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ORIGINAL ARTICLE

A glance at isotherapy to control weed germination

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Abstract

Currently more and more research is being done on integrated weed management to reduce or avoid herbicide use. Some growers are already using isotherapic dilutions to control weeds in organic farming. Isotherapy is different from homeopathy because it uses diluted and potentized (succussed) solutions of alcoholic macerate of the very pest causing health troubles. We set up a germination experiment to test if isotherapic dilutions of leaf macerate of annual ryegrass affect the dynamics of its seed germination in Petri dishes. Our results were diverse, from no effect to 10% more growing degree days necessary to reach 50% germination. It is doubtful that so low an effect will contribute to integrated weed management unless the slightly delayed germination triggers secondary effects at other life stages. This is in accordance with the scientific literature on that topic: two-fifths of the reports showed no effect, two-fifths resulted in positive responses and one-fifth had diverse responses for the criteria tested.

Keywords: germination, homeopathy, isotherapy, plant protection, review, ryegrass, weed control

Introduction

Organic farming must deal with pests, diseases and weeds while banning the use of synthetic agrochemicals so as to protect biodiversity and human health. For this purpose, it uses a variety of prevention methods and natural tools to preserve crop health and guarantee productivity and returns. These methods are related to those used in Integrated Pest Management, and do not use pesticides (Metcalf and Luckmann1982; Barzman *et al.* 2015) and in Biological Pest Control strategies (Stanhill 1990; Altieri *et al.* 2017). In addition to these techniques, there is a growing movement for using unconventional therapies as alternatives to chemical substances. On one hand, homeopathy treats diseased plants with that which would produce symptoms similar to those of the disease in a healthy plant. Several literature surveys are available that show the positive effects of such homeopathic treatments on diseased or predated plants (Betti *et al.* 2009; Majewsky *et al.* 2009; Jäger *et al.* 2011, 2015; Gama *et al.* 2015). On the other hand, isotherapy treats diseases with diluted and potentized (succussed) solutions of the very biological agent causing diseases. However, no literature survey is available and very few scientific papers have been published on this topic.

Since farmers engaged in organic farming are already using empirical isotherapic solutions to control weeds, and since no research paper has dealt with isotherapic weed control, we set up a simple experiment to determine if the seed germination process is affected by isotherapic dilutions. Furthermore, we have summarized information collected from research on isotherapy, including categorization of expected effects and results.

Materials and Methods

Seeds and treatment preparation

Seeds of *Lolium multiflorum* (annual ryegrass) were collected in 2008 in a wheat field in Eaux-Puiseaux in Champagne, France (48°07′15″ North, 3°53′30″ East) and then stored under room conditions (20–25°C, 50–60% humidity). This species was chosen because of its good germinating ability and easy germination under varied conditions. The experiments were carried out in fall 2014 and spring and fall 2016.

Young germinating seeds were kept on Petri dishes and placed in a growth chamber (20°C, 12 h light), and adult plants were obtained from a garden in 2014 and from a greenhouse in 2016 (15–20°C, 12 h light). At room temperature, 5 g of 5-day-old germinating seeds and 17.5 g of shredded aboveground parts of plants at bolting stage were hand crushed together using pestle and mortar. The mash was recovered in 102.5 g (122 ml) ethanol 70% (prepared with water warmed to 60°C, then cooled), then put in a flask and stored for 18 days in the dark. Thereafter, the macerate was filtrated through a Whatman 4V fluted filter paper. The resulting filtrate is called the mother tincture.

The filtrate was diluted in a centesimal Hahnemannian (CH) fashion, by diluting 0.1 ml of mother tincture in 9.9 ml ethanol 70% in a sealed flask. The flask was locked inside a rubber hammer, and then strongly shaken by 50 hammer blows on a solid bench, thus forming the 1 CH dynamized solution. We repeated the process using 0.1 ml of 1 CH to get 2 CH, and so on until we reached 15 CH. Aliquots of the dilutions of 7, 9 and 15 CH were mixed and labelled as CH to browse a large spectrum of potentially active dilutions as recommended by farmers following this weed management strategy. The procedure was replicated to get a second set of independent dilutions. In 2016, only one stock solution and dilution series was made. A control stock solution of the mother tincture was obtained through the same dilution scale, but it was not shaken (CT). A control stock solution containing only ethanol 70% was also used, not shaken in 2014 (CE) but shaken in 2016 (CES). A pure water control was used in 2014 (CW).

Germination experiment

Germination experiments were adapted from ISTA rules (1996), albeit without KNO_3 to avoid any interference. They were carried out on germination paper (Durieux®) in 14 cm Ø Petri dishes filled with small glass beads to facilitate water reserve, with 5, 7 or 10 Petri dishes for each treatment according to the experiment (Tables 1 and 2). Samples of 0.3 g seed (approximately 110 seeds) were deposited in each Petri dish filled with 40 ml of 0.2% dilution of stock solutions (CW, CE, CES, CT, CH). Dilutions were made by a technician who did not take part in the experiment. She colour labelled each solution for each experiment and kept it secret until the results were analysed. Thus the counting of the germinated seeds was performed in a blind manner. Petri dishes were randomly placed on a bench in a regulated greenhouse (15–20°C) in October and November 2014, and in an unoccupied, unheated laboratory in April, September and October 2016, in all cases with natural light. The temperature was recorded using automatic recorder facilities. These conditions were preferred to those of a controlled growth chamber because of probable interactions with electronic devices, as it has been shown in several works. The electromagnetic fields could modify the physico-chemical properties of water (Vallee 2004).

A seed was considered germinated as soon as the root was more than 1-mm long. Germination was recorded for 2 weeks, and then non-germinated seeds were checked for viability (presence of a pale grain inside the envelopes) and counted. Since the germination of annual ryegrass is rapid, non-regular record intervals were used. Growing degree days (*GDD*) were calculated as the sum of daily temperatures minored by the base temperature below which the species cannot germinate. The base temperature was taken as the mean value of the reported data in Moot *et al.* (2000) and Gundel *et al.* (2008), that is 3°C. Cumulative germination was regressed in terms of growing degree days (*GDD*) using a log-logistic model:

$$P = \frac{P_{\max}}{1 + \exp[b(\log GDD - \log GDD_{50})]},$$

where: P – the germination percentage, $P_{\max}$ – the maximum germination percentage, b – the slope of the curve at GDD_{50}, and GDD_{50} – the *GDD* value corresponding to 50% germination. The accuracy of the fit was given by the mean corrected *R*-square value. GDD_{50} values were considered different if their Wald 95% confidence interval did not overlap, and other point comparisons were performed using one-way ANOVA. Curves were fitted using Systat 13 (2009, Systat® Software Inc., Richmond, CA, USA).

Results

Seed viability was very good, with an average of 95.5% germination within two weeks, but it was somewhat less at the lowest temperature and in 2016 compared to 2014 (although the temperatures were not the

Table 1. Average (± SEM) total germination percentage at the end of each experiment (P_{max}). Mean temperature of the environment (and mini-maxi values in brackets) is indicated for each experiment

Treatments	Total germination percentage (P_{max})				
	2014		2016		
	October	November	April	September	October
	temperature [°C]		temperature [°C]		
	19.1 (15.0–24.0)	18.6 (14.8–22.2)	11.7 (10.0–14.0)	24.5 (22.0–25.5)	15.1 (13.4–18.0)
CW	98.1 ± 0.7	98.8 ± 0.3	–	–	–
CE	–	99.7 ± 0.2	90.3 ± 0.6	95.5 ± 0.8	95.1 ± 0.6
CES	–	–	91.1 ± 1.1	96.7 ± 0.6	94.5 ± 1.5
CT	–	–	88.9 ± 0.7	97.3 ± 0.5	95.0 ± 1.2
CH_1	97.4 ± 1.8	98.6 ± 0.4	89.2 ± 1.0	94.3 ± 1.1	93.4 ± 0.8
CH_2	99.2 ± 0.4	99.2 ± 0.4	–	–	–
No. Petri dishes	5	7	10	5	5

CW, CE, CES and CT – stock solutions, different kinds of control treatments (see text); CH – centesimal Hahnemannian dynamized treatments with a mixture of CH 7, 9 and 15

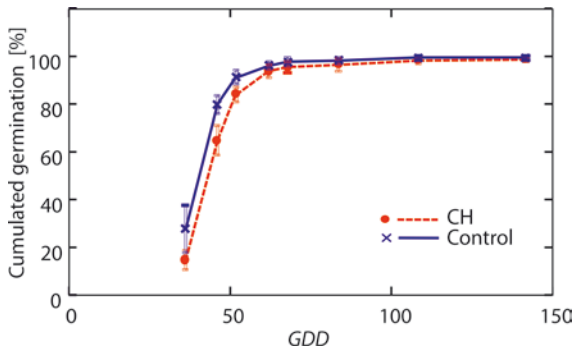

Fig. 1. Example of cumulative germination curve in terms of growing degree days (*GDD*) for the centesimal Hahnemannian dynamized treatment (mixture of CH 7, 9 and 15) and the alcoholic control (CE) in the November 2014 experiment

same both years), which could indicate some seed ageing with storage time (Table 1). No difference was found between CH and control solutions (*F* values with $p > 0.05$; Table 1). Cumulative germination showed typical S curve kinetics (Fig. 1). A slight shift toward delayed germination appeared for CH compared to control solutions in the 2014 experiments (Fig. 1), but all the curves matched each other exactly in 2016 (not shown). Cumulative germination of CH and control solutions were significantly different at the first and second records of the October 2014 experiment (*F* values with $p < 0.03$), and at the first, second and third records of the November experiment (*F* values with $p < 0.001$; Fig. 1). Log-logistic regression of cumulative

Table 2. Number of growing degree days corresponding to 50% germination (GDD_{50}) and its Wald 95% confidence limits (±), and mean slope (*b*) as estimated from non-linear regressions in the different experiments with a base temperature of 3°C. Mean temperature of the environment (and mini-maxi values in brackets) is indicated for each experiment

Treatments	GDD_{50} (for 50% germination)				
	2014		2016		
	October	November	April	September	October
	temperature [°C]		temperature [°C]		
	19.1 (15.0–24.0)	18.6 (14.8–22.2)	11.7 (10.0–14.0)	24.5 (22.0–25.5)	15.1 (13.4–18.0)
CW	40.5 ± 1.0	38.9 ± 0.4	–	–	–
CE	–	39.9 ± 0.4	46.1 ± 0.4	41.1 ± 0.4	43.9 ± 0.4
CES	–	–	46.4 ± 0.4	40.8 ± 0.5	43.2 ± 0.7
CT	–	–	46.5 ± 0.4	40.8 ± 0.4	43.5 ± 0.3
CH_1	43.5 ± 1.4	43.1 ± 0.4	46.4 ± 0.4	40.1 ± 0.5	43.7 ± 0.4
CH_2	44.4 ± 1.3	43.5 ± 0.5			
Mean slope (*b*)	−9.6 ± 1.4	−9.0 ± 0.4	−16.8 ± 0.9	−8.6 ± 0.4	−9.8 ± 0.5
No. Petri dishes	5	7	10	5	5

CW, CE, CES and CT – stock solutions, different kinds of control treatments (see text); CH – centesimal Hahnemannian dynamized treatments with a mixture of CH 7, 9 and 15

germination always showed mean corrected R-square higher than 0.948. The slopes (*b* parameter) were very similar within an experiment (Table 2). The 50% cumulative germination was obtained from 39 to 46.5 GDD, the highest values being observed for the lowest temperature conditions (Table 2). In both 2014 experiments and for the two independent CH replicates, the potentized CH treatments showed that on average four additional GDD were necessary to get 50% germination (Table 2, Fig. 1). In contrast, no significant difference was observed between treatments in 2016 (Table 2).

Discussion

Our results of the germination experiments are in complete accordance with previous literature on this topic for *Lolium multiflorum* (Gundel *et al*. 2008). The approximated base temperature from the literature, which may have been inappropriate for the used population, could have contributed to somewhat higher values than in Gundel *et al*. (2008), and temperatures

Table 3. Literature survey of papers dealing with isotherapy, excluding works on homeopathy, non-peer-reviewed papers, abstracts in conferences and technical reports. Effects of isotherapic solutions on stressed (diseases or pests) and healthy plants (NA): reduction (–), increase (+) or no effect (=) of the applied solutions at given dilutions on the tested criteria; GA3 – gibberellic acid

Classes	Stresses	Target plants	Origin of isotherapic solutions	Tested criteria (effect)	References
Diseases	*Phytophtora infestans*	*Solanum lycopersicum*	infested leaves (30CH)	infected area (=)	Diniz *et al.* (2006)
	Pseudomonas syringae	*Arabidopsis thaliana*	infested leaves (30CH)	colonies number (=)	Shah-Rossi *et al.* (2009)
	Alternaria solani	*S. lycopersicum*	fungi (30CH)	infected area (–)	Carneiro *et al.* (2010)
	P. infestans	*S. lycopersicum*	infested fruits (7CH)	disease scaling (–)	Berger *et al*. (2011)
	Septoria lycopersici	*S. lycopersicum*	infested leaves (24CH)	fungi dev. (–)	Modolon *et al*. (2012a)
Pests	*Dysaphis plantaginea*	*Malus domestica*	infested leaves (30CH)	aphid progeny size (–)	Wyss *et al*. (2010)
	Acromyrmex sp.	*Araucaria angustifolia*	ants (30CH)	ant movement (–)	Giesel *et al*. (2012, 2017)
	Anastepha fraterculus	*Prunus persica*	flies (6CH)	infested fruits and pest number (=)	Rupp *et al.* (2012)
Plants	NA	*Lemna gibba*	leaves of *Lemna minor* (30DH)	number and surface (=)	Scherr *et al*. (2007)
	NA	*L. gibba*	leaves of *Lemna minor* (30DH)	number and surface (=)	Scherr *et al.* (2009)
	NA	*Cyperus rotundus*	leaves (12CH)	biomass (=) emergence (+)	Silviera *et al*. (2009)
	NA	*S. lycopersicum*	leaves (24DH)	fruit composition (=)	Modolon *et al.* (2012b)
	NA	*Hordeum vulgare*	GA3 (30DH)	germination (+) length (=)	Hamman *et al.* (2003)
	NA	*Pisum sativum*	GA3 (18DH)	stem length (+)	Baumgartner *et al*. (2004)
	NA	*P. sativum*	GA3 (30DH)	stem length (+/=)	Baumgartner *et al.* (2008)
	NA	*L. gibba*	GA3 (30DH)	growth (–)	Scherr *et al.* (2009)
	NA	*Triticum aestivum*	GA3 (30DH)	germination (–)	Hartung *et al.* (2010)
	NA	*T. aestivum*	GA3 (30DH)	stem length (–/=)	Matzer *et al*. (2010)
	NA	*T. aestivum*	GA3 (30DH)	germination (–/=)	Endler *et al.* (2011)
	NA	*T. aestivum*	GA3 (30DH)	germination (=) stem length (–)	Pfleger *et al*. (2011)
	NA	*T. aestivum*	GA3 (30DH)	germination (–/=)	Kiefer *et al*. (2012)
	NA	*T. aestivum*	GA3 (30DH)	germination (=) stem length (–)	Hribar-Marko *et al*. (2013)
	NA	*L. gibba*	GA3 (30DH)	growth (+/–)	Majewsky *et al.* (2014)
	NA	*T. aestivum*	GA3 (30DH)	stem length (–/=)	Endler *et al*. (2015)

below the range recommended by ISTA (1996), namely 15–25°C, probably caused the highest GDD_{50}. As for the effect of the isotherapic treatments, only one round of the experiment provided positive differences, while the second one showed no difference at all. Obviously, it could be argued that the experimental conditions were different between the two series of experiments. Specifically, although the same quantity of plants from the same population and at the same growth stage were used for the 2014 and 2016 macerates, the quality of the plant material could have been different and therefore a major reason for the inconsistency of results. Indeed, mother tinctures were obtained from plants growing in a garden in 2014 whereas they were grown in a greenhouse in 2016. Furthermore, there were also differences in the experiment: 1) the seeds were 1.5 to 2 years older in 2016 than in 2014, possibly decreasing the total germination rate, 2) the difference between night and day temperatures was not as big in the laboratory in 2016 as in the greenhouse in 2014 because of the thermal inertia of the building compared to the greenhouse, 3) light conditions also were different.

One could say that more sophisticated experimental conditions must be set up to confirm the actual effect on germination. However, if the operational range of efficacy of isotherapy depended so much on the environmental conditions, or on the way the isotherapic solution is prepared, it would be of poor value or awkward to manage, and therefore difficult to apply under arable field conditions. This conclusion partially fits with the feeling of the farmers who are currently using isotherapy to control weeds but want more detailed information on CH dosage and spray conditions and timing. Four *GDD* are a minute proportion of the total *GDD* sum to achieve complete germination, so that it is doubtful that so low an effect will contribute to limiting weeds. However, it could trigger secondary effects at other life stages. For instance, a leverage effect among seedling cohorts interacting with the crop growth stage could occur; the later the weeds emerge, the less competitive and reproductive they are (Awan and Chauhan 2016; Ruehl *et al*. 2016). In fact, the very targets of the farmers on the date when they spray in the field are seedlings, so that further experiments should focus on the effects of isotherapic solutions on the early growth of seedlings, either alone or with the crop, in order to mimic field conditions. At present, there is more and more research on integrated weed management to reduce or avoid herbicide use, and any small contribution to making weeds less competitive is worthy.

Besides applied perspectives, the paucity of scientific literature on isotherapy on plants makes it difficult to reach any reasonable and generic conclusion on its effectiveness and the relevance of its application to agriculture. Indeed, after careful examination, we found only 23 articles dealing with isotherapic solutions on plants, which had clearly described and reproducible methods, and used statistics. Five studies dealt with fungus, four with pests and 15 with healthy plants, among which four used dilutions of leaf extracts and 12 used dilutions of gibberellic acid (GA3; one of these studies also dealt with leaf extracts). For a total of 29 criteria (Table 3), there were 13 cases with significant effects on the measured criteria or traits (reduction or increase), 10 without any effect, and six with diverse results. In these six studies, differences between significant effect and lack of response were attributed to seasonal interactions. For germination and emergence experiments, there were two cases with significant enhancing effect, one with delaying effect, two without any difference, and two with diverse responses among replicates (mix of significant delay or no difference). It is noteworthy that the effects observed in these studies were not observed under all the conditions of each subexperiment and seem to have depended on isotherapic preparations and experimental (climatic) conditions. Our study falls within the diverse responses observed in the literature, with both positive effect and lack of effect. Hence, such isotherapic approaches may be effective under some special conditions (growth stage, environment, and species), while under other conditions they may not. Therefore, it seems to be difficult to determine with absolute certainty whether it is effective or is not. At the best, sophisticated diagnosis of non-appropriate conditions is required. In addition, if the panel of appropriate conditions is so narrow, practical application in the field at the exclusion of other weed management methods is far too risky, which casts doubt on its interest in agriculture.

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