Abstract: Utilizing innovative agricultural practices that enhance the nutritional quality of staple foods such as potatoes provides farmers with tools to successfully meet the challenges of feeding a rising global population while sustaining organic food production. In the present study, we have demonstrated the potential of white mustard (Sinapis alba) seed meal extract to improve potato nutritional properties. Sinapis alba extract is a low-cost by-product of mustard oil extraction that contains a relatively high concentration of biologically active compounds. When applied to soil, S. alba extract had a positive impact on nutritional quality of potatoes. For example, total phenolic content in potatoes treated with S. alba extract increased by ~1.5 times, and potato nitrogen content increased from 1.52% to 1.73% with one application of S. alba extract. At the same time, application of S. alba extract had limited impact on the accumulation of anti-nutrients such as glycoalkaloids in potato tubers. The ability to boost the phenolics content of potatoes by applying an organic amendment is a valuable tool in organic farming as it creates more nutritional crop. To the best of our knowledge, this is the first study to examine the effect of S. alba extract on the nutritional quality of potatoes, or indeed of any food crop.

Keywords: Sinapis alba; potato quality; mustard biopesticide; organic agriculture

1. Introduction

Potatoes represent the third largest carbohydrate food source in the world, and they contribute key nutrients, antioxidants, and fibers to human diet [1]. Organic potatoes account for approximately 4.2% of the total potato market [2]. While there is a strong public demand for organic produce, there are many challenges associated with organic potato production. For example, one of these challenges is assessing the nitrogen sources for meeting potato plant nutrient requirements [2].

Seed meal of Brassicaceae crops can contain up to 5% nitrogen and thus can serve as an organic source of nitrogen in agricultural production systems [3,4]. For example, seed meal of white mustard (Sinapis alba) is a low cost by-product of mustard oil extraction, that is both affordable and easily accessible [5,6]. Mustard seed meal can be further extracted to obtain a concentrated product that can serve as a soil amendment to fulfill nitrogen requirements and provide a benefit of improving soil health [7–9]. In addition, concentrated white mustard (Sinapis alba) seed meal extract contains consistently high concentrations of the biologically active compounds that can potentially improve crop nutritional properties [8,10–12]. While the antioxidant activity of mustard meal is attributed to phenolic compounds, such as sinapine and benzoic and cinnamic acid derivatives, little is known about the effect of mustard meal extract on potato nutritional value [13–16].

Thus, the objective of the presented study was to evaluate the effect of S. alba extract on (1) the nutritional quality of potatoes; (2) phenolic content in potato tubers; and (3) accumulation of anti-nutrients such as glycoalkaloids. To accomplish this, we conducted a trial in a certified organic farm to reflect the typical organic production practice. To
the best of our knowledge, this is the first report studying the effect of *S. alba* seed meal extract on nutritional quality of organic potatoes. In addition to human health benefits, using *S. alba* extract in organic potato production can improve product marketability due to the zero-waste technology used for *S. alba* extract production and by focusing on sustainability, recovery, and reuse, and multiple high-value products [17–19]. Mustard itself is also a great rotational and cover crop that can be used to improve soil health and reduce pest pressure, which makes mustard production an environmentally sustainable soil amendment option [20,21].

2. Materials and Methods

2.1. Plant Materials

Mustard seed meal extract (MSME) was prepared using the procedure described previously from Organic Materials Review Institute (OMRI) certified *S. alba* (IdaGold variety) seed meal (Farm Fuel Inc., Watsonville, CA, USA) [7]. Briefly, cold press mustard meal was extracted with water at room temperature. Mustard meal sludge was pressed through the decanter centrifuge to remove most of the debris. Clarified extract was filtered through 100 µm filter and freeze dried. Certified Organic Yukon Gold seed potatoes were purchased from New Sprout Farms (Asheville, NC, USA).

2.2. *S. alba* Mustard Meal Extract Phytotoxicity Assay

Potato toxicity assays were conducted in 5 Ga (506.7 cm² surface area) pots that were filled with moist OMRI soil, and one seed potato was planted 5 inches deep in each pot. The pots were organized in a random block design with five replicates per treatment. Following treatments were used: *S. alba* extract applied (1) on the same day as planting, (2) 2 weeks after planting, (3) 4 weeks after planting, (4) or left untreated to act as a control. An application rate of 450 g/m² of *S. alba* extract was used for each of the aforementioned experimental groups by sprinkling the *S. alba* extract powder evenly over the surface of the soil and watering to incorporate it. Pots were kept in a greenhouse with a 14.5-h day length and maintained at 26/16 °C maximum/minimum temperatures. Soil was maintained moist by watering every other day using a lightly sprinkling hose attachment. Each week, percent damage was recorded for the potato plants in each pot. Percent damage was expressed based on total aboveground biomass conditions with 0% = unaffected and healthy-looking plant and 100% = completely dead plant. Damage was assigned the following ranges based on a visual observation: 1–30% minor burning of leaves and stems, 30–60% significant damage, and 60–90% major damage with extended necrosis of leaves and stems. When the potatoes were ready for harvest, fresh mass of potato yield was measured for each pot and averaged for each experimental group. A cross section of each tuber was manually measured.

2.3. Field Trials

The potato field trials were conducted on Soil Stewards Organic farm (Moscow, ID, USA), a certified organic farm in Northern Idaho. Soil Stewards farm has silty clay loam Mollisolls from two soil series: Latahco (Argiaquic Xeric Argicalbolls) and Thatune (Oxyaquic Argixerolls). Moscow, ID receives an average of 69 cm of rain and 124 cm of snow annually and has an annual average temperature of 8.3° (U.S. Climate Data, 2018). Twenty plots (90 × 60 cm²) were randomly assigned in one long row with a 30 × 60 cm² buffer zone. Within each experimental plot, four seed potatoes were planted. The potato row was watered via drip irrigation at a rate of 2.5 cm/week. Three weeks after planting, *S. alba* extract (4.5 t ha⁻¹) was applied once (1×), twice with a two-week interval (2×), or three times with a two-week interval (3×). Application was performed by top applying the dry *S. alba* extract on the surface of soil and irrigating afterwards. For the second and third application, when the shoots already appeared above the ground, *S. alba* extract was applied around the plant with 5–7 cm distance to avoid any potential plant damage. Untreated control received no *S. alba* extract.
2.4. Potato Chemical Analysis

Potato tubers were harvested, weighed to determine the yield, and visually evaluated for quality appearance. A cross section of each tuber was manually measured. Potato tubers were then freeze-dried. Dry tissues were pulverized using cyclone mill (UDY Corporation, Fort Collins, CO, USA) and kept at −20 °C until extraction and analysis. Standard plant analysis for macro and micronutrients was performed by Ward Laboratories, Inc., (Kearney, NE, USA). The total starch content of the potatoes was measured using a Megazyme® Total Starch Assay Kit. Corresponding antioxidant activity of potato extracts was assessed using a Folin-Ciocalteu assay [22].

2.5. Phenolics and Glycoalkaloids Analysis

Freeze-dried tuber tissues (1 g) were homogenized with 20 mL of methanol in Omni Prep homogenizer (Omni Int, Kennesaw, GA, USA) at 1500 rpm for 10 min. The suspension was centrifuged at 4000 × g rpm and 18 °C for 10 min and supernatant was collected. The extraction was repeated two more times. All supernatants were combined, evaporated until dry using a rotary evaporator, and reconstituted in 1 mL of methanol. Phenolics and antinutrient contents were evaluated as described previously using HPLC/MS method describe below [23,24]. Analysis of glycoalkaloids and phenolics were performed using an Agilent 1200 Series HPLC coupled to an Agilent G6230 ESI TOF MS (Agilent, Santa Clara, CA, USA). The chromatographic separation of glycoalkaloids was performed on an Extend-C18 3.5 µm, 2.1 × 100 mm (Agilent Technologies Inc., Santa Clara, CA, USA) reversed phase chromatographic column. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient program started with isocratic elution using 0% B for 1 min, followed by a linear gradient to 35% B from 2 to 11 min, followed by a linear gradient to 65% in 4 min, then organic solvent was increased to 100% B in 2 min, kept at 100% for 1 min, and re-equilibrated back to the initial mobile phase composition in 5 min. Column was maintained at 30 °C. The injection volume was 5 µL. The flow rate was 0.4 mL/min.

Electrospray ionization was operated in the positive and negative modes with absolute value for electrospray ionization potential at 3500 V. Collision-induced dissociation potential was set at 150 and 250 V to analyze spectra for molecular ion and fragmentation pattern, respectively. Gas temperature was 350 °C, drying gas (N₂) flow rate was 10 l/min, and nebulizer pressure was 2.4 × 10⁵ Pa. The analyses were conducted in a centroid mode within an m/z range from 100 to 1700 amu. Quantification of total glycoalkaloid concentration was done based on the external calibration curve constructed based on the pseudomolecular ion (Table 1). In the absences of analytical standards, glycoalkaloids were tentatively identified based on the literature data and in silico using open-source databases [24–26].

| Retention Time, min | Glycoalkaloid | Pseudomolecular Ion |
|---------------------|--------------|---------------------|
| 12.709              | Solanidatetraenol isomer | 862.446 |
| 12.865              | Solanidadienol | 866.525 |
| 12.980              | α-Chaconine isomer | 852.509 |
| 13.047              | Solanidenol | 884.487 |
| 13.059              | Dehydrochaconine isomer | 850.485 |
| 13.200              | Solanidatetraenol | 862.446 |
| 13.320              | Solanidenediol | 884.487 |
| 13.470              | Solanidatetraenol isomer | 862.446 |
| 13.592              | Solanidine | 1030.557 |
| 13.820              | Dehydrochaconine isomer | 850.485 |
| 13.854              | Solanidenetriol | 916.480 |
Table 1. Cont.

| Retention Time, min | Glycoalkaloid          | Pseudomolecular Ion |
|---------------------|------------------------|---------------------|
| 13.865              | Solanidadienol isomer  | 866.525             |
| 13.899              | α-Solanine             | 868.501             |
| 14.128              | α-Chaconine            | 852.509             |
| 14.763              | Solanidadienol isomer  | 866.525             |
| 14.790              | Leptinine II           | 884.487             |
| 15.616              | Leptine II             | 926.503             |

2.6. Data Analysis

Data were analyzed by analysis of variance (ANOVA) using JASP (University of Amsterdam, Amsterdam, The Netherlands), a graphical open-source software package for basic statistical procedures [27]. Pairwise comparisons were performed using Student’s t-test to assess the treatment differences and means were considered significantly different at $p \leq 0.05$.

3. Results

3.1. Plant Damage

Plants that were treated with *S. alba* extract at the same day of planting (0 days) exhibited minimum degree of stress and recovered shortly after (Figure 1). Plants that were applied with *S. alba* extract 14 days after planting were the most susceptible for damage as reflected by leaf wilt and yellowing. For example, one week after *S. alba* extract application, 24% of plant leaf surface was affected and the affected area has increased to 62% over the next seven days. Plants that were treated with *S. alba* extract 28 days after planting had significant damage with 35–54% of leaf area affected. While visible damage persisted for several days after *S. alba* extract application for potatoes treated 14 and 28 days after planting, plants did recover 14–21 days after.

![Figure 1. Damage in potatoes treated with *S. alba* extract at the time planting (0 days), 14 and 28 days after planting. Plant damage was calculated as a percentage of discoloured leaf area relative to the total area.](image)

3.2. Plant Nutrients

Potato tubers grown in *S. alba* extract applied plots were analyzed for essential nutrient content (Table 2). Potato starch content was not significantly affected by *S. alba* extract application and ranged from 52 to 58 g/100 g of potatoes on dry weight basis (Table 2). Similarly, no changes were observed for phosphorous content in potatoes treated with
S. alba extract with the average phosphorous content of 0.26%. Potato nitrogen content increased from 1.52% to 1.73% with one application of S. alba extract. However, no changes were observed with the additional applications of S. alba extract. Potassium, another critical plant nutrient, increased from 2.27 to 2.37% in potatoes treated with S. alba extract with only 2× application being statistically higher than the control treatment. Sulfur content significantly increased in potato tubers after S. alba extract application. One application of S. alba extract increased the concentration of sulfur by 3%, with each subsequent application bringing an additional 3% increase. For calcium and magnesium, one S. alba extract application resulted in a 13% and 2% increase, respectively. Concentrations of trace minerals (zinc, iron, manganese, copper, boron, and molybdenum) were not significantly altered by the addition of S. alba extract to potato plots.

Table 2. Selected nutritional qualities of potatoes treated with one, two, and three repeated application of S. alba extract under field conditions. Values ± standard errors are the average of five replicates. Values within the same row followed by a common letter are not significantly different (p ≤ 0.05).

| Antinutrients conc., mg/g | Control | 1× S. alba Extract | 2× S. alba Extract | 3× S. alba Extract |
|---------------------------|---------|--------------------|--------------------|--------------------|
| α-Solanine                | 0.6 ± 0.1 | 1.6 ± 0.3         | 2.6 ± 0.8         | 3.3 ± 0.9          |
| α-Chaconine               | 2.7 ± 0.4 | 4.1 ± 0.7         | 4.1 ± 0.5         | 3.6 ± 2.0          |
| Phenolics content, mg/g   | 0.6 ± 0.1 | 0.6 ± 0.1         | 0.6 ± 0.1         | 0.6 ± 0.1          |
| Total phenolics           | 0.6 ± 0.1 | 1.6 ± 0.3         | 2.6 ± 0.8         | 3.3 ± 0.9          |
| Caffeic acid              | 2.7 ± 0.4 | 4.1 ± 0.7         | 4.1 ± 0.5         | 3.6 ± 2.0          |
| Magnesium                 | 0.136 ± 0.01 | 0.138 ± 0.008   | 0.143 ± 0.003     | 0.144 ± 0.009     |
| Trace mineral, ppm        | Zinc     | 20.9 ± 3.0        | 21.0 ± 1.2        | 22.4 ± 1.8        | 24.0 ± 5.7        |
|                           | Iron     | 271 ± 122         | 231 ± 93          | 319 ± 106         | 299 ± 135         |
|                           | Manganese| 10.8 ± 1.6        | 10.0 ± 1.4        | 11.8 ± 1.6        | 11.8 ± 2.9        |
|                           | Copper   | 8.8 ± 0.9         | 8.4 ± 0.4         | 8.5 ± 0.8         | 8.5 ± 0.9         |
|                           | Boron    | 7.7 ± 0.6         | 7.2 ± 0.5         | 7.2 ± 0.5         | 7.2 ± 0.4         |
|                           | Molybdenum| 0.57 ± 0.20       | 0.67 ± 0.12       | 0.54 ± 0.09       | 0.43 ± 0.16       |

3.3. Potato Phenolics

Total phenolic content for control treatment with no S. alba extract application was 0.6 mg/g based on gallic acid equivalent (Table 2). After one application of S. alba extract, total phenolics content accounted for 1.6 mg/g. Each consequent application of S. alba extract resulted in the steady increase of phenolics content to 2.6 and 3.3 mg/g, respectively. Caffeic acid was identified as one of the major contributors to the overall phenolic content with the concentration increasing up to 1.6 times after S. alba extract application from 2.7 to 3.6–4.1 mg/g with no statistical difference between one, two, and three applications.

3.4. Potato Glycoalkaloids

Concentration of α-solanine, one of the major glycoalkaloids in potatoes, was 0.78–1.15 mg/g on dry weight basis when treated with S. alba extract with no statistical difference between one, two, and three applications. These values are not statistically different from α-solanine concentrations (0.80 mg/g) in potatoes from control plots. Concentrations of α-chaconine, another major glycoalkaloid, were 2.52–4.1 mg/g in potatoes treated with S. alba extract and 3.10 mg/g in potatoes not treated with S. alba extract. In addition to these two major glycoalkaloids, 15 other compounds assigned as glycoalkaloids were detected in all potato samples (Table 1). Due to the lack of analytical standards, exact concentrations of each compound in potatoes were not quantified. However, amounts of the glycoalkaloids
were expressed on relative amount basis to reflect the changes in their distribution under different treatments (Figure 2). Out of 15 additional glycoalkaloids, only five glycoalkaloids (α-chaconine isomer, dehydrochaconine, solanidadienol, solanidenediol, and leptinine) were sensitive to *S. alba* extract treatment (Figure 2). For example, α-chaconine isomer amounts in *S. alba* extract treated potatoes were higher as compared to non-treated control. Dehydrochaconine content, on the other hand, did decrease with the increase of *S. alba* extract application. Similarly, the relative amounts of solanidadienol and leptinine II were lower after repeated application of *S. alba* extract.

![Figure 2. Amounts of six glycoalkaloids that were sensitive to *S. alba* extract treatment. Values ± standard errors are the average of five replicates. Values within the same series followed by a common letter are not significantly different (p ≤ 0.05).](image)

### 4. Discussion

#### 4.1. Plant Damage

Along with biologically active compounds, such as sinapine and sinapic acid, *S. alba* extract contains ionic thiocyanate, a compound that has been shown to exhibit toxicity toward a range of plants by inhibiting germination and stunting plant growth [28,29]. Ionic thiocyanate is freely soluble in water and can be taken up by plant roots. As a result, the extent of ionic thiocyanate’s effect on a plant is determined by the compound concentrations in soil and the ability of plant to uptake it from soil pore water. The timing of *S. alba* mustard extract application is one of the factors that defines the ionic thiocyanate concentration in soils for plant uptake. For example, it was shown that two weeks after application, the concentration of ionic thiocyanate declines twofold in the top 5 cm of soil [30]. However, a corresponding increase is observed in deeper soil layer. Depending on the root system of the specific crop, the uptake of ionic thiocyanate will be highest when the migration of ionic thiocyanate within the soil profile coincides with the active root uptake zone. For potatoes, the depth of the rootzone depends on the seed planting depth, but generally falls in 10–25 cm [31]. This is consistent with the obtained results for potato that were applied with *S. alba* extract two weeks after planting and were the most susceptible for plant damage (Figure 1). At the same time, application of *S. alba* extract...
during the planting (0 days) inflicted minimum degree of stress on plants, presumably due to the misalignment between the ionic thiocyanate translocation in soils and the active uptake rootzone of potatoes. On the opposite side, delaying application of *S. alba* extract to four weeks after planting did inflict significant damage to plants. However, the timing of damage coincided with the potato senescence and tuber bulking, and it could not be differentiated purely from the observation. While visible damage persisted for several days after *S. alba* extract application for plants treated two and four weeks after planting, plants did recover 14–21 days after. However, the plant stress induced did result in significantly lower potato yields (Supplemental Material Table S1). For example, the yields for potatoes treated with *S. alba* extract at the same day as planting and four weeks after planting were 3.6 times lower than non-treated pots. While the number of potato tubers was not significantly different among the treatments, the size of potatoes treated with *S. alba* extract was 2.5–3 times lower compared to the control. Thus, based on the greenhouse data, the field application of *S. alba* two weeks after planting minimizes the effects of ionic thiocyanate on potato plants (Figure 1). However, the plant yields were still significantly affected by multiple *S. alba* extract applications with both the number and average size of potatoes being lower than for non-treated control. Previously, it was also reported that the use of mustard in potato did not improve total tuber yields or marketable yields [32].

### 4.2. Plant Nutrients

Several plant nutrients were monitored in potatoes treated with *S. alba* extract (Table 2). While starch content of potatoes was not affected, application of *S. alba* extract to soil resulted in the increase of minerals such as nitrogen, potassium, sulfur, and calcium contents in potatoes (Table 2). Nitrogen content was not significantly different when the application of *S. alba* was increased from one application to two or three applications during the growing season. While *S. alba* has relatively high nitrogen content (2.1% by weight), the uptake of nitrogen from soil is limited to the plant available form of nitrogen. Thus, while the absolute nitrogen concentrations were higher with the repeated application rates, they were not proportional to the increase in the nitrogen input through *S. alba* application. Phosphorous concentrations in tubers were not affected by the application of *S. alba* extract. While both organic and inorganic phosphorous in soil increased significantly after *S. alba* application, the translocation of phosphorous into tubers was not observed [9].

While the concentrations of potassium in potato tubers were increased up to 25% after *S. alba* extract application, the increase was not significantly different among three application rates suggesting that the plant uptake is limited by factors other than the plant available potassium content. In general, potatoes contain more potassium than other commonly consumed vegetables [33]. However, potassium shortages are associated with low protein content in potato tubers [34,35]. Thus, *S. alba* extract has potential to serve as an organic fertilizer to not only boost potassium content in potato tuber, but also positively affect other nutritional properties.

*S. alba* extract is sulfur rich due to the presence of sulfur-containing glucosinolates, that can be converted by enzyme myrosinase to inorganic sulfate, that, in turn, can be uptaken by plants. The potential of *S. alba* extract to increase sulfur content in potato tubers is advantageous as sulfur represents an essential dietary component for human diet and plays an important role in disease prevention [36]. In addition to the increased sulfur content of potato tubers, sulfur fertilization provides the benefit of improved micronutrients uptake, infection reduction in potato plants, and minimizing defects in potato tubers [37–39].

Based on the results from this study, *S. alba* extract can also act as an additional booster of calcium and magnesium in potato tubers (Table 2). The adequate calcium content in food, especially in gluten-free foods such as potatoes, is important for maintaining healthy bones and muscular systems [40].
4.3. Potato Phenolics

Total phenolic content in potatoes is generally one of the highest among other staple vegetables like carrots and onions [41]. The application of S. alba extract had a positive effect on the overall phenolics content in potatoes (Table 2). However, the total phenolic content value in potatoes from this trial was relatively low as compared to previously reported values [42,43]. While the lower value could be due to the specific variety used, it could also be due to the atypically dry growing season. Nevertheless, the application of S. alba extract resulted in more than a two-fold increase in total phenolic content of tubers. Every additional application of S. alba extract increased the phenolic content by ~1.5 times (Table 2). Phenolic compounds in potatoes are mostly represented by substituted hydroxycinnamic and hydroxybenzoic acids such as caffeic acid [44,45]. Caffeic acid represented the major phenolic compound in analyzed potatoes that increased after S. alba extract treatment, indicating the potential for boosting it in potatoes by using soil organic amendments. Caffeic acid has been shown to exhibit antioxidant and anticarcinogenic activity and its increase in potatoes is beneficial for human health [46].

4.4. Potato Glycoalkaloids

In addition to monitoring phenolics, potato glycoalkaloid content in potato tubers was measured. Glycoalkaloids are considered antinutrients as they are toxic to human and are generally undesirable [42,47]. At the same time, it has been shown that low concentrations of glycoalkaloid can exhibit anticancer activity [48]. The concentration of α-solanine, one of the major glycoalkaloids in potatoes, was not significantly different in the potatoes treated with S. alba extract compared to the control untreated potatoes. At the same time, concentrations of α-chaconine, another major glycoalkaloid, were statistically higher in potatoes after S. alba extract treatment but were not different for the three S. alba extract application rates. Still, the concentrations of α-chaconine were far below the established health-based maximum levels for human consumption [49]. The observed changes in other glycoalkaloids indicate that changes induced by S. alba extract are glycoalkaloid chemistry dependent.

5. Conclusions

To the best of our knowledge, this is the first study to examine the effect of S. alba extract on the nutritional quality of potatoes, or indeed of any food crop. S. alba extract is an organic amendment that has the benefit of boosting soil health and can potentially be beneficial for improving potato quality. Specifically, we have demonstrated that out of all measured nutritional properties, phenolic content of potatoes was the most impacted by the addition of S. alba extract. The ability to increase the phenolics content of potatoes by applying an organic amendment is a valuable tool in organic farming as it creates a more nutrient-dense product. At the same time, more research needs to be done to evaluate the overall applicability of S. alba extract in organic management practices to assure the productivity and sustainability of the system, especially regarding the yield reduction associated with the application of S. alba extract.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12112782/s1, Table S1: Potato plants above ground biomass and tuber yields after S. alba extract treatment for greenhouse and field trials. Data are average for six individual plants (greenhouse study) or five plots (field study). Values within the same set followed by a common letter are not significantly different (p ≤ 0.05).

Author Contributions: Conceptualization, I.P.; data curation, I.P.; formal analysis, I.P. and D.T.; funding acquisition, I.P.; investigation, D.T. and J.R.; methodology—D.T.; visualization, D.T.; writing—original draft, D.T.; writing—review & editing, D.T., J.R. and I.P. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by This project was supported by the Agriculture and Food Research Initiative competitive grant 2021-67021-34414 from the USDA National Institute of Food and Agriculture and Organic Farming Research Foundation grant.

Data Availability Statement: Data available upon request.

Acknowledgments: We would like to thank Emma Welch for help with potato analysis and Alison Detjens for help with field trials.

Conflicts of Interest: The authors declare no conflict of interest.

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