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Biodegradable mulching spray for weed control in the cultivation of containerized ornamental shrubs

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Abstract

Background: Weed control represents a major issue in plant cultivation in containers. Manual weed control is very expensive and the use of chemical herbicide or plastic mulch films has a large environmental impact. The aim of this study was to test the efficacy of an experimental biodegradable chitosan-based mulching spray in controlling weed growth in containers. This research also studied the effect of this mulch on the growth of *Viburnum lucidum* Mill. plants to test for possible phytotoxic effects.

Results: The study compared a total of six treatments derived from three types of weed control (no weed control; herbicide, oxadiazon; mulching spray) applied in containers either filled only with the sterile substrate or filled with the sterile substrate and then artificially inoculated with seeds of the weed species [*Sonchus asper* (L.) Hill subsp. *asper* and *Epilobium montanum* L.]. The mulch controlled the weeds effectively for more than 2 months after its application even under severe weed infestation. The mulching spray controlled the emergence of *S. asper* more efficiently than *E. montanum* plants, probably because the latter has a stronger capacity to penetrate the mulch film during emergence.

Conclusions: Three months after its application, the mulch started to degrade and this allowed some weeds to emerge in the containers, but, in general, the mulch performed better than the herbicide. The chitosan-based mulch did not have any negative effective on the growth of *V. lucidum* plants.

Keywords: Chitosan, *Viburnum lucidum*, *Sonchus asper*, *Epilobium montanum*, Plant biomass

Background

Weed control represents a major issue in plant cultivation [1]. Indeed, weeds can compete with cultivated plants for resources (light, water, nutrients, etc.) [2] and can represent a secondary host for crop pests [3]. In container cultivation, most of these negative effects may be amplified compared to open-field cultivations because of the reduced availability of growing media. Furthermore, the high fertility of the growth substrate often enhances weed growth rate compared to open-field cultivations [4]. Padgett and Frazier [5] estimated that manual weed control in nursery container cultivation requires 1542 h of labor per hectare. Currently, weed control is mainly chemical, but the most widely used herbicides, including those with broad-spectrum activity, cannot guarantee complete coverage against weeds [6]. This lack of full coverage forces operators to perform expensive manual weeding before marketing [1]. In addition, herbicides are phytotoxic [7] and dangerous for both the environment [8] and people [9]. Nowadays, plastic sheets are largely used for mulching in both open-field and container cultivations [10]. However, there is large concern about the use of plastic in agriculture, because of environmental issues [11]. It was estimated that around 700,000 tons of plastic are used annually for agriculture [12] and these

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materials are often incorrectly disposed of, being dumped in marginal areas or even burned in bonfires.

Lately, researchers have focused on the development of plastic materials with low environmental impact [10, 13]. This has allowed the introduction of biodegradable plastic sheets in open-field crops [14] that, depending on their specific weight can be fully biodegraded in the soil at the end of the plant growing cycle. Similarly, in container cultivation, discs made of different materials (coconut, geotextile fiber, and recycled paper) were introduced to cover container surfaces [10, 15]. Other techniques involve the use of plant materials, such as pine bark [13]. Immirzi et al. [16] developed sprays, made of sodium alginate, able to create a film on the soil resistant to weed stem penetration. The modality of applying this type of spray appeared to be suitable for container cultivation and also for use in nurseries. Sodium alginate-based sprays are soluble in water, but when calcium or other divalent cations is added to a water solution of sodium alginate, this compound gives rise to an insoluble gel. The strong cross-linked network occurring between the divalent cations and sodium alginate may cause the alginate to stiffen and withdrawn, causing surface cracking of the mulching which makes it more vulnerable to weed emergence [16]. Another way of developing these mulching sprays is the substitution of alginate with chitosan. Chitosan is a cationic carbohydrate biopolymer derived from chitin, the second most abundant polysaccharide present in nature after the cellulose. The main sources of chitin are the shell wastes of shrimp, lobsters, and crabs [17]. Due to its film-forming capacity, chitosan may be a suitable candidate when developing films for mulching applications in agricultural activities [18] especially in the case in which traditional films (polyethylene) or biodegradable ones cannot be used [19]. Chitosan readily dissolves in dilute solutions of most organic acids, like acetic acid, while it is insoluble in water. This feature is suitable for chitosan-based film applications on the soil; the water resistance of the films, in fact, could ensure their permanence on the soil for the required cultivation time, especially in such a case where it is not possible to use preformed traditional plastic coverages. Anifantis et al. [20] studied soil temperature under a traditional (LDPE black film), a biodegradable (polylactic film black) and two different black sprayable mulching materials (chitosan cellulosic fibers and galactomannans/agarose black) used in the cultivation of sunflowers in greenhouses. This study suggested that the sprayable mulching materials did not improve heat storage in the soil, whereas this effect is significant under traditional mulching films. Little is known about the performance of mulching sprays based on chitosan in weed control. The aim of this research was to study the effectiveness of an experimental mulching spray based on chitosan in controlling weeds during pot cultivation of an ornamental shrub.

Methods

Location and plant material

The trial was carried out at the experimental fields of the Department of Agricultural Sciences (University of Naples Federico II), located in Portici (Naples, Southern Italy; 40°49′11″N–14°20′28″E). On 01 July 2010, 1-year-old plants of *Viburnum lucidum* Mill. were transplanted in 5.5 L plastic containers (top diameter, 24 cm) filled with a steam-sterilized substrate mix containing 40% of coconut fiber, 40% of peat, and 20% of perlite. Before filling the pots, the substrate was homogeneously mixed with a controlled-release fertilizer $15-8-12+2\text{MgO}$ containing B, Cu, Fe, Mn, Mo, and Zn as trace elements (Basacote Plus, COMPO EXPERT Italia Srl) at the dose of 2 kg/m^3 (110 g/pot). No additional fertilization was provided to the plants throughout the rest of the experiment. The containers were placed on plastic mulching sheets. Drip-irrigation was provided daily with one emitter per container (4 L/h each)—located about 5 cm above the substrate—until the first drops of water came out from the pot drainage holes (substrate saturation). No pesticide application was required throughout the experiment.

Experimental design

The study compared a total of six treatments (Table 1) derived from three types of weed control (no weed control, NC; herbicide, H; mulching spray, M) applied in containers either filled with the sterile substrate and then artificially inoculated with seeds of the weed species (IS) or filled only with the sterile substrate that was left non-inoculated (NIS). Therefore, the six treatments were: non-inoculated substrate–no weed control (NIS–NC), non-inoculated substrate–herbicide (NIS–H), non-inoculated substrate–mulching spray (NIS–M), inoculated substrate–no weed control (IS–NC), inoculated substrate–herbicide (IS–H), and inoculated substrate-mulching spray (IS–M). The weed species selected were *Sonchus asper* (L.) Hill subsp. *asper* (*Asteraceae*) and *Epilobium montanum* L. (*Onagraceae*), because they are the most widespread in nurseries in the Campania region (A. Stinca, unpublished observation). The experiment had a complete randomized block design with six treatments and four replications. The experimental unit consisted of five plants (i.e., a total of 120 plants = 5 plants × 6 treatments × 4 replications).

Characteristics of the seed inoculum

Seed inoculum was prepared by mixing 50 achenes of *S. asper* subsp. *asper* and 50 seeds of *E. montanum* with 250 mL of sterile substrate. This mix was added to each inoculated container on top of the sterile substrate. The propagules were collected from plants in nature, air-dried and stored at 4 °C. Seed inoculum was applied in the same date of plant transplant (1 July 2010).

Characteristics of the herbicide

The herbicide used was a granular formulation of oxadiazon at 2% (Ronstar® Bayer Crop Science, Monheim, Germany) that is widely used in the nursery [21, 22]. Herbicide was distributed in each container according to the recommended doses (50 kg/ha) on the same day of plant transplant.

Characteristics of the mulching spray

The mulching spray was prepared as follows: 1.5 g of chitosan at 75% deacetylation degree was dissolved in 100 ml of acetic acid solution (3% vol), then 1.5 g of polyglycerol, 1.5 g of cellulosic fibers (used after sonication process) and 0.2 g of carbon black were added. To study the mechanical properties of the chitosan-based film, a film was prepared by spraying the mulching solution on polystyrene Petri dishes. The obtained film before testing on soil (thickness of 150 microns, a width of 4 mm, and a length of about 28 mm) was characterized by a Young modulus of 1301 ± 41 MPa, a stress at break of 101 ± 6 MPa, a strain at break of 10 ± 1%, and a biodegradability 8% higher than cellulose (unpublished results; biodegradability test was performed by Novamont following ASTM D5988-96 normative). Mechanical tests were performed on six specimens. The product was applied in the containers with a compressed air spray gun (outgoing hole diameter of 3.1 mm) in a quantity of 2 L/m^2 to create on the substrate a film with an estimated thickness of around 150 μm [20]. Before the spray treatments, the soil was moistened up to saturation to avoid any percolation of the sprays. The mulching spray was applied on 1 July 2010.

Similarly, the films were prepared to perform a puncture test. To follow the duration on the soil of the mulching materials, a different preformed disk of chitosan-based films was put on the soil in the same condition of the experimental plot. Six discs were picked up every 2 weeks and performed puncture test on them. This test was carried out with an Instron machine, (model 4301, Instron, Canton, MA, USA) [16, 23]. Briefly, the samples were cut with a punch cutter with a diameter of 42 mm and were trapped in cups fixed on the underside of the Instron instrument. They underwent the action of a force exerted by a spherical dart linked to a steel rod fixed on the upper side of the apparatus. The dart, moving down at a fixed rate of 2 mm/min, penetrated the sample until its rupture. The applied load was recorded as a function of the displacement; the parameters obtained were normalized with respect to the half of the area of the sphere and with respect to an estimated value of the displacement of 10 mm.

Table 1 Treatments used in the experiment and their abbreviations

Treatment	Abbreviation
Non-inoculated substrate–no weed control	NIS–NC
Non-inoculated substrate–herbicide weed control	NIS–H
Non-inoculated substrate–mulching weed control	NIS–M
Inoculated substrate–no weed control	IS–NC
Inoculated substrate–herbicide weed control	IS–H
Inoculated substrate–mulching weed control	IS–M

Measurements

Shoot growth of *V. lucidum* plants was measured on five days: 01, 14, and 29 July; 29 August; and 23 October 2010. The length of all the shoots produced by each plant was measured in all containers.

Weed infestation in the containers was evaluated on the same days when vegetative measurements of *V. lucidum* plants were carried out. In all containers, each live weed plant was counted and identified according to Pignatti [24] and Tutin et al. [25, 26]. On the last three measuring dates (29 July, 29 August, and 23 October), four containers per treatment (one per block) were sampled and all the weed plants collected, separated by species and their dry weight measured after they were dried to constant weight in a ventilated oven (set at 60 °C).

Air temperature, relative humidity, and rainfall were measured at a weather station located about two kilometers from the experimental site.

Statistical analysis

All statistical analyses were performed with SPSS software (SPSS Chicago, IL). The significance of the effects ($P < 0.05$) of the substrate seed inoculation (SSI), the type of weed control (TWC), the time (T) and the SSI × TWC, SSI × T, TWC × T, and SSI × TWC × T interactions on the measured parameters was evaluated by three-way ANOVA using the least significant difference (LSD) as a post hoc test for mean separation ($P < 0.05$).

Results and discussion

During the trial (from July to October), mean daily air temperature ranged between 11.2 and 28.9 °C (Fig. 1), whereas the maximum air temperature was 34.1 °C (16 July). In addition, mean air relative humidity ranged between 41 and 87% and total rainfall was 333.4 mm (with a total of 39 rainy days). Under these weather conditions, the chitosan-based mulching spray was effective in controlling weed emergence even under severe seed infestation (Fig. 2 and Table 2). Indeed, on the first measurement date after treatment application (14 July), 25% and 60% of substrate surface was occupied by weeds in IS–H and IS–NC containers, respectively (Fig. 2a), whereas in the other treatments, the percentage of infestation was either very low (NIS–NC and IS–M) or zero (NIS–H and NIS–M). In IS–H and IS–NC, containers reached maximum percentage of substrate surface covered by weeds on 29 July (around 40 and 70%, respectively), whereas in the containers of the other treatments, this parameter reached the maximum on 29 August (values between 6 and 18%). In all measuring dates between 14 July and the end of the experiment, the percentage of substrate surface covered by weeds was significantly higher in IS–NC than in the other treatments, whereas IS–H containers had intermediate values (significantly higher than IS–M, NIS–NC, NIS–H, and NIS–M containers). Furthermore, from 29 July to 23 October, the cumulated total dry weight of weed plants was significantly higher in IS–NC and IS–H containers than in the other treatments (Fig. 2b and Table 2). The dry weight of weed plants in NIS–H and NIS–M containers was either zero or very low throughout the experiment. These effects included both a delay in weed emergence and a decrease in total number and biomass of weeds per container (Fig. 3 and Table 2). The seasonal pattern of the dry weight of *S. asper* plants was very similar to that already described for the total dry weight of all the weeds (Fig. 3). On 14 and 29 July, the number of live *S. asper* plants counted in IS–NC was significantly higher than in the other treatments, whereas on the same

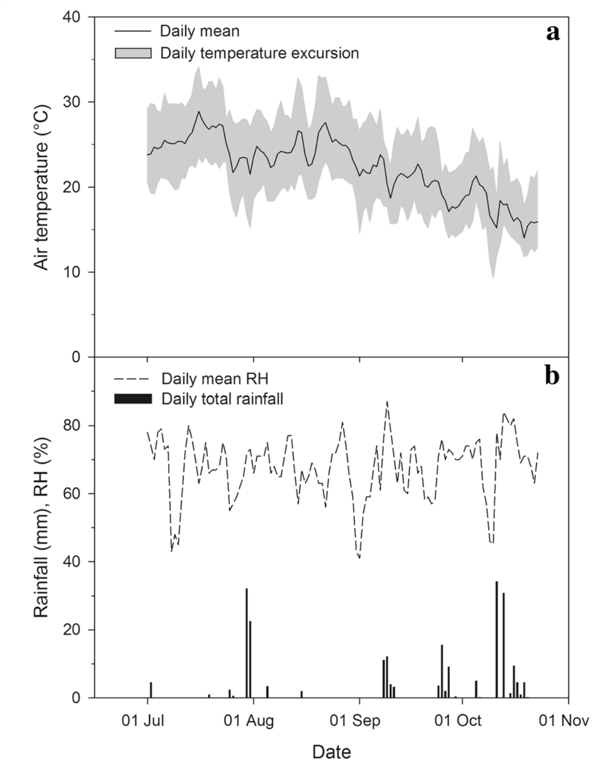

Fig. 1 Seasonal pattern of daily **a** mean, minimum and maximum air temperature and **b** relative air humidity and rainfall

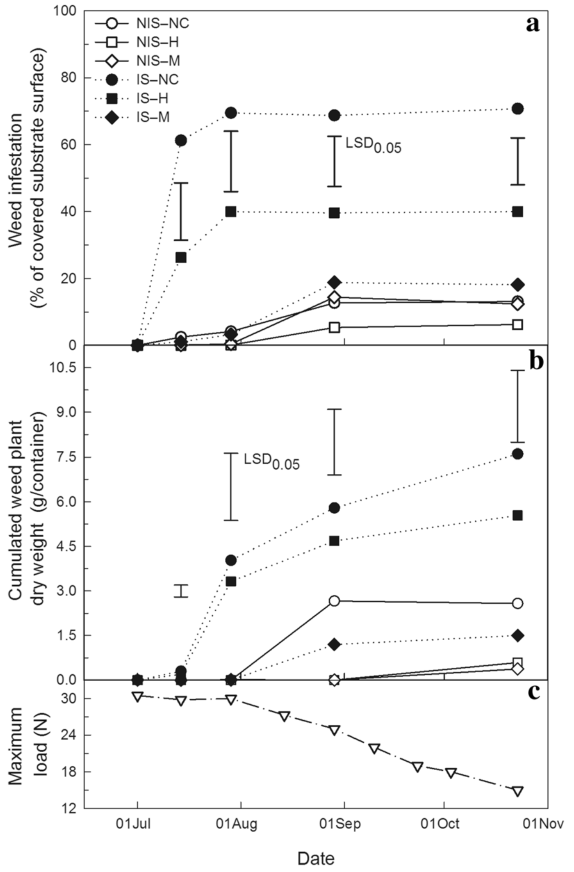

Fig. 2 Seasonal pattern of **a** the percentage of substrate surface infested by weeds and of **b** the cumulated total dry weight of weeds in containers with *Viburnum lucidum* plants grown on non-inoculated substrate (NIS) without weed control (NIS–NC), or with herbicide application (NIS–H), or with spray mulching application (NIS–M); or on substrate inoculated with *Epilobium montanum* and *Sonchus asper* subsp. *asper* seeds (IS) without weed control (IS–NC), or with herbicide application (IS–H), or with spray mulching application (IS–M); and **c** seasonal pattern of the resistance at the puncture test of mulching spray (each measure ± 3N)

days IS−H containers had an intermediate number of this weed plant (Fig. 3 and Table 2). On 9 September in IS−NC and IS−H containers, there was a sudden decrease in the number of alive *S. asper* plants, and this was followed by a second flush of plant germination in IS−NC containers. No differences were found in this parameter between the other treatments throughout the experiment. On 9 September, dry weight of *E. montanum* plants in IS−NC plants was significantly higher than the other treatments (Fig. 3), whereas on the last measuring date this parameter was highest in IS−NC and IS−H, intermediate in IS−M containers and around zero in all the other treatments. From 14 July to the end of the experiment, the number of alive *E. montanum* plants was higher in IS−NC containers than the other treatments (Fig. 3). The number of these weed plants was very similar in IS−H and IS−M containers. Even though previous studies demonstrated that the herbicide used in this study reduced by 96–97% the infestation of plants of the genus *Sonchus* in containers [27], the mulching spray appeared to be more efficient than oxadiazon in controlling these weed species (Fig. 3). The case of *E. montanum* (Fig. 3), which was controlled with similar efficiency by the herbicide and the mulch, was different. This was perhaps because the herbicide controlled *E. montanum* better than *S. asper* subsp. *asper* (Fig. 3), as previously reported [21]. The difference between the efficacy of the mulching spray in controlling the two inoculated weed species (Fig. 3 and Table 2) is quite interesting. Mulching spray fully controlled *S. asper* subsp. *asper* for more than 2 months (from 1 July to 9 September), whereas some *E. montanum* plants had already emerged in mulched containers on the second measuring date (14 July, Fig. 3). This difference in the time of emergence between weed species may be due to a greater capacity of *E. montanum* seedlings to break the polymeric structure created by the mulching sprays. This occurred even though at the beginning of the experiment the mulch was still characterized by high resistance to puncture (Fig. 2c). In this puncture test, the resistance of a dard to penetrate through the sprayable mulch was measured under controlled conditions. This test was designed to simulate the resistance of this material to weed growth. Mechanical performances play a fundamental role in mulching films, as they influence durability and functionality. The mulching films undergo, during the cultivation period, ageing processes due to weathering agents (solar radiations, high air temperature and relative humidity, etc.) and the employment of chemical substances. The prolonged exposure of films to these factors determines simultaneously progressive degradation and photooxidation processes, with a variation of physical and mechanical properties of the films [28]. In a previous study, chitosan-based spray mulch performed better in comparison to mulch based on sodium alginate [16]. Indeed, sodium alginate-based spray mulch was reported to present cracks already 1 month after application and that in this period of time some weeds grew through them [16]. The profile of the maximum load curve (Fig. 2c) is consistent with the loss of plasticizer previously reported [19]. As a result, the coating became more rigid and showed a lower resistance to rupture and displacement.

Some *S. asper* subsp. *asper* plants appeared in mulched containers on the last measuring date (23 October). This was probably (Fig. 2c) caused by the loss of resistance to puncturing on the film that became very rigid

Table 2 Significance of the effects ($P<0.05$) of the substrate seed inoculation (SSI), the type of weed control (TWC), the time (T) and the SSI × TWC, SSI × T, TWC × T, and SSI × TWC × T interactions on the measured parameters evaluated by three-way ANOVAs

Source of variation	Significance						
	% of substrate surface covered by weeds	Weed plant dry weight per container			Number of alive plants per container		Shoot length of *Viburnum lucidum*
		All weeds	*Sonchus asper*	*Epilobium montanum*	*Sonchus asper*	*Epilobium montanum*	
Substrate seed inoculation (SSI)	*< 0.001*	*0.045*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.012*
Type of weed control (TWC)	*< 0.001*	*0.001*	*0.012*	*0.020*	*0.007*	*< 0.001*	0.157
Time (T)	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
SSI × TWC	*< 0.001*	*0.036*	*0.013*	*< 0.001*	*0.002*	*< 0.001*	0.053
SSI × T	*< 0.001*	0.182	*< 0.001*	*< 0.001*	*< 0.001*	*0.001*	0.670
TWC × T	*< 0.001*	0.582	0.620	0.563	0.130	*0.006*	0.681
SSI × TWC × T	*< 0.001*	0.729	0.630	0.489	0.168	*0.006*	*0.025*

Significant effects are reported in italic and/or by the damage caused to the mulch structure by *E. montanum* plants. When mulch started to degrade, it also became partially vulnerable to root penetration of some weed species whose seeds were naturally disseminated on the mulch. This was particularly evident with of *Chamaesyce maculata* (L.) Small, *Erigeron sumatrensis* Retz., *Oxalis corniculata* L., and *Taraxacum officinale* Weber, that reached a maximum frequency higher than one plant per container. *Chamaesyce maculate* and *E. sumatrensis* are considered alien plants of the Italian vascular flora. The time of the first appearance of most of these weed species was after 29 July. On 23 October, the number of live *C. maculate* plants was significantly higher in NIS–NC and NIS–M containers than in the

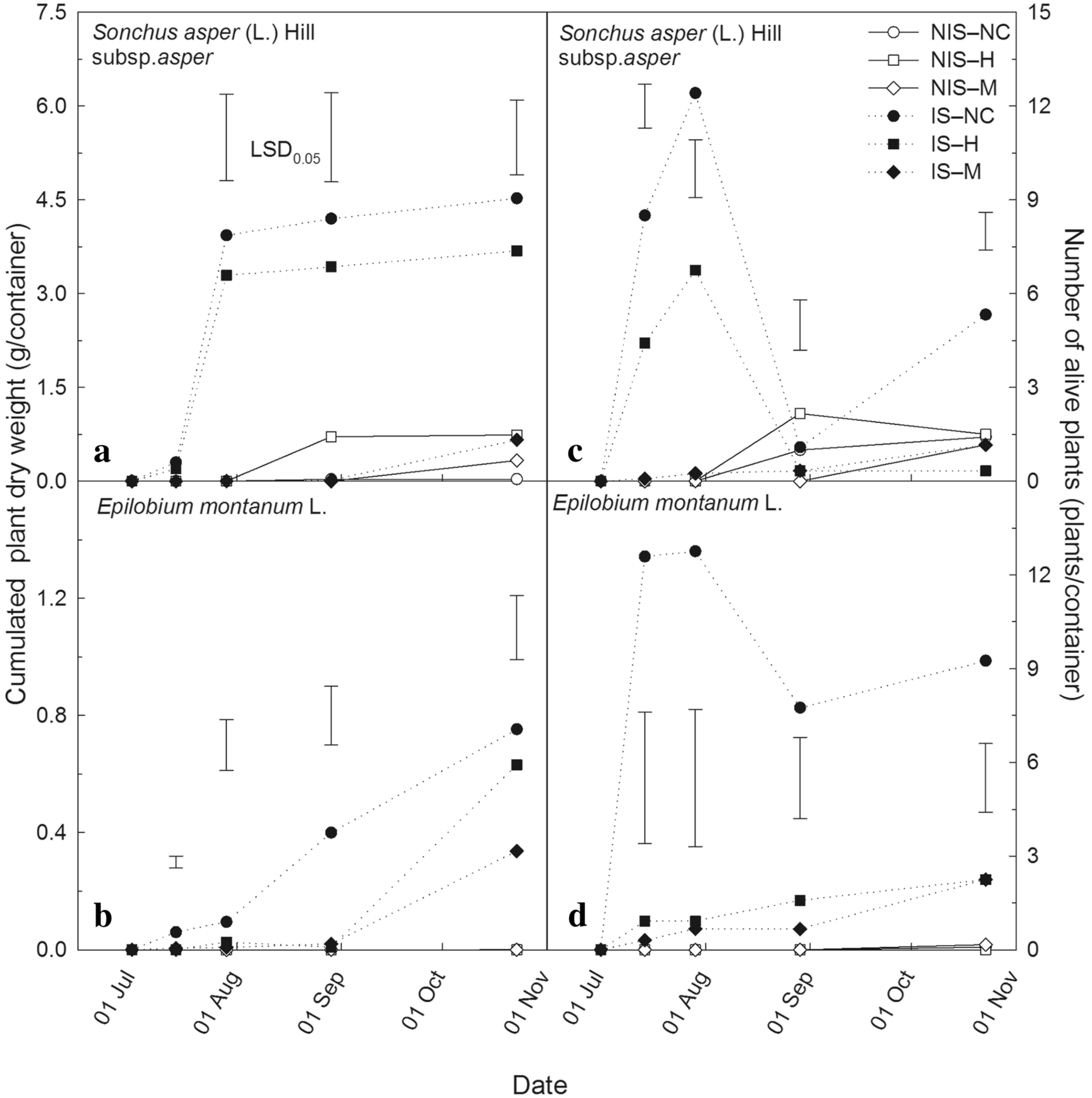

Fig. 3 Seasonal pattern of **a**, **b** dry weight and **c**, **d** number of alive plants of **a**, **c** *Sonchus asper* subsp. *asper* and **b**, **d** *Epilobium montanum* plants in containers with *Viburnum lucidum* plants grown on non-inoculated substrate (NIS) without weed control (NIS–NC), or with herbicide application (NIS–H), or with spray mulching application (NIS–M); or on substrate inoculated with *Epilobium montanum* and *Sonchus asper* subsp. *asper* seeds (IS) without weed control (IS–NC), or with herbicide application (IS–H), or with spray mulching application (IS–M)

other treatments. Similar, results were also found for *O. corniculata* (L.). On the last date of measurements, IS–M containers had the higher number of *E. sumatrensis* than the other treatments. Similarly, on the same date, NIS–M containers had the highest number of *Taraxacum officinale* plants.

In addition, the following weed species were found occasionally: *Portulaca oleracea* L. subsp. *oleracea*, *Veronica arvensis* L., *Poa annua* L., *Carduus pycnocephalus* L. subsp. *pycnocephalus*, *Cardamine hirsuta* L., *Silene gallica* L., *Mercurialis annua* L., and *Stellaria neglecta* Weihe.

In the last two measuring dates, plant growth of the cultivated shrub (*V. lucidum*) significantly decreased in inoculated containers when no weed control or herbicide was applied (IS–NC and IS–H, respectively) (Fig. 4). This negative effect on the growth of the cultivated plants can be the result of competition with weed plants for resources (light, water, and nutrients), but also of the release by weeds of root growth-inhibiting chemicals [4]. The latter is generally called allelopathy [29] and has been reported to be stronger in containers [4]. Another possible contribution to this negative effect can be due to the possible phytotoxicity of the herbicide. Different studies have reported that herbicides different from the one used here can have negative effects on the cultivated plant when used at high doses [30]. However, in this study, oxadiazon had no negative effect on *V. lucidum* plant growth when the herbicide was applied in non-inoculated containers (NIS–H treatment, Fig. 4). This suggests that oxadiazon did not have any important phytotoxic effect on the cultivated plant at least at the used recommended dose. Previous studies reported that some herbicides such as atrazine and trifluralin induce stress in the cultivated plant emphasizing the negative effects of allelopathy [31]. This can account for some of the negative effects on *V. lucidum* growth in IS–H containers. The mulching spray did not have any negative effect on the growth of the cultivated plants (Fig. 4).

In this research, the chitosan-based sprayable mulching was evaluated in open field under the typical summer conditions of a Mediterranean climate. The effectiveness of the sprayable mulching in controlling weed growth may also be affected by weather and soil microbiological conditions that may affect its degradation rate as previously reported for chitosan and for other biodegradable mulching materials [32, 33]. This may play an important role in the use of these mulching materials in open-field conditions. More studies are needed to test the performance of this chitosan-based material under different environmental and soil conditions.

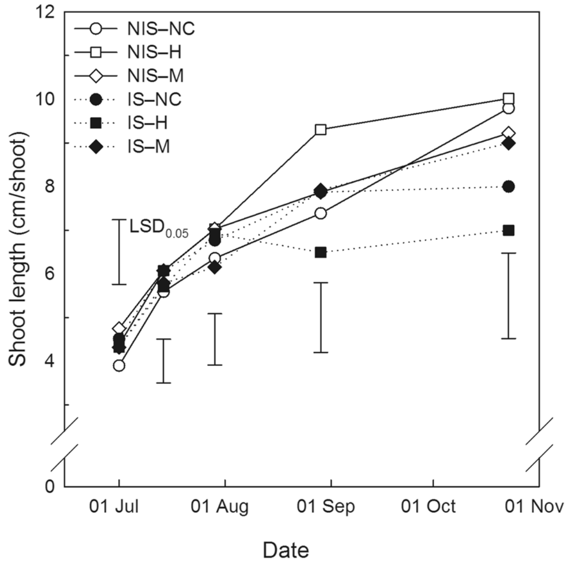

Fig. 4 Seasonal pattern of shoot growth of *Viburnum lucidum* plants grown on non-inoculated substrate (NIS) without weed control (NIS–NC), or with herbicide application (NIS–H), or with spray mulching application (NIS–M); or on substrate inoculated with *Epilobium montanum* and *Sonchus asper* subsp. *asper* seeds (IS) without weed control (IS–NC), or with herbicide application (IS–H), or with spray mulching application (IS–M)

Conclusions

For more than 2 months after its application, chitosan-based mulching spray efficiently controlled weed growth in containers even under severe weed infestation. The mulch inhibited the emergence of *S. asper* subsp. *asper* plants slightly better than *E. montanum* plants. Three months after its application, the mulch film started to degrade and this allowed the growth of some weed plants in the containers, but, in general, the mulch performed better than oxadiazon herbicide. This was also true because in the mulched containers, the cultivated *V. lucidum* plants grew better than in containers where weeds were not controlled or where the herbicide was applied.

Authors' contributions

All authors have contributed substantially to the work. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Data will not be available.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

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