tillage (Figure 3).

In general, the cumulative effect of herbicide treatments applied throughout the growing season affected the growth pattern of the weed by reducing the number of cohorts as well as the density and plant weight in each cohort. However, low efficacies were associated with the herbicide treatments florasulam + 2,4D, which partially or completely failed to effectively control the first cohort of HR *G. coronaria*. In cereal crops, the cross-resistance developed by *G. coronaria* to florasulam, even with low resistance levels [2,33] is likely to be the main cause explaining this failure. However, increased weed control (up to 80%) was reported in barley, suggesting that effective competitiveness of the crop compensated for low herbicide efficacies. Conversely, auxin herbicides resulted in effective control of the HR *G. coronaria* population, and they consistently reduced the final density of the weed in wheat, barley and rapeseed.

5. Conclusions

The results from our study showed a reduced weed control with ALS inhibitors (florasulam, in this case). However, herbicides belonging to groups 4 and 15 effectively controlled the HR *G. coronaria* population. On the other hand, the interaction between herbicide and barley crop is likely the best combination to control the weed competitiveness, resulting in low densities, low biomass accumulation and shorter plants.

This study provides important knowledge on the demographic traits and competitive ability of *G. coronaria* in different combinations of crops, tillage and herbicide modes of action. Farmers in the Bizerte region may plan an effective IWM program against HR *G. coronaria* using rapeseed or barley in crop rotation with durum wheat, and taking into consideration the history of weed infestation, previous weed management practices used and climatic features in the growing season. It is evident that further studies and experiments in a wider range of locations under a variety of climate conditions are needed to confirm our results.

Author Contributions: Z.H. and M.K. managed and directed the field trials at Fritissa farm/Mateur/Bizerte. Z.H., M.K. and T.S. designed the field experiments. Z.H. and H.J. sampled and worked on samples parameters. Z.H. analyzed the data and wrote the paper under the supervision of J.T., Y.M. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Institute of Field Crops (INGC) and a Ph.D. grant from the Ministry of Research and Higher Education of Tunisia. The article processing charge (APC) was funded the National Institute of Field Crops (INGC). Joel Torra acknowledges support from the Spanish Ministry of Science, Innovation, and Universities (grant Ramon y Cajal RYC2018-023866-I).

Data Availability Statement: Not applicable.

Acknowledgments: Special thanks to the INGC team. Authors thank also Aymen Nour for his technical assistance in field and Arbi boughanmi, Ilyess Ourari and Hajer Jeljli for their help in samplings and measurements of parameters.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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