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(4Z)-Lachnophyllum Lactone, an Acetylenic Furanone from *Conyza bonariensis*, Identified for the First Time with Allelopathic Activity against *Cuscuta campestris*

Mónica Fernández-Aparicio [1,*], Gabriele Soriano [2], Marco Masi [2,*], Pilar Carretero [3], Susana Vilariño-Rodríguez [4] and Alessio Cimmino [2]

[1] Department of Plant Breeding, Institute for Sustainable Agriculture (IAS), CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain
[2] Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte S. Angelo, Via Cintia, 80126 Naples, Italy; gabriele.soriano@unina.it (G.S.); alessio.cimmino@unina.it (A.C.)
[3] University of Córdoba, Campus de Rabanales, 14071 Córdoba, Spain; t72cargp@uco.es
[4] ALGOSUR S.A., Ctra Lebrija-Trebujena km 5.5, 41740 Sevilla, Spain; svilarino@algosur.com
* Correspondence: monica.fernandez@ias.csic.es (M.F.-A.); marco.masi@unina.it (M.M.)

Abstract: *Cuscuta* species are obligate parasitic plants that infect the stems of a wide range of hosts including many crop and weed species causing severe agricultural problems. Using in vitro experiments to screen organic extracts prepared from fifteen autotrophic weed species found in agricultural fields infested with *Cuscuta campestris*, we have identified for the first time a strong phytotoxic activity in *Conyza bonariensis* extract against *C. campestris*. Additional pot experiments revealed that seven day-old *Cuscuta* seedlings had reduced capacity to coil and properly attach on *Conyza* plants, leading to reduced parasitic weed infection. Via activity-guided fractionation of *Conyza* extracts, we isolated and identified the acetylenic furanone (4Z)-lachnophyllum lactone as the major active component, with a concentration required to achieve reduction of 50% *Cuscuta* seedling growth (IC_{50}) of 24.8 µg/mL. The discovery of (4Z)-lachnophyllum lactone bioactivity could aid the development of efficient and sustainable management strategies for *C. campestris*, whose control is limited or non-existent.

Keywords: dodder; parasitic weeds; bioherbicides; sustainable crop protection

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1. Introduction

Approximately 1% of angiosperms, distributed among 28 dicotyledonous families, are parasitic on other plants [1,2]. Some of these parasitic plants are obligate parasites that have abandoned key mechanisms that allow plants to function autotrophically and therefore depend on their host plants for nutrient acquisition, growth and reproduction. Among them, about 170 species of dodders (*Cuscuta* spp., Convolvulaceae) thrive at the expense of other plants in tropical, subtropical and temperate regions [3,4]. *Cuscuta* plants have no roots nor leaves and their seedlings coil around the stems of other plants, forming infective haustoria that withdraw nutrients and water through vascular connections [5,6]. Among dodder species, *Cuscuta campestris* Yunck. is one of the most damaging species for agricultural production, for which control in the majority of crops is limited or non-existent [7]. On one side, the intimacy of connections between *Cuscuta* and its crop host renders the available selective herbicides ineffective, and on the other side, there is a lack of development of resistant varieties against *Cuscuta* infection for the majority of crops affected [7–9]. In addition, the persistent *Cuscuta* seedbank and broad host range in the agricultural fields, which includes many species of crops and weeds, make the use of rotation ineffective for its control.

Elucidation of novel structures and modes of action of natural compounds with allelopathic activity against parasitic weeds is an alternative solution to provide efficacy and

sustainability in strategies for parasitic weed management [10–12]. Plants are a generous source of natural pesticides [13,14], but only a small fraction of plant metabolites has been screened for herbicidal activity [15]. From the screening of natural compounds produced by allelopathic plants, compounds with specific herbicidal activity against parasitic weeds have been previously discovered [10,11,16]. *Conyza* species (Asteraceae) are invasive weeds native to America, affecting more than 40 crops in 70 countries [17]. In Spain, three *Conyza* species, *Conyza bonariensis* (L.) Cronq., *Conyza canadensis* (L.) Cronq. and *Conyza sumatrensis* (Retz.) E. Walker, cause important problems in agricultural fields [18–20]. Allelopathy plays a part in their invasive success [21–24]. In this work, we used allelopathy assays to screen fifteen weedy species found in southern Spanish agricultural fields with soils infested with *Cuscuta.* This screening allowed us to identify for the first time the strong allelopathic activity of *C. bonariensis* dichloromethane extract against the growth of *C. campestris*. The bioactivity-guided purification of the *Conyza* extract led to the isolation of a main metabolite responsible for the phytotoxic activity. Using spectroscopic methods (essentially, ^{1}HNMR and ESI-MS), we identified this metabolite as (4*Z*)-lachnophyllum lactone, a phytotoxin with a potent activity against *C. campestris* growth never reported before.

2. Materials and Methods

2.1. General Experimental Procedures

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$ on a Bruker spectrometer (Karleshrue, Germany). The NOESY (nuclear overhauser enhancement spectroscopy) experiment was performed using standard Bruker microprograms. The same solvent was used as an internal standard. ESI mass spectra and liquid chromatography (LC)/MS analyses were performed using the LC/MS TOF system Agilent 6230B (Agilent Technologies, Milan, Italy), HPLC 1260 Infinity. The HPLC separations were performed with a Phenomenex (Bologna, Italy) LUNA (C18 (2) 5 µ 150 × 4.6 mm). Analytical and preparative thin-layer chromatography (TLC) was performed on silica gel (Kieselgel 60, F_{254}, 0.25 and 0.5 mm, respectively) plates (Merck, Darmstadt, Germany), and the compounds were visualized by exposure to UV light and/or iodine vapors or by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min.

2.2. Plant Material

Seeds from fifteen weed species from Amaranthaceae (*Amaranthus albus* L. and *Amaranthus retroflexus* L.), Asteraceae (*Conyza bonariensis* (L.) Cronq.), Boraginaceae (*Heliotropium europaeum* L.), Brassicaceae (*Capsella bursa-pastoris* (L.) Medik. and *Diplotaxis virgata* (Cav.) DC.), Convolvulaceae (*Convolvulus arvensis* L.), Malvaceae (*Malva sylvestris* L.), Papaveraceae (*Fumaria officinalis* L.), Polygonaceae (*Polygonum aviculare* L.), Portulacaceae (*Portulaca oleracea* L.), Solanaceae (*Datura stramonium* L. and *Solanum nigrum* L.), Urticaceae (*Urtica dioica* L.) and Zygophyllaceae (*Tribulus terrestris* L.) were collected during the season of 2016–2017 from a buckwheat field at Institute for Sustainable Agriculture (IAS-CSIC, Córdoba, southern Spain). Weed seeds were surface sterilized with 0.5% (*w*/*v*) sodium hypochlorite and 0.02% (*v*/*v*) Tween 20 for 5 min, rinsed thoroughly with distilled water and dried in a laminar airflow cabinet. Then, weed seeds were sown in a greenhouse in 1 L pots containing sand and peat (1:1, *v*:*v*) and grown for 40 days (23/20 °C, 16/8 h day/night). Then, the stem of each weed plant was cut 2–3 cm above the soil surface, and the roots were carefully washed, dried with filter paper, immediately frozen and then maintained at −80 °C until lyophilization.

Seeds of *Cuscuta* (*Cuscuta campestris* Yunck.) were collected in July 2019 from mature *Cuscuta* plants parasitizing pea plants in fields of IAS-CSIC. Dry *Cuscuta* seeds were separated from capsules using a winnowing with a fan and sifting with a 0.6 mm mesh-size sieve. *Cuscuta* seeds were stored dry in the dark at room temperature until use for this work in 2022.

2.3. Plant Extraction for Screening of Allelopathy in Weed Species

About 6 g of lyophilized tissue of each weed species described in Section 2.2 were milled in a Warry Blender and the resulting powder was macerated overnight in 200 mL of a mixture of methanol–distilled water (1:1, *v*:*v*) under stirring in the dark at room temperature. Then, the suspension was centrifuged at 7000 rpm for 1 h, at 4 °C. The supernatant was extracted with dichloromethane (3 × 200 mL). For each weed species, the organic extracts were combined, dried with sodium sulfate, filtered and evaporated under reduced pressure.

2.4. In Vitro Experiments for Screening of Allelopathy against Cuscuta Seedling Growth

Effects of dichloromethane extracts of fifteen weed species described in Section 2.2 were tested on growth of *Cuscuta* seedlings. To promote *Cuscuta* germination, the hard coat of *Cuscuta* seeds was eliminated by scarification with sulfuric acid during 45 min [25], followed by thorough rinses and air-dried. Then, five scarified *Cuscuta* seeds were manually placed using tweezers on 5 cm-diameter filter paper discs inside 5.5 cm-diameter Petri dishes. Stock solutions of each organic extract dissolved in methanol were diluted up to 100 µg weed extract/mL sterilized distilled water. The final concentration of methanol was 2%. Triplicate aliquots of 1 mL of each weed extract were applied to filter paper discs containing the scarified *Cuscuta* seeds. Triplicate aliquots of a treatment only containing 2% methanol and sterile distilled water was used as control. Treated *Cuscuta* seeds were incubated in the dark at 23 °C for 6 days. The seedling length was measured in each of the five *Cuscuta* seedlings for each of the three replicate filter paper discs per treatment. A second in vitro bioassay was performed to confirm the *Conyza* inhibitory activity identified in the first allelopathic screening. *Conyza* organic extract dissolved in methanol was applied at seven concentrations (100, 75, 50, 25, 10 and 5 µg *Conyza* extract/mL sterilized distilled water, maintaining the final concentration of methanol constant at 2%) to filter paper discs containing five scarified *Cuscuta* seeds as described before. Triplicate aliquots of a treatment only containing 2% methanol and sterile distilled water was used as control. After six days, *Cuscuta* seedling length was determined.

2.5. Pot Experiments for Validation of Conyza Allelopathic Activity

In a greenhouse, 40 pots containing sand and peat (1:1, *v*:*v*) were prepared for the validation of *Conyza* allelopathic activity against *Cuscuta campestris*. As a non-allelopathic control we used a subset of eight weed species from those that showed no allelopathic activity during the in vitro screening. Plants of *Amaranthus albus*, *Amaranthus retroflexus*, *Diplotaxis virgata*, *Convolvulus arvensis*, *Conyza bonariensis*, *Malva sylvestris*, *Polygonum aviculare*, *Portulaca oleracea* and *Solanum nigrum* were grown in pots at 23/20 °C, 16/8 h day/night. Each weed plant, at the stage of 4 leaves, was inoculated with pregerminated *Cuscuta* seeds. To promote *Cuscuta* germination, two days before inoculation, *Cuscuta* seeds were scarified with sulfuric acid for 45 min [25], rinsed thoroughly, and then spread in wet filter paper inside Petri dishes to allow their germination in the dark at 23 °C for 2 days. Then, nine pregerminated *Cuscuta* seedlings were manually placed using tweezers on the soil surface surrounding each weed plant at 1 cm distance from the weed stem. Seven days after germination, the *Cuscuta* seedlings were visually inspected and classified as either (i) unattached *Cuscuta* seedling or (ii) attached *Cuscuta* seedling. Fourteen days after inoculation, *Cuscuta* attached seedlings were classified as seedlings with adhesion disks (i) without posthaustorial growth, or (ii) with posthaustorial growth emerging at the *Cuscuta*–host interface.

2.6. Isolation and Identification of (4Z)-Lachnophyllum Lactone from Conyza Bonariensis Extracts

A measure of 27 g of *Conyza bonariensis*-lyophilized tissues obtained as described in Section 2.2 were extracted (1 × 150 mL) using a mixture of methanol–distilled water (1:1, *v*:*v*), 1% NaCl, under stirred conditions at room temperature for 24 h. The suspension was centrifuged, and the supernatant extracted using CH_2Cl_2 (3 × 150 mL). The residue (60

mg) of the organic extract, showing specific inhibitory activity against *Cuscuta campestris*, was purified by TLC eluted with EtOAc/*n*-hexane (6/4, *v*/*v*), yielding five homogeneous fractions which were screened for allelopathic activity against *Cuscuta* seedling growth as described in Section 2.7. The fraction with the strongest toxicity against *Cuscuta* was studied using spectroscopic methods (essentially ^{1}HNMR and ESI-MS).

2.7. Bioassays against Cuscuta Seedling Growth for Identification of (4Z)-Lachnophyllum Lactone Phytotoxic Activity

A third in vitro bioassay was used to guide the identification of the phytotoxic compound(s) during the fractioning of *Conyza* extract. Test fractions were dissolved in dimethyl sulfoxide and diluted up to 100 µg/mL sterilized distilled water. The final concentration of dimethyl sulfoxide was 2% in all treatments including the control. As described above, triplicate aliquots of 1 mL of each test fraction and control were applied to filter paper discs containing scarified *Cuscuta* seeds, and six days later, *Cuscuta* seedling length was determined. A subsequent screening was conducted to confirm the activity of (4Z)-lachnophyllum lactone and characterize its dose–response curve on *Cuscuta campestris*. Triplicate aliquots of 1 mL of (4Z)-lachnophyllum lactone dissolved in dimethyl sulfoxide was applied on *Cuscuta* scarified seeds at seven concentrations (100, 75, 50, 25, 10 and 5 µg/mL sterilized distilled water, maintaining the final dimethyl sulfoxide concentration constant at 2%). Triplicate aliquots of a treatment only containing 2% dimethyl sulfoxide and sterile distilled water was used as control. *Cuscuta* seedling length was determined six days later.

2.8. Statistical Analysis

All bioassays were performed using a completely randomized design. *Cuscuta* seedling length for each treatment was calculated relative to the *Cuscuta* seedling length of the corresponding control. Percentage data were approximated to normal frequency distribution by means of angular transformation (transformed value = $180/\Pi \times \text{arcsine}\,[\sqrt{(\%/100)}]$) and subjected to analysis of variance (ANOVA) using SPSS software for Windows (SPSS Inc., Chicago, IL, USA). The significance of mean differences among treatments was evaluated by Tukey test. Null hypothesis was rejected at the level of 0.05.

3. Results and Discussion

A first in vitro screening was conducted in order to identify candidate weed species as sources of allelochemicals that could be used for the control of *Cuscuta campestris*. Dichloromethane extracts obtained from fifteen weed species were individually applied to *Cuscuta* seeds at a concentration of 100 µg weed extract/mL sterilized distilled water and levels of *Cuscuta* seedling growth rated in comparison with the control (Figure 1). This first study revealed significant differences in phytotoxicity against *Cuscuta* growth among weed extracts tested (ANOVA, $p < 0.001$) and allowed us to identify an exceptional phytotoxic activity in the dichloromethane extract prepared from *Conyza bonariensis* while the dichloromethane extract prepared from the rest of the weed species showed no or negligible phytotoxicity.

In a second in vitro study, a dose–response screening was conducted to validate the effect on *Cuscuta* growth induced by *Conyza* extract in comparison with the growth of *Cuscuta* when treated with the control. This second study confirmed the phytotoxic activity of *Conyza* against *Cuscuta* and revealed an average of 99.3 ± 0.4% and 66.8 ± 1.8% inhibition of *Cuscuta* seedling length when, respectively treated with *Conyza* extract at 100 and 75 µg/mL. Negligible phytotoxicity was observed when *Cuscuta* seeds were treated with lower concentrations (ranged from 50 to 5 µg/mL) of *Conyza* extract (Figure 2).

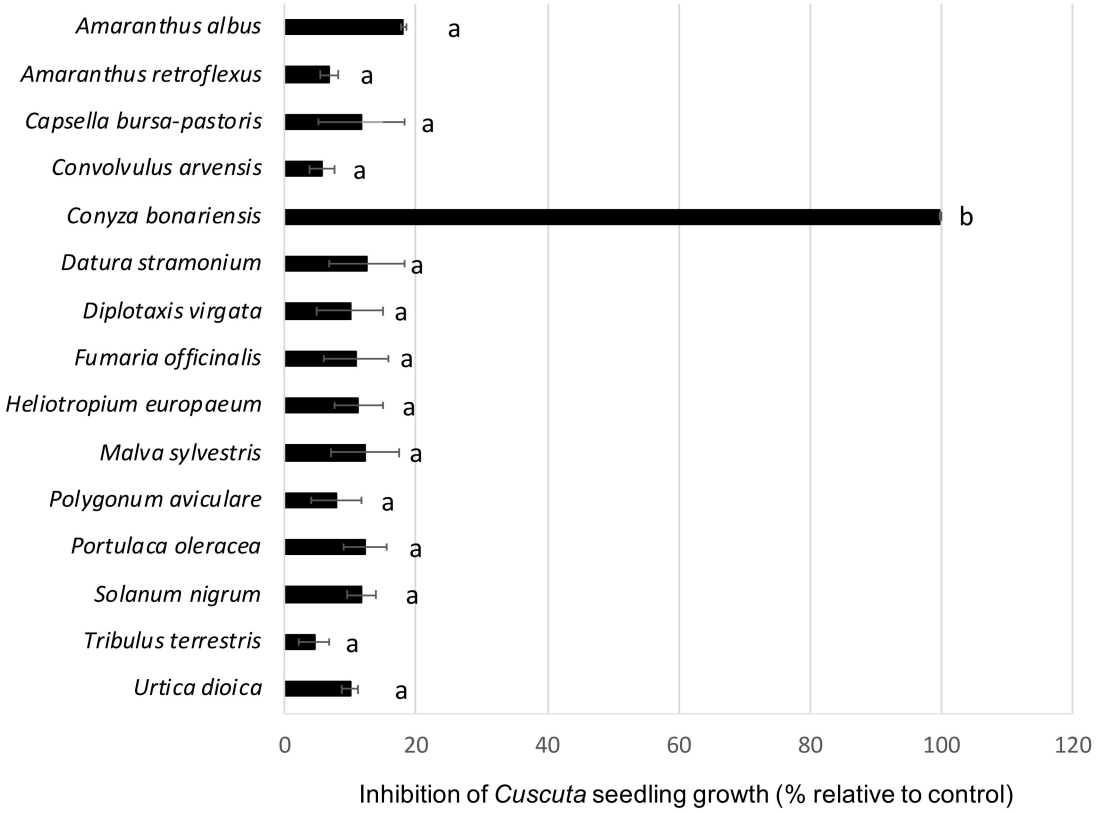

Figure 1. Allelopathic effects of dichloromethane extracts of fifteen weed species on growth of *Cuscuta campestris* seedlings expressed as percentage of inhibition compared to the control. Bars with different letters are significantly different using the Tukey test (p = 0.05). Error bars represent the standard error of the mean.

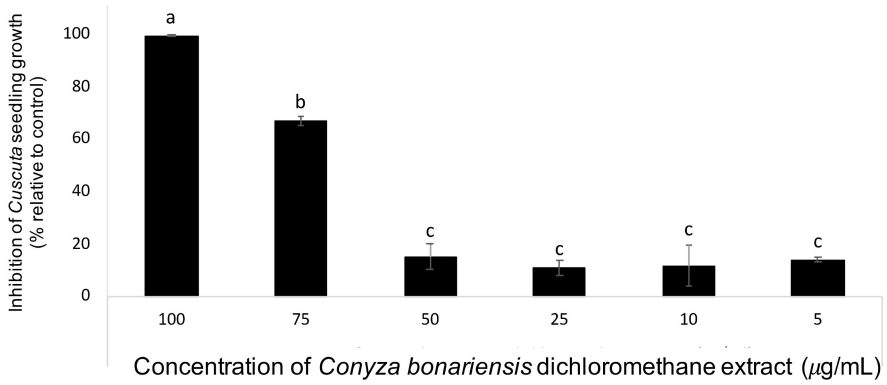

Figure 2. Dose–response screening of the phytotoxic activity on *Cuscuta campestris* seedling growth of *Conyza bonariensis* dichloromethane extract. Treatments with different letters are significantly different using the Tukey test (p = 0.05). Error bars represent the standard error of the mean.

Species of *Conyza* are sources of abundant phytotoxic compounds such as catechol, gallic acid, syringic acid and vanillic acid [24]. In vitro phytotoxicity of the closely related species *Conyza canadensis* against *Lactuca sativa* and *Agrostis stolonifera* was previously identified at 1 mg/mL [26]. To the best of our knowledge, there are no previous reports on phytotoxicity from any *Conyza* species against *Cuscuta* seedlings. On the contrary, Gaertner [25] described *Conyza canadensis* as a weed which *C. campestris* has the capacity to infect. Parasitic weeds can display preferences to infect different hosts in a species-specific manner [27]. To further explore in vivo the allelopathic potential of *C. bonariensis*

revealed by our in vitro screening, and also to confirm the differences between our results with *C. bonariensis* and those from Gaertner [25] with *C. canadensis*, we conducted a pot experiment to observe the interaction between our candidate allelopathic species *C. bonariensis* for *Cuscuta* control and *Cuscuta* plants. The interaction between *C. bonariensis* and *Cuscuta* was compared with the interaction between *Cuscuta* and a control group of eight non-allelopathic weed species whose dichloromethane extracts showed no phytotoxicity against *Cuscuta* in the first in vitro screening. Plants of *Conyza* and the eight control weeds were cultivated in a greenhouse and individually inoculated with pre-germinated *Cuscuta* seeds. Without host infection, *Cuscuta* seedling viability expires in 3–7 weeks depending on the photosynthetic capacity of the *Cuscuta* species considered [28]. In our work with the species *Cuscuta campestris*, unattached *Cuscuta* seedlings did not show visual evidence of photosynthetic activity and their viability expired in two weeks without attachment to a host plant. Therefore, we determined success of *Cuscuta* coiling on the host at seven days after *Cuscuta* germination (Figure 3) and the success of infection at fourteen days after *Cuscuta* germination (Figure 4).

Cuscuta seedlings explore the environment searching for a host to which they can coil using a rotative movement guided by host-derived volatiles and far-red light [29–31]. *Cuscuta* seedlings also coil nonspecifically around inert objects, such as metal or plastic sticks they accidentally encounter during their rotative movement. Therefore, reduced coiling can be a sign of allelopathic activity. Figure 3 shows that the success of coiling of seven day-old *Cuscuta* seedlings was significantly affected by the weed species considered (ANOVA, $p = 0.03$), with the percent of *Cuscuta* seedlings that coiled and established proper contact with *Conyza* plants being significantly lower (34.2 $\pm$ 9.9%) than the percent of coiling around the stems of the eight non-allelopathic control weed species (percent of coiling ranged from 75.6 $\pm$ 12.4% in *Amaranthus albus* to 93.8 $\pm$ 3.8% in *Portulaca oleracea*).

Once *Cuscuta* coils around the stems of its hosts, tactile signals, light spectrum and phytohormones promote the development of an haustorium that enables infection [32–35]. The haustorium invades the host stem, connecting the host xylem to withdraw nutrients and water used by *Cuscuta* to develop posthaustorial stems [3]. In our pot experiment, the success of infection was observed as the percent of coiled *Cuscuta* seedlings that were able to develop posthaustorial stems from the site of attachment (Figure 4). There were not significant differences among the infection success of *Cuscuta* on stems of *Conyza* (57.5 $\pm$ 10.9%) and the infection success of *Cuscuta* on stems of the non-allelopathic weed species, which ranged from 36.1 $\pm$ 7.3% in *A. albus* to 88.9 $\pm$ 7.9% in *Polygonum aviculare* (data for the rest of species are not shown).

Despite the capacity of *Cuscuta* to infect plants of *Amaranthus* and *P. oleracea* observed in our work (Figures 3 and 4) and by that from Orkić et al. [36], a previous work by Gaertner [25] described *A. retroflexus* and *P. oleracea* as weed species on which *Cuscuta campestris* would not be able to survive. On the contrary, *Cuscuta campestris* was reported to have high binding ability on *Conyza canadensis* by Orkić et al. [36], however, our work revealed that *Cuscuta* seedlings had reduced capacity to coil and properly attach to *Conyza bonariensis* plants, but those few *Cuscuta* seedlings able to attach on *Conyza* had the capacity to infect and grow for at least fourteen days. Orkić et al. [36] obtained the results through observations of field infections which could be influenced by a high *Cuscuta* density because these authors did not distinguish between success in coiling and success in infection as we did in our work (Figures 3 and 4). In addition, field observations could not distinguish whether the infection was produced by either *Cuscuta* seedlings or by mature *Cuscuta* stems originated in nearby plants (capacity of infection could differ between primary infection of prehaustorial *Cuscuta* seedlings and secondary infection of mature posthaustorial *Cuscuta* stems). Our results indicate that *Cuscuta* seedlings had a reduced capacity to coil and attach on *Conyza bonariensis* plants (Figure 3D) in comparison with the non-allelopathic control weed species (Figure 3), but those *Cuscuta* seedlings that were able to properly attach to *Conyza* had the capacity to infect (Figure 4C), indicating that *Conyza* does not seem to impose resistance mechanisms against the invasion of the attached haustorium

and subsequent parasitic growth of *Cuscuta* seedlings up to at least an age of 14 days old. Resistance to *C. campestris* haustorium invasion and subsequent parasitic growth have been previously described in other plant species [7,8].

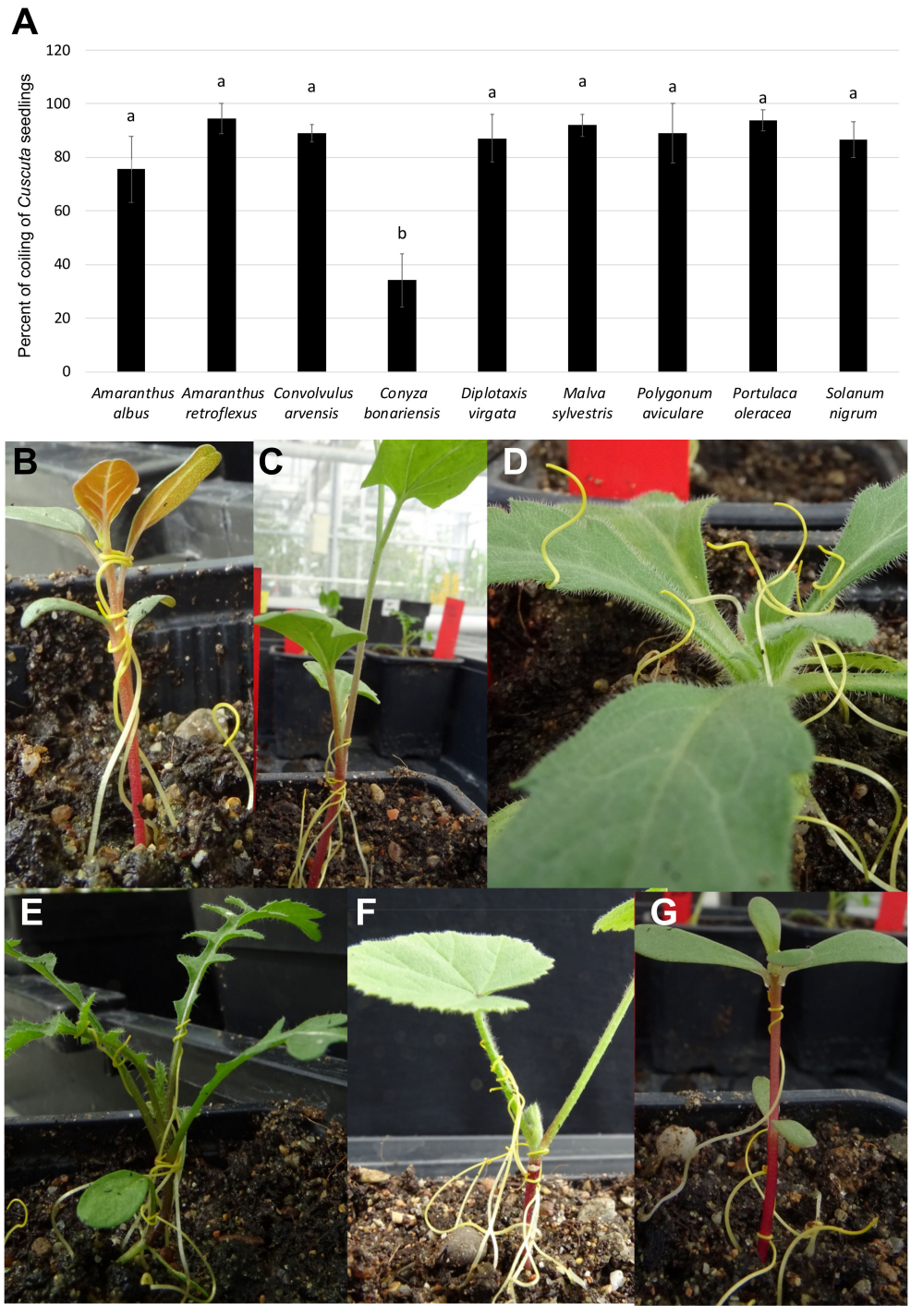

Figure 3. Compatibility of seven day-old *Cuscuta campestris* seedlings with a collection of 9 weed species. (**A**) Percentage of *Cuscuta* seedlings that coiled around weed plants, and illustrative photographs showing the coiling of *Cuscuta* seedlings on the stems of (**B**) *Amaranthus albus*; (**C**) *Convolvulus arvensis*; (**D**) *Conyza bonariensis*; (**E**) *Diplotaxis virgata*; (**F**) *Malva sylvestris*; (**G**) *Portulaca oleracea*. Treatments with different letters are significantly different according to the Tukey test ($p = 0.05$). Error bars represent the standard error of the mean.

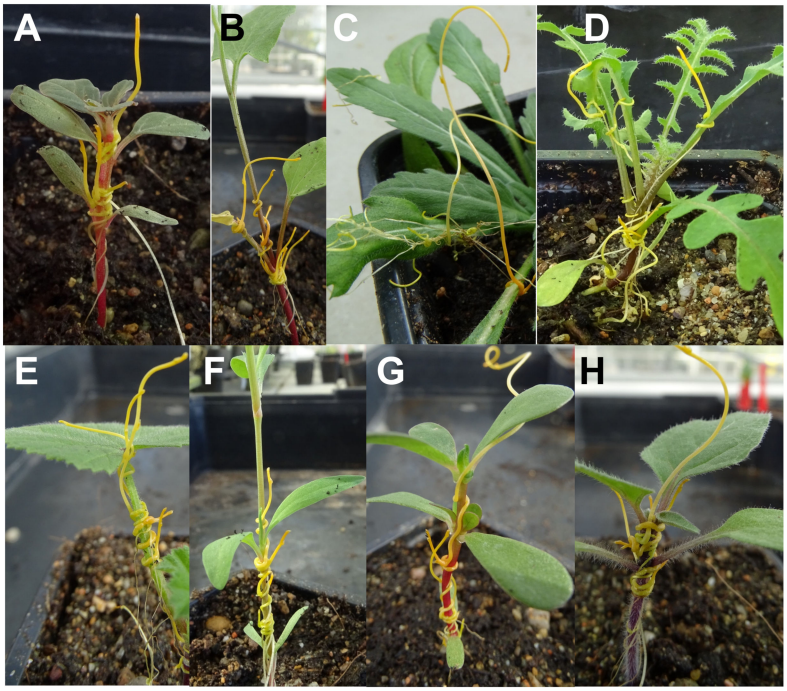

Figure 4. Illustrative photographs of *Cuscuta campestris* posthaustorial growth at the site of attachment on (**A**) *Amaranthus albus*; (**B**) *Convolvulus arvensis*; (**C**) *Conyza bonariensis*; (**D**) *Diplotaxis virgata*; (**E**) *Malva sylvestris*; (**F**) *Polygonum aviculare*; (**G**) *Portulaca oleracea*; (**H**) *Solanum nigrum*.

To identify the compound(s) responsible for the allelopathic activity against *Cuscuta* in *Conyza bonariensis* dichloromethane extract, an increased amount of *Conyza*-lyophilized tissue was extracted. The resulting organic extract was subjected to fractionation using TLC as reported in the Materials and Methods Section, yielding five homogeneous fractions (CBA, CBB, CBC, CBD and CBE). Phytotoxicity screening revealed that, among the five fractions of the *Conyza* extract, the CBB fraction caused the strongest phytotoxicity in seedlings of *Cuscuta* (Figure 5). This phytotoxicity was observed as the abnormal growth of the *Cuscuta* seedling with a length reduction in comparison with the control seedlings.

The investigation of the active fraction CBB, by the study of the ^{1}H NMR and ESI-MS spectra, revealed that it consisted in a pure compound, which was identified as (4*Z*)-lachnophyllum lactone, the (*Z*)-5-(hex-2-yn-1-ylidene)furan-2(5*H*)-one (Figure 6, R_f = 0.76, 5.10 mg). Its structure was confirmed by comparison of the ^{1}H-NMR data with those reported in the literature [26,37,38]. The configuration of the double bond was deduced from the presence of coupling between H-5 with H-3 and H-2 in the NOESY spectrum (Figure S1). In addition, the chemical shifts of H-5 (δ = 5.33) and C-5 (δ = 94.5) were very similar to those previously reported for lachnophyllum lactone and other natural furanones, with an α *Z*-disubstituted vinyl group, substantially differing from those having a *E*-vinyl group [38–41]. This structure was confirmed by the data of its ESI-MS spectrum which showed the sodiated adduct $[2M + Na]^+$ and protonated $[2M + H]^+$ dimers, and protonated $[M + H]^+$ ions at *m/z* 347, 325 and 163, respectively. This lactone with unspecified configuration was previously reported from different plant species [26,37,38,42]. The ^{1}H NMR data of the (*Z*) and (*E*) isomers of the acetylenic lactone were reported when both the compounds were isolated from *Baccharis paniculata*. A clear upfield shift of proton H-5 was observed for the *Z*-isomer [38].

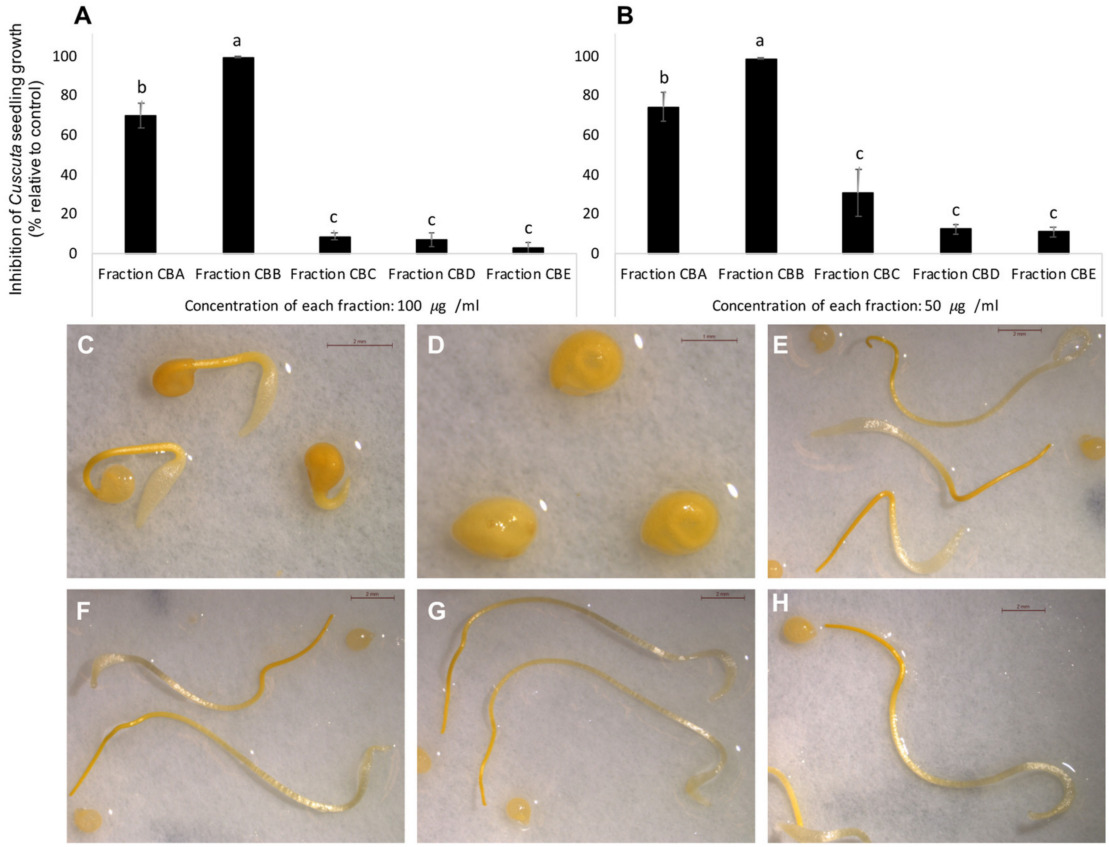

Figure 5. Allelopathic effects of five homogeneous fractions obtained from *Conyza bonariensis* dichloromethane extract on growth of six day-old *Cuscuta campestris* seedlings applied at (**A**) 100 µg/mL and (**B**) 50 µg/mL. (**C**–**H**) Photographs illustrating the development of *Conyza* seedlings when treated with: (**C**) *C. bonariensis* first fraction CBA; (**D**) *C. bonariensis* second fraction CBB; (**E**) *C. bonariensis* third fraction CBC; (**F**) *C. bonariensis* fourth fraction CBD; (**G**) *C. bonariensis* fifth fraction CBE; and (**H**) control treatment. In each Figure 5A,B, treatments with different letters are significantly different using the Tukey test ($p = 0.05$). Error bars represent the standard error of the mean.

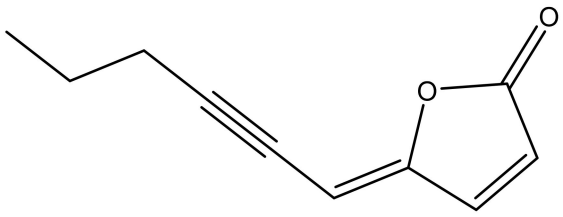

Figure 6. Structure of (4*Z*)-lachnophyllum lactone.

A subsequent dose–response screening was conducted to validate the phytotoxicity of (4*Z*)-lachnophyllum lactone, confirming the inhibitory activity of *Cuscuta* seedling growth at concentrations ranged from 100 to 10 µg/mL (Figure 7). The concentration required to achieve reduction of 50% *Cuscuta* seedling growth (IC_{50}) was observed at 24.8 µg/mL.

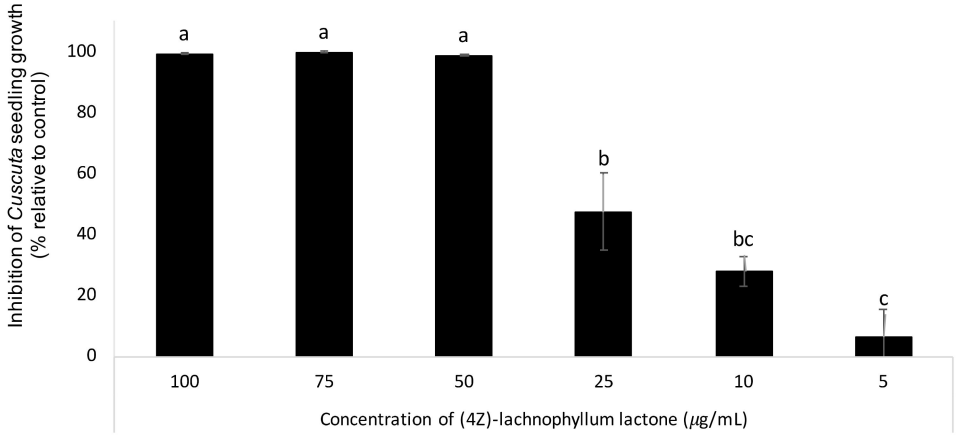

Figure 7. Dose–response screening of the phytotoxic activity on *Cuscuta campestris* seedling growth of (4Z)-lachnophyllum lactone. Treatments with different letters are significantly different using the Tukey test (p = 0.05). Error bars represent the standard error of the mean.

Previously, (4Z)-lachnophyllum lactone isolated from *Conyza canadensis* showed phytotoxic activity against *Lactuca sativa*, *Agrostis stolonifera* and *Lemna paucicostata* [26]. Furthermore, (4Z)-lachnophyllum lactone has reported fungitoxic activity against the fungi causing postharvest diseases in strawberry, i.e., *Colletotrichum acutatum*, *C. gloeosporioides* and *C. fragariae* [26], and causing postharvest diseases in citrus, i.e., *Penicillium digitatum* [43]. Fungitoxic activity against *Pyricularia oryzae* was identified in lachnophyllum lactone isolated from *Erigeron apiculatus* [44]. In addition, a repellent activity against *Monotonda neritoides* was identified in lachnophyllum lactone with unspecified configuration isolated from *Erigeron sumatrensis* [37]. These biological activities could be related to the presence in the structure of (4Z)-lachnophyllum lactone of an α,β-unsaturated carbonyl group, a known structural feature involved in nucleophilic Michael addition reaction mechanism frequently reported for bioactive natural compounds [40,45]. However, further studies are needed to elucidate the specific mode of action of this acetylenic furanone on *Cuscuta* development identified in this work.

4. Conclusions

From the allelopathy screening of dichloromethane extracts obtained from fifteen weed species, we identified that *Conyza bonariensis* extract causes strong phytotoxicity against *Cuscuta campestris*, a parasitic weed that causes worldwide agricultural problems and for which control is limited or non-existent. Sources of allelopathic activity have been previously identified in autotrophic weeds for control of parasitic weed species, such as *Orobanche* and *Phelipanche* species [46,47], however, to the best of our knowledge, this is the first report of such type of allelopathy screening of weed species against *Cuscuta* species, resulting in the identification of *Conyza bonariensis* as a source of compounds that can lead to the development of new bioherbicides. The bioactivity-guided fractionation of *Conyza* extract lead us to the isolation of the acetylenic furanone (4Z)-lachnophyllum lactone as the responsible compound for the allelopathic action of *C. bonariensis* against *C. campestris*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12060790/s1, Figure S1: NOESY spectrum of (4Z)-lachnophyllum lactone recorded in $CDCl_3$ at 500 MHz; Figure S2. ^{1}H-NMR spectrum of (4Z)-lachnophyllum lactone recorded in CDCl3 at 500 MHz; Figure S3. ^{13}C-NMR spectrum of (4Z)-lachnophyllum lactone recorded in CDCl3 at 125 MHz; Figure S4. ESI MS spectrum of (4Z)-lachnophyllum lactone recorded in positive modality.

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M.F.-A., G.S., M.M. and A.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Nickrent, D.L.; Malécot, V.; Vidal-Russell, R.; Der, J.P. A revised classification of Santalales. *Taxon* **2010**, *59*, 538–558. [CrossRef]
2. Westwood, J.H.; Yoder, J.I.; Timko, M.P.; de Pamphilis, C.W. The evolution of parasitism in plants. *Trends Plant Sci.* **2010**, *15*, 227–235. [CrossRef] [PubMed]
3. Dawson, J.H.; Musselman, L.J.; Wolswinkel, P.; Dörr, I. Biology and control of *Cuscuta*. *Rev. Weed Sci.* **1994**, *6*, 265–317.
4. Nickrent, D.L. Parasitic angiosperms: How often and how many? *Taxon* **2020**, *69*, 5–27. [CrossRef]
5. Christensen, N.M.; Dörr, I.; Hansen, M.; van der Kooij, T.A.W.; Schulz, A. Development of *Cuscuta* species on a partially incompatible host: Induction of xylem transfer cells. *Protoplasma* **2003**, *220*, 131–142. [CrossRef] [PubMed]
6. Dörr, I. The haustorium of *Cuscuta*—New structural results. In Proceedings of the Fourth International Symposium on Parasitic Flowering Plants, Marburg, Germany, 2–7 August 1987; Weber, H.C., Forstreuter, W., Eds.; Phillips-Universitat: Marburg, Germany, 1987; pp. 163–170.
7. Goldwasser, Y.; Miryamchik, H.; Sibony, M.; Rubin, B. Detection of resistant chickpea (*Cicer arietinum*) genotypes to *Cuscuta campestris* (field dodder). *Weed Res.* **2012**, *52*, 122–130. [CrossRef]
8. Córdoba, E.M.; Fernández-Aparicio, M.; González-Verdejo, C.I.; López-Grau, C.; Muñoz-Muñoz, M.V.; Nadal, S. Search for Resistant Genotypes to *Cuscuta campestris* Infection in two legume species, *Vicia sativa* and *Vicia ervilia*. *Plants* **2021**, *10*, 738. [CrossRef]
9. Nadler-Hassar, T.; Shaner, D.L.; Nissen, S.; Westra, P.; Rubin, B. Are herbicide-resistant crops the answer to controlling *Cuscuta*? *Pest Manag. Sci.* **2009**, *65*, 811–816. [CrossRef]
10. Cimmino, A.; Fernández-Aparicio, M.; Andolfi, A.; Basso, S.; Rubiales, D.; Evidente, A. Effect of fungal and plant metabolites on broomrapes (*Orobanche* and *Phelipanche* spp.) seed germination and radicle growth. *J. Agric. Food Chem.* **2014**, *62*, 10485–10492. [CrossRef]
11. Fernández-Aparicio, M.; Cimmino, A.; Evidente, A.; Rubiales, D. Inhibition of *Orobanche crenata* seed germination and radicle growth by allelochemicals identified in cereals. *J. Agric. Food Chem.* **2013**, *61*, 9797–9803. [CrossRef]
12. Fernández-Aparicio, M.; Delavault, P.; Timko, M.P. Management of infection by parasitic weeds: A Review. *Plants* **2020**, *9*, 1184. [CrossRef] [PubMed]
13. Dayan, F.E.; Duke, S.O. Natural compounds as next-generation herbicides. *Plant Physiol.* **2014**, *166*, 1090–1105. [CrossRef] [PubMed]
14. Macías, F.A.; Molinillo, J.M.; Varela, R.M.; Galindo, J.C. Allelopathy-a natural alternative for weed control. *Pest Manag. Sci.* **2007**, *63*, 327–348. [CrossRef] [PubMed]
15. Westwood, J.H.; Charudattan, R.; Duke, S.O.; Fennimore, S.A.; Marrone, P.; Slaughter, D.C.; Swanton, C.; Zollinger, R. Weed Management in 2050: Perspectives on the Future of Weed Science. *Weed Sci.* **2018**, *66*, 275–285. [CrossRef]
16. Cimmino, A.; Masi, M.; Rubiales, D.; Evidente, A.; Fernández-Aparicio, M. Allelopathy for parasitic plant management. *Nat. Prod. Commun.* **2018**, *13*, 1934578X1801300307. [CrossRef]
17. Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. *World Weeds: Natural Histories and Distribution*; John Wiley and Sons: New York, NY, USA, 1997; pp. 226–235.
18. González-Torralva, F.; Cruz-Hipolito, H.; Bastida, F.; Mülleder, N.; Smeda, R.J.; De Prado, R. Differential susceptibility to glyphosate among the *Conyza* weed species in Spain. *J. Agric. Food Chem.* **2010**, *58*, 4361–4366. [CrossRef]
19. 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]
20. Heap, I. International Survey of Herbicide Resistant Weeds. Available online: www.weedscience.com. (accessed on 10 February 2022).

21. Djurdjević, L.; Mitrović, M.; Gajić, G.; Jarić, S.; Kostić, O.; Oberan, L.; Pavlović, P. An allelopathic investigation of the domination of the introduced invasive *Conyza canadensis* L. *Flora* **2011**, *206*, 921–927. [CrossRef]
22. Gao, X.; Li, M.; Gao, Z.; Zhang, H.; Sun, Z. Allelopathic effects of *Conyza canadesis* the germination and growth of wheat, sorghum, cucumber, rape and radish. *Alellopathy J.* **2009**, *23*, 287–296.
23. Hu, G.; Zhang, Z.H. Aqueous tissue extracts of *Conyza canadensis* inhibit the germination and shoot growth of three native herbs with no autotoxic effects. *Planta Daninha* **2013**, *31*, 805–811. [CrossRef]
24. Shaukat, S.S.; Munir, N.; Siddiqui, I.A. Allelopathic responses of *Conyza canadensis* (L.) Cronquist: A cosmopolitan weed. *Asian J. Plant Sci.* **2003**, *14*, 1034–1039.
25. Gaertner, E.E. Studies of seed germination, seed identification, and host relationship in Dodders, *Cuscuta* spp. *Mem. Cornell Agric. Exp. Stn.* **1950**, *294*, 1–56.
26. Queiroz, S.C.N.; Cantrell, C.L.; Duke, S.O.; Wedge, D.E.; Nandula, V.K.; Moraes, R.M.; Cerdeira, A.L. Bioassay-directed isolation and identification of phytotoxic and fungitoxic acetylenes from *Conyza canadensis*. *J. Agric. Food Chem.* **2012**, *60*, 5893–5898. [CrossRef] [PubMed]
27. Fernández-Aparicio, M.; Sillero, J.C.; Rubiales, D. Resistance to broomrape species (*Orobanche* spp.) in common vetch (*Vicia sativa* L.). *Crop Prot.* **2009**, *28*, 7–12. [CrossRef]
28. Heide-Jørgensen, H.S. *Parasitic Flowering Plants*; Brill Leiden: Leiden, The Netherlands, 2008.
29. Holmes, M.G.; Smith, H. The function of phytochrome in plants growing in the natural environment. *Nature* **1975**, *254*, 512. [CrossRef]
30. Orr, G.L.; Haidar, M.A.; Orr, D.A. Small seed dodder (*Cuscuta planiflora*) phototropism toward far-red when in white light. *Weed Sci.* **1996**, *44*, 233–240. [CrossRef]
31. Runyon, J.B.; Mescher, M.C.; De Moraes, C.M. Volatile chemical cues guide host location and host selection by parasitic plants. *Science* **2006**, *313*, 1964–1967. [CrossRef]
32. Dörr, I. Feinstruktur intrazellular wachsender *Cuscuta*-Hyphen. *Protoplasma* **1969**, *67*, 123–137. [CrossRef]
33. Haidar, M.A.; Orr, G.L.; Westra, P. Effects of light and mechanical stimulation on coiling and pre-haustoria formation in *Cuscuta* spp. *Weed Res.* **1997**, *37*, 219–228. [CrossRef]
34. Hegenauer, V.; Körner, M.; Albert, M. Plants under stress by parasitic plants. *Curr. Opin. Plant Biol.* **2017**, *38*, 34–41. [CrossRef]
35. Vaughn, K.C. Attachment of the parasitic weed dodder to the host. *Protoplasma* **2002**, *219*, 227–237. [CrossRef] [PubMed]
36. Orkic, I.; Stefanic, E.; Antunovic, S.; Zima, D.; Kovacevic, V.; Stefanic, I.; Dimic, D. Host range of field dodder (*Cuscuta campestris* Yuncker) in sugar beet fields: Example from Northeastern Croatia. *Listy Cukrov. Reparske* **2019**, *135*, 198–203.
37. Nawamaki, T.; Sakakibara, T.; Ohta, K. Isolation and identification of lachnophyllum lactone and osthol as repellents against a sea snail. *Agric. Biol. Chem.* **1979**, *43*, 1603–1604.
38. Rivera, A.P.; Arancibia, L.; Castillo, M. Clerodane diterpenoids and acetylenic lactones from *Baccharris paniculata*. *J. Nat. Prod.* **1989**, *52*, 433–435. [CrossRef]
39. Cheng, W.; Zhu, C.; Xu, W.; Fan, X.; Yang, Y.; Li, Y.; Chen, X.; Wang, W.; Shi, J. Chemical constituents of the bark of *Machilus wangchiana* and their biological activities. *J. Nat. Prod.* **2009**, *72*, 2145–2152. [CrossRef]
40. Masi, M.; Di Lecce, R.; Marsico, G.; Linaldeddu, B.T.; Maddau, L.; Superchi, S.; Evidente, A. Pinofuranoxins A and B, bioactive trisubstituted furanones produced by the invasive pathogen *Diplodia sapinea*. *J. Nat. Prod.* **2021**, *84*, 2600–2605. [CrossRef]
41. Takeda, K.I.; Sakurawi, K.; Ishii, H. Components of the Lauraceae family—I: New lactonic compounds from *Litsea japoncia*. *Tetrahedron* **1972**, *28*, 3757–3766. [CrossRef]
42. Bohlmann, F.; Knauf, W.; King, R.M.; Robinson, H. Ein neues diterpen und weitere inhaltsstoffe aus Baccharisarten. *Phytochemistry* **1979**, *18*, 1011–1014. [CrossRef]
43. Terao, D.; Queiroz, S.C.N.; Maia, A.D.H.N. Bioactive compound isolated from *Conyza canadensis* combined with physical treatments for the control of green mould in Orange. *J. Phytopathol.* **2022**, *170*, 158–166. [CrossRef]
44. Vidari, G.; Abdo, S.; Gilardoni, G.; Ciapessoni, A.; Gusmeroli, M.; Zanoni, G. Fungitoxic metabolites from *Erigeron apiculatus*. *Fitoterapia* **2006**, *77*, 318–320. [CrossRef]
45. Cimmino, A.; Masi, M.; Evidente, M.; Superchi, S.; Evidente, A. Fungal phytotoxins with potential herbicidal activity: Chemical and biological characterization. *Nat. Prod. Rep.* **2015**, *32*, 1629–1653. [CrossRef] [PubMed]
46. Fernández-Aparicio, M.; Cimmino, A.; Soriano, G.; Masi, M.; Vilariño, S.; Evidente, A. Assessment of weed root extracts for allelopathic activity against *Orobanche* and *Phelipanche* species. *Phytopathol. Mediterr.* **2021**, *60*, 455–466. [CrossRef]
47. Soriano, G.; Fernández-Aparicio, M.; Masi, M.; Vilariño-Rodríguez, S.; Cimmino, A. Complex mixture of arvensic acids isolated from *Convolvulus arvensis* roots identified as inhibitors of radicle growth of broomrape weeds. *Agriculture* **2022**, *12*, 585. [CrossRef]