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Pasture Management to Reduce the Risk of *Acer pseudoplatanus* Poisoning While Preserving Ecological Sustainability

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Abstract: In spring, grazing equids may suffer from a severe rhabdomyolysis syndrome named atypical myopathy. This environmental intoxication results from ingestion of toxins contained in *Acer pseudoplatanus* seedlings. The aim of this study was to investigate the effectiveness of herbicide spraying and mowing to reduce the toxic pressure of sycamore seedlings. In a first experiment, the efficacy of three herbicides to eradicate seedlings was compared to mowing. In a second experiment, the influence of the mowing timing on pasture productivity was determined. In both experiments, sycamore seedling counting, grass height and botanical composition were determined. In experiment 2, the final harvest biomass and its nutritional value were also determined. Herbicides and mowing both reduced the number of seedlings, which nevertheless disappear naturally over time without intervention (i.e., in control areas). As opposed to mowing, herbicide spraying altered the composition of the pasture flora. Both sprayed and mowed seedlings remain toxic until full decomposition. Early mowing (i.e., early April) did not affect the harvest yield. Late mowing (i.e., end of April) reduced the harvest yield but its nutritional value fitted the horses' need. In conclusion, mowing is the best strategy to reduce the risk of *Acer pseudoplatanus* poisoning in grazing equids while preserving ecological sustainability and nutritional value of pastures.

Keywords: atypical myopathy; hypoglycin A; *Acer pseudoplatanus*; pasture management; herbicides; mowing

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1. Introduction

The use of pesticides has drastically increased crop yield and plant protection [1]. However, their application has adverse effects on life (i.e., intoxicating farmers [2], companion animals [3] and wildlife [4]) and on the environment (i) by delaying and decreasing the flowering [5]; (ii) by selecting resistant species [6]; and (iii) by increasing pollution [7]. Nevertheless, the environment itself can produce toxic compounds affecting both human and animal health [8–10]. For instance in the USA, 4000 people are affected each year by plant poisoning [8]. Pasturing animals such as cattle, sheep and equids are also affected by plant poisoning [9].

Grazing equids can develop a deadly seasonal rhabdomyolysis called atypical myopathy (AM) [11]. This environmental poisoning is caused by the ingestion of the toxins, hypoglycin A (HGA) and methylenecyclopropylglycine (MCPrG) [12], produced by some *Acer* species and specifically by the sycamore maple (*Acer pseudoplatanus*) [13,14]. All parts of the tree are toxic [13,15,16]. However, autumnal and spring outbreaks of AM have been respectively associated with the presence of samaras and seedlings in pastures [13,15] with three quarters of AM cases occurring in autumn and the remainder in spring [11,17,18]. Although fewer cases occurred in the spring, the use of grassland during this season might represent a serious risk of poisoning for all equids including broodmares and their future foals given the fact that AM toxins can be transferred to milk [19–21].

If seedlings are, or may be, present at pasture, it is advised to stable horses between 1 March and 31 May where 94% of spring cases occurred [16]. Keeping a horse in the stable all day round may be difficult, and it is not considered as a good practice with regard to animal welfare. It is worth noting that the limitation of grazing time to a few hours per day was found to be a protective factor for AM [11,18] suggesting that the risk can be decreased by reducing contact with toxic plant material. Therefore, techniques to destroy seedlings or at least to reduce their number are needed. The usefulness of herbicide spraying or mowing have been investigated in a previous study [22]. However, these interventions were applied belatedly in the season (i.e., mid-May to mid-June) [22], with regard to the occurrence of most of the AM spring cases (i.e., 1 March until 31 May) [11,17,18] and the pasture biomass production [23]. To the author's knowledge, the efficacy of herbicide spraying or mowing applied earlier in the high-risk season to reduce the pasture toxicity has never been investigated.

The aim of this study was to investigate the effectiveness of these two interventions to eradicate sycamore seedlings as soon as they have emerged in the beginning of the high-risk season. In addition to the number of seedlings, the HGA concentration, the flora composition and the pasture productivity were evaluated.

2. Materials and Methods

2.1. Experimental Conditions

The effects of herbicide spraying and mowing were investigated in flat pastures containing sycamore seedlings during the spring of 2019 in Belgium. The procedures were tested on separate blocks delineated with bamboo canes on the pasture (Figure 1); each block contained at least 25 sycamore seedlings per square meter. Two experiments were conducted into two different pastures. Experiment 1 tested the efficacy of herbicide spraying and mowing to reduce the toxic pressure of sycamore seedlings. Experiment 2 evaluated the influence of the mowing timing on pasture productivity. Each experiment was performed in four blocks allowing each experimental condition to be repeated four times. Figure 1 illustrates one block in experiment 1 (Figure 1A) and experiment 2 (Figure 1B), respectively.

2.1.1. Experiment 1—Pasture 1

Experiment 1 started when the seedlings had two cotyledons but no leaf (i.e., on 17 April 2019). Each experimental block was subdivided into five lanes, three of which were for herbicide treatment, one for mowing and one as a control (Figure 1A). The allocation of the different interventions was randomly distributed within the four blocks. The three herbicides tested were all based on triclopyr (i.e., an anti-dicotyledon recommended to be used in spring). Herbicides were Doxstar® containing 216 g/L fluroxypyr-meptyl and 209 g/L triclopyr-butotyl (Dow Agrosciences B.V, Antwerpen, Belgium); Genoxone® containing 103.6 g/L triclopyr and 93 g/L 2,4-D ethyl-hexyl ester form (Arysta Lifescience Benelux SPRL, Seraing, Belgium) and Garlon Super® containing 240 g/L triclopyr and 30 g/L aminopyralid (Dow Agrosciences B.V, Antwerpen, Belgium). Herbicides were sprayed with a portable hand pressure pump spray, in compliance with the manufacturer's instructions. In dedicated lanes, mowing was performed with a mowing machine with a cutting height of 6 cm (Figure 1) and without removing the mow (i.e., the mowed material was left in place). In the control lane, no intervention was undertaken. Counting of sycamore seedlings was performed within a 4 m^2 area inside each lane. The soil condition was silty-stony with good drainage. The grassland was pastured by horses for May until December.

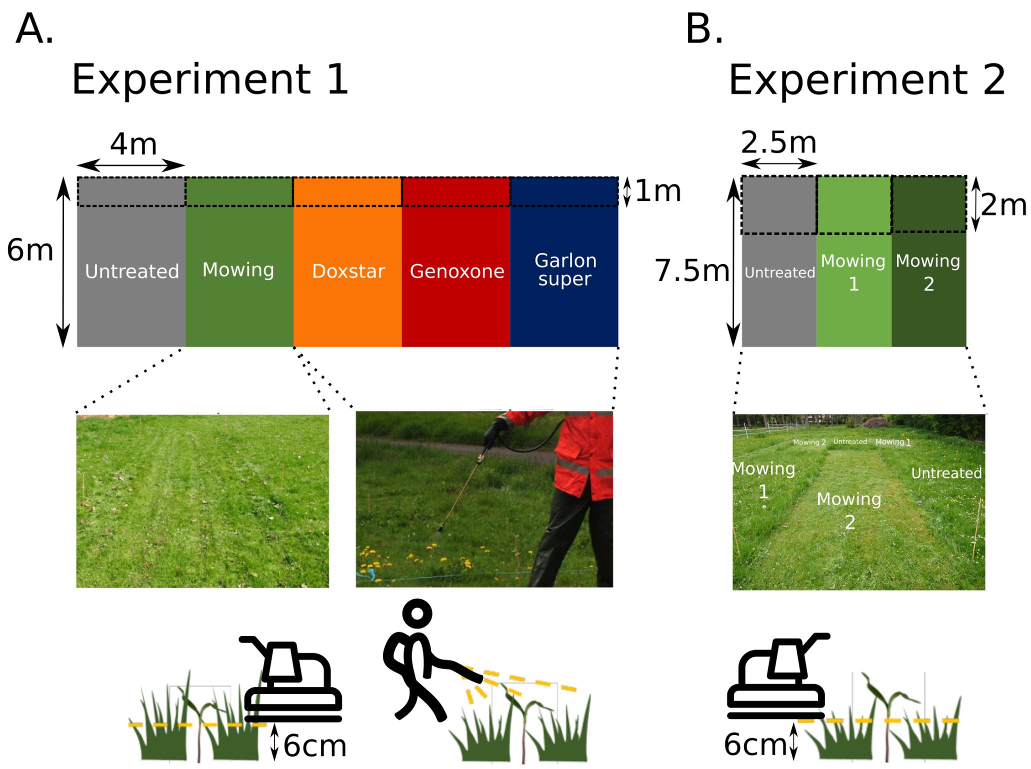

Figure 1. Illustration of a block in experiments 1 and 2. (**A**) Experiment 1—pasture 1: each block was subdivided in five lanes illustrated in grey (untreated area), in green (mowed area), in orange (Doxstar®), in red (Genoxone®) and in blue (Garlon Super®). Each lane measured 6 m height and 4 m width and within each lane, a zone was dedicated to counting of seedlings represented by a dotted zone; (**B**) Experiment 2—pasture 2: each block was subdivided in three lanes illustrated in grey (untreated, i.e., no mowing), in light green (mowing performed on 5 April 2019) and in dark green (mowing performed on 29 April 2019). Each lane measured 7.5 m height and 2.5 m width and within each lane, a zone was dedicated to counting of seedlings represented by a dotted zone. Pictures in real condition are displayed. In both experiments, the cutting height of the mowing machine was 6 cm.

2.1.2. Experiment 2—Pasture 2

In a second experiment, performed in another pasture, the influence of the mowing timing was investigated using a mowing machine with a cutting height of 6 cm at two different dates: 5 April 2019 and 29 April 2019, i.e., on short and tall grass, respectively. At the second mowing date, most seedlings had their first pair of leaves. At the first mowing date, the mowed material was left to decompose, whereas, at the second date, it has been removed from the pasture. An additional lane served as control. Inside each lane, counting of sycamore seedlings was performed within a 5 m^2 area. The soil condition was silty without rock with a medium drainage. The grassland was used for forage production in the first instance and then was pastured by horses.

2.2. Evaluated Parameters

2.2.1. Experiment 1—Pasture 1

The evaluated parameters were sycamore seedling counting, the concentration of HGA in sprayed seedlings, grass height and flora. Measurements were performed every week starting from 17 April (day 0) to 16 May (day 29). Flora was sampled a few hours before any intervention on 17 April and on 16 May.

2.2.2. Experiment 2—Pasture 2

The evaluated parameters were sycamore seedling counting, grass height, botanical composition, the final harvest biomass and its nutritional value. Measurements were performed on 5 April (day 0; mowing 1), on 29 April (day 24; mowing 2), and on 17 May (day 42). All measurements were performed before any eventual intervention at each point. In addition, final harvest biomass and its nutritional value were measured on 20 June (i.e., two days before harvesting).

2.3. Hypoglycin A Concentration

Between 25 to 30 sycamore seedlings were sampled in control and herbicidal treated lanes, except in cases where fewer of them remained. The entire shoots including the root were mechanically ground and mixed. Samples were aliquoted in 5 g and stored at −80 °C up to analysis. Aliquots were freeze-dried and weighed to calculate dry matter. Briefly, samples were mixed with 40 mL of pure methanol (VWR International, Leuven, Belgium). Then, samples were gently agitated for 24 h at 50 °C. The supernatant was removed and evaporated. The residue was dissolved in 3 mL of pure water and centrifuged at 4500 *g* for 5 min. High performance thin layer chromatography analysis (Merck, Darmstadt, Germany) was performed as previously described [16]. Results were normalized to dry matter obtained from each aliquot.

2.4. Botanical Composition of the Pasture

Botanical composition was characterized using a visual estimation method [24] and was performed by the same experienced person all along the experiments. Ten handfuls of grass were randomly and blindly sampled. For each sample, dominant species were ranked visually from one to three. Rare species were marked as "+". The data were merged to assess the proportion of cover in grass, legumes and forbs.

2.5. Grass Height, Final Harvest and Nutritional Value

Grass height was measured by taking ten measurements with a manual plate meter (Jenquip, New Zealand). The fresh mass was divided by the area harvested. Samples were sent to a laboratory (Centre de Michamps asbl, Bastogne, Belgium) to determine nutritional values by using near infrared spectrometry. The concentration of phosphorus and minerals (i.e., potassium, sodium, calcium and magnesium) were monitored using colorimetry and atomic absorption spectrometry, respectively. Final biomass yield was converted to dried mass/ha (DM/ha) using dry mass at 103 °C.

In order to evaluate the impact of treatment on the quality of the pasture, the nutritional value of forage fed to horse (UFC; kg DM^{-1}) was forecasted using three equations from works of Martin-Rosset [25–27]:

$$dMO = 67.98 + 0.07088 \times MAT - 0.000045 \times NDF - 0.12180 \times ADL \quad (1)$$

$$MOD = dMO * MO \quad (2)$$

$$UFC = -0.124 + 0.0003 \times GC + 0.0013 \times MOD \quad (3)$$

where:

dMO = enzymatic digestibility (%)
MAT = total nitrogenous matter (g/kg DM)
NDF = total cell walls (g/kg DM)
ADL = lignin (g/kg DM)
MOD = digestible organic matter (g/kg DM)
MO = organic matter (g/kg DM)
GC = cytoplasmic carbohydrates (g/kg DM)

Horse digestible nitrogenous matter (*MADC*; g/kg DM) was calculated by means of an equation for natural pastures:

$$MADC = -67.1 + 0.861 \times MAT + 0.105 \times CB \quad (4)$$

where:

MAT = total nitrogenous matter (g/kg DM)
CB = crude fiber (g/kg DM)

2.6. Statistical Analysis

Statistical analyses were performed using R (version 3.6.3, R Core Team, Vienna, Austria). Normality and heteroscedasticity of the data were tested using Shapiro–Wilk and Levene tests. Statistical significance was set to $p < 0.05$. For experiment 1, a Wilcoxon test with a Holm post-hoc test was performed to investigate sycamore seedlings/m². A Kruskal–Wallis test followed by a Dunn test were performed for legume cover before (day 0) and after (day 29) intervention. For experiment 2, a two-way ANOVA with a Tukey post-hoc test was performed to investigate sycamore seedlings/m². Other variables were investigated using a one-way ANOVA followed by a Tukey test.

3. Results

3.1. Experiment 1: Evaluation of Herbicide Spraying and Mowing

3.1.1. Eradication of Sycamore Seedlings

Seedlings naturally disappeared with time in untreated areas (Figure 2). Indeed, the number of seedlings decreased from 51 ± 8 to 11 ± 5 sycamore seedlings/m² after 29 days (Figure 2B). The same observation was made in the second experiment.

All treatments reduced the number of sycamore seedlings. Indeed, at the end of the experiment (i.e., after 29 days), less than 0.5 sycamore seedlings/m² were present in treated areas, while 10 ± 2 sycamore seedlings/m² ($p < 0.05$) were present in the untreated areas (Figure 2A). Mowing as well as Genoxone® significantly decreased the number of sycamore seedlings after seven days ($p < 0.05$) while a significant decrease was observed with Garlon Super® and Doxstar® after 15 and 29 days, respectively ($p < 0.05$) (Figure 2A). It is worth noting that, after seven days, the number of sycamore seedlings/m² was more reduced by mowing (mean = 1.5) in comparison to herbicides (mean = 14.83), given the fact that sprayed seedlings disappeared progressively by decomposing. Nevertheless, it should be remembered that the mowing material was left in place (i.e., the seedlings are no longer visible but are still in the mown grass).

3.1.2. Hypoglycin A level

Hypoglycin A was detected in all sprayed seedlings (i.e., with all herbicide spraying interventions), whatever their level of decomposition. Results are given as mean ± standard deviation. Seven days after the treatment, the concentrations of HGA were 605.6 ± 304.76 µg/g and 1084.5 ± 87.22 µg/g, in untreated seedlings and sprayed seedlings, respectively. Fourteen days after the treatments, these concentrations increased to 2424.2 ± 246.80 µg/g and 2305.6 ± 224.22 µg/g, respectively. Twenty-one days after the treatment, the concentrations of HGA were 1647.8 ± 90.64 µg/g and 4160 ± 2066.00 µg/g, respectively.

3.1.3. Grass Height

Grass height was not significantly different in any lane before mowing and herbicide spraying. Mowing and herbicides decreased the grass height on days 7, 15 and 22. However, after 29 days, grass height was not significantly different between the different interventions.

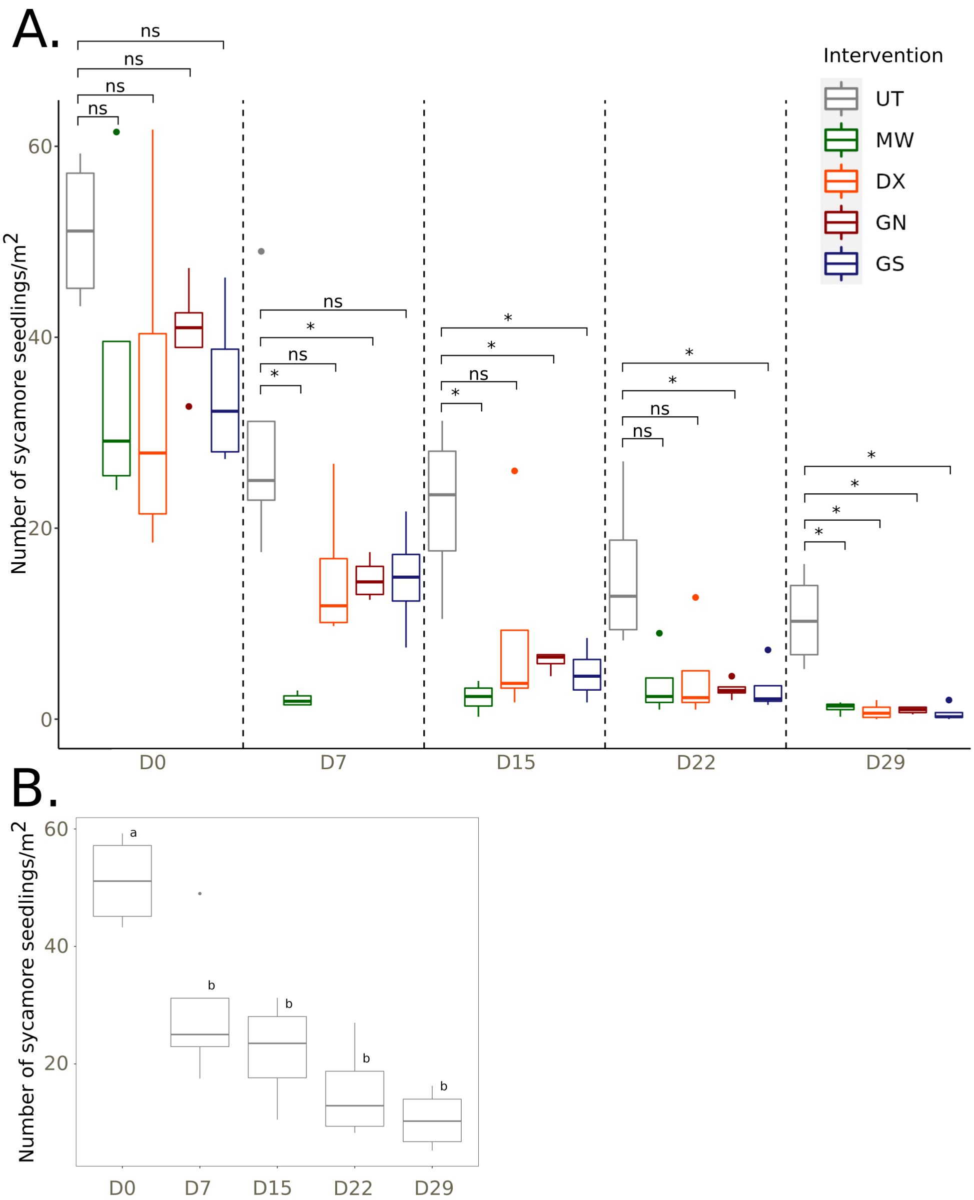

Figure 2. Results of experiment 1: mean number of sycamore seedlings before mowing and herbicide treatments (D0) and every week post intervention. (**A**) The intervention consisted of mowing (MW), Doxstar® (DX), Genoxone® (GN), Garlon Super® (GS), in green, orange, dark red and dark blue, respectively. Untreated condition (UT) is illustrated in grey. Outliers are represented with a dot. Dashed lines separate the different days of seedlings counting; (**B**) mean number of sycamore seedlings over time in the untreated area. Letters indicate results of Tukey test. ns: non-significant; *: $p < 0.05$.

3.1.4. Botanical Composition of the Pasture

Pasture cover did not significantly differ in any lane before mowing or herbicides spraying (Figure 3A). Four weeks after herbicide spraying, the pasture cover was significantly changed (Figure 3B). Indeed, legumes and forbs cover was reduced to 8.9 ± 6.1% compared to 39.4 ± 14.9% in the untreated area ($p < 0.001$; Figure 3B.). In fact, the legume cover was significantly reduced by herbicide spraying from 12.3 ± 8.5% to 0.1 ± 0.2% in comparison with the untreated area ($p < 0.01$). In the same manner, forbs was decreased by Genoxone® and Garlon Super® to 1.6 ± 2.4% and 12.3 ± 9.2%, respectively, in comparison to 27.1 ± 8.8% in the untreated area. However, in this counting, no significant change was observed with Doxstar®. As a result of these changes, grass cover was significantly higher in herbicide spraying (91.1 ± 6.1%) compared to the untreated area (60.6 ± 14.9%; $p < 0.001$; Table S1). By contrast, none of these changes were observed with mowing.

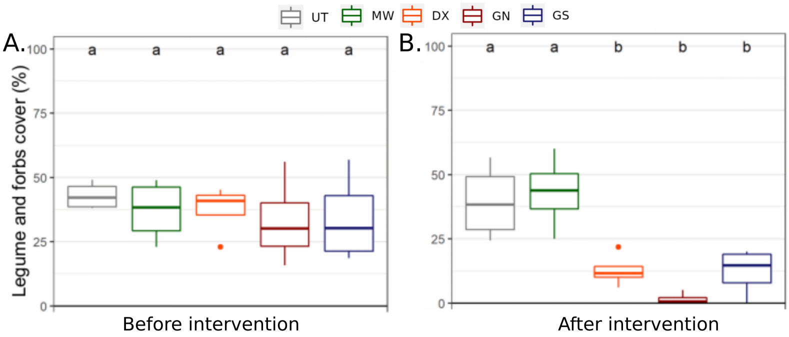

Figure 3. Results of experiment 1: botanical composition before (**A**) and after (**B**) four weeks of mowing or herbicide spraying: untreated (UT), mowing (MW), Doxstar® (DX), Genoxone® (GN) and Garlon Super® (GS). Letters indicate results of Tukey test (after intervention $p < 0.001$). Outliers are represented with a dot.

3.2. Experiment 2: Evaluation of Different Mowing Dates

3.2.1. Eradication of Sycamore Seedlings

As observed in experiment 1, seedlings naturally disappear in the untreated area (Figure 4). However, after 24 and 42 days, no significant difference in sycamore seedling/m² was observed between the three conditions (Figure 4). It should be noted that mowing 2 (done on the 29 April 2019) was conducted when the number of seedlings was already low due to the significant natural decline of seedlings. As a consequence, after 42 days, almost no sycamore seedlings where found in mowed areas as opposed to the untreated area where a few were found (Figure 4).

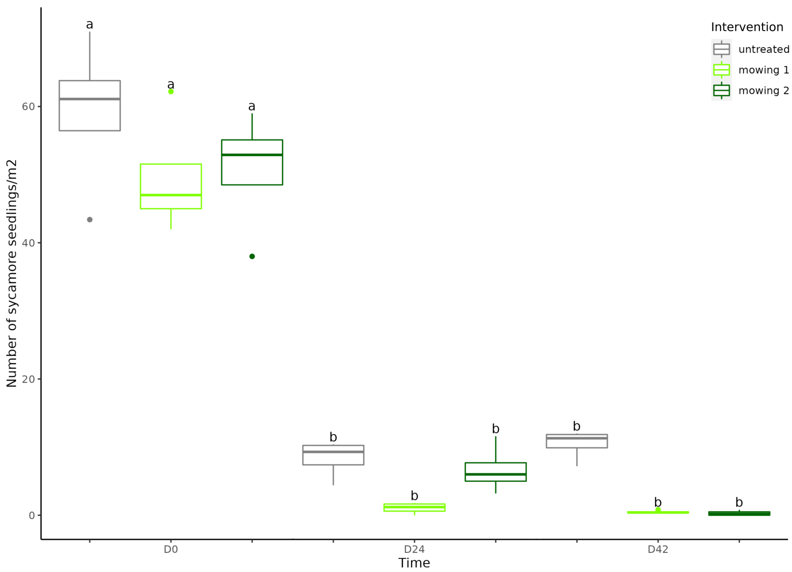

Figure 4. Results of experiment 2: mean number of sycamore seedlings before any intervention (D0, i.e., 5 April 2019) and 24 (D24, i.e., 29 April 2019) or 42 (D42, i.e., the 20 June 2019) days later. Untreated condition, mowing 1 (done on 5 April 2019) and mowing 2 (done on 29 April 2019) are represented in grey, light and dark green, respectively. Letters indicate significantly different results of the two-way ANOVA with a post-hoc Tukey test with $p < 0.05$. Outliers are represented with a dot.

3.2.2. Grass Height

Results are displayed using mean ± standard deviation. Grass height was not significantly different between lanes at the start of the experiment. On day 24, measurements were taken before applying mowing 2 and grass was therefore significantly taller (10.6 ± 1.7 cm) than in the mowing 1 lane (7.5 ± 0.3 cm; $p < 0.05$) but with no significant differences with the untreated condition (9.2 ± 1.5 cm). On day 42, grass was significantly shorter in mowing 2 (6.2 ± 0.1 cm) compared to mowing 1 and the untreated condition (11.8 ± 1.0 cm and 13.5 ± 2.6 cm, respectively; $p < 0.001$).

3.2.3. Botanical Composition of the Pasture

Before harvesting (i.e., the 20th of June), legume cover was significantly increased by mowing 1 (10.2 ± 2.5%) compared to other conditions (4.6 ± 1.9%; $p < 0.01$; mean of mowing 1 and 2, Figure 5). However, no significant change in grass and forbs cover was detected.

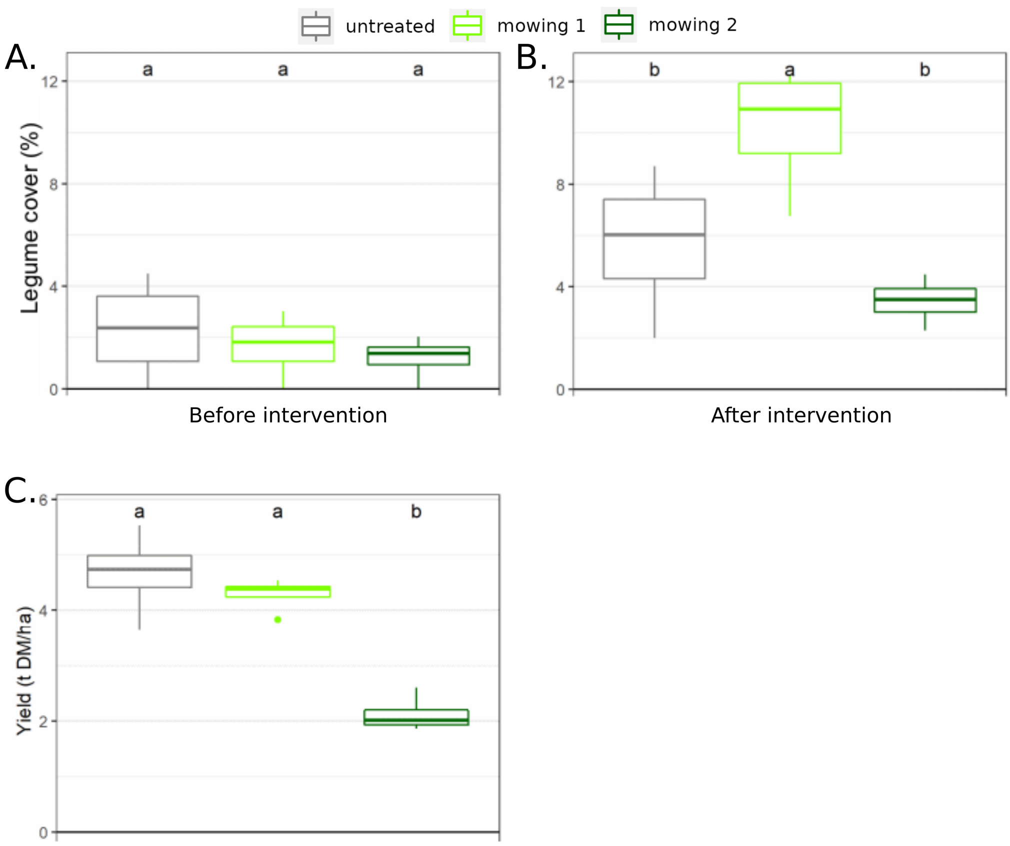

Figure 5. Experiment 2: legume cover before (**A**) and after (**B**) different mowing dates which was significantly different for mowing 1 ($p < 0.01$). (**C**) Harvest yield of the pasture for the different mowing dates which was significantly lower in the mowing 2 condition ($p < 0.001$). Letters indicate results of the Tukey test.

3.2.4. Harvest

A significantly smaller harvest was obtained with mowing 2 (2.1 ± 0.3 t DM/ha) in comparison to the untreated condition (4.7 ± 0.8 t DM/ha; $p < 0.001$; Figure 5C). By contrast, the harvest was not affected in mowing 1 (4.3 ± 0.3 t DM/ha) as the grass was smaller than the cutting height of the mowing machine.

3.2.5. Nutritional Value

No significant difference in energy (i.e., UFC) among conditions was observed. Horse digestible nitrogenous matter was significantly higher in mowing 2 (52.6 ± 10.6 g MADC/kg

DM; $p < 0.05$) compared to the untreated condition and mowing 1 (35.7 ± 5.9 and 34.5 ± 4.4 g MADC/kg DM, respectively). As a result, the MADC/UFC ratio was significantly higher ($p < 0.01$) for mowing 2 (75.0 ± 13.9 g MADC/UFC) in comparison to the untreated condition and mowing 1 (50.1 ± 7.9 and 48.8 ± 5.9 g MADC/UFC), respectively. With regard to minerals, phosphorus was significantly higher in mowing 2 (3.3 ± 0.3 mg P/kg DM) compared to other treatments (2.7 ± 0.2 mg P/kg DM; $p < 0.05$). No significant differences were observed between treatments for potassium, sodium, calcium and magnesium.

4. Discussion

The effect of several interventions (i.e., three different herbicides and mowing) on the reduction of the toxic pressure induced by sycamore seedlings in pastures and their ecological footprint (e.g., botanical composition, nutritional values, harvesting yield, etc.) has been investigated in this longitudinal study. To do so, herbicide spraying and mowing were studied into two pastures containing sycamore seedlings in spring 2019 in Belgium. Case of AM reached a record in Belgium in autumn 2018 with 191 reported cases [11]. Occurrence of autumnal cases are, among others, linked to sycamore seeds production. In the same way, spring cases are met following large autumnal outbreaks with the germination of the samaras. As a consequence, 49 AM cases were reported in spring 2019 due to seedlings present in a sufficient amount to intoxicate horses but also to perform this study [11]. Although the conditions were specific to this study (e.g., location in Belgium, climate, meteorological condition, soil compositions, etc.), some fundamental principles might be extrapolated from this study to help horse owners and farmers in pasture management.

The major finding was the natural disappearance of sycamore seedlings (Figure 2B). Indeed, after three to four weeks, 78–86% of sycamore seedlings had spontaneously disappeared. This natural decay of sycamore seedlings has already been observed in temperate forest between April and October 2017 [28]. However, in the above-mentioned study, the seedlings mortality was about 60% [28]. The higher mortality rate observed in our study could be explained by the dense vegetation of grassland which may be more competitive than the vegetation on the woodland floor, even if pasture may benefit from better light access in comparison with temperate forest. The rate of natural disappearance of seedlings might be specific to pasture characteristics as some of them have been identified as risk factors for AM (e.g., quality of the grassland, the slope and the humidity of the pastures, etc.; [11,29]). This natural decay is not enough to prevent spring outbreaks. *Acer pseudoplatanus* is a medio-European with sub-Atlantic tendency tree, naturally distributed in Austria, Belgium, Czech republic, Denmark, France, Germany and Italy [30–32]. This species has a high reproductive capacity and a wide ecological amplitude [33] explaining its expansion to some European areas (e.g., Sweden, Norway) [30,33]. In addition, their concentration is increasing in some areas of Europe such as England [34]. In view of the invasive nature of the sycamore maple, its area of distribution and climatic change, it cannot be excluded that, in the coming years, other herbivores (e.g., cattle) grazing in the vicinity of this tree may be at risk of poisoning [35]. Very recently, maple poisoning has been observed in zoo-kept species [36–39] suggesting that domestic ruminants might also be at risk.

Owners of horses often ask how AM cases can be prevented at the pasture level [11]. To address this demand from the field, mowing and three commonly used herbicides (i.e., Doxstar®, Genoxone®, Garlon Super®) were evaluated regarding their ability to reduce the toxic pressure (i.e., to eliminate/kill seedlings) in spring. The mowing treatment aimed to kill the sycamore seedlings by cutting off the cotyledons, thus depriving the plants of photosynthesis. Herbicides based on triclopyr aimed to kill the sycamore seedlings by mimicking a specific plant growth hormone called auxin [40] causing uncontrolled plant growth and plant death [41]. These herbicides were sprayed with a portable hand pressure pump spray. Both methods of intervention are compatible with large areas to be processed. All tested methods reduced significantly the number of sycamore seedlings in comparison with the untreated condition (Figure 2A) but *in fine*, only a few seedlings remained in the control lanes. However, herbicide spraying favoured the disappearance of all legumes and most forbs (Figure 3). This disappearance of legumes and forbs left gaps in the pasture cover. These gaps might be colonized by weeds. To reduce the impact of this effect, an oversowing of grasses and legumes suitable for grazing can be used [42]. By contrast, mowing did not impact the legume and forbs percentage cover. Thus, mowing had a lower footprint in terms of pasture flora composition in comparison with herbicide spraying.

Apart from the counting of sycamore seedlings, the concentration of HGA in sprayed seedlings was monitored in this study. However, the presence of MCPrG, another toxin in AM [12] was not evaluated in this study. Even though a strong relationship between MCPrG and HGA was observed in the blood of AM horses exposed to sycamore maple [12], it could be interesting to develop the dosage of MCPrG in plant extract [43,44]. In addition, HGA could not be measured in mowed seedlings due to the difficulty to sample the remaining seedling stems among grass stems. Nevertheless, sprayed seedlings did contain HGA even after 21 days of treatment. Moreover, HGA concentration in sprayed seedlings increased with time. Similar results were observed by Gonzalez-Medina and collaborators [22]. In addition, in their study, HGA was also present in grass surrounding the sycamore seedlings [22]. Therefore, sprayed seedlings as well as the neighbouring grass might remain toxic until the full decomposition of the seedlings. By contrast, mowing enables the removal of the mowed seedling and grass. As mowed seedlings still contain HGA [22], the mow from the pasture should be removed whenever possible, depending on the pasture configuration. Furthermore, it is necessary to wait at least two weeks for the full decomposition of the seedling stems. Of course, this period may vary according to climatic conditions.

To go further, the influence of two mowing moments was investigated in the second experiment. Mowing 1 was performed in the beginning of April (i.e., on the 5th) while mowing 2 occurred belatedly at the end of April (i.e., on the 29th). In experiment 2, it was found that the mowing of seedlings was impeded by short grass due to the airflow of the machine leading to the inclination of the seedlings and their escaping of the blade. By contrast, in taller grass, the seedlings were trapped in the grass facilitating the mowing. Thus, when the grass is short, it might be necessary to reduce the cutting height of the mowing and/or to go back and forth several times. The number of reported cases in spring in Europe follows a Gaussian curve from 1 March until 31 May [11]. With regard to this Gaussian distribution, mowing 1 was applied at the beginning of the dramatic increase while mowing 2 was performed close to the median when the curve starts to decrease. At these moments, the height of the grass was 4.8 ± 0.5 cm and 10.6 ± 1.7 cm before mowing 1 and 2, respectively. The later mowing induced a loss of yield of 2.5 ± 1.0 t DM/ha (mean $\pm$ sd) (Figure 5C), given the fact that a significant height of grass was removed. In comparison with the untreated condition and mowing 2, mowing 1 slightly favoured legumes (Figure 5A). The growth of legumes depends upon the light access and the heat. Therefore, this result may vary with climate, which differs according to years and location. However, the harvest of mowing 2 presented a better MADC and a better balance of MADC/UFC with regard to recommendations [45]. Indeed, recommendations are 50 g MADC/kg DM and 60–70 g MADC/UFC for horses with regular activity or not physically active and 78 g MADC/UFC for young horses with regular activity [45]. This higher value of MADC can be caused by the presence of younger grass [46], as this intervention did not induce higher legume cover (Figure 5A). Thus, mowing should be applied as soon as the seedlings are taller than the minimal cutting height of the mowing machine during the risky season [11]. By doing so, the impact on the harvest will be minimized. By contrast, the quality of the harvest (i.e., MADC/UFC) will be impacted by sooner mowing.

5. Conclusions

Herbicides and mowing have reduced the number of seedlings, which nevertheless disappear naturally over time without intervention. Herbicide spraying may not be recommended as these treatments impact the composition of the pasture flora. In addition, as the sprayed seedlings remain toxic until full decomposition, there is no time saving compared to the natural death of the seedlings. Mowing strategies have reduced the number of sycamore seedlings more rapidly than herbicide spraying and did not affect the pasture flora. In addition, early mowing (i.e., beginning of April) did not affect the harvest yield. Late mowing (i.e., end of April) has reduced the harvest yield, but the nutritional value of this harvest was more adapted for horses. In conclusion, mowing is the best strategy to reduce the risk of *Acer pseudoplatanus* poisoning in grazing equids while preserving ecological sustainability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land11081345/s1, Table S1: List of the five species with highest cover in experiments 1 and 2, before (D0-D24) and after (D42 or D29) treatments; Table S2: Number of sycamore seedlings in experiment 1; Table S3: Grass height in experiment 1; Table S4: Concentration of hypoglycin A in sycamore seedlings in experiment 1; Table S5: Number of sycamore seedlings in experiment 2; Table S6: Grass height in experiment 2; Table S7: Harvest yield in experiment 2; Table S8: Nutritional values of the harvest from experiment 2.

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Abbreviations

The following abbreviations are used in this manuscript:

ADL	Lignin
AM	Atypical myopathy
CB	Crude fibre
DM	Dried mass
dMO	Enzymatic digestibility
GC	Cytoplasmic carbohydrates
HGA	Hypoglycin A
MADC	Horse digestible nitrogenous matter
MAT	Total nitrogenous matter
MCPrG	Methylenecyclopropylglycine
MO	Organic matter
MOD	Digestible organic matter
NDF	Total cell walls
UFC	Forage fed to horse

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