



# Deciphering field-based evidences for crop allelopathy in weed regulation. A review

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## Abstract

It is now essential to reduce the negative impacts of weed management and especially herbicide use. Weed-suppressive crop species/varieties hold promise for integrated and sustainable weed regulation. Competition for resources and allelopathy are the two main underlying mechanisms. Unlike competition, which is well studied and established, allelopathy by living crops remains a contentious mechanism. A major difficulty to demonstrate the effects of allelopathy in the field is to dissociate them from those of competition. Here, we systematically and quantitatively review the literature, searching for field-based evidence of the role of allelopathy (by root exudation of living crops) in weed regulation, independently of competition, focusing on studies comparing different varieties of a given crop species. Our critical literature analysis also aims to identify weaknesses and strengths in methodology, providing insights on optimal experimental designs and avenues for future research. Our main conclusions are: (1) in most articles, the role of crop competition is disregarded or not exhaustively studied. Consequently, contrary to authors' conclusions, it cannot be determined whether weed regulation is due to allelopathy and/or to competition. (2) Few articles provided convincing evidence of the presence/absence of allelopathy in the field. (3) To further investigate allelopathy in the field we recommend to (i) finely characterize crop competition by measuring traits in the field, (ii) assess crop allelopathic potential with complementary experiments in controlled conditions or by quantifying allelochemicals in the field, and (iii) quantify the contribution of each studied trait/mechanism in explaining weed regulation in the field with multiple regression models. In conclusion, the consistent use of the suggested guidelines, as well as alternative approaches (e.g., creation of varieties with deactivated allelopathic functions, development of process-based simulation models), may provide a basis for quantifying the role of allelopathy in the field and, subsequently, for designing weed management strategies promoting weed biological regulation.

**Keywords** Cultivar · Genotype · Competition · Biological control · Biocontrol · Allelopathic compounds · Allelochemicals · Plant traits

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## 1 Introduction

Weeds can greatly reduce yields and harvest quality, mainly by competing with the crop for resources (Oerke 2006). Different practices can regulate or control weed dynamics, but their efficacies are not complete. This is the reason why herbicides, with their high level of efficiency and their low cost, generally play a key role in ensuring crop production in conventional cropping systems. However, reducing the use of herbicides has become necessary in view of their harmfulness for the environment and public health ([statistiques.developpement-durable.gouv.fr](http://statistiques.developpement-durable.gouv.fr)) and the technical deadlock of herbicide resistance.

The use of weed-suppressive crop species/varieties appears as a promising option (among others) to promote biological weed regulation (Andrew et al. 2015; Petit et al. 2018). Competition for resources (light, nutrients, or water) is the most frequently cited underlying mechanism and has been exhaustively studied so far (Zimdahl 2007). For example, it is established that crop canopies with a rapid soil coverage and/or high nitrogen uptake limit the growth of weeds (den Hollander 2007; Corre-Hellou et al. 2011; Schappert et al. 2019).

Allelopathy has also been suggested to play a role in weed regulation by living crops. Despite a high number of studies, allelopathy remains a controversial scientific subject in non-cultivated and cultivated areas. Although it is recognized that plants emit a large number of substances – about 200 were identified by Jabran and Farooq (2013) and Aslam et al. (2017) – the effective role of these molecules is very challenging to demonstrate (Inderjit and Del Moral 1997). Restricting the definition to crop-weed interference, allelopathy refers here to any direct harmful effect by one plant on another through the production of chemical compounds (allelochemicals) exuded by roots (Rice 1974). Several asteraceae,

brassicaceae, fabaceae, poaceae, and polygonaceae crops have been screened for their allelopathic potential against weeds, and differences between species and varieties of a given species have been reported (Wu et al. 1999; Tesio and Ferrero 2010; Jabran et al. 2015). Nevertheless, the allelopathic effect of crops on weeds has mainly been observed in laboratory conditions (Kato-Noguchi and Salam 2013), while field experiments are scarce. Characterizing allelopathy only in the lab seems insufficient. Indeed, even if a variety has shown high allelopathic potential in a laboratory experiment, the allelopathic effect can be ineffective in field conditions due to complex interactions in agroecosystems (Stowe 1979; Khanh et al. 2005). Indeed, the allelochemicals released in the environment can be lixiviated, bound and immobilized by soil organic matter and/or clay, or degraded by soil microbial communities (Blum et al. 1999; Zeng 2014).

Two main reasons explain why it is challenging to prove allelopathy in the field. (1) The effects linked to allelopathy are difficult to dissociate from those linked to competition for resources (light, nutrients, water). Plants have to be close enough (i.e., same spatial niche) so that allelochemicals released by the donor are absorbed by the receiver. But with such a proximity, plants are competing for limiting above- and belowground resources. As light is unidirectional, it is a limiting resource, and hence, the shading of one plant influences growth of neighboring plants by modifying the quantity and quality of light transmitted into the canopy (Holt 1995). Competition for nutrients and/or water may also occur, when plants are simultaneously exploiting a common limiting resource pool in a limited space. (2) While competition for light systematically occurs in canopies (as soon as leaves of neighboring plants overlap or fast-growing plants exceed their neighbors), the allelochemical production and the sensitivity of the receiving plant vary depending on the plant species, the plant stage, and the environment (Weidenhamer 1996; Inderjit and Del Moral 1997; Blum et al. 1999; Duke 2015; Jabran 2017; Mwendwa et al. 2018; Gerhards and Schappert 2020). Therefore, allelopathy and competition may occur concomitantly, making it difficult to determine whether only allelopathy by crop plants can significantly affect weed growth in the field (Fig. 1).

To preclude the effects of crop competition as much as possible, one possible option is to study the allelopathic effects of different varieties of a given crop species. Indeed, different varieties can judiciously be chosen to have (1) similar morphology and growth dynamics (attested by plant trait measurements; Violle et al. (2007)), and thus a comparable competitive effect against weeds (Andrew et al. 2015), but (2) different allelopathic properties (characterized in controlled conditions). In this situation, varietal differences in weed-suppressive effect in the field that correlate with differences in allelopathic properties can be assumed to provide evidence of allelopathy, independent of competition (Fig. 2). Alternatively, varieties can be chosen to have different trait values related to competition, in addition to



**Fig. 1** Differences in weed suppression between two winter wheat (*Triticum aestivum* L.) varieties sown at the same date and density (the left-hand variety, i.e., var. Nogal, was more suppressive than the right-hand variety, i.e., var. Renan). The contribution of allelopathy and competition in this differential weed suppression remains to be investigated. Photograph courtesy of Loïc Prieur © CREAB, 2014.

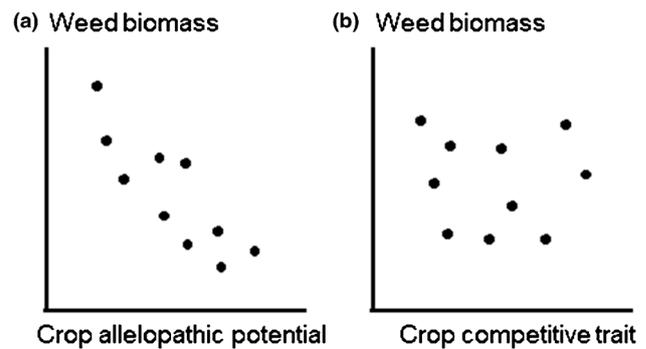
different allelopathic properties. In this case, varietal differences in weed-suppressive effects in the field that correlate with differences in allelopathic properties (Fig. 2a) but not with differences in competitive trait values (Fig. 2b) can be assumed also to provide evidence of allelopathy, independently of competition.

In the light of this, the aim of this article was to search the literature for scientific evidence of weed regulation by crop allelopathy in field conditions. A focus was made on studies comparing different varieties of a given species, in order to disentangle the effects of allelopathy from those of competition. We restricted our analysis to root exudation by living plants (excluding studies on allelopathy by crop residues). Our approach consisted in performing a systematic review. Contrary to the narrative review aiming at discussing a broad range of issues within a given topic, a systematic review consists in conducting an exhaustive search of all the articles falling in the scope of the research topic (Collins and Fauser 2005). This approach allowed us to provide an exhaustive, reproducible, and also a quantitative and critical analysis of the literature. The identification of weaknesses and strengths in the methodological aspects related to the characterization of allelopathy in field conditions allowed to provide insights into the optimal experimental design and avenues for future research.

## 2 Materials and methods

### 2.1 Literature search

A first stage identified a corpus of articles related to the research topic. Articles were collected by searching the Web

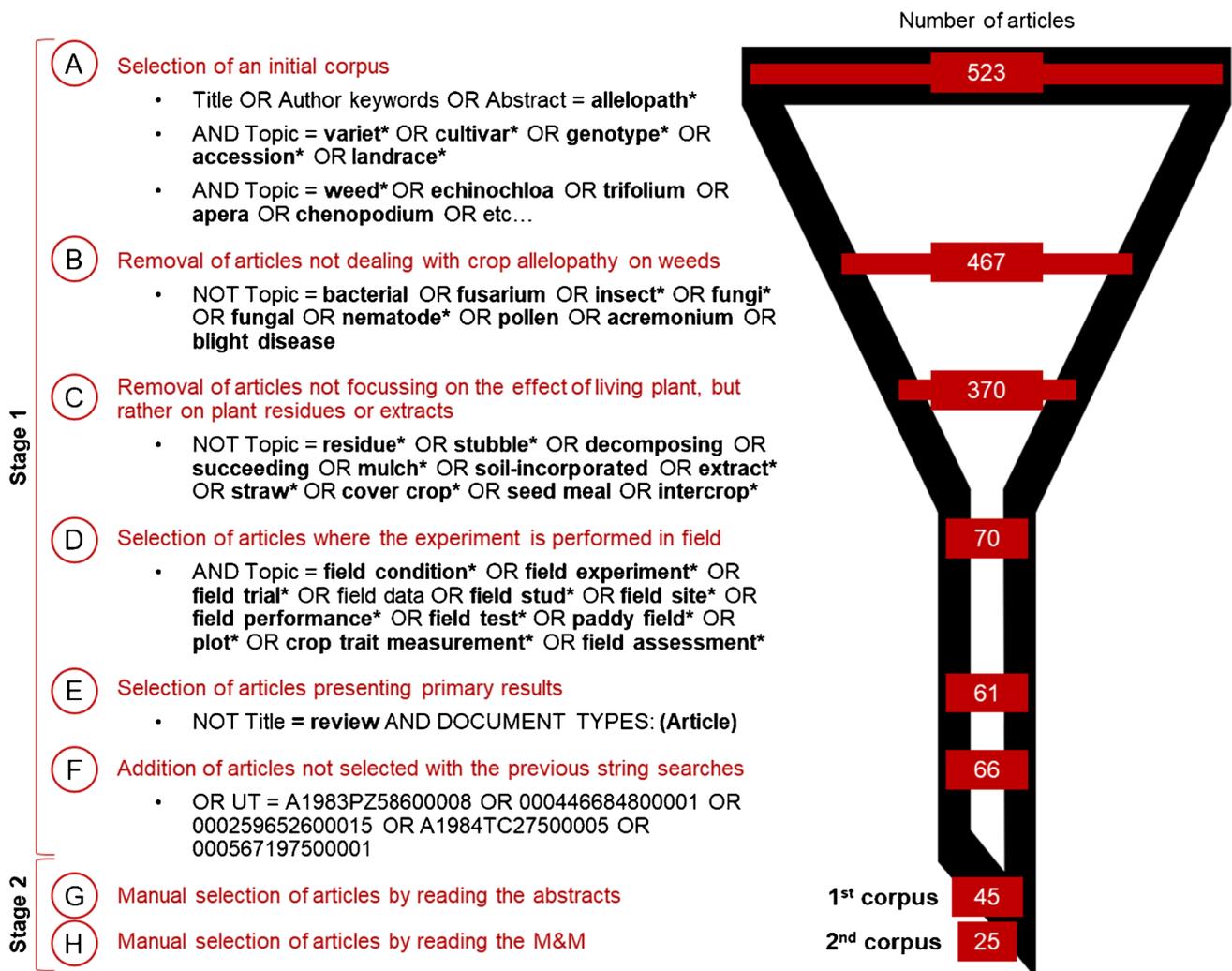


**Fig. 2** Schematic example of convincing evidence of the role of crop allelopathy (independently of competition) in weed regulation by comparing different varieties of the same crop species. Correlation between weed biomass and the crop allelopathic potential (a), without any correlation between weed biomass and the crop competitive trait values (b). Correlations are calculated independently for several crop competitive traits. One point represents one crop variety. For both situations described in the Introduction section – varieties with similar or contrasting crop competitive trait values – both figures (a) and (b) are applicable, with only a difference in the X-axis scale for crop competitive trait values (i.e., larger range of values when considering varieties with contrasting competitive crop traits).

of Science from 1956 onwards (last date of search: March 2021). A preliminary search was carried out to define the terms related to the research question. Articles studying the allelopathic effect of different varieties on weeds were selected when containing the terms “allelopath\*”, “variet\*”, or a synonym (“cultivar\*”, “genotype\*”, “accession\*”, etc.), and “weed\*” (Fig. 3, step A). We associated a list of several weed species to catch articles where the term “weed” was not used. We focused on non-parasitic weeds, as they represent the majority of weeds in arable crops. Articles not directly related with our search question were first removed by excluding a list of terms corresponding to organisms that can induce allelopathic effects on weeds (e.g., “bacterial”, “insect\*”, and “fungal”) (Fig. 3, step B). As we focused on the allelopathic effect induced by living plants only, articles were excluded when the title contained terms related to residues or plant extracts (Fig. 3, step C). Articles were then limited to experiments performed in the field, by including terms such as “field condition\*”, “field stud\*”, or “plot\*” (Fig. 3, step D). At each step, title and abstract of the removed articles were briefly screened to check that they did not fall in our scope. Finally, reviews were excluded, and five articles that could not be extracted with the search query (articles selected by the first search strings but deleted at some subsequent steps, or articles without abstract or keywords) were manually added to the corpus (Fig. 3, steps E and F).

### 2.2 Screening of the search results

A second stage screened all the articles by reading their abstract to select those that answer our research question.



**Fig. 3** Representation of the search strings and the number of articles retained. The “Topic” field returns articles selected either by title, abstract, author keywords, or keywords Plus (keywords automatically generated and added by Web of Science).

Firstly, articles not related to our question, despite the positive wording with the search query, were discarded. This corpus was termed hereafter “first corpus” (Fig. 3, step G). Secondly, as we were interested in allelopathic effects on weeds and in discriminating allelopathy from resource competition, the articles had to provide quantitative data on weed pressure and competitive traits of the crop. So, papers were removed when weed pressure was not quantified (neither weed biomass nor density), or when competition was disregarded, not studied, or not presented with quantitative data. This corpus was termed hereafter “second corpus” (Fig. 3, step H). The whole two-stage procedure (Fig. 3) was repeated with the SCOPUS database (instead of Web of Science), but this did not yield any additional articles in the second corpus.

### 2.3 Detailed analysis of the articles belonging to the second corpus

Detailed information was retrieved from the articles of the second corpus, related to the studied crop and weed species, the experimental design, the pedoclimatic conditions, the plant measurements, the results, and the authors’ conclusions. This information is gathered in Supp.Mat. 1. Some data of the experiment of Kashif et al. (2015) cited in the retrieved article of Kashif et al. (2016) was also included. Available quantitative data was extracted whenever possible from the papers, and when needed, authors were contacted to provide more data. Whenever possible, we analyzed data from each article graphically and/or statistically to investigate relationships between weed infestation on the one hand, and

competitive traits and allelopathic potential of the crop on the other hand. For each article, we quantified the level of variation of each trait (e.g., crop height, leaf area index) between varieties with the formula:

$$\text{Variation (\%)} = 1 - \frac{V_{\min}}{V_{\max}} \times 100 \quad (1)$$

where  $V_{\min}$  and  $V_{\max}$  are the values of the measured crop trait for the variety having the lowest and the highest value, respectively.

If no correlations were provided in the article, the correlations between the weed infestation and the different measured variables (crop competitive traits or allelopathic potential), as well as their significance, were calculated using Pearson's correlation coefficients with the function `cor.test()` (R Core Team 2017).

### 3 Results and discussion

#### 3.1 Characterization of past research on the topic

##### 3.1.1 Only a few field studies explicitly consider competition in addition to allelopathy

Analyzing the main bottlenecks in the corpus selection provided an illustration of the main weak points in the past research. Among the different search strings of the query (Fig. 3), the “field experiment” criterion was the most restrictive one, reducing the number of articles by 81% (step D). This finding confirms quantitatively that, in most of the studies on allelopathy, experiments were performed in controlled conditions and were thus out of scope of this review.

Another key point was the bottleneck from the first to the second corpus, reducing the number of papers by approximately 45% (Fig. 3, step H). Despite the necessity to differentiate allelopathy from competition (see “Introduction” section), about one-third of the articles of the first corpus ignored competition by crop (orange section in Fig. 4). Surprisingly, for most of them, authors assumed that allelopathy was the only mechanism explaining the variation in weed infestation between crop varieties, which is unlikely (see “Introduction” section). In a few articles, the authors deemed that the tested crop varieties have similar morphological traits (and therefore competition trait values) without, however, any quantitative data to support their statements. Thus, our analysis highlighted the difficulty in explicitly considering both competition and allelopathy in field studies. A few articles (grey section Fig. 4) did not present any quantitative data on weed pressure, which does not allow to compare crop varieties on that response variable. Overall, only 25 articles studied the differential effects of crop varieties on

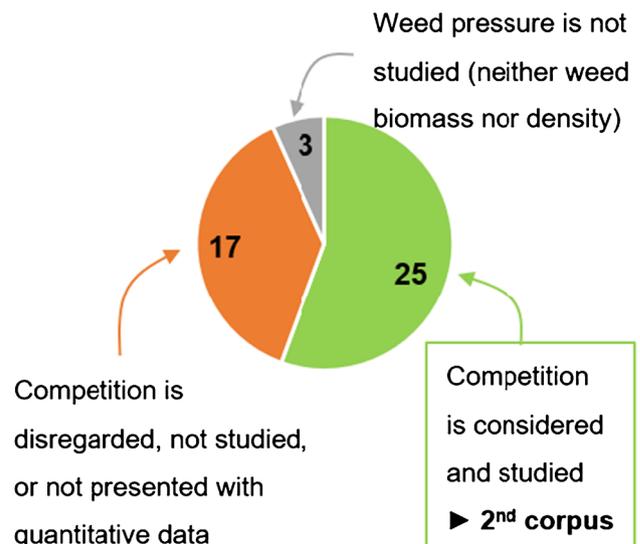


Fig. 4 Classification of the 45 articles of the first corpus based on step H (Fig. 3).

weed pressure, explicitly considering allelopathy and competition (green section in Fig. 4). Thereafter, only this second corpus was analyzed.

##### 3.1.2 The main features of the studies within our scope

The analysis of the characteristics and methodologies used in the 25 retrieved articles provided an overview on the research conducted on allelopathic crop varieties targeting weed regulation.

**When, where and which species?** Studies were published between 1994 and 2020, with an increase in publication since 2009. Crop allelopathy appears to be a worldwide topic, as experiments have been carried out in countries from all continents (e.g., Australia, Denmark, China, Pakistan, and USA). The most common crop species studied by far is rice (*Oryza sativa* L.), followed by wheat (*Triticum aestivum* L.) (Fig. 5a), which is in agreement with the review of Belz (2007). From 2 to 5000, crop varieties were tested in each experiment (median of 7).

**Which characterization of weed infestation?** Weeds were seeded in the experimental plots in about 80% of the studies. Otherwise, weeds were from spontaneous infestations.

The levels of weed infestation were assessed mainly via aboveground weed biomass (Fig. 5c). In some studies, a visual estimation was performed, either using a score (Asaduzzaman et al. 2014) or a rating comparing the weed infestation with a crop-free control plot (Gealy and Duke 2017).

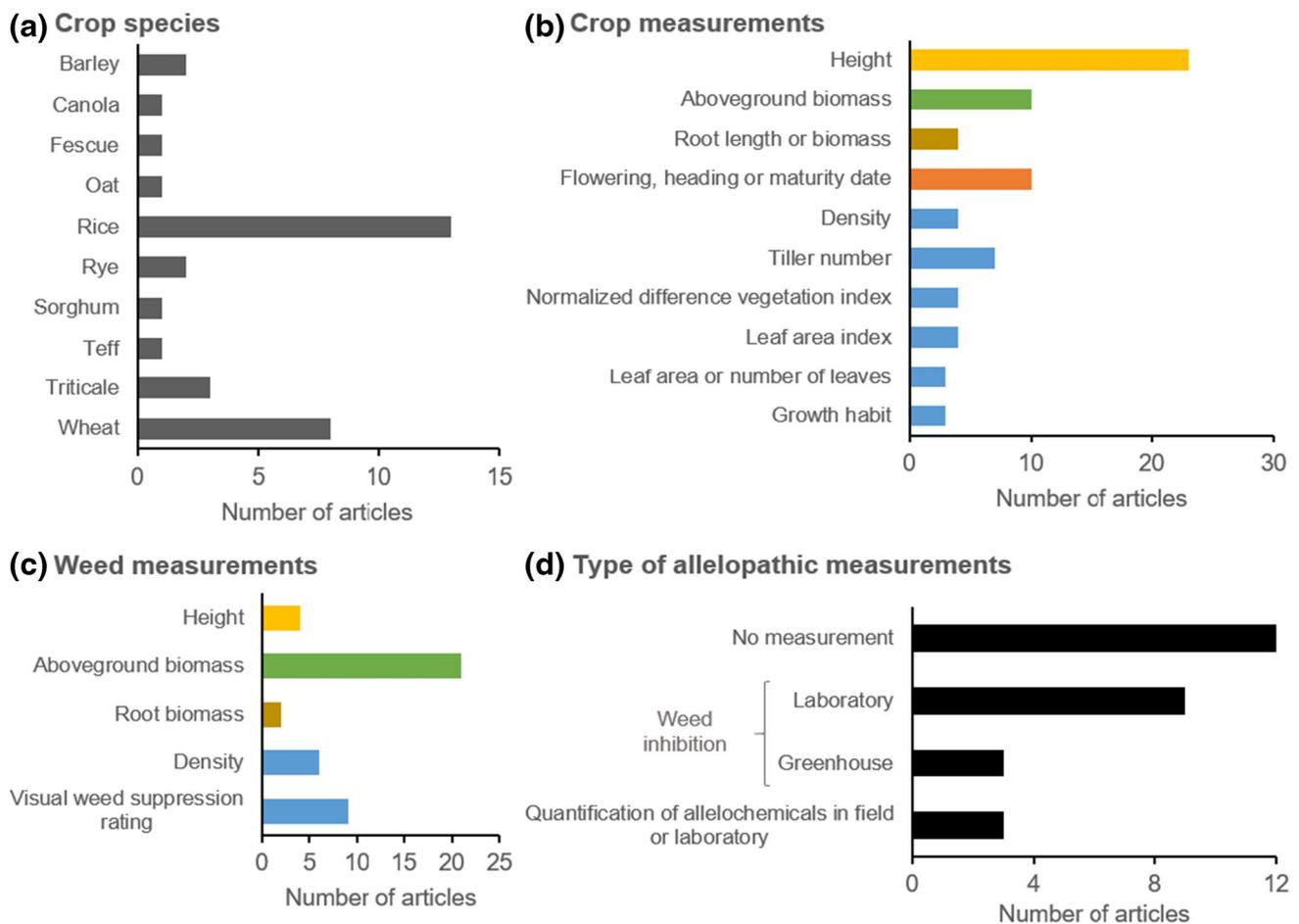
**Which characterization of crop allelopathic effect?** No measurements of allelopathy were made in half of the articles, neither in the field nor in the laboratory. Most of the authors have hypothesized that weed infestation not explained by competitive traits can, by default, be attributed to allelopathy, which is a strong assumption.

To characterize allelopathic effects, complementary experiments or measurements were done in 13 articles (Fig. 5d), most commonly in laboratory, mainly with the “agar-based bioassay” method (Bertholdsson 2011). This method consists in growing weed seedlings with (treatment) and without (control) crop seedlings in a nutrient-free agar media and comparing weed growth parameters in both situations (usually root length after 7–10 days of co-culture). An inhibition percentage is then calculated using the formula:

$$\text{Inhibition (\%)} = 1 - \frac{\text{treatment}}{\text{control}} \times 100 \quad (2)$$

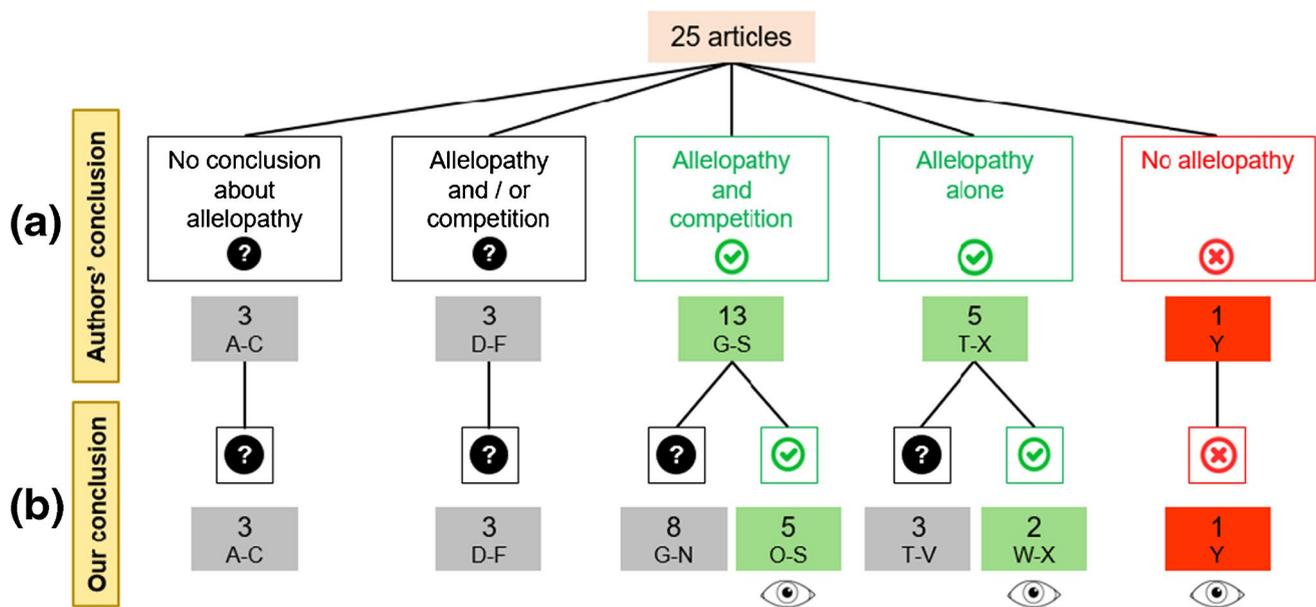
where treatment and control are the trait values of weed seedlings (root length, area or biomass, or shoot length, depending on the article) measured, respectively, in the presence and absence of crop seedlings.

Some greenhouse experiments were a simple co-culture of crop and weed in a same pot (Junaedi et al. 2012). After 2 weeks of co-culture, allelopathic activity was then calculated using Eq. 2, with the control treatment without crop. Nevertheless, one limitation of this approach is that competition for resources cannot be totally excluded. To overcome this problem, an interesting experimental design has been developed: the “staircase device”, where weed and crop are grown in separate pots with the leachates of the crop pot irrigating the weed pot (Al-Bedairy et al. 2013). The device enables the transfer of crop allelochemicals to weeds with the exclusion of competition for light, although competition for nutrients can occur if nutrients are limiting. Weed growth is then compared with a control device where the crop pot



**Fig. 5** Main characteristics of the 25 articles of the second corpus. Studied crop species (a), measurements on crop varieties (b), and weeds (c) and type of allelopathic measurements made in addition to field experiments (d). Crop and weed measurements are grouped by:

aboveground vertical occupation (yellow bars), aboveground biomass production (green bars), root distribution (brown bars), plant phenology (orange bars), and ground cover (blue bars).



**Fig. 6** Consistency between the authors' conclusions (a) and our conclusions (b) about evidence of crop allelopathy in weed regulation for the 25 articles (second corpus). Figures represent the number of articles, divided into: absence of evidence of allelopathy (black question mark), evidence of the absence (red cross) and the presence (green check) of allelopathy. Capital letters refer to the articles (Supp.Mat. 1): A (Gealy et al. 2003), B (Gealy et al. 2014), C (Gealy et al. 2019), D (Gealy and Duke 2017), E (Gealy and Moldenhauer 2012), F (Seavers and Wright 1999), G (Asaduzzaman et al. 2014), H

(Bertin et al. 2009), I (Gealy et al. 2013a), J (Gealy et al. 2013b), K (Gebrehiwot et al. 2020), L (Khanh et al. 2009), M (Olofsdotter et al. 1999), N (Pheng et al. 2009), O (Al-Bedairy et al. 2013), P (Bertholdsson 2005), Q (Bertholdsson 2011), R (Reiss et al. 2018a, b), T (Dilday et al. 1994), U (Junaedi et al. 2012), V (Kong et al. 2011), W (Bertholdsson 2010), X (Kashif et al. 2016), Y (Worthington et al. 2015). Categories of articles followed by an eye icon are further analyzed in Section 3.3.

is empty (filled only with the soil media), but still transferring excessive fluids from the crop-free pot to the weed pot.

In three experiments, allelochemicals released by crop varieties were quantified in field soil or in crop plants (i.e., stems and roots) grown in field or in laboratory, by spectrophotometry (Kashif et al. 2016) and mass spectrometry (Reiss et al. 2018a, b).

**Which characterization of crop competitive effects?** The competitive ability of varieties against weeds was evaluated by the measurements of traits (hereafter termed as competitive traits). Measured competitive traits differed among studies, but crop height was measured in nearly all studies (Fig. 5b). The aboveground soil cover was assessed in 15 out of 25 studies by measurements of tiller number for graminaceous species or leaf area index (blue bars in Fig. 5b). Less frequently, the aboveground biomass or root biomass and length were studied.

On average, crop competitive ability was assessed by two to three different traits per study, often belonging to different categories in Fig. 5b. These results highlight that crop competition is often not exhaustively studied, and it may not be possible to characterize and compare the competitive ability of varieties with so few measurements. It is noteworthy that

these competitive traits could be measured at different crop development stages: early stage, flowering, or harvest. Furthermore, in only nine articles (Supp.Mat. 1), the competitive crop traits were measured in weed-free plots, indicating competitive potential against weeds (also called “effect traits” (Violle et al. 2007)). When competitive traits are measured in weedy plots (i.e., in the 16 remaining articles), they are the result of an interference between crop and weeds and are therefore influenced by the presence of weeds and the tolerance of the variety to the presence of weeds (they are also called “response traits”).

### 3.1.3 What did authors conclude?

We classified authors' conclusion from the 25 articles in five categories (Fig. 6a). Five articles stated that allelopathy alone was occurring and explained the observed differences in weed infestation between crop varieties. This conclusion was nuanced in three other articles, where authors concluded that the observed differences in weed infestation among varieties can be due to allelopathy and/or competition. Authors considered that both mechanisms occurred in 13 articles. In only one article, authors demonstrated the absence of allelopathy, namely that competition alone

**Table 1** Main weak/strong points of the 17 articles represented in grey squares in Fig. 6b that failed to provide convincing evidence of the presence or the absence of allelopathy. Weak points to demonstrate allelopathic effect are represented in red and strong

points in green. For the first two columns Yes means that the measure was done, and for the last column Yes means that at least one correlation was significant (\* figures are not congruent with the corresponding text).

Reference	Early competitive traits measurements	Soil coverage measurements	Significant correlation between some competitive traits and weed infestation
Gealy et al. (2014)	Yes	Yes	Yes
Gealy et al. (2019)	Yes	Yes	Yes
Gealy et al. (2013a)	Yes	Yes	Yes
Olofsdotter et al. (1999)	Yes	Yes	Yes
Seavers and Wright (1999)	Yes	Yes	Yes
Bertin et al. (2009)	No	Yes	No
Gealy and Duke (2017)	No	Yes	Yes
Gealy et al. (2013b)	No	Yes	Yes
Gebrehiwot et al. (2020)	No	Yes	?*
Dilday et al. (1994)	No	No	No
Gealy and Moldenhauer (2012)	No	No	No
Junaedi et al. (2012)	No	No	No
Khanh et al. (2009)	No	No	No
Kong et al. (2011)	No	No	No
Pheng et al. (2009)	No	No	No
Asaduzzaman et al. (2014)	No	No	Yes
Gealy et al. (2003)	No	No	Yes

explained the differences in weed infestation between crop varieties. Finally, for three articles (on the left-most square in Fig. 6a), no conclusion about allelopathy was presented. Indeed, for these articles, authors had objectives different from ours (e.g., comparison of different cropping systems (Gealy et al. 2003, 2019)).

### 3.2 Only few articles characterize allelopathic effects independently of competition

We analyzed which of the 25 articles really provided convincing evidence of the presence or the absence of allelopathy, independently of competition (Fig. 6b). We logically did not find any support of evidence of allelopathy in the six articles where the authors either were unable to differentiate allelopathy from competition or did not conclude about allelopathy (two leftmost squares in Fig. 6b). Among the 18 articles reporting an allelopathic effect (combined with competition or alone; green squares in Fig. 6a), we identified insufficient evidence to support this conclusion in 11 cases (articles G-N and T-V, Fig. 6b). Overall, three main reasons did not make it possible to conclude on an effect of allelopathy for the 17 articles represented in grey squares in Fig. 6b (Table 1). Two reasons were methodological. (1) There were no measurements of soil coverage by crop varieties (8 articles), which is an important trait describing the crop competitiveness for light. (2) No competitive traits were measured at early crop

stages (12 articles), although early vigor has been shown to be instrumental in variety competitiveness against weeds (Andrew et al. 2015; Mwendwa et al. 2020). As shown by Worthington et al. (2015), the effect of one competitive trait can be missed if not measured at the right stage (i.e., one trait can be correlated with weed infestation at a specific crop stage, but not before or after). (3) The third reason was related to the findings. For some articles, we identified a correlation (presented by the authors or based on our own analysis of their data) between some competitive traits of the crop and weed infestation (9 articles). As allelopathy and competition could not be differentiated, we were unable to conclude on allelopathy. Four articles made exception (Bertholdsson 2005, 2011; Reiss et al. 2018a, b), by using multiple regression models based on both allelopathic and competitive traits of the crop varieties to explain differences in weed infestation. This statistical approach helped to dissociate allelopathic and competitive traits. According to our analysis, only seven and one articles provided evidence of the presence and the absence of allelopathy, respectively (green and red squares Fig. 6b).

These differences of conclusion between our analysis and authors' analysis are partly explained by differences of objectives. Some studies focused on the overall performance of crop varieties (not aiming to discriminate competition from allelopathy) and set up breeding programs to develop new varieties with enhanced weed suppression and high yield (e.g. Kong et al. 2011).

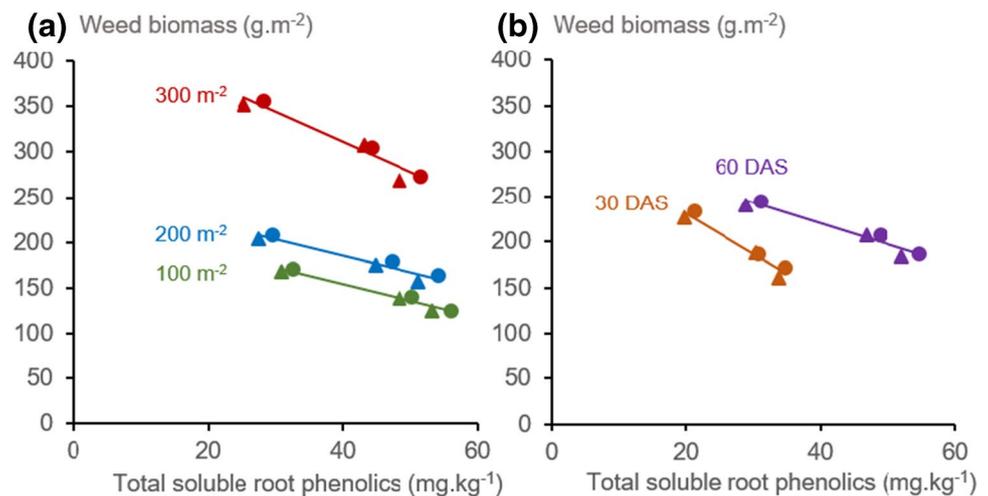
**Table 2** Overview of the characteristics of the 8 articles providing convincing evidence of the presence or the absence of allelopathy, regarding the field experiment, the allelopathic potential assessment and the competitive traits measured. The “convincing evidence” column refers to the two categories of articles described in Section 3.3. <sup>1</sup>Methods are described in Section 3.1.2.3.; <sup>2</sup>mixture of *Trifolium pratense*, *T. repens*, *Phleum pratense*, *Festuca pratense*, and *Lolium perenne*; <sup>3</sup>first year/the following 3 years

	Experimental design			Allelopathic potential			Competitive traits			Approach used to dissociate allelopathy and competition		
	Number of varieties	Number of study sites	Study duration (years)	Weed species studied in field	Complementary experiment in controlled conditions	Quantification of allelochemicals	Number of competitive traits measured	Competitive traits measured in weed-free plots	Early competitive traits studied	Ground cover studied	Varieties with similar competitive traits	Use of multiple regression models
Spring wheat	3	1	2	<i>Phalaris minor</i>	agar-based bioassay <sup>1</sup>	phenolic compounds	2	x	x	x	x	x
Bertholdsson 2005	20	1	2	spontaneous	agar-based bioassay <sup>1</sup>		3		x			x
Bertholdsson 2010	5	1	2	mixture of grasses and clover <sup>2</sup>	agar-based bioassay <sup>1</sup>		2		x			x
Reiss et al. 2018a	10	1	1	<i>Chenopodium album</i> , <i>Avena fatua</i> , <i>Sinapis alba</i>		group of benzoxazinoids	3			x		x
Winter wheat	12	1	2	<i>Apera spica-venti</i>	agar-based bioassay <sup>1</sup>		4		x	x		x
Reiss et al. 2018b	4	2	2	spontaneous		group of benzoxazinoids	3			x		x
Worthington et al. 2015	8	2	2	<i>Lolium multiflorum</i>	agar-based bioassay <sup>1</sup>		5	x	x	x		x
Spring barley	20/12 <sup>3</sup>	1	4	spontaneous	agar-based bioassay <sup>1</sup>		3		x			x
Rye	4	2	2	spontaneous		group of benzoxazinoids	3			x		x
Sorghum	2	1	2	spontaneous	staircase device <sup>1</sup>		3			x	x	x
Spring triticale	2	1	1	<i>Chenopodium album</i> , <i>Avena fatua</i> , <i>Sinapis alba</i>		group of benzoxazinoids	3			x		x
Winter triticale	4	2	2	spontaneous		group of benzoxazinoids	3			x		x

**Table 3** Variation of the competitive traits, the allelopathic potential and the weed infestation between varieties for the eight articles providing convincing evidence of the presence or the absence of allelopathy. The variations were calculated with Eq. 1. Articles studying different crop species were divided into several rows. Blank cell: trait not measured in the study, nd not determined ('nd' is mentioned in the articles); NA not available (neither calculated by authors nor calculable from retrieved data); \* significant at  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ ; NS not significant. <sup>1</sup>Crop biomass and early shoot length were measured 1–2 weeks before heading in Bertholdsson (2005), 2 weeks before heading in Bertholdsson (2010), and at early stem extension in Bertholdsson (2011) and crop biomass was measured at harvest in Al-Bedairy et al. (2013) and Reiss et al. (2018a). <sup>2</sup>Leaf area index (green leaf area per unit ground area); <sup>3</sup>normalized difference vegetation index (a proxy for vigor and vegetation amount, and calculated from differences in light canopy reflectance); <sup>4</sup>weed biomass with the staircase device (detailed in Section 3.1.2.3.); <sup>5</sup>weed inhibition percentage in laboratory bioassay calculated with Eq. 2; <sup>6</sup>concentration of allelochemical compounds in crop or soil; <sup>7</sup>no information about the level of significance; <sup>8</sup>variation is not calculated from weed inhibition percentage data but from raw data on root and shoot length/biomass; <sup>9</sup>one variety (that was an influential variety, see Supp.Mat.2) was removed, so the analysis was performed with 4 varieties instead of 5; <sup>10</sup>only one replicate available; <sup>11</sup>the significance was calculated including two triticale and two rye varieties, that have distant values from those of wheat, explaining the high level of significance; <sup>12</sup>fall/spring; <sup>13</sup>averaged across the two study sites; <sup>14</sup>pooled sites: Caswell 2012, Piedmont 2012, and Caswell 2013; <sup>15</sup>weed seed heads  $m^{-2}$  (which have been reported to be correlated to the ratio between weed biomass and wheat biomass in a previous experiment (Worthington et al. 2013)) instead of weed biomass; <sup>16</sup>the difference is significant if two varieties with exceptional high variance are removed; <sup>17</sup>averaged across the three planting densities of sorghum (6.6, 13.3, and 26.6 plant. $m^{-2}$ )

	Variation in competitive traits of crop										Variation in allelopathic potential				Variation in weed infestation	
	Biomass <sup>1</sup>	Early shoot length <sup>1</sup>	Final plant height	Tiller number	LAI <sup>2</sup>	NDVI <sup>3</sup>	Root length	Root biomass	Staircase device <sup>4</sup>	Agar-based bioassay <sup>5</sup>	Allelopathic compounds in crop <sup>6</sup>	Allelopathic compounds in soil <sup>6</sup>	Density	Biomass		
Spring wheat																
Kashif et al. 2011		4% * <sup>7</sup>	40% ***	NS					46% <sup>8</sup> * <sup>7</sup>	42% * <sup>7</sup>	20% * <sup>7</sup>			26% * <sup>7</sup>		
2012		3% *	3% *	2% NS						39% *	18% *			26% *		
Bertholdsson 2001	50% ***	18% *	22% nd						44% ***					66% *		
2005	38% **	22% NS	21% ***						68% ***					74% **		
Bertholdsson 2007	26% NS	10% NS	16% *						53% ***					21% NS		
2010	34% NS	6% NS	18% NS											37% *		
Bertholdsson 2007	26% NA	10% NA	16% NA						51% NA					21% NA		
2010 <sup>9</sup>	31% NA	6% NA	18% NA											37% NA		
2017		40% ***	40% ***		49% ***	9% ***				84% NA <sup>10</sup>	85% NA <sup>10</sup>			100% NS		
Reiss et al. 2018a																
Winter wheat																
Bertholdsson 2011	34% *** <sup>11</sup>	38% *** <sup>11</sup>	30% *** <sup>11</sup>		16% <sup>12</sup>	29% <sup>12</sup>			35% *** <sup>11</sup>					41% *** <sup>11</sup>		
2009	49% *** <sup>11</sup>	31% *** <sup>11</sup>	23% *** <sup>11</sup>				21% *** <sup>11</sup>							45% *** <sup>11</sup>		
Reiss et al. 2016	22% NS	22% NS	31% NS		12% ***	4% NS					68% NS			55% NS		
2018b <sup>13</sup>	NA	17% ***	22% ***		16% ***	14% ***				57% NA <sup>10</sup>	40% NS			73% *		
Worthington et al. 2015		NA	NA		NA	NA			80% * <sup>7</sup>				18% NS	27% <sup>15</sup> * <sup>7</sup>		
Spring barley																
Bertholdsson 1999	51% **		38% **						33% ***					73% NS <sup>16</sup>		
2000	40% *	43% ***	28% **						26% ***					61% NS <sup>16</sup>		
2001	44% ***	43% ***	37% ***						30% ***					50% NS <sup>16</sup>		
2002	35% *	12% NS	13% NS						52% ***					63% NS <sup>16</sup>		
Reiss et al. 2016	20% NS	11% NS	10% NS		19% ***	6% ***					73% NS			34% NS		
2018b <sup>13</sup>		6% **	3% ***		4% NS	11% NS				26% NA <sup>10</sup>	39% NS			86% NS		
Sorghum																
Al-Bedairy et al. 2013 <sup>17</sup>	9% NS	0% NS	0% NS		9% NS								25% NS	44% * <sup>7</sup>		
2010	8% NS	10% NS	10% NS		9% NS								23% NS	30% * <sup>7</sup>		
Reiss et al. 2018a	NA	11% **	11% **		3% NS	10% ***				10% NA <sup>10</sup>	5% NA <sup>10</sup>			59% NS		
Winter triticale																
Reiss et al., 2016	7% NS	28% NS	26% ***		22% ***	9% ***					64% NS			33% NS		
2018b <sup>13</sup>	NA	14% ***	19% ***		9% ***	3% NS				38% NA <sup>10</sup>	32% NS			84% NS		

**Fig. 7** Correlations between weed biomass (*Phalaris minor* Retz.) and the phenolic compounds of the three wheat varieties (a) measured at 60 days after sowing (DAS) for three sowing densities of *Phalaris minor* Retz. (100, 200 and 300 m<sup>-2</sup>) and (b) for two sampling dates (30 and 60 DAS) averaged between the three sowing densities. First year of the experiment in triangles and second year in circles. All the regressions were significant with *p* values < 0.05. Data extracted from Kashif et al. (2016).



### 3.3 Some convincing evidence of the presence/absence of allelopathy

The eight articles providing convincing evidence of the presence or absence of allelopathy (articles O-S, W-X, and Y in Fig. 6) can be separated into two categories. (1) Articles comparing the suppressive ability of crop varieties having similar morphological traits (attested by trait values) and different allelopathic properties. Variety competitiveness is hypothesized to be similar; thus, the observed differences in weed biomass can be attributed to allelopathy. (2) Articles comparing varieties differing in competitive traits and using correlations or multiple regression models to assess the contribution of competition (through field traits measurements) and allelopathy (through quantification of allelochemicals, laboratory bioassays or greenhouse experiments). Table 2 summarizes the convincing evidence of the presence or the absence of allelopathy for the eight articles and discusses their methodological strengths and weaknesses. For example, for some articles, the plot size was quite small, or the crop competitive ability was characterized by only a few traits, questioning the validity of the results (see Supp. Mat. 2 for more details on each of the eight articles). The methodological biases of the allelopathic measurements are presented in Supp. Mat. 3. Note that, unfortunately, we were not able to find crop varieties common to different studies, which would have allowed a comparison between studies and strengthened evidence in case a given variety was identified as allelopathic in independent studies.

#### 3.3.1 Articles comparing crop varieties with similar competitive traits

Two articles fell into this category. In the experiments of Al-Bedairy et al. (2013) and Kashif et al. (2016), we considered that varieties with similar morphological traits were

compared, since the variation in competitive traits was not significant or very low (Table 3). In addition, the varieties differed significantly by their allelopathic potential. Finally, the variation in weed biomass was significant between varieties, suggesting that allelopathy may be the main mechanism explaining the difference in crop-weed interference.

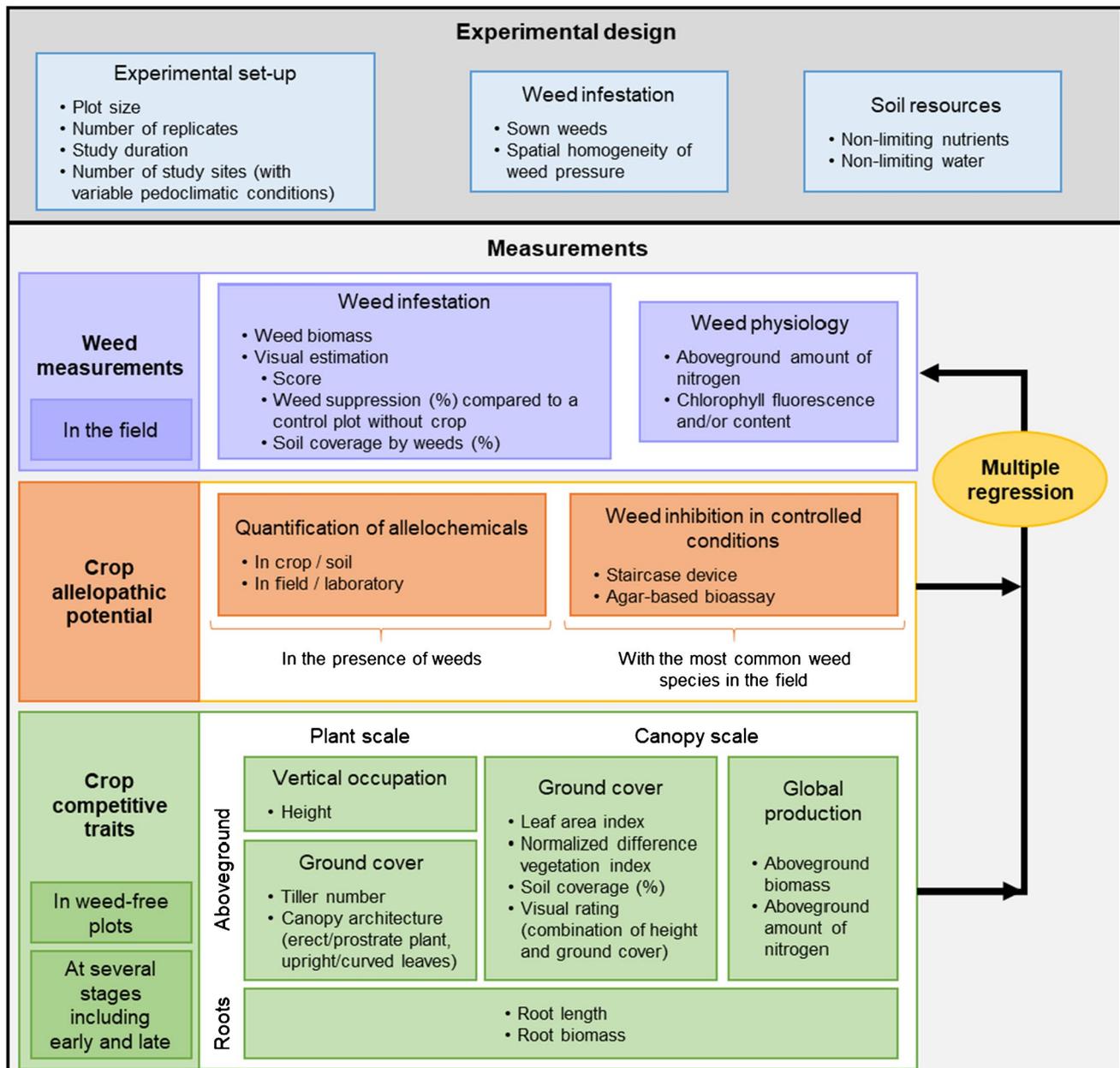
Al-Bedairy et al. (2013) compared two sorghum varieties (*Sorghum bicolor*) at different sowing densities. They showed that the most weed suppressive variety in the field (i.e., having the lowest weed biomass for each sowing density) was also the most weed-suppressive one in the staircase device (i.e., having the lowest biomass of *Portulaca oleracea*, a species observed in the field) (Supp. Mat. 2), suggesting the role of allelopathy in weed suppression.

Kashif et al. (2016) compared three wheat varieties (*Triticum aestivum* L.). They showed that the varieties were similarly ranked for both their weed suppression and allelochemical production in the field as well as laboratory experiment. The variety having the lowest weed biomass (*Phalaris minor* Retz.) in the field also had the lowest root and shoot length or biomass of weed plants (again *Phalaris minor* Retz.) in the agar-based bioassay. This variety also had the highest content of allelochemicals (namely phenolic compounds) in roots and soil assessed in the field experiment and in roots and stems assessed in the laboratory experiment (Supp. Mat. 2). Finally, weed biomass in the field was highly correlated to the allelochemical content in roots and soil for each of the sowing densities of *Phalaris minor* Retz. (Fig. 7a) and sampling dates (Fig. 7b). All these results tend to show the role of allelopathy in weed suppression.

However, both articles suffered from an insufficient characterization of the competitiveness of crop varieties: only a few competitive traits were measured and none at early stages, so that the similarity of crop-variety morphology may be questioned (Table 2). In addition, no measurements were made to ensure that soil resources were not limiting,

**Table 4** Correlations between weed biomass and the competitive traits or the allelopathic potential of the crop varieties for the 8 articles providing convincing evidence of the presence or the absence of allelopathy (cells shaded grey when correlations were not presented in the article, but calculated using the available data). For Worthington et al. (2015) the correlations were made with the weed seed heads.m<sup>-2</sup> instead of the weed biomass. Articles studying different crop species were divided into several rows. Blank cell: trait not measured in the study; nd not determined ('nd' is mentioned in the articles); NA not available (neither calculated by authors nor calculable from retrieved data); \* significant at  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ ; NS not significant. <sup>1</sup>Crop biomass and early shoot length were measured 1–2 weeks before heading in Bertholdsson (2005), 2 weeks before heading in Bertholdsson (2010) and at early stem extension in Bertholdsson (2011) and crop biomass was measured at harvest in Al-Bedairy et al. (2013) and Reiss et al. (2018b); <sup>2</sup>leaf area index (green leaf area per unit ground area); <sup>3</sup>normalized difference vegetation index (sensitive to the vigor and quantity of vegetation, and calculated from differences in light canopy reflectance); <sup>4</sup>visual estimation (1 for erect plant and 9 for prostrate plant); <sup>5</sup>combination of plant height and ground cover (1-to-9 scale with 1 the most vigorous plant); <sup>6</sup>weed biomass with the staircase device (detailed in Section 3.1.2.3.); <sup>7</sup>weed inhibition percentage in laboratory bioassay calculated with Eq. 2; <sup>8</sup>concentration of allelochemical compounds in crop or soil; <sup>9</sup>one variety (that was an influential variety, see Supp.Mat.2) was removed, so the analysis was performed with 4 varieties instead of 5; <sup>10</sup>fall/spring; <sup>11</sup>*Sinapis alba/Lolium perenne*; <sup>12</sup>averaged across the two study sites; <sup>13</sup>pooled sites: Caswell 2012, Piedmont 2012, and Caswell 2013; <sup>14</sup>at late tillering/stem extension; <sup>15</sup>at stem extension/heading; <sup>16</sup>at early/late tillering; <sup>17</sup>at late tillering; <sup>18</sup>at early/late tillering/heading; <sup>19</sup>only 2 varieties tested.

		Competitive traits of the crop										Allelopathic potential			
		Crop biomass <sup>1</sup>	Early shoot length <sup>1</sup>	Final plant height	Tiller number	LAI <sup>2</sup>	NDVI <sup>3</sup>	Growth habit <sup>4</sup>	Vigor <sup>5</sup>	Root length	Root biomass	Staircase device <sup>6</sup>	Agar-based bioassay <sup>7</sup>	Allelopathic compounds in crop <sup>8</sup>	Allelopathic compounds in soil <sup>8</sup>
Spring wheat	Kashif et al. 2016			-0.15 NS	-0.20 NS										
	Bertholdsson 2001	-0.51 *	-0.01 NS	-0.06 NS											
	Bertholdsson 2005	-0.25 NS	-0.20 NS	-0.08 NS											
	Bertholdsson 2007	0.78 NS	0.14 NS	-0.88 *											
	Bertholdsson 2010	0.68 NS	0.34 NS	-0.17 NS											
Bertholdsson 2007		0.84 NS	0.08 NS	-0.83 NS											
	Bertholdsson 2010 <sup>9</sup>	0.72 NS	0.37 NS	-0.17 NS											
Reiss et al. 2018a	2017			0.31 NS		-0.01 NS	0.20 NS						0.14 NS	0.62 *	
Winter wheat	Bertholdsson 2011	-0.58 *	-0.58 *	0.16 NS			0.02/ NS/ -0.65 <sup>10</sup> *		0.33 NS	-0.11 NS			-0.65/ 0.33 <sup>11</sup> NS		
	Reiss et al. 2018b <sup>12</sup>	2016	0.02 NS	-0.22 NS	-0.08 NS		-0.06 NS	-0.08 NS							-0.12 NS
	Worthington et al. 2015	2017		0.22 NS	0.44 ***		-0.14 NS	-0.59 ***						0.96 *	0.29 *
		13		-0.77/ -0.76 <sup>14</sup> *	0.56 NS		-0.83/ NA <sup>15</sup> NS	0.81 <sup>17</sup> *	0.5/0.82 NS/ <sup>18</sup> /0.58 /NS				0.48 NS		
Spring barley	Bertholdsson 2005	1999	-0.63 **	nd	-0.09 NS										
		2000	-0.78 **	-0.62 *	-0.71 **										
		2001	-0.56 *	-0.41 NS	-0.20 NS										
		2002	-0.56 *	-0.42 NS	0.44 NS										
Rye	Reiss et al. 2018b <sup>12</sup>	2016	-0.09 NS	0.01 NS	-0.08 NS		-0.14 NS	-0.59 ***							-0.10 NS
		2017		0.29 *	0.10 NS		-0.05 NS	-0.19 NS							0.28 *
Sorghum	Al-Bedairy et al. 2013	2 years	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>
Spring triticale	Reiss et al. 2018a	2017		NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>	NA <sup>19</sup>
	Reiss et al. 2018b <sup>12</sup>	2016	0.04 NS	0.11 NS	-0.22 NS		-0.18 NS	0.01 NS							-0.04 NS
Winter triticale	Reiss et al. 2018b <sup>12</sup>	2017		-0.17 NS	-0.33 **		-0.20 NS	-0.01 NS							0.03 NS
		2017													0.14 NS



**Fig. 8** Main methodological points to consider when implementing an experiment aiming at characterizing crop allelopathic effects in the field by comparing crop varieties.

although plots were fertilized with nitrogen and phosphorus. Therefore, an effect of competition for resources cannot totally be excluded.

### 3.3.2 Articles comparing crop varieties with different competitive traits

Six articles (Bertholdsson 2005, 2010, 2011; Worthington et al. 2015; Reiss et al. 2018a, b) reported a significant correlation between the level of weed infestation in the field and the allelopathic potential (assessed by agar-based bioassay

or by quantification of allelochemical compounds in soil and roots of varieties in the field) (Table 4).

Through the use of multiple regression models, four articles (Bertholdsson 2005, 2011; Reiss et al. 2018a, b) were able to determine which of the studied crop traits (related to allelopathy and/or competition) best explained the weed biomass variations.

For example, Bertholdsson (2005) studied barley (*Hordeum vulgare* L.) varieties during 4 years and, using a backward multiple regression analysis, showed that early crop biomass and allelopathic potential assessed by agar-based bioassay best explained field weed biomass ( $R^2 > 44\%$ , Supp.

Mat. 2). The author concluded that both early vigor (above-ground biomass and shoot length at early stages) and allelopathy explain the weed suppressive ability of varieties. Reiss et al. (2018b) used a partial least squares regression to evaluate the contribution of competitive traits and allelopathic measurements (namely concentration of benzoxazinoids in soil) to explain the variance in weed biomass for wheat, triticale, and rye varieties. They showed that several competitive traits (i.e., crop height, leaf area index, and normalized difference vegetation index) and allelopathic measurements (e.g., APO and AMPO concentration, molecules previously shown to be allelopathic (Jabran 2017; Mwendwa et al. 2018)) were equally important to explain crop weed suppression.

Two articles (Bertholdsson 2010; Worthington et al. 2015) used simple regressions; i.e., correlations were analyzed trait by trait. For example, Worthington et al. (2015) compared eight wheat varieties with detailed measured competitive traits (five traits measured at different growth stages). They determined that only growth habit, height, early vigor, and leaf area index at advanced tillering and stem extension stages were correlated to weed infestation, but not the allelopathic potential (assessed by agar-based bioassay). This is the only article of the second corpus that illustrates the absence of allelopathy in the field (while it was attested in the agar-based bioassay on the same wheat varieties and weed species).

However, correlations must be interpreted with caution, as there is not necessarily a causal relationship between correlated variables (Sheather 2011). Another variable may drive the two studied variables and explain the observed relationship. Furthermore, when multiple regression models were used, *p*-values or partial R squared (partial  $R^2$ ) for the explanatory variables were not always presented in the articles. It may thus not always be possible to correctly estimate the relative contribution of each variable to the weed suppressive ability of crop varieties.

Surprisingly, while rice was the most common crop species studied (Fig. 5a), no experiments were retained showing convincing evidence of allelopathy. Indeed, the protocols used did not allow dissociating allelopathy and competition. Yet, breeding programs have been carried out based on allelopathic potential, and a few allelopathic rice varieties are already released on the market (Jabran 2017; Gfeller et al. *in press*).

As it was the case for the articles comparing crop varieties with similar competitive traits, no measurements were made to check that soil resources were not limiting. Overall, it is difficult to assess this assumption a posteriori as for some experiments no fertilization was applied in the plots or the information is not provided in the articles.

### 3.4 Avenues for designing an optimal experiment

Based on our critical analysis of the literature, this section discusses appropriate experimental designs to characterize

allelopathy when comparing the weed-suppressive ability of varieties in the field.

#### 3.4.1 Previous criteria to demonstrate the role of allelopathy

Fuerst and Putnam (1983) proposed a list of criteria for compelling proof of occurrence of allelopathy, built on the Koch's postulates used to demonstrate a causative relationship between a microbe and a disease: (1) demonstrate the existence of a crop-weed interference by identifying symptoms on the weed, (2) isolate the chemical responsible of the interference, characterize and synthesize it, (3) apply the synthesized chemical on weeds at rates present in nature and check if the same symptoms (as described in step 1) are observed, and (4) monitor the release, the movement, and the uptake of the chemical from the crop to the weed.

Although interesting for the scientific rigor, those criteria are unrealistic, or at least, extremely difficult to set up, and do not provide an appropriate methodology or framework to prove allelopathy in the field. Weidenhamer (1996) and Blum et al. (1999) pointed out that those criteria do not take the degradation of the released allelochemical (that may be more or less toxic than the precursor), its possible immobilization by the soil solution and the interactions among allelochemicals into account. Indeed, root exudates are a complex assemblage of chemicals, and one unique chemical is unlikely to cause the whole interference (Mwendwa et al. 2018).

#### 3.4.2 Measurements and data analysis

The weaknesses and strengths of the articles identified in the present systematic review highlighted key methodological aspects and allowed us to list a number of criteria to consider in an optimal protocol for assessing the contribution of allelopathy in weed regulation by crop varieties in the field (Fig. 8). There are no straight answers to optimal parameters of the protocol (e.g., number of replicates, observations and measurement dates, plot sizes or number of competitive traits measured), as all those parameters are a trade-off for each experiment and depend mainly on human, technical and financial resources. Nevertheless, they determine the statistical power and the reliability of the results afterwards.

**Experimental design** Our analysis substantiates the importance of conducting allelopathy experiments during several years and in several sites under variable pedoclimatic conditions. Indeed, environmental conditions partly influence the weed suppressive ability of crop varieties. We reported year to year variations of allelopathic and competitive traits (Tables 3 and 4), which corroborates previous reviews (Belz 2007; Worthington and Reberg-Horton 2013; Mwendwa

et al. 2018). For example, in the experiment of Bertholdsson (2005), correlations between weed biomass and allelopathic potential assessed by agar-based bioassay were significant in only two out of four years (Table 4). The author explains the absence of significance for 2 years by huge infestations of *Sonchus asper* (Supp.Mat. 2).

It is particularly advisable to sow weeds to ensure a homogenized weed pressure between plots, or at least to ensure that natural weed pressure is homogeneous. It should also be checked that soil resources are not limiting (i.e., water and nutrients) to minimize competition (Romeo 2000). A measurement of crop aboveground amount of nitrogen can be used to check that plants do not suffer from nitrogen deficiencies and a nitrogen nutrition index can also be calculated (Perthame et al. 2020; Louarn et al. 2021). Moreover, water seems necessary in the establishment of the allelopathic effect, since allelochemicals have to be released in the soil media, and as for all molecules, absorption of allelochemicals by the root system of the receiving plant is dependent on the soil water content. On the other hand, little is known on the effect of excessive water, and we can wonder whether it might lixiviate allelochemicals.

**Crop and weed measurements** In order to assure that the proportion of weed infestation variance not caused by competition can (partly) be explained by allelopathy, three categories of measurements should be made: weed measurements in the field, crop competitive traits in the field, and assessment of crop allelopathic potential in the field or in the lab (“Measurements” box in Fig. 8). These last two categories of measurements should then be analyzed by multiple regressions to assess their respective contribution in explaining weed biomass. Only looking at significance levels is insufficient, and quantification of the importance of explanatory variables is needed. The relative contribution of the significant explanatory variables should also be assessed with partial  $R^2$  (i.e., the variability in weed biomass explained by each variable) based on type III sum of squares needed to evaluate each variable taking account of all other variables. Significant variables can be chosen by different selection processes, e.g., stepwise (successively adding less and less important variables) or backward (successively deleting less and less important variables after starting with the full complement). A different approach can be classification and regression trees (Breiman and Ihaka 1984) and random forests (Breiman 2001), which are particularly well adapted for interacting and/or correlated explanatory variables. The trees could be used to predict weed biomass from the explanatory variables (“predictors”) by recursively splitting the dataset into two subsets along a threshold value of a predictor in order to maximize the difference between subsets. Branches are combinations of predictor values that lead

to predicted weed biomass contained in leaf nodes. The trees calculate, for each predictor, the variable importance, which can be used to calculate the equivalent of partial  $R^2$ . Another approach would be the use of multivariate regression models, such as partial least squares regression (PLSR), as done in Reiss et al. (2018a, b). Multivariate regression models are particularly suitable for situations where predictor variables are correlated with explanatory variables, which is common for datasets resulting from chemical analysis of allelopathic compounds. The output of PLSR are principal components that reflect the covariance of predictor (weed biomass) and explanatory variables (e.g., concentration of chemical compounds) and allow for a ranking of the explanatory variables according to the part of the variability of the model they describe (Mehmood et al. 2012; Mevik et al. 2020).

As mentioned in the “Introduction” section, the sensitivity of one plant to allelochemicals depends on its development stage. It seems that allelopathy affects seed germination less than plant growth (Zhang et al. 2021). This result is corroborated by Worthington et al. (2015) and Al-Bedairy et al. (2013) who found no significant difference in weed densities between crop varieties but a significant difference in weed biomass (Table 3). Therefore, efforts should be focused on studying weed growth rather than weed numbers. Ideally, weed infestation should be assessed by weed biomass, otherwise by a visual estimation (purple squares in Fig. 8). In addition to weed growth measurements, complementary measurements that characterize the plant functioning could be relevant in the field, although it has classically only been done under controlled conditions so far. Some allelochemicals are inhibitors of photosystem II, and for example, Bouhaouel et al. (2018) have shown that chlorophyll content and fluorescence of weeds (*Bromus diandrus* Roth) were modified when weeds were grown in substrate containing allelochemicals exuded by barley (namely a substrate where barley have been previously grown). Besides, measurements of aboveground amount of nitrogen can be used to estimate weed nitrogen status (Perthame et al. 2020; Louarn et al. 2021) and exclude potential effects of nitrogen competition.

There are two ways to estimate the allelopathic potential of a variety (orange squares in Fig. 8): quantifying allelochemicals or measuring weed inhibition in greenhouse or laboratory experiments. Furthermore, allelopathy may be a defense mechanism induced in response to plant interference. Crop production of allelochemicals has indeed been shown to increase in the presence of weeds, indicating that plants may detect the presence of neighboring plants (Kong et al. 2006; Kato-Noguchi 2011; Kashif et al. 2016; Zhang et al. 2020; Gfeller et al.). That is why, it may not be relevant to measure allelochemical production in crop plants grown in the absence of weeds. Weed inhibition in controlled

conditions should also be assessed using the main weed species observed in the field, as allelopathy may be selective and the effect may depend on the weed species (Blum et al. 1999; Wu et al. 2001). Main biases in estimating allelopathic potential are detailed in Supp.Mat. 3.

The competitive traits presented in green squares in Fig. 8 are not an exhaustive list of measurements that have to be made, but rather a “tool box” from which researchers can choose measurements adapted to their staff availability, material, and financial resources. Nevertheless, the number of traits should be maximized to ensure a good characterization of crop competitiveness, including measurements at early and late stages. Traits should be chosen to cover different scales: plant scale (e.g., height, tiller number) and canopy scale, which is more integrative (i.e., combination of several plant traits), and to characterize the crop vertical occupation, the ground cover as well as the global biomass production. Some traits are substitutable when they provide broadly the same information, as leaf area index and soil coverage, for example. Furthermore, as discussed in Section 3.1.2.4., it is advisable to measure crop competitive traits in weed-free plots to assess the crop competitive potential against weeds. As mentioned in Section 3.2., the timing of crop traits measurement may influence the characterization of crop competitiveness. Some studies included in our review (Bertholdsson 2005, 2011; Worthington et al. 2015) highlighted that crop early vigor has a high contribution to the crop-weed competition later in the season. Hence, some competitive traits should be measured at early crop development stage (e.g., 1 or 2 weeks before heading as in Bertholdsson (2005, 2010)).

### 3.4.3 Additional technical approaches

Beyond the findings in our systematic review, additional technical approaches may be used to help differentiating allelopathy from resource competition. Four of them are discussed below:

- (1) Approaches **neutralizing the allelochemicals in soil**, mainly by adsorbents as activated carbon. Nevertheless, Lau et al. (2008) drew attention to the fact that the allelopathic effect can be confounded by experimental artifacts. Indeed, activated carbon appears to affect the nutrient availability and plant growth. Again, a validation of the method seems necessary (in this case, proof that weed growth is not modified by activated carbon, in the absence of a crop).
- (2) The “**inhibitory-circle method**” developed by Li et al. (2015) where crop and weed seedlings are grown in concentric circles for 7 days, in pots filled with field soil. This method mimics field conditions better than

laboratory bioassays and is much faster and more practical than field experiments. Attention should be paid to ensure that soil resources are not limiting and that light competition is reduced.

- (3) The **combination of different treatments in pot trials**: the use of nets to limit competition for light and/or the use of plastic bags to separate the rhizosphere of the two studied species (Falquet et al. 2014; Kong et al. 2018). Pots are filled with field soil and are regularly watered with liquid fertilizer, so that competition for water or nutrients is excluded. By comparing weed growth in different treatments it is possible to show the separate effects of competition for light and allelopathy by root exudation. Moreover, some control pots are included to assess that weed growth is not impacted by the experimental design in the absence of crop plants (condition with net and plastic bag in the presence of crop plant vs condition in the absence of net and plastic bag and in the absence of crop plant).
- (4) The **use of nets** in field experiments to limit competition for light (Gfeller et al. 2018) by producing different shading levels that allow to study weed response. If shading level does not affect weed response (e.g., biomass), aboveground competition can be considered not to be the primary mechanism of weed growth repression. Nevertheless, belowground competition cannot be excluded.
- (5) The nearly perfect evidence of allelopathy in the field would be to compare two crop varieties, indistinguishable in terms of morphology, and differing only by the ability to produce allelochemicals. A few articles have created such varieties using **genetic manipulation** and have provided strong evidence of the role of crop allelopathy in weed growth (Xu et al. 2012; Yoshida et al. 2017). Unfortunately, these experiments were only conducted under laboratory conditions and it remains to confirm whether these results would also be conclusive in the field. Moreover, mutation of a gene may alter other plant functions and result in unintended effects (e.g., modification of competitive traits) (Duke 2015). Thus, it should also be checked whether the allelochemicals do not play a role in other key plant functions.

## 4 Conclusion

### 4.1 Novelty and strong points

Past reviews on crop allelopathy were usually narrative, broad, and did not focus on allelopathy by living plants and/or on field experiments (Belz 2007; Narwal and Haouala 2013; Jabran et al. 2015; Jabran 2017; Mwendwa et al. 2018). Moreover, given the difficulty to disentangle them,

the discrimination between the effects of allelopathy and competition in field is rarely considered or even discussed.

To our knowledge, we conducted the first systematic review on the role of crop allelopathy providing quantitative data of field-based weed regulation. As we focused on crop variety differences, we did not consider all the published articles on allelopathy and may have excluded some articles that do provide convincing evidence on allelopathy in fields but not by comparing varieties. Nevertheless, this choice can also be considered as a strength, as this focus allowed disentangling allelopathic from competitive effects, which is the most critical step to prove allelopathy in the field. Our study also provided an exhaustive analysis of the literature that allowed objectifying research on allelopathy, which is huge but confusing (2596 articles retrieved with Web of Science using the equation in Fig. 3 step A, when the term “variet\*” and synonyms were removed). The criterion of field experiment drastically reduced the corpus, but this focus is essential to identify options to implement cropping systems promoting weed biological regulation.

## 4.2 New insights on weed regulation by allelopathic crop varieties in the field

As in previous studies (Fuerst and Putnam 1983), we highlighted a misuse of the term allelopathy: most articles demonstrate a crop-weed interference (any adverse effect of one plant on the growth of another plant, either via competition and/or allelopathy), but not the cause of this interference, let alone that allelopathy is the cause. Moreover, studies investigating allelopathy suffer from methodological inadequacies, despite no ideal method exists to assess the effect of allelopathy. This is why caution is needed when reading articles on the topic, to make sure that the observed effects cannot be explained by other mechanisms, such as competition. We finally did not include articles that do not assess allelopathic potential, such as showing convincing evidence of allelopathy. Thus, the evidence of allelopathy by default (when no competitive traits explain the weed infestation, the weed suppression can be assumed to originate from allelopathy) was not retained. Therefore, the most convincing evidence of allelopathy is provided when combining several methods (field measurements on weeds and crop varieties and assessment of allelopathic potential in field or laboratory, linked by a multiple regression), when results are consistent. We identified key methodological points that must be considered for future works to investigate crop-weed interactions in the field. This methodology could also be used to investigate the allelopathic potential of different crop species to regulate weeds in field, since their competitive traits are studied in detail.

Our analysis only identified few articles showing convincing evidence of the presence ( $N = 7$  articles) and the absence

( $N = 1$ ) of allelopathy in weed regulation by living crop varieties in the field. This small number, compared to the total number of articles published on allelopathy, is mainly due to protocols not adapted to answer this question accurately, as well as to the authors' objectives. Although being convincing, we discussed some methodological weaknesses of the eight studies.

## 4.3 Perspectives for future research

Our analysis illustrated the difficulty of providing experimental evidence and quantifying the role of allelopathy in weed regulation in the field. We suggested approaches to improve the disentangling of the effects of allelopathy and competition. Similarly to genetically modified varieties, virtual experimentations could be interesting to be developed. Indeed, through mechanistic (i.e., process-based) cropping system models of weed dynamics, it would be possible to selectively switch submodels related to allelopathy and competition for resources on or off (e.g., light, nitrogen). However, this approach requires sufficient beforehand knowledge on underlying mechanisms of crop allelopathy, namely (1) the production of allelochemicals by the donor plant (chemical compounds, quantity, depending notably on plant growth stage); (2) the mode of release of the allelochemicals, their fate in the environment, and their mode of uptake by the receiving plant (maximal spatial distance between the two plants, favorable conditions, degradation, and interactions with the soil media); and (3) responses of the receiving plant (mode of action of the allelochemicals, intensity of the response, depending on the quantity, the nature of the allelochemicals and the plant growth stage). Some formalizations of modelling allelopathy have already been developed (An et al. 1993, 2003), and they could be integrated in models that simulate crop-weed competition (Colbach et al. (2014, 2021)). In combination with field and laboratory/greenhouse experiments, this original approach could help to assess the role of allelopathy in weed suppression in the field.

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**Code availability** Not applicable

## Declarations

**Ethics approval** Not applicable

**Consent to participate** Not applicable

**Consent for publication** Not applicable

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