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Physiological and Ethological Effects of a Pelargonic Acid Herbicide Used by Non-professional Persons: A Study on Ants as Biological Models

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Research Article

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ABSTRACT

Objective: Nowadays, the environmental including natural water is polluted by products used by humans. Among them, pharmaceutical drugs and pesticides are largely studied. Investigations on herbicides are less numerous, though the most toxic one has been banned. Using ants as models, we examined the potential harmful effects of an herbicide still authorized for gardeners, the active substance of which is pelargonic acid.

Methods: We used the experimental protocols we are accustomed to use for examining the physiological and ethological impacts of products, drugs, or situations. We worked on ants as biological models, as usually, and we explain in the paper why we work according to this way.

Results: We found that this substance reduced the ants' food intake, activity, orientation ability, audacity, tactile perception, social relationships, cognition, learning and memorization. The ants did not adapt themselves to these side effects. After having no longer been in contact with the herbicide, the survived ants recovered in a total of 24 hours according to a quadratic function. Using herbicides the active substance of which is pelargonic acid imperils thus the life or at least the health of the soil fauna, and although having been duly weeded, the treated areas are deprived of their essential fauna.

Conclusion: We recommend using pelargonic acid herbicides for weeding only not cultivated lands (paths, alleys, sidewalks) and never for weeding arable lands. Also, we recommend to pay attention to the users' health, as well as to not pollute natural water.

INTRODUCTION

Pollution of natural water by humans' use of pharmaceutical drugs, insecticides, pesticides, and plastics has been and is still now largely studied; it is obvious that all these products impact all the animal species and the ecological processes [1-4]. Pollution due to herbicides has only little been examined. However, these substances are often used by professional growers as well as by gardeners, and since they prevent plants from living, they may also impact the survival or at least the health of animals, invertebrates and vertebrates including humans. This is why we intended to study, on ants as biological models, the impact of an herbicide commonly used by gardeners, i.e. Herbatak®, on several physiological and ethological traits. Before relating our experimental work, we here under give general information about herbicides (their diversity, their potential impact on the environment) and about the product here studied, and we then explain why we used ants as models.

Information available about herbicides

There exist numerous kinds of herbicides. They are classified according to their general mode of action (selective herbicides, total herbicides, desiccants), their chemical structure (the various synthetic herbicides, among others), and their precise activity on the plants (disruption of the photosynthesis, inhibition of the synthesis of lipids or of amino acids, disruption of the cell division). The herbicides lead to a decrease of the amount and the biodiversity of weeds and consequently of birds. They pollute the surface water as well as the groundwater [5-8]. Humans using herbicides suffer from depressions, cancers, respiratory problems, the Parkinson's disease, and birth defects. Using herbicides at sub lethal doses induces the emergence of resistant plants what accentuates the 'weeds' eliminating work [9-15]. Since January 2019, the most dangerous herbicides have been banned for non-professional users (for common gardeners). Plenty of internet links detail the herbicides, as well as the pesticides and insecticides, still nowadays authorized in different countries. The aim of the present work was to study, on ants as models, the potential adverse effects of a novel kind of herbicide nowadays authorized and largely used, namely Herbatak®. This product is an aqueous solution of 500 g of pelargonic acid. Its official number is G/B detained by Belchim Crop Protection nv/sa (Technologielaan, 7, B-1840, Londerzeel, Belgium). Information on its mode of action, its use, and its disadvantages can be found on its package as well as on several websites. Pelargonic acid is present in nature. It affects the cellulose wall of the plant cells which consequently lose their water. It irritated the skin and especially the eyes. It is toxic to aquatic animals, and of course to any useful cultivated plants. While using this herbicide, gardeners must thus be careful to avoid skin and eyes pain, to not contaminate surrounding cultivated plants, and to not pollute natural water. Except these advices, nothing is mentioned as for the potential impact of Herbatak® on terrestrial animals living in the ground (annelids, spiders, insects, larvae) or flying above the treated areas (butterflies, mosquitoes, bumblebees, bees). In other words and briefly, the here above cited website clearly states that the kind of herbicide the active substance of which is pelargonic acid has toxicity, but it does not give any other details. Therefore, our aim was to study the potential adverse impacts of Herbatak® on terrestrial animals using ants as models.

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Why using ants as models; which species we used, and which traits we examined

Most biological mechanisms are identical for all the animal species, e.g. genetic, protein synthesis, muscles contraction, nerve impulses, and respiration [16].

This is why biological and medicinal investigations are firstly performed in animals as models, e.g. fruit flies, cockroaches, bees, mice, and monkeys, before being made in humans [17]. Insects are often used due to their rapid development and easy maintenance in a laboratory [18]. Hymenoptera, among others, and thus ants can be used [19]. Ants present complex behavior and many evolved skills. They build complex nest, communicate with congeners thanks to pheromones, mark the different parts of their territories, navigate using memorized cues, take care of their brood and queens, clean their nest and manage cemeteries at the frontier of their territory [20].

Here, we worked on the species *Myrmica sabuleti,* Meinert, 1861, the biology of which we well know. Among others, we have studied their eyes morphology, visual perception, conditioning ability, recruitment strategy, navigation system, as well as the ontogenesis of some of their abilities [21,22]. These ants recognize themselves in a mirror [23]. The distance and size effects, as well as the Weber's law can be applied to their perception [24,25]. They detain many numerosity abilities [26-28], to cite only few of their abilities. The effects of products on some of their abilities can thus be examined. The results of these studies can then allow making similar investigations in humans what could improve the knowledge of the side effects of the examined products on the humans' health.

We here aimed to examine the effects of the herbicide Herbatak® on the ants' food intake, general activity, locomotion, orientation ability, tactile (pain) perception, social relationships, stress, cognition, conditioning ability and memory, as well as the ants' potential adaptation to side effects and the decrease of the effect of Herbatak® after the ants were no longer in contact with it.

MATERIALS AND METHODS

The here used experimental materials and methods are identical to those already many times used and explained in published papers [29-31] among others since we have until now studied the side effects of 51 products or situations used by humans. They are thus here rather briefly related, but some self-plagiarism is inevitable.

Collection and maintenance of ants

The experiments were performed on two colonies of *M. sabuleti* collected in May 2021 from an abandoned quarry located in the Aise Valley (Ardenne, Belgium). These colonies were living under stones and in grass, and contained about 600 workers, a queen and brood. They were maintained in the laboratory in one to three glass tubes half filled with water, a cotton plug separating the ants from the water. The nest tubes of each colony were set in a tray (34 cm × 23 cm × 4 cm) which served as a foraging area. In these trays, pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were delivered three times per week, and a tube plugged with cotton containing an aqueous solution of sugar (15%) was permanently provided. The lighting varied between 330 lux and 110 lux, the ambient temperature equaled 20°C, the humidity equaled c80%, and the electromagnetism equaled 2 μWm^2. Such conditions are suitable to the species *M. sabuleti*. The ants are here often named workers, nest mates or congeners as do researchers on social insects.

e-ISSN: 2347-7830
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Solution of Herbatak® spread on the ants' area

Herbatak® can be bought in most shops provided with products used for gardening. The aqueous solution of 500 g pelargonic acid per liter must be diluted for being correctly used. The recommended doses are 35 ml or 45 ml of the latter concentrated solution diluted into 1 liter of water. We opted for 40 ml to dilute in 1 liter of water. Working on ants and thus needing only a few ml, we decided to use a solution of 4 ml diluted onto 100 ml of water, and made in fact a solution of 2 ml of the bought concentrated solution diluted into 50 ml tap water. The recommended solutions of 35 ml or 45 ml diluted into 1 liter of water are to be used for treating an area of 20 m^2. For conducting our experiments on ants, we needed to treat an area of 20 cm^2 for each two used colonies. Therefore, for each colony, we used 1,000 ml/1,000=1 ml and spread this 1 ml onto an area of 20,000 cm^2/1,000=20 cm^2 as follows. We spread 0.2 ml all around the tube containing sugar water, 0.2 ml all around the meat food, and 0.6 ml all-round the nest entrance, these three locations being the most frequented ones by the ants (Figures 1 and 2). Herbatak®, i.e. pelargonic acid, is rapidly degraded, and the areas treated with this product can be used for cultivating useful plants one day after the end of the treatment. Consequently, we kept the solution '2 ml of the concentrated bought solution into 50 ml tap water' at –15°C, and we spread again the three here above cited zones of the ants' area with respectively 0.2 ml, 0.2 ml, and 0.6 ml of that solution.

The control experiments were firstly made on ants living in not treated areas. Then, we conducted the test experiments relative to the effects of Herbatak® on ants' physiological and ethological traits. This lasted eight days. After that, for studying the loss of the effect of the herbicide after having no longer been in its contact, each two colonies were relocated into a novel tray, being so no longer in the presence of Herbatak®, and a physiological trait affected by the herbicide was assessed over time until it had again its control value (Figures 1-3).

Figure 1. Successively, above the chemical structure of pelargonic acid, a package of Herbatak® (the kind we here used), and below: the realization of the solution used for spreading some parts of the ants' environment, in order to study the impact of Herbatak® on several ants' physiological and ethological traits.

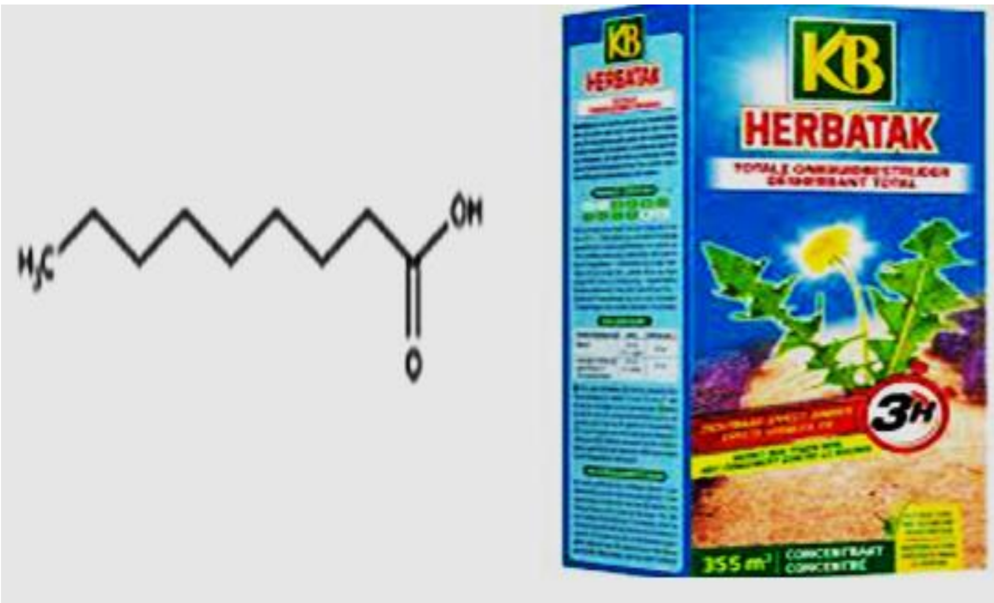

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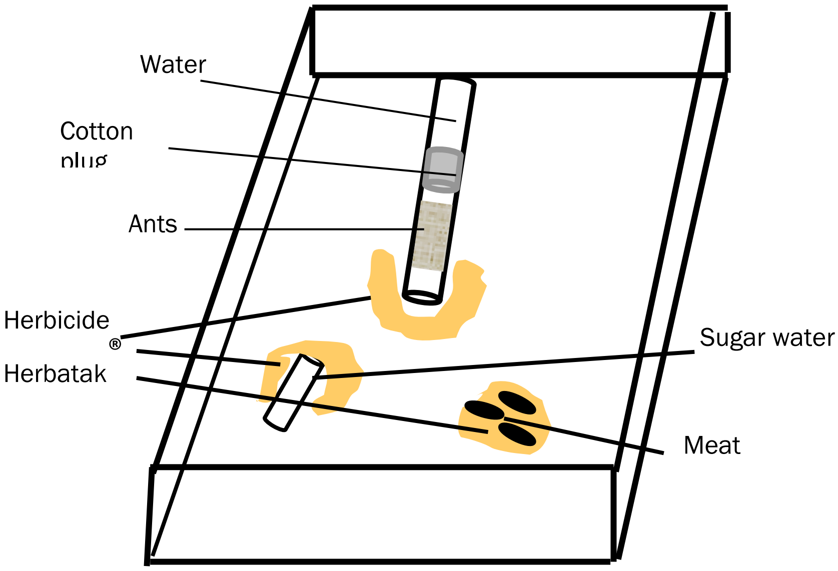
Figure 2. Experimental protocol for making the solution of Herbatak® which must be used for setting the ants under the same conditions as those of animals present in the treated zones.

Figure 3. Experimental design used for examining, on ants as models, the impact of the herbicide Herbatak® (pelargonic acid) on several physiological and ethological traits.



Assessment of ethological and physiological ants' traits

Food intake, general activity: The ants present on the meat food, present at the entrance of the sugar water tube, and being active at any place of their environment (foraging area, food sites, inside of the nest) were counted four times per day for each colony, and this was run during six days. For each of the three kinds of count, and for each kind of diet (normal and with the herbicide), the mean of these 4 times × 2 colonies=8 daily counts was established (Table 1, the six first lines). The means of the six daily means were also calculated for each kind of count and each kind of diet (sample size=48; Table 1). The six daily means obtained for each kind of count for ants living in the presence of Herbatak® were compared to the six corresponding daily means obtained for ants living under normal maintenance using the non-parametric test of Wilcoxon [32], the level of probability being set at 0.05.

Linear and angular speeds, orientation: These traits were assessed on ants moving in their foraging area, the speeds in the course of an experiment without stimulating them, the orientation in the course of another experiment while stimulating them with a nest mate tied to a piece of paper. Such a tied nest mate emits its attractive alarm mandibles glands pheromone. To assess the ants' speeds then orientation, 40 foragers' trajectories were recorded and analyzed thanks to adequate software [33] set up on the basis of the following definitions. The linear speed (in millimeter per second = mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in angular degrees per cm = ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in angular degrees= ang. deg.) towards a location is the sum of successive angles made by the direction to the location and the direction of the trajectory, divided by the number of angles measured. An orientation value lower than 90° signifies that the animal orients itself towards the location; an orientation value larger than 90° signifies that the animal tends to avoid the location.

For each variable, i.e. linear speed, angular speed, and orientation, the median and quartiles of the 40 recorded values were established (sample size for each variable: 40, Table 2. The distributions of the values obtained for ants having Herbatak® on their area were compared to the corresponding distributions obtained for ants normally maintained by using the non-parametric x^2 test [32].

Audacity: This trait was evaluated through the ants coming onto a risky unknown apparatus. A cylindrical tower (height=4 cm; diameter=1.5 cm) tied to a squared platform (9 cm^2), both parts made of Steinbach® paper, was set in the ants' tray, and the workers of the two colonies present on this apparatus were counted 10 times over 10 min. The mean and extremes of the recorded numbers were established (sample size: 20, Table 2). Then, the numbers obtained for the two colonies were correspondingly added, and the sums obtained for every two successive minutes were added, what provided five sums of numbers. The five sums obtained for ants having Herbatak® on their area were compared to the five sums obtained for ants normally maintained by using the non-parametric test of Wilcoxon [32].

Tactile (pain) perception: Ants perceiving the rough character of a substrate walk on it with difficulty, slowly, sinuously, and often touch it with their antennae. Ants which poorly perceive the rough character of a substrate walk on it easily, rather rapidly, not very sinuously, and seldom touch the substrate with their antennae. Therefore, to estimate the ants' tactile (pain) perception, we quantified their linear and angular speeds while they walked on a rough substrate. A piece (3 cm × 2+7+2=11 cm) of n° 280 emery paper duly folded was set in a tray (15 cm × 7 cm × 4.5 cm) dividing this tray in three zones, a small 3 cm long one, a second 3 cm long one covered with the emery paper, and a last 9 cm long zone. For making an experiment on a colony, 12 ants were deposited in the first small 3 cm long zone of the apparatus, and their trajectories were recorded while they walked in the zone covered with the emery paper. Their linear and angular speeds were then quantified as explained in an above subsection. Twenty four values of linear and of angular speeds were so obtained, and for each speed, the median and quartiles of the obtained values were established (sample size for each speed: 24, Table 2). For each speed, the distribution of values obtained for ants having herbicide on their area was compared to that obtained for ants living under normal maintenance using the non-parametric x^2 test [32].

Brood caring: For each colony, a few larvae were removed from the nest and deposited near its entrance. For each colony, five of these larvae were observed during five minutes. Only five larvae were observed since they had to be so simultaneously. Also, the experiment was not repeated because removing brood out of the nest induced a strong perturbation to the colony and may imperil the life of the brood. The ants' behavior in front of the 2 × 5 tracked larvae was cautiously observed and the larvae not yet re-entered in the nest after 30 seconds, 1, 2, 3, 4 and 5 minutes were counted. The six numbers obtained for the two colonies were correspondingly summed (sample size: 10, Table 3), and the six sums obtained for ants living with herbicide on their area were compared to those obtained for ants normally maintained using the non-parametric test of Wilcoxon [32].

Social relationships: Ants belonging to the same colony are not aggressive towards one another. Therefore, to examine the impact of Herbatak® on this peaceful relationship, five dyadic encounters were conducted for each colony, each one in a cup (diameter=2 cm, height=1.6 cm) the inner borders of which having been slightly covered with talc. During each encounter, one ant of the pair was observed for 5 minutes, and the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped the other ant (level 3), and tried to sting or stung it (level 4) were counted. The numbers obtained for each ant and each colony were correspondingly added.

The distribution of these recorded numbers obtained for ants having e herbicide on their area was compare to that obtained for ants living in the absence of herbicide by using the non-parametric x^2 test [32]. In addition, for each kind of diet, the ants' social relationship was evaluated thanks to a variable 'a' (global aggressiveness) which equaled the number of aggressiveness levels 2+3+4 divided by the number of aggressive levels 0+1 (sample size: 10, Table 3).

Stress and cognition: These traits were appreciated through the ants' ability to escape from an enclosure. Indeed, for doing so, they must not stress, stayed calm, and research for an exit; they must also have some intact cognitive abilities. To assess these traits, six ants of each colony were enclosed under a reversed polyacetate cup (=the enclosure; height=8 cm, bottom diameter=7 cm, ceiling diameter=5 cm, the inside surface having been slightly covered with talc) deposited in their foraging area. A notch (3 mm height, 2 mm broad) had been created in the rim of the bottom of the cup giving so to the ants the possibility of escaping. For each colony, the ants escaped after 2, 4, 6, 8, 10 and 12 minutes were counted and the numbers obtained for the two colonies were correspondingly added (sample size: 12+12, Table 3). The six successive sums obtained for ants having the herbicide on their area were compared to the six ones obtained for ants having not this product by using the non-parametric Wilcoxon test [32].

Cognition: This trait was evaluated through the ant's ability to cross a twists and turns path. Two pieces of paper (Steinbach®, 12 cm × 4.5 cm) duly folded were inserted in a tray (15 cm × 7 cm × 4.5 cm) with the aim to create a twists and turns path between a 2cm long zone in front of this "difficult" path and a 8 cm long zone beyond that path. Such an apparatus was constructed for each colony. To make an experiment on a colony, 15 ants were deposited in the zone lying in front of the twists and turns path, and those still there as well as those having reached the zone lying beyond the difficult path were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were correspondingly added (sample size: 12+12, Table 3). For each of the two zones of the apparatus, the six sums obtained for ants having some herbicide on their area were compared to those obtained for ants maintained without herbicide by using the non-parametric Wilcoxon test [32].

Learning and memory: For each of the two colonies having Herbatak® on their area, a blue hollow cube made in strong paper (Canson®) was deposited above the nest entrance at a recorded time o'clock. Since that time, the ants underwent operant visual conditioning. The control experiment was previously made on another similar colony of *M. sabuleti*, collected at the same time and from the same site than the two experimented colonies, and continuously maintained without herbicide on tier area. This must be done because as soon as a worker ant has acquired conditioning to a stimulus, it keeps it during minimally two to three days and even after having lost its conditioning, it more quickly than usually again acquires it. This having been conditioned ant can thus no longer be used for quantifying its acquisition of conditioning. To come back to the present experiment, in the course of the ants' conditioning acquisition, as well as after having removed the green cube, i.e. in the course of the ants' loss of conditioning, the ants of each three colonies were tested in an own Y-apparatus (made of strong white paper, its sides having been slightly covered with talc) deposited in a separated tray. A blue hollow cube was inserted in randomly either the left or the right branch of the Y-apparatus. For making a test on a colony, 10 ants were one by one deposited in the area of the Y-maze lying in front of its two branches, and the ants' first choice of one or the other branch of the Y-apparatus was recorded. Choosing the branch containing the blue cube was considered as giving the correct response. After having been tested, each ant was kept in a cup until 10 ants of its colony were tested for avoiding testing twice the same ant. After having tested, the 10 ants of each colony were transferred back into their foraging area. Tests were performed after several time periods, and for each of these periods, the 10 responses obtained for the two experimented colonies were added. Then, the proportions of correct responses (=the conditioning scores) was each time established for the 'control colony' and for the two experimented ones (sample size: 6+12, Table 4). Having no distribution of values but only a conditioning score at our disposal, the Wilcoxon test was used for comparing the 'colonial' scores of ants having Herbatak® on their area to the 'colonial' scores previously obtained for ants normally maintained [28].

Adaptation to side effects: An individual adapts itself to a situation inducing side effects when it less and less suffers from these adverse effects over time. To study such an adaptation, a trait affected by the situation must be assessed twice, firstly soon, then later after some time of the situation occurrence, and the two assessments must be compared to one another. In the present work, a trait largely impacted by Herbatak® was the ants' sinuosity of movement. Therefore, this trait was assessed after the ants had the herbicide on their area since 1 then since 5 days (sample size: 40), and the data recorded during these two assessments were statistically compared using the non-parametric x^2 test [32].

Ants' recovering after no longer being in contact with the herbicide: This recovering was studied after the ants had the herbicide on their area since eight days. The physiological trait used for studying this decrease was the ants' angular speed (sinuosity of movement). It was once more assessed, at 't=0', as it had been previously assessed except that not 40 but only 20 ants' trajectories were recorded and analyzed. Doing so allowed making the required successive assessments all along the decrease of the effect of the herbicide on the ants' angular speed. After this assessment, each two colonies were transferred into a novel intact area, this act defining a kind of weaning, i.e. the start of the decrease of the effect of Herbatak® on the ants' physiology. Since that time, the ants' sinuosity was assessed every two hours, until this trait became similar to that of ants living under normal maintenance (=the control value). For each record, the median and quartiles of the 20 obtained values were established (sample size: 20 for each time, Table 6). The distributions of values obtained at each testing time were compared to the distribution obtained at t=0 and to the control distribution using the non-parametric x^2 test for independent samples

(Table 6) [32]. The mathematical function best describing the decrease of the effect of Herbatak® on the ants' sinuosity was established using Statistica v10 software, and graphically represented in Figure 6.

RESULTS

Food intake, general activity

The herbicide affected each of these three physiological traits (Table 1), and this was statistically significant. Indeed, Herbatak® reduced the ants' meat intake, sugar water consumption as well as general activity at a significant level of probability (for each examined trait: N=6, T= -21, P=0.016). In the course of the experimental work, we checked if these impacts still subsisted, and they obviously did so (Table 1).

Table 1. Impact of Herbatak® on ants' food intake and general activity. The table gives the mean numbers of ants counted each day on their meat, at the entrance of their tube filled with sugar water, and being active at any place, as well as the mean of these six means. The herbicide largely and significantly impacted these three physiological traits.

Days	Normal maintenance			With Herbatak® on the ants' area		
	meat	sugar water	activity	meat	Sugar water	activity
I	1.00	1.75	13.63	0.25	0.25	6.88
II	1.25	1.75	15.00	0.25	0.25	7.12
III	1.25	1.88	14.75	0.25	0.38	5.75
IV	1.38	2.00	21.25	0.25	0.25	5.25
V	1.25	1.50	19.88	0.25	0.25	6.25
VI	1.63	1.25	21.38	0.25	0.25	5.87
I-VI	1.29	1.52	12.82	0.25	0.27	6.18

Linear and angular speeds: These traits were largely impacted by the presence of Herbatak® on the ants' area. Indeed, under the latter environmental condition, the ants walked with difficulty, trebling, turning all the time, walking backwards, stopping, and moving very slowly and very sinuously. This was obvious to observers, and clearly revealed by the recorded data (Table 2). This was also confirmed by the statistical results: the ants' linear and angular speeds presented while having herbatak® on their area were statistically different from their speeds while moving on their not treated area: linear speed: x^2 = 56.26, df = 2, P < 0.001; angular speed: x^2 = 80.00, df = 1, P< 0.001. It was examined if the ants could adapt themselves to this impact of the herbicide on their locomotion after having had it on their area during five days (see below the subsection relative to the ants' adaptation).

Orientation: The presence of Herbatak® largely affected the ants' orientation capability (Table 2). While under normal maintenance, the ants very well oriented themselves towards a tied nestmate, when they were in the presence of the herbicide, they could no longer done so, i.e. they did not avoid the tied nestmate, but they poorly found their way towards it. The difference of orientation capability between the ants maintained with and without the herbicide on their area was significant: x^2 =19.61, df=2, P<0.001. This may result from an impact of Herbatak® on the ants' olfactory perception, a presumption checked thanks to subsequent experiments (see the subsections relative to tactile perception and brood caring).

Audacity: Herbatak® impacted this ethological trait (Table 2). While normally maintained ants came with only little hesitation on the presented unknown risky apparatus, those having the herbicide on their area were not inclined to act in this manner. The difference as for the numbers of ants counted on the presented apparatus between the ants living under one and the other kinds of maintenance was statistically significant: N = 5, T= -15, P = 0.031. This resulted from the ants' difficulty walking while having Herbatak® on their area, but could also be due to some decrease of the ants' tendency to perform tasks. The latter hypothesis was checked thanks to two following experiments (see the subsections relative to the ants' stress and cognition, and cognition) (Tables 2,3).

Table 2. Impact of Herbatak® on five ants' physiological and ethological traits. The Table gives the median (and quartiles) or the mean (and extremes) of the recorded data. The herbicide affected each of these physiological and ethological traits, and these five effects were statistical significant (see details in the text). mm/s=millimeter per second, ang.deg./ cm=angular degree per centimeter, ang.deg.=angular degree, n° =number.

Traits	Normal maintenance	With Herbatak® in ants' area
Linear speed (mm/s)	9.7 (8.8-10.6)	5.6 (5.1-6.0)
Angular speed (ang.deg./cm	106 (95-121)	252 (227-287)
Orientation (ang.deg)	36.3 (26.9-44.7)	65.8 (52.1-89.3)
Audacity (n°)	2.90 (1-4)	1.00 (0-2)
Tactile (pain) perception		
linear speed and angular speed on a rough substrate	4.0 (3.7-4.5) and 322 (276-346)	5.8 (5.1-6.3) and 229 (213-247)

Table 3. Effect of Herbatak on four ants' ethological and physiological traits. The Table gives the numbers of counted larvae, levels of aggressiveness, and ants for ants maintained with or without the herbicide on their area. The herbicide impacted the four traits, i.e. the social interactions the cognition. Nevertheless, the ants' state of stress was not affected.

Traits	Normal maintenance	With Herbatak® on ants' area
Brood caring: n° of not re-entered larvae over time	30s 1 2 3 4 5 min 6 4 2 0 0 0	30s 1 2 3 4 5 min 10 10 10 10 10 10
Social relationships: n° of aggressive levels 0 to 4, 'a'	levels 0 1 2 3 4 'a' 65 60 9 0 0 0.07	levels 0 1 2 3 4 'a' 27 32 51 0 0 0.86
Stress and cognition: n° of ants escaped over time	2 4 6 8 10 12 min 2 5 7 10 11 12	2 4 6 8 10 12 min 1 1 2 3 4 4
Cognition: n° of ants in front and beyond a twists and turns path over time	min: 2 4 6 8 10 12 front 24 18 15 12 8 7 beyond 0 1 2 5 7 8	min: 2 4 6 8 10 12 front 28 23 19 18 16 16 beyond 0 1 0 1 1 2

Tactile (pain) perception: This physiological trait was impacted by the presence of Herbatak® on the ants' area (Table 1). This was obvious to observers. Under normal maintenance, the ants walked far more slowly and far more sinuously on the rough substrate than on their foraging area (for their linear as well as for their angular speed: x^2=64.00, df=1, P<0.001). Ants which were in contact with Herbatak® walked on the rough substrate nearly as they walked on their treated area. They even walked a little less slowly and less sinuously, but this amelioration was not statistically significant.

The difference between their linear and angular speed on a rough substrate on one hand and on their foraging area on the other hand was not significant: linear speed: x^2 = 2.28, df=1, 0.10<P<0.20; angular speed: x^2 = 0.12, df=1, P ~ 0.70. This decrease, even loss, of sensory perception accounted for the ants' impacted orientation to a tied nestmate (see the above subsection relative to the ants' orientation). Let us add that such a nearly loss of sensory perception may affect all the animals present on the areas treated with Herbatak®, and must thus be taken into account when planning using this herbicide on some areas (see the discussion and the conclusion sections).

Brood caring: Herbatak® largely affected this ethological trait (Table 3). Ants under normal maintenance rapidly found the larvae experimentally removed from the nest, took them in their mandibles and quickly transported them into the nest. When having the herbicide on their area, the ants did not found the larvae, and if nevertheless approaching them, they did not take them in their mandibles. None of the 10 larvae removed from the nest were re-entered at the end of the five experimental minutes. The difference between the ants maintained without or with the herbicide on their area was statistically significant: N=6, T= -21, P=0.016. This may be due to an impact of the herbicide on the sensory organs (eyes, antennae) of the ants, what would be in agreement with the ants' poor orientation in the presence of this herbicide, and their decrease, even loss, of tactile perception (see the above subsections relative to the ants' orientation and tactile (pain) perception).

Social relationships: This ethological trait was highly affected by the presence of Herbatak® on the ants' area (Table 3). Ants under normal maintenance stayed peacefully side by side or in front of each other, and made antennal contacts. Ants having the herbicide on their area only seldom stayed near each other, and when doing so, they generally stayed head to tail and often opened their mandibles. This difference of behavior assessed through the numbers of observed levels of aggressiveness was highly significant: x^2=51.95, df=2, P< 0.001. This observation was in agreement with that made for the ants' brood caring (see the above subsection relative to 'brood caring'), and may be due, at least partly, to the ants' affected sensory perception, an effect already observed in the course of three here above experiments, i.e. 'orientation', 'tactile perception', 'brood caring'.

The present observation was obvious to observers: in their nest, the ants poorly care of their brood, and in their foraging area, they often opened their mandibles when encountering a congener. It can be concluded that the herbicide impacted the individuals' social interactions, this being partly due to their decrease of sensory perception, and maybe also to some other ethological and/or cognitive impairments. The latter presumption was investigated thanks to the three following experiments (see the subsections relative to the ants' stress and cognition, cognition, as well as learning and memory).

Stress and cognition: These traits assessed through the ants' ability in escaping from an enclosure was partly affected by the presence of the herbicide on the ants' area (Table 3). Ants being not in contact with the herbicide could escape from the enclosure: they did not stress and had their cognitive abilities not impacted. Ants having the herbicide on their area appeared to not stressing and walked calmly, though with difficulty, along the rim of the enclosure. They had some difficulty in perceiving the provided exit and guessing that they could escape through it. However, a few ants were able to escape, this act not requiring the use of the antennae which seemed altered, damaged by the herbicide.

Nevertheless, the difference of the numbers of escaped ants over time between the ants having or not the herbicide on their area was significant: N=6, T= -21, P= 0.016. It remains to know if the ants' cognitive ability were affected by the herbicide.

Cognition: This ethological and physiological trait was, at least partly, affected by some contact with Herbatak® (Table 3). Ants normally maintained could cross the presented difficult path: After the 12 experimental minutes, only 7 ones were still in front of this path while 8 ones were beyond it.

Ants having the herbicide on their area were less able to do so: After the 12 minutes, 16 ones were still in front of the path while only 2 ones were beyond it. The difference between the ants maintained with or without the herbicide on their area as for their numbers present over time in front and beyond the path was slightly significant (in front: N = 6, T = +21, P=0.016; beyond: N=4, T= -10, P = 0.063). Such a result was in agreement with those on the ants' audacity and escaping ability, and the impact of Herbatak® on the ants' cognitive abilities was again examined. Having the cognitive abilities affected could result from damage to sensory organs, and ultimately to general health.

Learning and memory: Herbatak® affected these physiological traits (Table 4). Normally maintained ants reached a visual conditioning score of 70% and 85% after respectively 31 and 72 training hours. Ants living in the presence of the herbicide on their area never reached a conditioning score higher than 45%. The difference between the two kinds of ants as for their conditioning acquisition was significant: N=6, T= -21, P=0.016. Herbatak® affected thus the ants' short-term memory.

Since the ants living in the presence of the herbicide acquired no conditioning, their loss of condition, i.e. their middle-term memory could not be examined. The present result revealing an impact of the herbicide on the ants' brain functioning was in agreement with those of the two previous experiments relative to the ants' cognition (see the two above subsections relative to the ant's stress and cognition, and cognition). Such an impact may have adverse effects on the ants' life and social tasks performing.

Adaptation to side effects: The ants did not adapt themselves to the impact of Herbatak® on their locomotion (Table 5. After having been in contact with the herbicide during five days, the ants still walked very slowly and very sinuously as when they had been in such a contact during one day. The difference of linear speed value between the ants having lived for five days and those having lived for one day in the presence of the herbicide was not significant: x^2=0.34, df=1, 0.50<P<0.70. The difference of angular speed value between these two kinds of ants was slightly significant, with the value for ants in contact with the herbicide for five days somewhat larger than that for ants in such a contact for one day: x^2 = 4.71, df=1, 0.02<P<0.05. Consequently, the ants did not at all adapt themselves to the effect of the herbicide on their sinuosity of movement; on the contrary, this effect seemed to increase over time (see also, in the subsection relative to the ants' recovering, the value of angular speed obtained at t=0). The physiological trait 'sinuosity of movement' was thus chosen for studying the loss of the effect of the herbicide on the ants' health after the ants were no longer in contact with it (see the under subsection relative to the ants' recovering).

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Supplementary observations: Contrary to usually maintained colonies, after having been maintained during five days with Herbatak® on some parts of their area, the experimented colonies had several dead workers on their foraging area, and moreover, these dead ants had not been transported onto the cemeteries of the colonies. Also, the corpses of these dead ants looked differently: those of ants having never been in contact with the herbicide were located in a cemetery, had intact antennae and an usual 'ant' appearance; those having been in contact with the herbicide were not located in a cemetery, had damaged antennae and an unusual appearance as if they had suffered. We could thus presume that the herbicide impacted in a first time the sensory organs of the individuals, and then, finally, their general health, even leading them to death (Figure 4) (Tables 4 and 5).

Figure 4. Potential effects of the herbicide Herbatak® on ants' physiological and ethological traits. Some views of the experiments made to know potential effects of the herbicide Herbatak® on ants' physiological and ethological traits. **1:** Ants under normal maintenance; **2:** Ants having the herbicide on some parts of their area. **A**: Ants orienting themselves to a tied nestmates in the absence of the herbicide, and poorly doing so in its presence; **B**: Ants coming onto a risky apparatus in the absence of the herbicide and poorly doing so in its presence; **C**: Ants walking with difficulty on a rough substrate in the absence of the herbicide, and more easily in its presence, poorly perceiving then the uncomfortable character of the substrate; **D**: An ant under normal maintenance taking care of a larva, and an ant in contact with the herbicide not doing so; **E**: Two nestmates under normal maintenance, peacefully contacting each other with their antennae, and two nestmates in contact with the herbicide staying head to tail, slightly opening their mandibles. **F**: An ant under normal maintenance escaping from an enclosure, and an ant living with Herbatak® on its area not doing so.

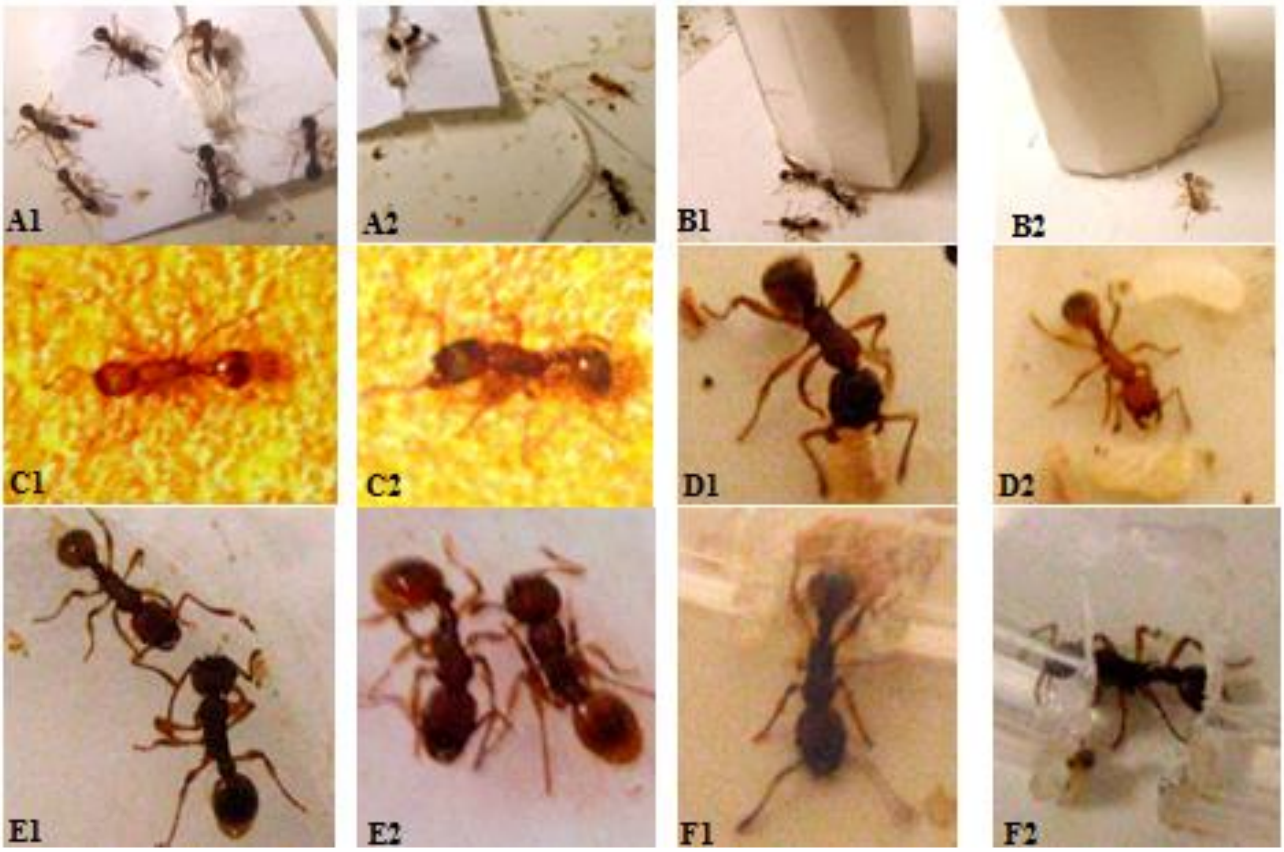

Table 4. Impact of Herbatak® on the ants' conditioning acquisition and memorization. The Table gives the numbers of correct *versus* wrong responses given by 10 tested ants of each colony, and the final corresponding proportions of correct responses (= the ants' conditioning scores). While having Herbatak® on their area, the ants were unable to acquire conditioning. Their short-term memory was thus impacted. Having learned nothing, their middle term memory could not be studied.

Time (hours)	Without herbicide colony C	With herbatak® on ants' area colony A colony B score
7 h	6 *vs.* 4 60%	4 *vs.* 6 5 *vs.* 5 45%
24 h	6 *vs.* 4 60%	4 *vs.* 6 4 *vs.* 6 40%
31 h	7 *vs.* 3 70%	5 *vs.* 5 4 *vs.* 6 45%
48 h	7 *vs.* 3 70%	5 *vs.* 5 4 *vs.* 6 45%
55 h	8 *vs.* 2 80%	6 *vs.* 4 3 *vs.* 7 45%
72 h	9 *vs.* 1 85%	5 *vs.* 5 4 *vs.* 6 45%
cue removal 7 h	9 *vs.* 1 85%	-
24 h	8 *vs.* 2 80%	final score = 45%
31 h	8 *vs.* 2 80%	thus, impossible to study
48 h	8 *vs.* 2 80%	-
55 h	8 *vs.* 2 80%	-
72 h	8 *vs.* 2 80%	-

Table 5. Ants' potential adaptation to the impact of Herbatak® on their locomotion. After having been in presence of the herbicide for five days, the ants still walked very slowly and very sinuously. They thus did not adapt themselves to the effect of the herbicide on their locomotion. Their sinuosity was even larger after five than after one day of life in the presence of the herbicide.

Trait	No herbicide	Herbicide for 1 day	Herbicide for 5 days
Linear speed (mm/s)	9.7 (8.8-10.6)	5.6 (5.1-6.0)	5.0 (4.4-5.6)
Angular speed (ang.deg./cm)	106 (95-121)	252 (227-287)	271 (254-294)

Ants' recovering after having no longer been in contact with the herbicide: Numerical and statistical results are given in Table 6, and this recovering is graphically presented in Figure 6. Four hours after having no longer been in contact with the herbicide, the ants presented a sinuosity of movement already slightly different from that presented while being in its contact ($P<0.05$). After six hours, this difference was highly significant ($P<0.001$). However, the ants' sinuosity of movement stayed highly different from that presented in the absence of the herbicide until 10 hours after no more contact with the herbicide ($P<0.001$), still though less different after 12 hours ($P<0.01$), and also but slightly different after 14 hours ($P<0.05$). It is only after 16 hours with no contact with the herbicide that the ants' sinuosity no longer statistically differed from that presented in the absence of the herbicide ($P<0.20$).

This sinuosity became identical to that exhibited without the herbicide after a total of 24 hours following the ants' setting in an area having never contained the herbicide. This decrease was not linear; it was more rapid during the first hours and slower thereafter (Figure 5).

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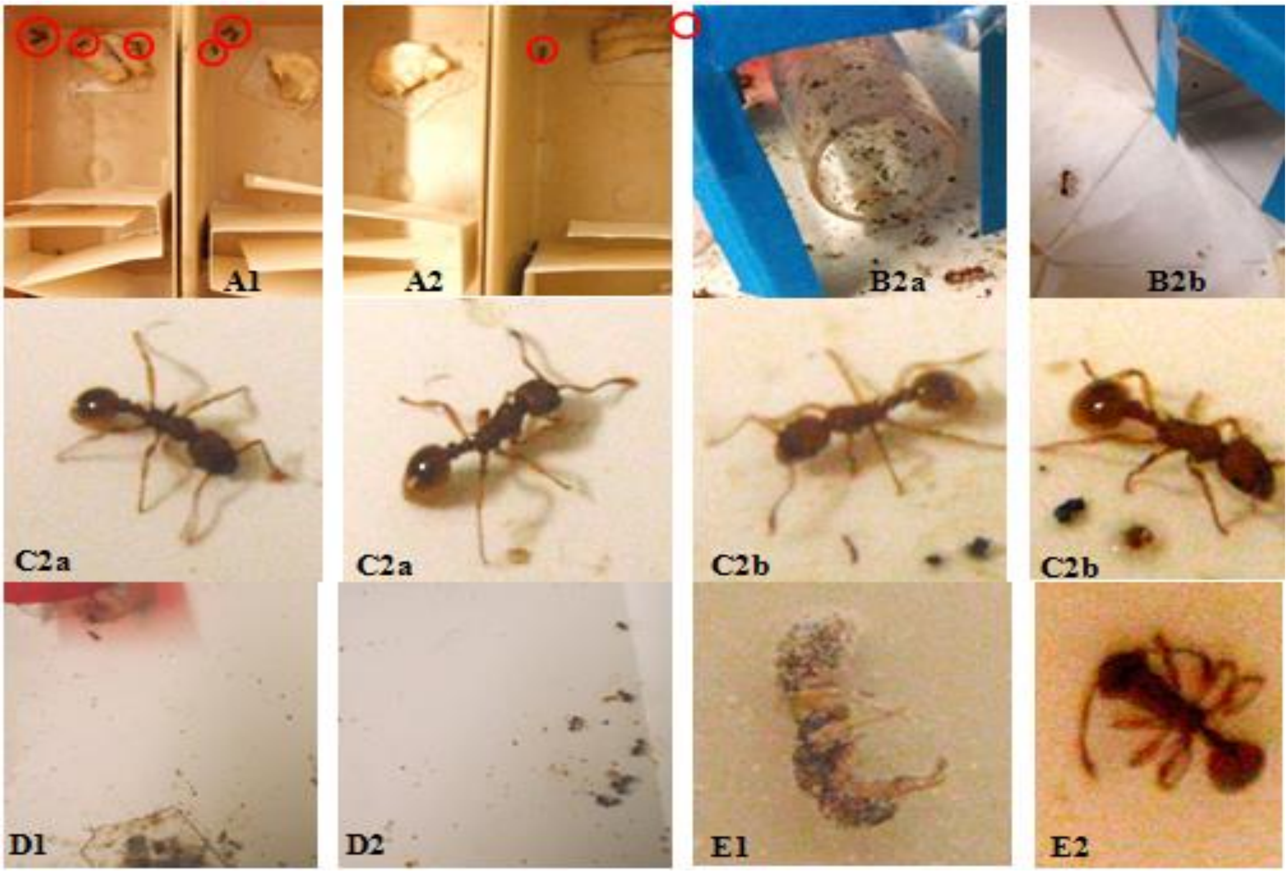
Figure 5. Some views of the experiments made for knowing the potential impact of Herbatak® on the ants' cognition (**A**), conditioning acquisition (**B**), adaptation to side effects of this herbicide (**C**), and death of ants (**D, E**). **1**: Ants under normal maintenance, **2**: Ants having the herbicide on some parts of their area. **A:** When living in the presence of Herbatak®, the ants were less able to cross the presented twists and turns path than when living in its absence. **B2**: Ants living with Herbatak® on their area conditioned to a blue hollow cube; **a**: Training, **b**: Testing: The ant gave the wrong response. **C2**: In contact with the herbicide since 1 (**a**) and 5 (**b**) days: The ants went on presenting difficulties in moving, thus not adapting themselves to the adverse effect of the herbicide on their locomotion. **D**: In presence of the herbicide, several ants died. **E**: The aspect of the corpses allows presuming the ants having been in contact with the herbicide had their antennae damaged and suffered.



It could be best described by the following quadratic function of the running time:

$$E_t = E_i - 15.15t + 0.299t^2$$

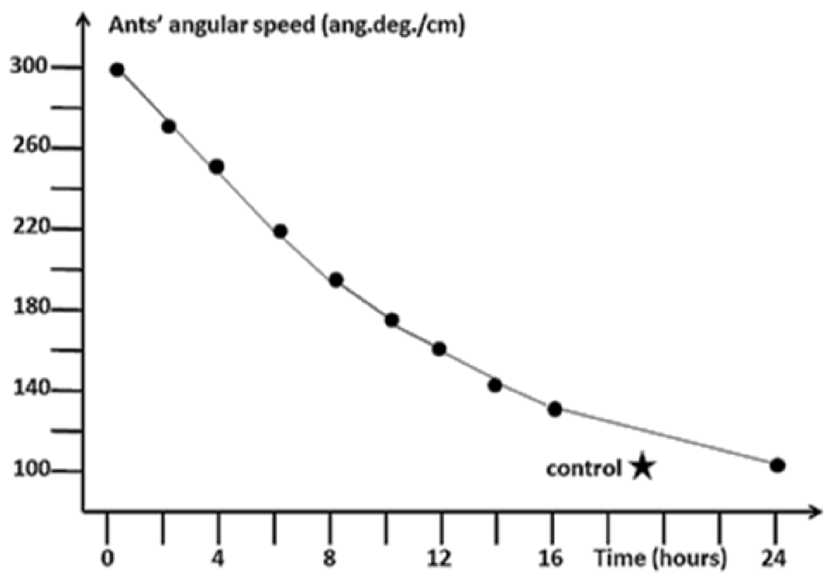
With E_t = effect of the herbicide on the ants at time t, E_i = initial effect, t = time

Note that not all the ants recovered. Several ones died while being in the presence of the herbicide (see the subsection Supplementary observations). Treating areas with Herbatak® imperils thus the health of the organisms living in the ground (see the discussion section) (Table 6 and Figure 6).

Table 6. Decrease of the effect of Herbatak after having no longer been in contact with it. The ants fully recovered after a total of 24 hours. After six hours, their angular speed was already significantly lower than when the ants were in contact with the herbicide, but until after 14 hours, this sinuosity of movement was still significantly larger than that presented in the absence of the herbicide. This recovering is graphically presented in Figure 6, and details can be found in the text.

Time (hours)	Angular speed ang.deg./cm	*vs.* t = 0			statistics *vs.* control		
		χ^2	df	P	χ^2	df	P
t = 0	300 (280-324)	-	-	-	60.00	1	<0.001
2 h	268 (239-296)	2.16	2	< 0.50	60.00	1	<0.001
4 h	244 (222-278)	6.41	2	< 0.05	60.00	1	<0.001
6 h	215 (192-232)	25.25	2	<0.001	27.78	1	<0.001
8 h	197 (160-228)	21.46	2	<0.001	18.00	1	<0.001
10 h	175 (131-190)	15.17	1	<0.001	22.29	1	<0.001
12 h	164 (146-183)	21.54	1	<0.001	7.33	1	<0.01
14 h	144 (129-166)	26.66	1	<0.001	4.58	1	<0.05
16 h	133 (115-165)	32.73	1	<0.001	2.13	1	<0.20
24 h	107 (96-115)	40.00	1	<0.001	0.73	1	<0.50
control	106 (95-121)	60.00	1	<0.001	-	-	-

Figure 6. Ants' recovering after having been no longer in the presence of Herbatak®. Numerical results are given in Table 6, and details in the text. Briefly, the ants' sinuosity of movement stayed different from that presented in the absence of the herbicide until 14 hours after no more contact with it, though it became different from that presented in the presence of the herbicide six hours after no more contact with it. The ants' recovering could be best described by a quadratic function of time. Not all the ants recovered; several ones died while being in contact with the herbicide.



DISCUSSION

Pollution due to pharmaceutical drugs and pesticides is nowadays more and more examined; that resulting from the use of herbicides is not so much studied. We investigated on the still authorized pelargonic acid herbicide, and discovered that it largely affected several physiological and consequently ethological traits. Using ants as models, we showed that this kind of herbicide decreased these insects' food consumption, orientation ability, audacity, sensory perception, social interrelations, cognition, learning and memorization, with no adaptation to these side effects. The results of each of these experiments were in agreement with one another. The ants had apparently their antennae, legs, maybe mouth parts and eyes damaged; several ants died in four to six days. After having no longer been in contact with the herbicide, the survivors recovered in a total of 24 hours. In addition to the assessed side effects of Herbatak®, we saw several dead ants on the foraging area of the experimented colonies, and the aspect of these corpses suggested that the ants had their antennae damaged and had suffered before dying.

In consequence, herbicides, the active substance of which is Pelargonic acid, should be used only for uncultivated soils since the soil fauna of weeded areas would at least partially be destroyed by such a product. Indeed, the soil fauna must be preserved to ensure good crops. The soil fauna includes microscopic animals (protozoa, nematodes, rotifers, tardigrades), invertebrates visible to the naked eye (annelids, mites, centipedes), larvae of insects (Lepidoptera, coleopteran), and adult insects (coleopteran, ants). The soil fauna usually reaches 2.5 tons per hectare. It is the intermediary between the ground and the plants. It assures the ground balance, i.e. its aeration, the plants' assimilation of nutritional elements, and an adequate amount of minerals. The herbicides destroy or at least damage the soil fauna, making the ground at least partly unsuitable for cultivating.

Pelargonic acid herbicides are very efficient [34,35]. In the last cited reference, the authors explain that, without using herbicides, 50% of the crops could be lost, and showed that, compared to two other non-synthetic products, pelargonic acid herbicides are the most efficient ones. Novel herbicides derived from pelargonic acid could nowadays be created [36]. Also, the efficiency of pelargonic acid herbicides can be enhanced by adding organic acid [37]. However, such efficient herbicides have harmful side effects. We have revealed several ones in the present work. In the Introduction section, we have mentioned their toxicity for aquatic animals and have reported some of their adverse effects in humans. A study of the pelargonic acid herbicides toxicity for fishes has been made by Techer et al. [38]. Their general toxicity has been examined by a large number of researchers who published their results in the EFSA journal in 2021 [39]. It follows that further research must be carried out and that, presently, attention must be paid while using these efficient pelargonic acid herbicides. Let us add that, in fine, these herbicides, though useful and less toxic than the previously used, nevertheless contribute to the environment pollution which is nowadays a crucial increasing problem [40,41].

CONCLUSION

To conclude, using herbicides is required for easily cultivating useful plants. However, these products are toxic. The very toxic previously used herbicides are nowadays no longer authorized. The presently largely used ones, the active substance of which is pelargonic acid, are less toxic, but have nevertheless several harmful impacts on the fauna and on the users. We clearly proved so in the present work, and this is also shown by several other researchers, though herbicides are far less investigated than insecticides and humans' medicinal drugs. On the basis of our results and of information available on pelargonic acid, we advise to use the pelargonic acid herbicides only for paths, alleys, et, and not for cultivated areas because this acid imperils the survival of the soil fauna which is necessary to the growth of useful cultivated plants. Also, attention must be paid to the users' skin and eyes, as well as to not pollute natural water with the herbicide.

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We are sincerely grateful to Dr. Roger Cammaerts who helped us defining the function which best described the ants' recovering over time after having no longer been in contact with the herbicide.

DECLARATION OF INTEREST STATEMENT AND ETHICAL CONSIDERATIONS

We affirm having no conflict of interest as for the use of pelargonic acid herbicides or any other kinds of herbicides. We receive no money for making our research. We work on ants, e.g. on their behavior and cognitive abilities. We also use them as biological models (as in the present work), and even if they are social insects and not vertebrates ,we maintain them in the best possible environmental conditions

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