agronomy

MDPI

Article

Herbicidal Effect of Different Alternative Compounds to Control *Conyza bonariensis* in Vineyards

Carlos Cabrera-Pérez *, Aritz Royo-Esnal and Jordi Recasens

Department of Horticulture, Botany and Landscaping, ETSEA, AGROTECNIO-CERCA Center, University of Lleida, Avinguda Rovira Roure 191, 25198 Lleida, Spain; aritz.royo@udl.cat (A.R.-E.); jordi.recasens@udl.cat (J.R.)
* Correspondence: carlos.cabrera@udl.cat

Abstract: *Conyza bonariensis* (L.) Cronquist is a widespread noxious weed with high fecundity, associated with no-till systems such as vineyards and other perennial crops in Mediterranean climates. Seeds germinate in staggered flushes, which leads to a great variation in the growth stage between individuals in the same field, and chemical control becomes challenging. Besides, *Conyza* species have evolved resistance to herbicides worldwide, particularly to glyphosate. Even though tillage is expected to provide weed-free fields, it negatively affects vineyards, causing erosion, loss of soil structure and a reduction in organic matter or vine growth (shallow roots can be affected), among other effects. Fuel consumption of this management is also very high because recurrent interventions of in-row tiller are required. In this context, bioherbicides, defined as environmentally friendly natural substances intended to reduce weed populations, are a potential tool for integrated weed management (IWM). In this work, the herbicidal effect of the following six products is tested on a glyphosate-resistant *C. bonariensis* population present in commercial vineyards: T1, mixture of acetic acid 20% and the fertilizer N32; T2, mixture of potassium metabisulfite and pelargonic acid 31%; T3, pelargonic acid 68%; T4, humic-fulvic acid 80%; T5, hydroxy phosphate complex; and T6, potassium metabisulfite. The results showed high field efficacy for T1 and T4 (>80% biomass reduction). For the rest of the products, high efficacy was obtained only in dose–response greenhouse experiments. The present work demonstrates the potential of certain bioherbicide compounds to manage herbicide-resistant weed species, such as *C. bonariensis*. Therefore, bioherbicides could be successfully incorporated into vineyards for IWM.

Keywords: bioherbicides; no-till; conservation agriculture; sustainable weed management; organic viticulture

check for updates

Citation: Cabrera-Pérez, C.; Royo-Esnal, A.; Recasens, J. Herbicidal Effect of Different Alternative Compounds to Control *Conyza bonariensis* in Vineyards. *Agronomy* **2022**, *12*, 960. https://doi.org/10.3390/agronomy12040960

Academic Editor: Fabrizio Araniti and Konstantinos M. Kasiotis

Received: 26 February 2022
Accepted: 13 April 2022
Published: 15 April 2022

1. Introduction

Conyza bonariensis (L.) Cronquist (hairy fleabane) is one of the most problematic weed species throughout the world [1], and in Spain it is considered one of the most competitive introduced noxious weeds [2] that harms crops and leads to yield loss [3,4], particularly under soil conservation management [5]. In fact, the increase in *C. bonariensis* prevalence has been associated with changes from conventional tillage to minimum tillage or no-till, as reducing soil disturbance favours seed germination and the establishment success of this species [6,7]. Apart from their adaptability to undisturbed crops, *Conyza* species have evolved resistances to herbicides worldwide [8], particularly to glyphosate, which has been widely applied in Spain for weed control in citrus orchards, olive groves, grape vineyards, and others perennial and annual crops [9,10]. Synthetic herbicides are important weed management tools in intensive cropping systems, but the numerous herbicide-resistant weed biotypes and environmental concerns provide limited lifespan to these chemical tools [11]. This situation has been worsened with the lack of new herbicide modes of action discovery in the past few decades [12].

Conyza bonariensis has a high fecundity, producing over 100,000 non-dormant seeds per plant [13]. Seeds germinate in staggered flushes throughout the year, depending on the environmental conditions, and consequently there is a great variation in the growth stage between individuals in the same field, and chemical control becomes challenging [14], especially in perennial irrigated crops. For example, in Mediterranean vineyards, mainly in those with dry or semiarid climates as is the case in North-Eastern Spain, this weed can be established in high densities in the in-row area of the vine, competing for water and nutrients [15]. This competition is aggravated if glyphosate-resistant biotypes are present in a particular field, because these are more competitive against young vines than glyphosate-susceptible biotypes [16].

Tillage is expected to provide weed-free fields, including *Conyza* species, in Mediterranean vineyards, but it negatively affects vines [17], mainly damaging the young ones, in part, because tillage decreases the presence of grapevine roots in the topsoil [18]. Tillage also causes erosion and a loss of soil structure and reduces the organic matter content [19]. Furthermore, the fuel consumption of this management method doubles the carbon footprint of pesticides or fertilizers [20], because recurrent interventions of in-row tiller along the season are required to effectively manage weeds in vineyards.

Against this background, and considering new challenges related to economic, environmental and social concerns for more sustainable and environmentally friendly weed management [21], the development of alternative weed control tools is mandatory, and especially for *C. bonariensis* in Mediterranean vineyards. Hence, bioherbicides, defined as substances of natural origin intended to reduce weed populations without damaging the environment [22], are a potential tool for integrated weed management. One of the challenges still faced in bioherbicide production is the low herbicidal activity compared to the effects of chemical herbicides [23]. Thus, Bioherbicides are currently underused, and few products have been launched on the market [24]. Nevertheless, the development of new bioherbicides is compelling as these products lag far behind those for pests other than weeds [25]. Furthermore, there are strong needs for any new weed management technology because of the rapid evolution and spread of herbicide resistance, and because weed management is the most difficult (and expensive) pest management problem in organic agriculture [26]. Natural substances face several opponents since there are doubts regarding the registration processes of natural products due to the lack of relevant toxicological data for their use at a commercial scale [27]. Although these concerns might exist, there is evidence that most essential oils and their main compounds are not necessarily harmful to human health [28]. Such natural herbicides are sometimes less hazardous for environmental and human health in comparison to the commercial synthetic herbicides. Some commercial products, such as acetic or pelargonic acid, have already been used as weed control agents. Acetic acid ($C_2H_4O_2$), sold as horticultural vinegar, is not persistent in either soil or water and has a low to medium oral toxicity to most biodiversity. However, it is highly corrosive and so may damage anything it comes into contact with. Pelargonic acid ($C_9H_{18}O_2$) is a saturated fatty acid naturally occurring as esters in the essential oil of *Pelargonium* spp. and can be derived from the tissues of various plant species. Toxicity tests on non-target organisms, such as birds, fish, and honeybees, revealed little or no toxicity [29]. To our knowledge, other products such as a hydroxy phosphate complex and humic-fulvic acid, widely used as organic fertilizers in many crops; potassium metabisulfite, a preservative, antioxidant and bleaching agent in food, especially in acidic foods, such as wine; or N32, a synthetic fertilizer, have never been used as herbicides.

The aim of this study is to assess the mentioned products in order to identify alternative compounds for use as herbicides, which could be incorporated in weed management programs in vineyards, while considering *C. bonariensis* as the main weed. In this study, the suppressive effect of six products on this weed is evaluated in comparison to an untreated control.

2. Material and Methods

The *Conyza bonariensis* population from vineyards located in Raimat, Lleida (NE Spain) was studied. The site is known to have a history of weed-control failures because of field manager complains about the impossibility to control *C. bonariensis* with glyphosate. Seeds were collected from a treated field with high *C. bonariensis* density and stored during summer 2018 as a potentially herbicide-resistant population.

2.1. Characterisation of the Herbicide Resistance

In autumn 2018, a dose–response experiment was set up with the Raimat population and with a sensitive (SP) population from Argentina, as it was deemed very unlikely to find SP in the region. Seeds were sown in peat and after seven days, seedlings were transplanted to 7 × 7 × 8 cm plastic pots filled with a mixture of silty loam soil 30% (*w*/*v*), sand 20% (*w*/*v*), and peat 50% (*w*/*v*). Four seedlings were transplanted per pot. When populations reached BBCH 12–13 (Weiber et al., 1998), Glyphosate 360 g a.i. L^{-1} (Roundup; Bayer CropScience, Valencia, Spain) was applied at 90, 180, 360 (1×), 720 and 1440 g a.i. ha^{-1}, with a precision bench sprayer delivering 200 L ha^{-1} at a pressure of 215 kPa. Seven replicates (pots) were included for each population and dose. Pots were placed in a greenhouse at the University of Lleida (UdL), Spain, and watered regularly. Four weeks after treatment, the above ground part of the plants from each dose was harvested to measure the dry weight. Samples were oven dried at 65 °C for 48 h and weighted with a precision weigher (Mettler Toledo AB54-S, Barcelona, Spain). For the Raimat population, the results obtained for the percentage of reduction for dry weight, with respect to the control, were 10%, 30%, 56%, 65% and 80% at doses of 0.25×, 0.5×, 1×, 2× and 4×, respectively (ED_{50} = 1.057). On the contrary, the percentages of reduction in dry weight for the SC population were 60% at 0.25×, 80% at 0.5× and 100% at 1×, 2× and 4× (ED_{50} = 0.176), thereby confirming a resistance factor of 6 in the population from Raimat.

2.2. Bioherbicide Field Trials

A field trial was carried out from February to June in Raimat, Lleida (NE Spain) in an herbicide-managed commercial vineyard (Raventós-Codorníu S.L.). The field trial was repeated in three seasons (2019, 2020, 2021), but the location within the vineyard was changed for each repetition (Table 1). The climatic classification of this area is cold semiarid (BSk) [30], with an average annual precipitation of 342 mm, and annual mean temperature of 14.1 °C (average min of 8.1 °C and average max of 20.7 °C). Weather data were collected from a nearby meteorological station (https://meteocat.cat, accessed on 15 September 2021).

Table 1. Field characteristics by season. Vine variety, Caber: Cabernet Sauvignon, Chard: Chardonnay; Coordinates, Lat.: Latitude, Long.: Longitude; Vine spacing; Soil texture; pH, O.M.: Organic matter; Initial infestation level of *Conyza bonariensis*.

	Vine	Coordinates ETRS89		Spacing (m)		Soil Texture (%)				(%)	Initial
Season	**Variety**	**Lat.**	**Long.**	**Between**	**Within**	**Sand**	**Silt**	**Clay**	**pH**	**O.M.**	**Infestation**
2019	Caber	41°39′26.8″ N	0°31′10.3″ E	2.7	1.7	59.5	28.1	12.4	8.4	3.18	Low
2020	Caber	41°39′16.5″ N	0°30′51.3″ E	2.7	1.7	28.4	47.7	24.2	8.4	1.61	High
2021	Chard	41°40′42.9″ N	0°27′51.0″ E	3	1.5	27.9	38.9	33.2	8.2	2.32	Medium

The trial locations were drip irrigated regularly throughout the growing season and vines were trained as bilateral cordons. The vineyard alleyways were maintained with a spontaneous cover crop that was shredded 2–3 times per season. The soil at this site was classified as a Petrocalcic Calcixerept; the specific field and vineyard characteristics are shown in Table 1, where three different previous levels of *C. bonariensis* infestation are indicated.

The following six treatments were studied by combining different compounds (Table 2) to test their herbicidal effect on *C. bonariensis*: T1, mixture of acetic acid 20% (BioEmpe-20, Bodegas Dinastia S.L., Tomelloso, Spain) and the fertilizer N32 (YaraVita LAST N, Yara Iberian S.A., Madrid, Spain) (70 and 30% *v*/*v*, respectively); T2, mixture of potassium metabisulfite (AGROVIN S.A., San Juan, Spain) and pelargonic acid 31% (Finalsan RTU, W. Neudorff GmbH KG, Valencia, Spain); T3, pelargonic acid 68% (Kalina, Comercial Química Massó S.A., Barcelona, Spain); T4, humic-fulvic acid 87% (Herbiz, PRO&Garden, Barcelona, Spain); T5, hydroxy phosphate complex (Xekator, Aldamus Hispania, S.L., Madrid, Spain); T6, potassium metabisulfite (AGROVIN S.A., San Juan, Spain). The herbicidal effect of these compounds occurs through contact and for this reason, their effect is immediate (1–2 days).

Table 2. Compounds tested, application dose and application volume.

Treatment	Compounds	Application Dose	Application Volume (L/ha)
T1	Acetic Acid 20% (1) +N32 (2)	(1) 122.5 L/ha (2) 52.5 L/ha	175
T2	Potassium metabisulfite (1) +Pelargonic acid 31% (2)	(1) 70 kg/ha (2) 17.5 L/ha	500
T3	Pelargonic acid 68%	16 L/ha	200
T4	Humic-Fulvic acid	35 L/ha	700
T5	Hydroxy phosphate complex	15 L/ha	150
T6	Potassium metabisulfite	60 kg/ha	250

Previous essays with T1 and T4 were carried out at the UdL to choose the best application dose (Montull et al., 2019). For T2 and T6, these previous essays were performed by the winery. For T3 and T5, the application doses were chosen according to the manufacturer recommendations.

Each year, a completely randomize design was established with six treatments and four replicates. In 2019 and 2020, the treatments were an untreated control, T1, T2, T3, T4 and T5. In 2021, T5 was excluded due to the low efficacy observed in the previous years, and a new treatment (T6) proposed by the winery was added. Thus, treatments were an untreated control, T1, T2, T3, T4 and T6. The treated area was along the space within three vines (3.0 m or 3.4 m) always with a width of 0.6 m. In 2019 and 2020, treatments were applied four times, between February and May (2019) and from March to May (2020). In 2021, treatments were applied three times, from April to May, when the growth stage of the plants was between BBCH 11–12 (first application) and BBCH 31–32 (last application). All plots were treated the same day in each application with a manual hand sprayer at mid-day on sunny days. A *C. bonariensis* assessment was made before each application to estimate the initial weed coverage, and another one was performed two days after treatment (DAT) to evaluate the herbicidal effect. In July 2020 and 2021, the above ground biomass of *C. bonariensis* plants from each plot was harvested, oven dried at 65 °C for 48 h, and the dry weights were measured.

2.3. Dose–Response Experiment

Seeds of *C. bonariensis* from the Raimat population were sown in peat and, after seven days, seedlings were transplanted to 7 × 7 × 8 cm plastic pots filled with a mixture of silty loam soil 30% (w/v), sand 20% (w/v), and peat 50% (w/v). Four seedlings were transplanted per pot, placed in a greenhouse at the UdL, and watered regularly. The experiment was carried out for T1, T2, T3, T4 and T6 at two different phenological stages (PS) of the weed, namely when seedlings achieved BBCH 12–13 and when they achieved BBCH 14–15. Five replicates (pots) were included for each treatment, PS and dose. Pots were treated with the following doses: (T1) 0, 21.9, 43.8, 87.5, 175 L/ha at BBCH 12–13 and 0, 43.8, 87.5, 175, 350 L/ha at BBCH 14–15; (T2) 0 + 0; 8.75 + 2.19, 17.5 + 4.37, 35 + 8.75, 70 + 17.5, 140 + 35 kg/ha + L/ha, respectively for each compound of the mixture, at both BBCH 12–13 and BBCH 14–15; (T3) 0, 4, 8, 16, 32 L/ha at BBCH 12–13 and BBCH 14–15; (T4) 0, 1.1, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 12–13 and 0, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 14–15; T6) 0, 5, 10, 20, 40, 80, 160 kg/ha at BBCH 12–13 and 0, 20, 40, 60, 80, 100, 160 kg/ha at BBCH 14–15. Treatments were applied using a manual hand sprayer. Four weeks after treatments, the above ground part of the plants was harvested, oven dried and weighted as in point 2.1, to measure their dry weight.

2.4. Weather Conditions

The highest observed mean temperature (T_m) during the potential emergence period of *C. bonariensis* in Raimat (grey arrows in Figure 1) was in 2020 (14.5 °C), followed by 2021 (14 °C), and it was lowest in 2019 (13.5 °C). Both 2020 and 2021 were also warmer than the historical average (13.5 °C). The years 2019 and 2021 were similar in terms of precipitation during the whole growing season, with 102 mm and 110 mm, respectively, and below the historical average (162 mm), while in 2020 the growth season was very wet (248 mm).

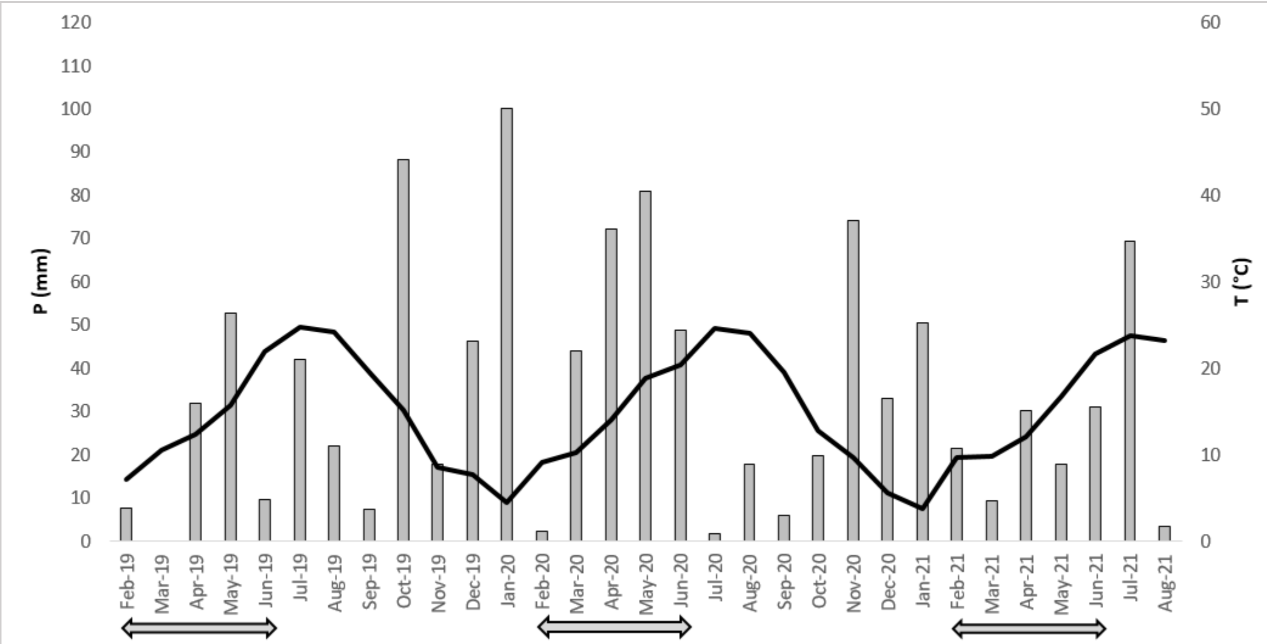

Figure 1. Weather conditions of the experiment period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (Tm). Arrows represent the growing season each year.

2.5. Statistical Analyses

The field efficacy results of each treatment were expressed as cover reduction after the Henderson–Tilton formula [31]. After testing for normality (Shapiro–Wilk) and homoscedasticity (Leven's test) requirements for a parametric analysis, both coverage and above ground biomass data were subjected to a one-way ANOVA, followed by multiple comparisons of treatment effects with Tukey's HSD-test ($p < 0.05$). In the case of heteroscedasticity, the variance was analysed by the Kruskal–Wallis *H* test. Data from dose–response experiments were analysed using a nonlinear regression model (1). The treatment curve was fitted with a four-parameter logistic function:

$$y = c + \frac{d - c}{1 + (\frac{x}{EC_{50}})^b} \quad (1)$$

where y is the response expressed as the percentage of reduction with respect to the untreated control, c is the lower level of the curve, d is the upper level of the curve, b is the slope, EC_{50} indicates the concentration that causes a 50% growth reduction, and x is the treatment dose (independent variable). Data were analysed using JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC, USA. SAS Institute Inc.) and SigmaPlot 12.0 (Sistat Software, Inc., San José, CA, USA).

3. Results

3.1. Field Efficacy Trials

Significant differences were found in all sampling dates between treatments (Table 3). Most of the compounds tested succeeded in decreasing the cover of *C. bonariensis* (Table 4), mainly in 2019, when lower overall cover of the weed was observed, and the maximum values of the untreated plots occurred in June (10.8%). All treatments significantly reduced *C. bonariensis* cover compared to the untreated control, except T5, which showed unsatisfactory efficacy in all application dates (between 13% and 36%). The efficacy of T1 and T2 was very high from April until June (>85%) and, in the case of T4, the efficacy was very high from February until June (>90%), while T3 always ranged between 52% and 77%. In contrast, in 2020, the highest *C. bonariensis* cover was observed, coinciding with the wettest and hottest season, and the untreated plots showed 83% weed cover (on average) by May. Again, lower efficacy was observed in T5, which never exceed 7.5%; in T1 and T3, the efficacy was also low (15% and 56%, respectively); while in T2 and T4, the efficacy was high and close to 80% or 90% for most sampling dates. This trend repeated in 2021, but T6 was incorporated instead of T5, and efficacy varied between 64% and 85% depending on the application date; T1 and T3 continued to present low efficacy (11% and 36%, respectively). The biomass measured (g/plot) after the last application date in 2021 supported the weed cover results, with the lowest values obtained by T2 (10.4), followed by T4 (45.6) and T6 (53.2), although significant differences were observed only for T2 with respect to T3 (244.0) and the untreated control (393.8), and for T4 and T6 compared to the untreated control (Table 5). In 2020, with higher *C. bonariensis* emergences and cover, no significant differences were found in biomass, although a lower weight was also measured for T2 and T4.

Table 3. Significance of the one-way ANOVA or Kruskal–Wallis test. F/H and *p* values of each sampling date.

Date		F/H	*p*
2019	February	4185	0.014
	April	20,963	<0.001
	May	1962	<0.001
	June	21,448	<0.001
2020	March	4358	<0.001
	April	70,444	<0.001
	May	69,285	<0.001
	June	21,488	<0.001
2021	April	31,403	<0.001
	May	20,329	<0.001
	June	22,273	<0.001

3.2. Dose–Response Curves

The equation parameters of the best fitted models, based on the coefficient of determination (r^2) and the EC_{50} values, are shown in Table 6 and represented in Figure 2. The obtained r^2 values were always above 0.8, and most were above 0.9, which indicates the suitability of this function to describe the growth response of *C. bonariensis* to different concentrations of the tested compounds. Biomass reduction was greatly influenced by the phenological stage of the treated plants, while a 100% biomass reduction was achieved in BBCH 12–13 in almost all treatments, although this value was more difficult to reach in BBCH 14–15. On the other hand, the EC_{50} value at least doubled as compared to that of BBCH 12–13 when the population of *C. bonariensis* was in BBCH 14–15.

Table 4. Treatments efficacy in each application date expressed as % after Henderson–Tilton formula. Mean values ± standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

2019				2020				2021			
Applic. Date/BBCH	**Treat.**	**Pre-Spray Cover (%)**	**Cover Reduction (%)**	**Applic. Date/BBCH**	**Treat.**	**Pre-Spray Cover (%)**	**Cover Reduction (%)**	**Applic. Date/BBCH**	**Treat.**	**Pre-Spray Cover (%)**	**Cover Reduction (%)**
7 February	Control	0.6	0.0 ± 0.0 b	11 March	Control	26	0.0 ± 0.0 c	15 April	Control	5	0.0 ± 0.0 b
BBCH 11–12	T1	0.5	64.3 ± 20.6 ab	BBCH 11–12	T1	21	30.4 ± 7.4 b	BBCH 11–12	T1	3	11.3 ± 6.6 b
	T2	1	76.8 ± 16.3 ab		T2	20	37.5 ± 7.9 b		T2	4	89.6 ± 3.6 a
	T3	2	76.8 ± 14.1 ab		T3	19	15.4 ± 3.1 b		T3	4	13.4 ± 7.7 b
	T4	2.4	91.7 ± 4.5 a		T4	21	66.9 ± 7.3 a		T4	4	90.6 ± 3.6 a
	T5	1.9	34.5 ± 5.6 ab		T5	33	2.1 ± 2.5 c		T6	4	74.6 ± 7.8 a
16 April	Control	3	0.0 ± 0.0 b	15 April	Control	51	0.0 ± 0.0 d	11 May	Control	9	0.0 ± 0.0 c
BBCH 11–15	T1	2	87.5 ± 7.5 ab	BBCH 11–15	T1	34	56.4 ± 12.6 b	BBCH 11–15	T1	8	28.3 ± 6.4 b
	T2	2.3	100 ± 0.0 a		T2	26	91.4 ± 3.0 a		T2	1	60.0 ± 10.0 ab
	T3	5.5	73.8 ± 4.7 ab		T3	36	31.8 ± 9.6 bc		T3	6	34.7 ± 8.3 b
	T4	2	100 ± 0.0 a		T4	32	85.6 ± 6.1 a		T4	2	75.4 ± 10.5 a
	T5	4	23.8 ± 10.3 ab		T5	59	7.2 ± 2.8 cd		T6	3	85.3 ± 4.0 a
23 May	Control	7	0.0 ± 0.0 b	7 May	Control	64	0.0 ± 0.0 c	1 June	Control	23	0.0 ± 0.0 d
BBCH 11–31	T1	1.9	98.3 ± 1.7 a	BBCH 11–18	T1	38	39.7 ± 13.0 b	BBCH 11–31	T1	12	10.7 ± 4.3 cd
	T2	1.1	86.7 ± 8.1 ab		T2	16	85.5 ± 5.4 a		T2	2	87.1 ± 5.3 a
	T3	9.5	53.2 ± 8.1 ab		T3	34	26.5 ± 6.9 bc		T3	11	36.3 ± 15.8 bc
	T4	1.9	97.5 ± 2.5 a		T4	26	78.5 ± 7.2 a		T4	3	88.1 ± 4.7 a
	T5	7.4	36.2 ± 14.7 ab		T5	66	7.6 ± 0.4 c		T6	5	64.4 ± 8.9 ab
13 June	Control	10.8	0.0 ± 0.0 c	20 May	Control	83	0.0 ± 0.0 c				
BBCH 11–31	T1	0.4	92.5 ± 7.5 a	BBCH 11–32	T1	49	24.7 ± 12.0 abc				
	T2	0.3	90.0 ± 5.8 a		T2	11	81.4 ± 1.6 a				
	T3	3	52.1 ± 12 b		T3	44	26.4 ± 4.7 abc				
	T4	0.9	100 ± 0.0 a		T4	26	69 ± 8.5 ab				
	T5	4	13.1 ± 9.4 c		T5	76	6.6 ± 0.2 bc				

Table 5. Dry weight (g) biomass of *C. bonariensis* in July 2021. Mean values ± standard errors of the mean. Different letters denote significant differences among treatments at $p < 0.05$. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

Treatment	g/Plot	
	2020	2021
Control	308.8 ± 14.4	393.8 ± 106.5 a
T1	271.8 ± 10.0	161.9 ± 65.5 abc
T2	216.9 ± 18.9	10.4 ± 5.2 c
T3	262.2 ± 41.5	244.0 ± 103.5 ab
T4	231.5 ± 30.3	45.6 ± 18.7 bc
T5	278.1 ± 47.7	-
T6	-	53.2 ± 23.6 bc

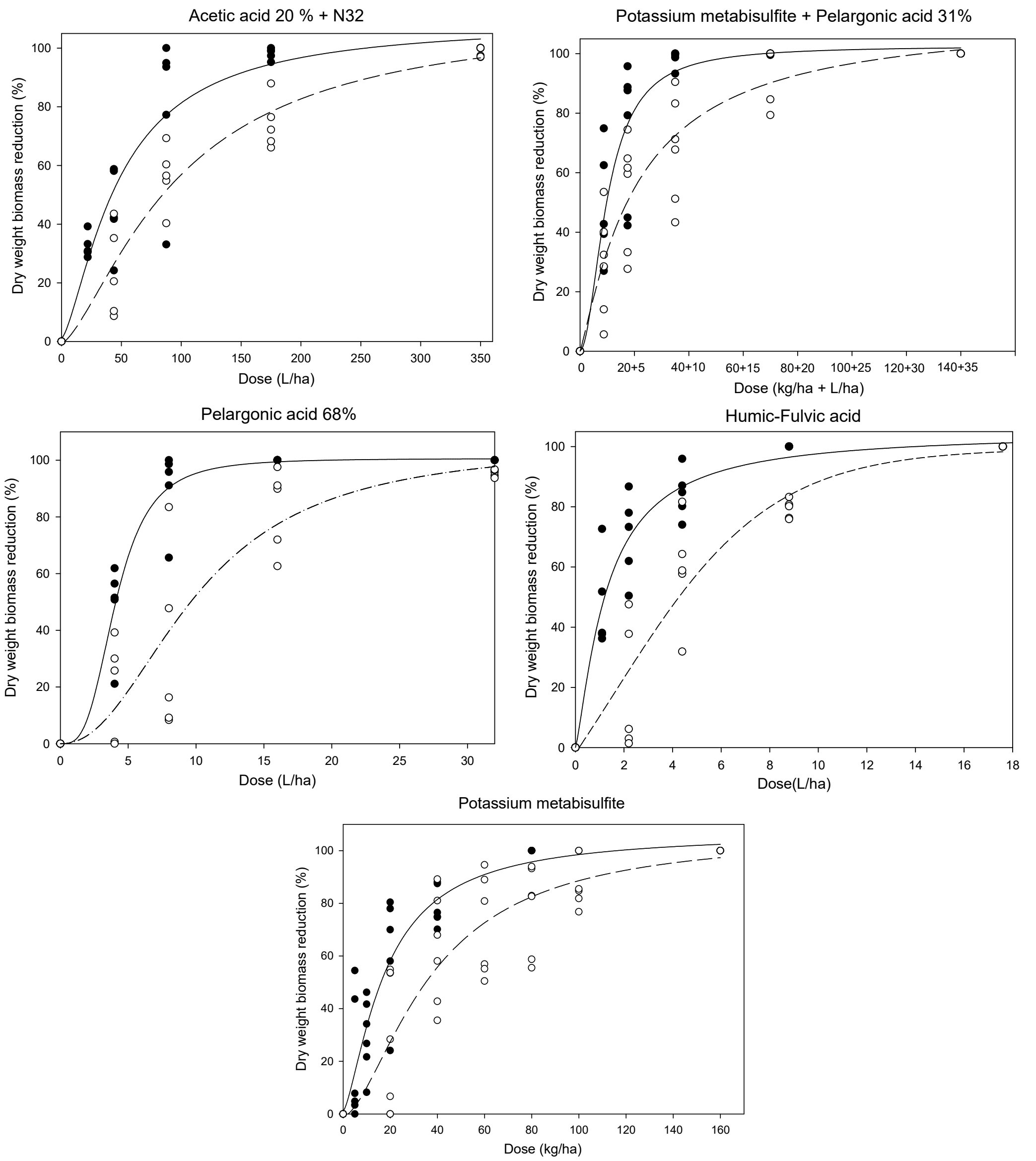

Figure 2. Dose–response curves of T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%; T4: humic-fulvic acid, T6: potassium metabisulfite. Values are presented as dry weight biomass reduction (%) of the no-treated control. Black points (•) and solid lines (—), BBCH 12–13; white points (○) and dashed lines (– –), BBCH 14–15.

Table 6. Parameters of dose–response curves represented in Figure 2. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%; T4: humic-fulvic acid, T6: potassium metabisulfite.

Treatment	Compounds	BBCH	r^2	EC_{50}	Slope (*b*)
T1	Acetic Acid 20% + N32	12–13	0.900	47.62 (L/ha)	1.44
		14–15	0.940	96.92 (L/ha)	1.45
T2	Potassium metabisulfite + Pelargonic acid 31%	12–13	0.909	10.06 + 2.52 (kg/ha + L/ha)	1.92
		14–15	0.887	21.02 + 5.26 kg/ha + L/ha)	1.15
T3	Pelargonic acid 68%	12–13	0.954	4.10 (L/ha)	3.25
		14–15	0.827	10.07 (L/ha)	2.2
T4	Humic-Fulvic acid	12–13	0.939	1.30 (L/ha)	1.25
		14–15	0.906	4.12 (L/ha)	2.01
T6	Potassium metabisulfite	12–13	0.892	17.84 (L/ha)	1.43
		14–15	0.812	37.32 (L/ha)	1.64

4. Discussion

The *C. bonariensis* populations from the Raimat vineyards were found to be resistant to glyphosate with a resistance factor of 6. This species is known to easily evolve resistant biotypes as several cases are reported in the literature. For example, Travlos and Chachalis [32] observed 4- to 7-fold resistance levels in *C. bonariensis* growing in Greek perennial crops, including vineyards. Similarly, Urbano et al. [4] established a 7- to 10-fold resistance level in *C. bonariensis* collected from Spanish olive fields, and more recent studies have showed a 27-fold resistance level for this weed in South African vineyards [33]. The cropping systems of the above-mentioned examples are similar to that of this study, and they share common features such as a long and repeated history of glyphosate use, and a lack of crop and herbicide rotation.

According to Bailey [22], bioherbicides are products of natural origin that are useful for weed control, and that can be either living organisms or products derived from living organisms. All the tested compounds fulfil that definition, except N32 (synthetic fertilizer) and potassium metabisulfite. Nevertheless, the last compound was tested because its use in winemaking is very common and, according to the International Chemical Safety Cards (ICSCs), the environmental effects of potassium metabisulfite has no significant effects, according to the current knowledge. Furthermore, T2, T4 and T6 stand out from the rest, as high field efficacy was observed along the application dates (Table 4), which was confirmed with the harvested above ground biomass in 2021 (Table 5), but not in 2020, where emergences were more abundant and constant until July, probably due to a higher initial presence of seeds with the combination of an extraordinarily wet spring. Furthermore, differences in 2020 biomass between high-effective treatments (T2 and T4) and low-effective ones (T1, T3 and T5) could have been diminished because of intraspecific competition of *C. bonariensis* plants in the latter treatments, as higher weed cover was observed but with smaller plants. The efficacy of T1, T3 and T5 was unequal and not always sufficient to maintain low *C. bonariensis* cover. The lack of efficacy of T5 is likely due to a harmless effect on the plant rather than in the applied dose. Conversely, the burning effect of the acetic (T1) and pelargonic (T3) acids are highly effective in early rosette stages of this species; nonetheless, some individuals showed green growth regions in the centre of the rosette after applications, which eventually developed inflorescences and disseminated achenes in the field trials. Pline et al. [34] observed a variation in pelargonic acid efficacy from only 6% up to 65%, depending on the annual weed species and similar to Travlos et al. [35], who reported an efficacy of between <20% up to >90%. Webber III et al. [36] observed good grass control (>80%) and fair (>70%) broadleaf control (without *Conyza* spp.), and Kanatas et al. [37] attributed the low weed control efficacy of pelargonic acid in olive fields to the presence of *C. bonariensis*, which indicates tolerance of this species to this acid, similar to the observations in the present study. The presence of buds in the *C. bonariensis* taproot allowed for rapid regrowth after clipping [38]. Variations in efficacy are also found in the literature with acetic acid, as Webber et al. [39] observed an efficacy ranging from 4.5% up to 100%, depending on the weed species, when acetic acid at 20% was applied at 187 L/ha. In the current study, acetic acid (T1) obtained good efficacy only in 2019, with low *C. bonariensis* cover (probably due to the low number of emergences) and when all plants could be treated in an early phenological stage.

Similar to synthetic herbicides, the effect of the tested compounds may rely on dosage, the phenological stage of the target weed, and on the environmental conditions [40,41]. In fact, the most effective treatments, potassium metabisulfite (T2) and humic-fulvic acid (T4), obtained better results when applied in April, although in 2019 and 2020 there was a previous application in February and March, respectively, and according to dose–response results (Figure 2, Table 5), it would therefore be expected to be more effective, as *C. bonariensis* rosettes were smaller. This contradictory result can be explained by the weather conditions. In April 2019 and 2020, the temperature was higher than in February 2019 and March 2020, which is known to improve herbicide efficacy [42]. Waltz et al. [43] attributed this enhanced effect with higher temperatures to a change in the epicuticular wax that facilitates the herbicidal effect. This statement would lead to the conclusion that the treatments' effect should improve during spring, but *C. bonariensis* plants that survived the firsts applications of low-effective treatments (T1, T3 and T5) were in an advanced phenological stage by May, so despite the high temperatures, the efficacy was lower, especially in 2020, when there was an abundant emergence of the weed which could hinder droplet contact with the leaves.

The herbicide's efficacy is clearly influenced by the phenological stage of *C. bonariensis* [4,33], with sensitivity or injury decreasing as the growth stage advances. This has been confirmed by the dose–response curves in all treatments (Figure 2). When plants grow from BBCH 12–13 to BBCH 14–15, the EC_{50} doubles in all treatments (Table 5). In the dose–response experiment, nearly 100% of the biomass reduction was achieved in BBCH 12–13 at some dose in all treatments, compared to the untreated control, demonstrating the potential of these compounds to control *C. bonariensis*. Plants showed visible injury ranging from chlorosis, going through necrosis, to eventually complete the wilting of plants. In general, the observed injury symptoms increased with increasing compound concentrations. The same symptoms were observed in the field trials two days after treatments. However, long-term control for *C. bonariensis* is challenging because of its germination and emergence characteristics, with overlapped cohorts along the season. *Conyza* species can potentially germinate at any time throughout the year [44], and irrigated crops such as drop-irrigated vineyards ease this process. For this reason, contrasting reports about the main emergence season for *Conyza* spp. are found in the literature, as it is sometimes considered in terms of winter annuals [38] and at other times in terms of summer annuals [3]. Moreover, Valencia-Gredilla et al. [45] observed the highest germination percentage of *C. bonariensis* at 22 °C, but they also reported that the biotypes from the Lleida region had more germinated seeds at lower temperatures than biotypes from warmer regions. Thus, the application of a control method (either bioherbicide or synthetic chemical) in the homogeneous phenological stage is extremely difficult, and explains differences found in the efficacy between greenhouse experiments of dose–response curves and field efficacy trials.

Consequently, although the available bioherbicides are promising compounds for weed control, few have achieved long-term commercial success in the field [24]. According to our results, bioherbicides may display their potential when addressed to specific species in early phenological stages, rather than during their widespread use to many species. The increase in *C. bonariensis* prevalence had created an urgent need to find alternatives for their control. In this sense, new herbicidal compounds may be incorporated as tools for integrated weed management (IWM). In fact, none of the individual techniques on their own can be expected to provide acceptable control levels, but when combined with other tools, successful results can be achieved [44]. So, the combination of different techniques such as cover crops, mulching and bioherbicides could facilitate a decrease in *C. bonariensis* infestations in no-till viticulture. It is important to know that in the Mediterranean climate, earlier cohorts of *C. bonariensis* contribute most to the following generations, therefore, they should be preferably targeted when designing control strategies [46]. Predicting the emergence of *C. bonariensis* with already developed models based on climate parameters, such as those from Zambrano-Navea et al. [2], can also contribute to a decision support system for optimum application timing.

Although many naturally occurring materials, such as most of the tested compounds, have herbicidal properties, there is controversy around whether they should be permitted in organic crop production systems [47,48]. Therefore, producers need to know the regulation policies that cover their organic, natural, or sustainable crop production. Finally, given the necessity of reducing the carbon footprint caused by tillage, and the lack of new modes of actions in synthetic herbicides, innovations for bioherbicides are much needed [49], so that they can be successfully incorporated in vineyards for IWM in the short-term.

5. Conclusions

To date, no studies have focused on the herbicide potential of alternative compounds, specifically for *C. bonariensis* in commercial vineyards. The findings of the present study revealed that, despite most of the tested compounds being able to control the weed in the greenhouse dose–response experiment, only the potassium metabisulfite + pelargonic acid 31% (T2), the humic-fulvic acid (T4) and the potassium metabisulfite (T6) obtained high field efficacy throughout the application dates and were able to maintain an acceptable *C. bonariensis* cover.

Author Contributions: Conceptualization, C.C.-P. and J.R.; methodology, C.C.-P. and J.R.; software, C.C.-P.; validation, C.C.-P., J.R. and A.R.-E.; formal analysis, C.C.-P. and J.R.; investigation, C.C.-P. and J.R.; resources, J.R.; data curation, C.C.-P.; writing—original draft preparation, C.C.-P.; writing—review and editing, J.R. and A.R.-E.; visualization, J.R. and A.R.-E.; supervision, J.R. and A.R.-E.; project administration, J.R.; funding acquisition, J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish State Research Agency, grant number AGL2017-83325-C4-2-R, and by the MACMHER "Grups Operatius" promoted by Departament of Agriculture of the Catalan Government (DARP) in 2018. The first author obtained a PhD grant from the University of Lleida (PhD grants).

Acknowledgments: We would like to acknowledge Maria Casamitjana, Bruna Català and Irene Llobera for their help in the field work carried out, and the facilities and technical support supplied by the company Raventós–Cordorníu to carry out the field trials in their vineyards in Raimat.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bajwa, A.A.; Sadia, S.; Ali, H.H.; Jabran, K.; Peerzada, A.M.; Chauhan, B.S. Biology and management of two important *Conyza weeds*: A global review. *Environ. Sci. Pollut. Res.* **2016**, *23*, 24694–24710. [CrossRef] [PubMed]
2. Zambrano-Navea, C.; Bastida, F.; Gonzalez-Andujar, J.L. A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Res.* **2013**, *53*, 213–220. [CrossRef]
3. Davis, V.M.; Johnson, W.G. Glyphosate-resistant Horseweed (*Conyza canadensis*) emergence, survival, and fecundity in no-till soybean. *Weed Sci.* **2008**, *56*, 231–236. [CrossRef]
4. Urbano, J.M.; Borrego, A.; Torres, V.; Leon, J.M.; Jimenez, C.; Dinelli, G.; Barnes, J. Glyphosate-resistant Hairy Fleabane (*Conyza bonariensis*) in Spain. *Weed Technol.* **2007**, *21*, 396–401. [CrossRef]
5. Wu, H.; Walker, S.; Rollin, M.J.; Tan, D.K.Y.; Robinson, G.; Werth, J. Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* [L.] Cronquist). *Weed Biol. Manag.* **2007**, *7*, 192–199. [CrossRef]
6. Storrie, A. Distribution and Identification of *Conyza bonariensis* Species. In *Proceedings of 2nd Fleabane Workshop*; Walker, S., Werth, J., Widderick, M., Eds.; Department of Primary Industries and Fisheries: Toowoomba, Australia, 2007; pp. 10–11.
7. Shrestha, A.; Hembree, K.; Wright, S. *Biology and Management of Horseweed and Hairy Fleabane in California*; University of California, ARN Publication: Davis, CA, USA, 2008; Volume 8314, pp. 1–9.

8. Heap, I. The International Herbicide-Resistant Weed Database. 2008. Available online: http://weedscience.org/Home.aspx (accessed on 1 December 2021).
9. González-Torralva, F.; Cruz-Hipolito, H.; Bastida, F.; Mülleder, N.; Smeda, R.J.; Prado, R. De Differential Susceptibility to Glyphosate among the *Conyza weed* Species in Spain. *J. Agric. Food Chem.* **2010**, *58*, 4361–4366. [CrossRef]
10. González-Torralva, F.; Gil-Humanes, J.; Barro, F.; Domínguez-Valenzuela, J.A.; Prado, R. De First evidence for a target site mutation in the EPSPS2 gene in glyphosate-resistant Sumatran fleabane from citrus orchards. *Agron. Sustain. Dev.* **2014**, *34*, 553–560. [CrossRef]
11. Heap, I.; Duke, S.O. Overview of glyphosate-resistant weeds worldwide. *Pest Manag. Sci.* **2018**, *74*, 1040–1049. [CrossRef]
12. Peters, B.; Strek, H.J. Herbicide discovery in light of rapidly spreading resistance and ever-increasing regulatory hurdles. *Pest Manag. Sci.* **2018**, *74*, 2211–2215. [CrossRef]
13. Wu, H.; Walker, S. Fleabane biology and control. In *Proceedings of Fleabane Workshop*; Walker, S., Widderick, M., Wu, H., Eds.; Queensland DPI: Toowoomba, Australia, 2004; pp. 5–610.
14. Wu, H.; Walker, S.; Robinson, G.; Coombes, N. Control of flaxleaf fleabane (*Conyza bonariensis*) in Wheat and Sorghum. *Weed Tech.* **2010**, *24*, 102–107. [CrossRef]
15. Oerke, E.C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43. [CrossRef]
16. Alcorta, M.; Fidelibus, M.W.; Steenwerth, K.L.; Shrestha, A. Competitive Effects of Glyphosate-Resistant and Glyphosate-Susceptible Horseweed (*Conyza canadensis*) on Young Grapevines (*Vitis vinifera*). *Weed Sci.* **2011**, *59*, 489–494. [CrossRef]
17. Prosdocimi, M.; Cerdà, A.; Tarolli, P. Soil water erosion on Mediterranean vineyards: A review. *CATENA* **2016**, *141*, 1–21. [CrossRef]
18. Smart, D.R.; Schwass, E.; Lakso, A.; Morano, L. Grapevine rooting patterns: A comprehensive analysis and a review. *Am. J. Enology Vitic.* **2006**, *57*, 89–104.
19. Smith, R.; Bettiga, L.; Cahn, M.; Baumgartner, K.; Jackson, L.E.; Bensen, T. Vineyard floor management affects soil, plant nutrition, and grape yield and quality. *Calif. Agric.* **2008**, *62*, 184–190. [CrossRef]
20. Jradi, S.; Chameeva, T.B.; Delhomme, B.; Jaegler, A. Tracking carbon footprint in French vineyards: A DEA performance assessment. *J. Clean. Prod.* **2018**, *192*, 43–54. [CrossRef]
21. Lechenet, M.; Bretagnolle, V.; Bockstaller, C.; Boissinot, F.; Petit, M.S.; Petit, S.; Munier-Jolain, N.M. Reconciling Pesticide Reduction with Economic and Environmental Sustainability in Arable Farming. *PLoS ONE* **2014**, *9*, e97922. [CrossRef] [PubMed]
22. Bailey, K.L. *The Bioherbicide Approach to Weed Control Using Plant Pathogens, Integrated Pest Management: Current Concepts and Ecological Perspective*; Abrol Dharam, P., Ed.; Elsevier (Academic Press): New Delhi, India, 2014; pp. 245–266.
23. Bordin, E.R.; Frumi Camargo, A.; Stefanski, F.S.; Scapini, T.; Bonatto, C.; Zanivan, J.; Preczeski, K.; Modkovski, T.A.; ReichertJúnior, F.; Mossi, A.J.; et al. Current production of bioherbicides: Mechanisms of action and technical and scientific challenges toimprove food and environmental security. *Biocatal. Biotransform.* **2021**, *39*, 1–14. [CrossRef]
24. Cordeau, S.; Triolet, M.; Wayman, S.; Steinberg, C.; Guillemin, J.P. Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. *Crop Prot.* **2016**, *87*, 44–49. [CrossRef]
25. Seiber, J.N.; Coats, J.; Duke, S.O.; Gross, A.D. Biopesticides: State of the art and future opportunities. *J. Agric. Food Chem.* **2014**, *62*, 11613–11619. [CrossRef] [PubMed]
26. Bolda, M.P.; Tourte, L.; Klonsky, K.M.; De Moura, R.L. *University of California Cooperative Extension*; Bulletin ST-CC-06-O; Davis, CA, USA, 2016.
27. Pavela, R.; Benelli, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Sci.* **2016**, *21*, 1000–1007. [PubMed]
28. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef] [PubMed]
29. European Food Safety Authority. Conclusion on the peer review of the pesticide risk assessment of the activesubstance Fatty acids C7 to C18 (approved under Regulation (EC) No 1107/2009 as Fatty acids C7 to C20). *EFSA J.* **2013**, *11*, 3023. [CrossRef]
30. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World Map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
31. Henderson, C.F.; Tilton, E. Test with acaricides against the brown wheat mite. *J. Econ. Ent.* **1995**, *48*, 157–161. [CrossRef]
32. Travlos, I.S.; Chachalis, D. Glyphosate-Resistant Hairy Fleabane (*Conyza bonariensis*) Is Reported in Greece. *Weed Technol.* **2010**, *24*, 569–573. [CrossRef]
33. Okumu, M.N.; Vorster, B.J.; Reinhardt, C.F. Growth-stage and temperature influence glyphosate resistance in *Conyza bonariensis* (L.) Cronquist. *South African J. Bot.* **2019**, *121*, 248–256. [CrossRef]
34. Pline, W.A.; Hatzios, K.K.; Hagood, E.S. Weed and Herbicide-Resistant Soybean (Glycine max) Response to Glufosinate and Glyphosate Plus Ammonium Sulfate and Pelargonic Acid1. *Weed Tech.* **2000**, *14*, 667–674. [CrossRef]
35. Travlos, I.S.; Rapti, E.; Gazoulis, I.; Kanatas, P.; Tataridas, A.; Kakabouki, I.; Papastylianou, P. The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. *Agronomy* **2020**, *10*, 1687. [CrossRef]
36. Webber, C.L., III; Merritt, J.T.; Shrefler, J.W. Weed control in yellow squash using sequential postdirected applications of pelargonic acid. *Hort Tech.* **2014**, *24*, 25–29. [CrossRef]
37. Kanatas, P.; Antonopoulos, N.; Gazoulis, I.; Travlos, I.S. Screening glyphosate-alternative weed control options in important perennial crops. *Weed Sci.* **2021**, *69*, 704–718. [CrossRef]

38. Wu, H. The biology of Australian weeds 49. *Conyza bonariensis* (L.) Cronquist. *Plant Prot. Q.* **2007**, *22*, 122–131.
39. Webber, C.L., III; White, P.M.; Shrefler, J.W.; Spaunhorst, D.J.; Webber, C.L. Impact of Acetic Acid Concentration, Application Volume, and Adjuvants on Weed Control Efficacy. *J. Agric. Sci.* **2018**, *10*, 1. [CrossRef]
40. Ghorbani, R.; Seel, W.; Hassan Rashed, M.; Leifert, C. Effect of Plant Age, Temperature and Humidity on Virulence of Ascochyta Caulina on Common Lambsquarters (*Chenopodium Album*). *Weed Sci.* **2006**, *54*, 526–531. Available online: http://www.jstor.org/stable/4539425 (accessed on 10 December 2021). [CrossRef]
41. Hallett, S.G. Where are the bioherbicides? *Weed Sci.* **2005**, *53*, 404–415. [CrossRef]
42. Stewart, C.; Nurse, R.; Sikkema, P. Time of Day Impacts Postemergence Weed Control in Corn. *Weed Tech.* **2009**, *23*, 346–355. [CrossRef]
43. Waltz, A.L.; Martin, A.R.; Roeth, F.W.; Lindquist, J.L. Glyphosate Efficacy on Velvetleaf Varies with Application Time of Day. *Weed Technol.* **2004**, *18*, 931–939. [CrossRef]
44. Zambrano-Navea, C.; Bastida, F.; Gonzalez-Andujar, J.L. A cohort-based stochastic model of the population dynamic and long-term management of *Conyza bonariensis* in fruiting tree crops. *Crop Prot.* **2016**, *80*, 15–20. [CrossRef]
45. Valencia-Gredilla, F.; Supiciche, M.L.; Chantre, G.R.; Recasens, J.; Royo-Esnal, A. Germination behaviour of Conyza bonariensis to constant and alternating temperatures across different populations. *Ann. Appl. Biol.* **2020**, *176*, 36–46. [CrossRef]
46. Zambrano-Navea, C.; Bastida, F.; Gonzalez-Andujar, J.L. Demography of *Conyza bonariensis* (Asteraceae) in a ruderal Mediterranean habitat. *Phytoparasitica* **2018**, *46*, 263–272. [CrossRef]
47. Webber, C.L., III; Shrefler, J.W.; Brandenberger, L.P. Organic Weed Control. In *Herbicides—Environmental Impact Studies and Management Approaches*; IntechOpen: London, UK, 2012. [CrossRef]
48. Dayan, F.E.; Cantrell, C.L.; Duke, S.O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034. [CrossRef] [PubMed]
49. Charudattan, R.; Dinoor, A. Biological control of weeds using plant pathogens: Accomplishments and limitations. *Crop Prot.* **2000**, *19*, 691–695. [CrossRef]