Research Article

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# Effects of methyl jasmonate seed treatments on adult oviposition preference and larval performance of seed corn maggot (*Delia platura*) in corn (*Zea mays*)

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## Abstract

**BACKGROUND: Eliciting host plant resistance using plant hormones such as jasmonates has the potential to protect seeds and seedlings against insect pests; however, several hurdles exist for adapting it for pest management. This includes determining a dose that promotes resistance without limiting plant growth, an application method that growers could use, and ensuring the plants are responsive in the abiotic conditions when the pest occurs. In laboratory and field assays, we tested if treating corn seeds with multiple concentrations of methyl jasmonate would reduce the preference of ovipositing seed corn maggot adults and the performance of larvae feeding on seeds.**

**RESULTS: We found that corn seeds soaked in aqueous 0.2 mM methyl jasmonate solution showed marginally lower seedling growth, but the adult oviposition preference was ~60% lower on these seeds compared to control water-soaked seeds. Seeds that were treated with methyl jasmonate using a conventional polymer-based seed coating showed no effect on seedling growth but reduced adult oviposition preference. In no-choice bioassays with adult flies, we found reduced oviposition on seeds soaked with aqueous methyl jasmonate compared to controls. Larval survival to pupation was also lower in methyl jasmonate-treated seeds. Lastly, the methyl jasmonate-induced resistance also occurred at the lower temperatures typical of the spring soil conditions when this fly is most damaging.**

**CONCLUSION: Methyl jasmonate seed treatment in aqueous solution or using conventional polymer-based technology, has the potential to deter adult oviposition and reduce maggot performance in spring temperature conditions with minor effects on seed germination and growth.**


*Supporting information may be found in the online version of this article.*

**Keywords:** seed corn maggot; methyl jasmonate; seed treatment; corn; induced plant resistance

## 1 INTRODUCTION

The use of the plant hormone jasmonic acid (JA) and its derivative methyl jasmonate in foliar induction of host plant resistance to insect herbivory has been studied extensively,[1] yet there is less known about its potential as a seed treatment. Exogenous foliar applications of JA and its derivative methyl jasmonate have been shown to increase endogenous levels of JA in plants, and subsequently increase plant resistance to insect herbivores feeding on leaves, roots, stems and flowers. In the last decade methyl jasmonate and JA also have been shown to have potential as a seed treatment inducing resistance to insect herbivores in a range of plants, including tomato, cabbage and rice.[2–4] For example, working in the leguminous crop, Andean lupin (*Lupinus mutabilis*), Erazo-Garcia *et al*. found that methyl jasmonate-treated seeds were less preferred by seed corn maggot (*Delia platura*) adult flies for oviposition and larval performance was lower on methyl jasmonate-treated seeds.[5] In addition, rice seeds that were soaked in methyl jasmonate or JA were resistant to rice weevil damage.[4] We evaluated the potential for this technique in controlling the seed and seedling pest, seed corn maggot (*D. platura*), in corn. Open questions remain about establishing a jasmonate dose that provides resistance with minimum cost in terms of plant growth, developing an application technique that could be used by growers, and testing whether the plant-induced response occurs in the abiotic conditions of seed germination.

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The use of methyl jasmonate as a seed treatment could have a net positive effect on plant growth and yield by reducing herbivory. Many studies show the benefits of induced resistance in decreasing the preference and performance of herbivorous insects,[6,7] and increasing the dose can result in increased resistance, but the benefits level off at higher doses and eventually become disruptive and toxic to the plant. One of the negative side effects of jasmonate-induced response can be through reduced seed germination which can delay seedling growth. Some of these costs may be the direct result of energetic investments in induction, whereas other costs may be indirect, arising from regulation of growth by defense signaling pathways.[8] However, it may be possible to activate resistance at a sufficiently low level to obviate stress associated with growth costs to germinating seeds. For agricultural applications, it is important to determine a dose of methyl jasmonate that promotes resistance without a significant cost in terms of germination or early seedling growth which could affect stand formation or crop yield. Treatment of seeds with methyl jasmonate is done before planting while the seeds are quiescent so that induction of host plant resistance occurs as the seeds become metabolically active after sowing during imbibition and germination. Jasmonates have been shown to negatively affect the likelihood of germination by interacting with the abscisic acid pathway which can promote seed dormancy.[4,9] However, recent studies show that it is possible to establish doses of methyl jasmonate that induce resistance without hampering seed germination or seedling growth. For example, Erazo-Garcia *et al.* (2021) showed that lupin seeds treated with 0.1 mM concentrations of methyl jasmonate induced resistance against *D. platura* and did not affect seed germination or seedling growth.[5]

Many of the studies on treating seeds with methyl jasmonate have been conducted by soaking the seed in an aqueous solution containing jasmonate. While soaking seeds with methyl jasmonate to induce plant resistance can be relevant in planting crops such as rice, most field crop seeds are not hydrated before sowing. Field corn seeds are commercially treated with the application of plant protectants for early season pest management. The plant protectants are mixed with a film-coating polymer to achieve uniformity of application and adherence of seed treatment active ingredients to the seed surface, and commonly applied using rotary pan seed treatment technology.[10] The same commercial film-coating polymer formulations applied with rotary pan technology can be performed on a laboratory-scale.[11]

Although these commercial coating methods are used extensively for pesticide applications, they have not yet been adapted to coat seeds with methyl jasmonate. There are many factors that determine if a chemical seed treatment can permeate through the seed coat and/or seed maternal covering layers and diffuse to the embryo. The primary physicochemical properties that determine the relative systemic uptake into seeds are molecular charge, lipophilicity and molecular size, with molecular weight being <500 Da.[12] Corn seeds were demonstrated to be permeable only to nonionic compounds, whereas ionic compounds were restricted by the pericarp-testa.[13] Many species have this differential permeability to nonionic *versus* ionic compounds termed selective seed coat permeability.[14] Jasmonic acid is an ionic compound and therefore would not be able to be taken up into corn seeds passively using the conventional polymer-based seed-coating technique. By contrast, methyl jasmonate is a nonionic compound and based on its molecular charge can diffuse into the embryo. The second property is the lipophilicity measured by the partition coefficient of a compound between water and octanol, termed log $K_{ow}$.[12] A similar log $K_{ow}$ of the organic compound and the seed coat permeability would have the greatest uptake potential of that compound. The log $K_{ow}$ of methyl jasmonate is 2.6 (https://chemicalize.com), whereas the optimum log $K_{ow}$ for uptake for the pericarp-testa of corn is 2.2–3.8.[12] Collectively, the uptake of methyl jasmonate is in the optimal range for diffusion into corn seeds and therefore we used it to coat the corn seeds using the conventional polymer-based seed-coating technique in this study. There are, however, a few key differences between using methyl jasmonate to coat corn seeds using the conventional polymer-based seed treatment *versus* soaking seeds in aqueous methyl jasmonate solution. Seeds that are soaked in aqueous methyl jasmonate solution perceive the plant hormone while it is germinating and so are metabolically active; conversely, seeds are physiologically dormant when methyl jasmonate is applied using the conventional polymer-based seed treatment. Therefore, the perception of methyl jasmonate into the seeds using these two delivery methods may be physiologically varied, and therefore may not have exactly similar effects with respect to plant growth and resistance. Another important consideration while using seeds treated with methyl jasmonate using the conventional polymer-based coating technique is the possible outward diffusion and leaching of methyl jasmonate into the soil after the sowing of seeds. With these factors in mind, we treated corn seeds using both delivery methods and measured plant growth and resistance to seed corn maggots.

Although most studies of induced plant responses have been conducted at warm temperatures, the seeds of many crops are planted in the spring when soil temperatures are cool. Several studies have shown that induction of plant resistance is temperature-dependent, at least at higher temperatures.[15] Although little research has been conducted on induction at cool temperatures, it may be lower owing to an overall lower rate of plant metabolism.[16,17] For example, foliar treatment with JA has been shown to be temperature-dependent in soybean where soybean aphids performed better on JA-treated plants at 25 °C compared with plants that were induced and grown at 17 °C.[18] However, little is known about how lower temperatures may affect induction of host plant resistance by jasmonates in seeds. Therefore, we also measured the effect of low temperature on seed germination, seedling growth and adult oviposition preference on seeds that were treated with methyl jasmonate.

The seed corn maggot (*D. platura*) is a polyphagous belowground pest with a diverse host range of >50 species.[19] The larvae of seed corn maggots feed on the cotyledons of the seeds during germination and the roots of emerging seedlings.[20] In the United States, corn is a major commodity crop and seed treatment with pesticides such as neonicotinoids is a common way to control early season belowground herbivores such *D. platura*. However, several recent studies have shown the devastating effect of neonicotinoids on nontarget beneficial insects such as insect predators of herbivorous insects, bees and several bird species.[21–24] Therefore, it is imperative that we seek alternatives for early seedling pests. One of the most damaging generations of seed corn maggots occurs in early spring after they emerge from diapause which coincides with planting season for corn in the temperate corn-growing regions of the United States. The mean soil temperatures in early spring in such regions can be as low as 15–20 °C.

In this study we explored the use of methyl jasmonate as a seed treatment to induce host plant resistance to seed corn maggot. Specifically, we (i) tested the effect of five different concentrations



  

of methyl jasmonate on corn seed germination and seedling growth after soaking seeds in aqueous methyl jasmonate and by using a conventional polymer-based seed treatment method in the laboratory. (ii) Using doses of both types of seed treatment method that do not limit germination, we conducted laboratory- and field-based choice, and laboratory-based no-choice oviposition preference assays with adult flies, and measured the performance of the seed corn maggot larvae. (iii) We tested the effect of methyl jasmonate seed treatment on germination, seedling growth and fly oviposition preference at cool temperatures.

## 2 MATERIALS AND METHODS

### 2.1 Plant material and insects

We used the corn hybrid variety 410 with a maturity time of 91 days obtained from Prairie Hybrids Seeds, Deer Grove, Illinois (IL, USA) and this seed lot was not treated by the seed company. Adult flies and larva of seed corn maggots (*D. platura*) were collected from the corn fields in Tompkins County, New York and were brought back to the laboratory. Adult flies were reared on 0.5% sucrose solution along with dry yeast extract powder and a dry powder diet consisting of 10 parts casein protein, 10 parts sucrose, 1 part Brewer's yeast and 1 part soy protein as food source[25] (Rooney *et al*., in review). Organic lima bean seeds were used to feed the larvae.

### 2.2 Methyl jasmonate seed treatment of corn seeds

Corn seeds were treated either by soaking them overnight in methyl jasmonate solution or methyl jasmonate was applied with laboratory-scale seed treatment equipment and a commercial film-coating polymer, L-650. For the wet soaking method, 200 corn seeds were soaked in 150 mL of 0.2, 0.4, 0.8, 1 and 10 mM methyl jasmonate solution overnight (for 14 h). The control seeds were soaked in water. The detergent Tween-20 was added to both the water-treated controls and methyl jasmonate solutions at the concentration of 45 parts per billion as a surfactant. Because soaking the corn seeds in 0.2 mM methyl jasmonate had minimal effect on plant growth and no effect on germination rate, we used this concentration for our subsequent oviposition bioassays with the wet-soaking method. To simulate conventional polymer-based seed treatment technology, we used a commercial, seed film-coating polymer, L650 from Incotec (Urbandale, IA, USA). For 100 g corn seeds, 1 mL coating suspension was used which was composed of 100 µL L650 and 900 uL water or water + methyl jasmonate. Seeds were treated in a Hege 11, seed treater (Wintersteiger, Salt Lake City, UT, USA) for 0.5 min, allowed to air-dry overnight and later used for insect bioassays. The amount of methyl jasmonate needed to coat the seeds was determined by calculating the equivalent amount of methyl jasmonate that is absorbed by the corn seeds when soaked in a 0.2, 0.4 or 0.8 mM methyl jasmonate solution, respectively, overnight. For the corn variety that we used, 1 g corn seed absorbed 0.2895 g water overnight. Therefore, in a 0.2 mM methyl jasmonate solution, the corn seeds would absorb 12.98 µg methyl jasmonate. Based on the density of methyl jasmonate (0.998 g $mL^{-1}$), we used 13.00 nL methyl jasmonate per g corn seed to treat the seeds. For our experiments, we treated 1000 seeds (~220 g corn seeds) with a subset of the concentration of methyl jasmonate from the aqueous treatments to save on costs. We used 0, 0.2, 0.4 and 0.8 mM equivalent amounts of methyl jasmonate, and the amount of methyl jasmonate, L650 and water used to coat the seeds are summarized in Table 1. In the 0 mM film coat control treatment, corn seeds were treated with L650 without any methyl jasmonate.

### 2.3 Seed germination assays

In order to determine the effect of methyl jasmonate seed treatment on germination of seeds and seedling growth, we soaked 200 corn seeds in 150 mL aqueous methyl jasmonate solutions of 0.2, 0.4, 0.8, 1 and 10 mM concentration, and in water as control. Seeds were soaked in a shaker incubator at 24 °C for 14 h with constant shaking at 200 rpm. Seed germination cups were set up as shown in Fig. S1 with 20 soaked seeds placed in sand in each cup. Ten cups were set up for each seed treatment ($n = 10$). All cups were placed in growth chamber with 14 h:10 h, light:dark photoperiod, with a temperature of 24 °C during the light cycle and 16 °C for the dark cycle. Placement of the germination cups containing seeds treated with different concentrations of methyl jasmonate or control untreated seeds were randomized. The number of seeds that germinated each day was measured daily for 7 days for seeds that were soaked in methyl jasmonate solution. We also performed end-point seed germination assays using the same set-up for seeds treated with methyl jasmonate using the conventional polymer-based seed treatment method. In this assay, we used a subset of methyl jasmonate concentrations (0.2 , 0.4 and 0.8 mM) along with the control seed treatment that consisted of seeds treated with the polymer matrix only. For the seeds treated with methyl jasmonate using conventional polymer-based seed treatment method, the total number of seeds that germinated after 7 days was measured. The height of seedlings that emerged from seeds were measured after 14 days of sowing for both seeds that were soaked in aqueous methyl jasmonate or treated with methyl jasmonate using conventional polymer-based seed treatment.

### 2.4 Adult oviposition assays

For oviposition bioassays, 20 corn seeds that were treated with methyl jasmonate (overnight soaked or in polymer-based seed treatment) or control seeds (soaked in water only or coated with polymer only) were placed on sand in 237-mL (8 oz) cups. The sand was kept moist by threading a cotton wick into the cup with

**Table 1.** Calculation of the amount of MeJA used to coat corn seeds using L-650

| Concentration of methyl jasmonate | Weight of methyl jasmonate (mg) | Volume of methyl jasmonate (µL) | Volume of L-650 (µL) | Volume of water (µL) |
|---|---|---|---|---|
| 0 mM (Film coat control) | 0 | 0 | 220 | 1980 |
| 0.2 mM | 220 × 12.98 = 2855.6 µg = 2.855 | 2.86 | 220 | 1977.14 |
| 0.4 mM | 220 × 12.98 × 2 = 5.71 | 5.72 | 220 | 1974.28 |
| 0.8 mM | 220 × 12.98 × 4 = 11.42 | 11.44 | 220 | 1968.56 |

sand that was wetted with water from a cup below it as demonstrated in Supporting information Fig. S1. For two-choice oviposition assays in the laboratory, 30 male and female flies were selected from the laboratory colony that were the same age and were ≥2 weeks post-eclosion. The flies were then released in 30 × 30 × 30 cm plastic cages with two cups containing corn seeds treated with methyl jasmonate or control untreated seeds. For two-choice assays performed in the field, 58 cm × 28 cm mesh cage was placed above the two cups with seeds and 30 flies in each cage. A total of 16 replicates were set up for the two-choice assays in the laboratory in a walk-in growth chamber. The growth chamber was set at 25 °C with a 14 h:10 h, light:dark photoperiod for the laboratory oviposition assays. All cages were placed under the light source and the vertical height from the light source to each cage was equal. For the field assays, 10 replicates each were set up in the first week of July 2023 and then again in the first week of September 2023 at Homer C. Thompson Vegetable Research Farm at Freeville, New York. After 5 days the number of eggs in each cup were counted and the percentage of eggs deposited on water-soaked or methyl jasmonate-soaked seeds was calculated for each cage. The average daytime temperature at the research farm during the course of the experiment was 28 °C in both July and September, and the average night-time temperature was 17.9 °C in July and 17.2 °C in September. To count the number of eggs deposited by the flies in each cup, the contents of the cup were thoroughly mixed in 30% glycerol solution and then set aside at room temperature for 30 min. Thereafter, the clear glycerol solution containing the eggs was decanted and sieved through a 1-μm sieve, and the number of eggs was counted. For oviposition assays that were performed at low temperatures, the growth temperature was set at 15 °C for the light cycle and 5 °C for the dark cycle (14 h:10 h, light:dark photoperiod).

For the no-choice oviposition assays in the laboratory, a similar set-up was used as the two-choice assays, except that the flies were offered only seeds soaked in 0.2 mM methyl jasmonate or water-soaked seeds. Ten cages were set up for each seed treatment and after 5 days, the cages that received the methyl jasmonate-treated seeds, were given water-treated seeds and *vice versa*. Therefore, each cage containing 30 flies had the choice to oviposit on water-soaked seeds first and then on methyl jasmonate-soaked seeds or *vice versa*. The order of the seed-type presentation was randomized. The total number of eggs deposited in each cup was counted as described above.

### 2.5 Synchronized seedling growth stage bioassay

Because we found delayed germination in the seeds treated with methyl jasmonate using the soaking method, we checked whether this could have caused the increase in oviposition on the control seeds. It is possible that the flies could only oviposit on seeds once they begin to germinate, essentially increasing the window of time available for oviposition in the control treatment compared to methyl jasmonate treatment. We tested this by germinating corn seeds soaked with 0.2 mM methyl jasmonate 2 days before control water-soaked seeds to synchronize their stage of germination. The two-choice bioassays were set up as before in the growth chamber at 25 °C with 30 flies in each cage. A total of 12 replicates were set up for this bioassay.

### 2.6 Larval performance bioassay

We measured the performance of seed corn maggot larvae on corn seeds treated with aqueous methyl jasmonate by measuring the percentage of larvae that pupated. Ten 1st-instar (2-day-old) seed corn maggot larva were placed in 237 mL (8-oz) cups with 10 seeds soaked in 0.2 mM aqueous methyl jasmonate or in water. The number of pupa emerging was counted after 2 weeks.

### 2.7 Statistical analyses

We examined the effects of seed treatment on the percentage of germinated seeds and seedling height using a one-way ANOVA and performed Tukey's *post hoc* test ($\alpha = 0.05$) to compare means between different methyl jasmonate concentrations. We examined the effect of methyl jasmonate seed treatment (fixed effect) on oviposition preference by adult flies in the two-choice assays and larval performance using a one-way ANOVA. To examine the effects of seed treatment on the oviposition preference in the no-choice assay, we fitted a generalized linear mixed effects model (GLMM) with the number of eggs in each oviposition cup as the response, seed treatment and order in which each cage received either of the two treatments as the fixed effects, and cage as the random effect. We used a Poisson error distribution with a log link function. The model was fitted via the *glmmtmb()* function in the R GLMMTMB package.[26] We checked the model assumptions using quantile residuals generated from the function *simulateResiduals()* in the R DHARMA package (Hartig, 2022). We used the likelihood ratio test to assess predictor significance using the *Anova()* function in the R CAR package.[27] All analyses were performed in R v4.3.1 (R Core Team 2023).

## 3 RESULTS

### 3.1 Seeds soaked in aqueous methyl jasmonate slowed germination and early seedling growth, but it was not affected in seeds treated with methyl jasmonate using conventional polymer-based treatment

We compared the rate of germination in corn seeds treated with 0.2, 0.4, 0.8, 1 and 10 mM of aqueous methyl jasmonate to control seeds. Seeds treated with 0.2, 0.4, 0.8 and 1 mM methyl jasmonate had no difference in total germination 7 days post-treatment compared to controls while seeds treated with 10 mM methyl jasmonate showed <20% germination [Fig. 1(a)]. However, there was a 2-day delay in germination post-treatment with 0.2, 0.4, 0.8 and 1 mM methyl jasmonate at compared with control seeds [Fig. 1(a)]. Because we did not observe any difference in germination 7 days post sowing with seeds soaked in aqueous methyl jasmonate for any concentration other than 10 mM methyl jasmonate, we measured germination in an endpoint assay at 7 days post sowing with seeds treated with methyl jasmonate using conventional polymer-based seed treatment at concentrations of 0.2, 0.4 and 0.8 mM. The seeds treated with methyl jasmonate using conventional polymer-based seed treatment did not show differences in seed germination 2 weeks post-treatment compared with seeds with a control coating without methyl jasmonate [Fig. 1(c)].

The delay in the germination of seeds soaked in 0.2, 0.4, 0.8 and 1 mM aqueous methyl jasmonate was reflected in reduced seedling height after 14 days [$F_{3,20} = 27.34$, $P < 0.001$; Fig. 1(b)]. Seedlings emerging from seeds treated with 0.2 and 0.4 mM methyl jasmonate showed the least amount of growth reduction (~12–15%) when compared to control water-soaked seeds, so we used 0.2 mM methyl jasmonate-treated corn seeds to perform our subsequent bioassays. There was no difference in seedling growth when treated with methyl jasmonate using the conventional polymer-based seed treatment method [Fig. 1(d)].

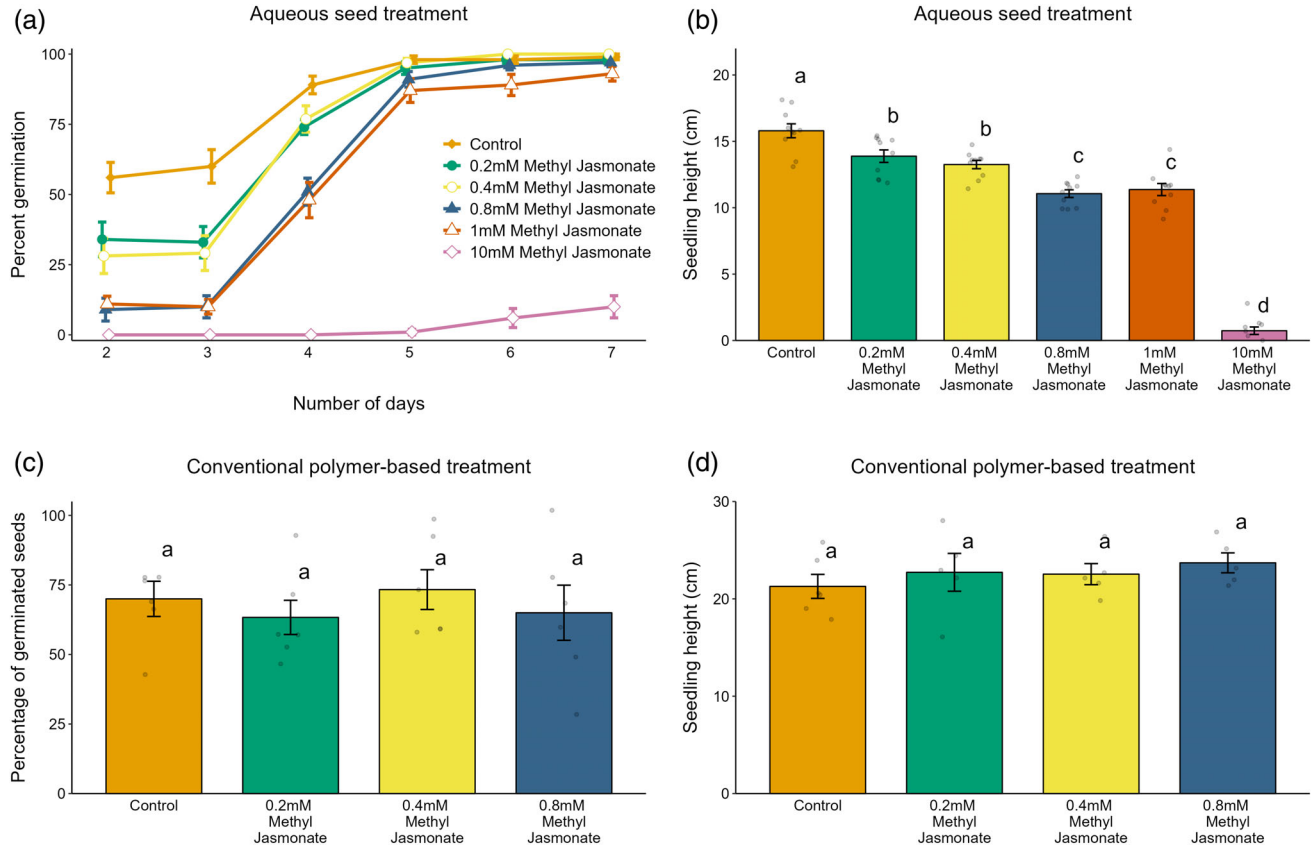


**Figure 1.** Germination rate and seedling height of corn seedlings post seed treatment with methyl jasmonate. The seed germination rate of corn seeds soaked in 0.2–10 mM methyl jasmonate (MeJa) solution and water (control) was measured for 7 days (a) and the percentage of germinated seeds with conventional seed treatment with methyl jasmonate was measured after 7 days (c). The height of seedlings emerging from both seeds treated with methyl jasmonate using the wet soaking method (b) and conventional seed treatment method (d) was measured 14 days post sowing. One-way ANOVA was performed to measure significant difference among treatments at $P = 0.05$. Means that are different from each other are denoted by different letters using means separation by Tukey's *post hoc* test. Error bars indicate standard error around the mean.

### 3.2 Methyl jasmonate-treated seeds were less preferred by adult flies and had lower larval performance compared to controls

When given the choice to oviposit between water-treated and corn seeds soaked in methyl jasmonate in two-choice oviposition assays, the adult flies of seed corn maggots laid ~60% fewer eggs on 0.2 mM methyl jasmonate-treated seeds compared to water-soaked seeds [$F_{1,14} = 14.64$, $P < 0.001$; Fig. 2(a)]. We also performed this two-choice oviposition assay in the field setting where methyl jasmonate-treated seeds had ~20% lower oviposition by adult flies compared with water-soaked seeds [$F_{1,18} = 12.046$, $P = 0.0027$; Fig. 2(b)]. Likewise, in a two-choice assay with seeds treated with methyl jasmonate using conventional polymer-based seed treatment, we found that seeds that were treated with 0.2 or 0.4 mM methyl jasmonate had ~20% fewer eggs deposited on them compared to control-treated seeds [Fig. 2(c,d)].

The two-choice oviposition assay shows that adult flies prefer to lay eggs on untreated seeds over methyl jasmonate-treated seeds, yet once adapted for commercial use, flies would encounter only methyl jasmonate-treated seeds in the field. Therefore, in order to understand how the adult flies lay eggs on the two seed treatments independently, we performed no-choice oviposition assays. In a no-choice assay, when the adult flies were exposed to seeds soaked in either water or 0.2 mM aqueous methyl jasmonate solution in the laboratory, the average number of eggs laid on methyl jasmonate-treated seeds was half to that laid on water-treated seeds [$\chi^2 = 90.5$, $df = 1$, $P < 0.001$; Fig. 3(a)].

In our germination and growth bioassay, the seeds soaked in 0.2 mM aqueous methyl jasmonate germinated marginally slower than control water-soaked seeds at Days (D)3 and (D)4 [Fig. 1(a)]. This delay in germination and plant growth falls within the 5-day time period that we used for our oviposition bioassays with the adult flies. This added the possibility that earlier emergence of control water-treated seeds may have increased the window of time during which adult flies could oviposit in them compared with methyl jasmonate-treated seeds in our subsequent two-choice assays [Fig. 2(a),(b)]. Therefore, we also performed oviposition preference bioassays that were matched for developmental stage for these two treatments. In stage-matched seeds, we did not find increased oviposition on control seeds; instead we found increased oviposition on the methyl jasmonate-treated seeds [Fig. 3(b)], indicating that the increased oviposition on the control seeds is not to the consequence of a longer window of availability for oviposition caused by differences in germination rate.

In order to understand if methyl jasmonate had a direct effect on the development of the seed corn maggot larvae, we fed corn seeds soaked in water or in 0.2 mM aqueous methyl jasmonate solution to freshly hatched 1$^{st}$-instar larva of seed corn maggots. Thirty percent fewer seed corn maggot larvae successfully pupated when fed on seeds soaked in methyl jasmonate compared to water-soaked seeds [$F_{1,8} = 5.444$, $P = 0.0479$; Fig. 3(c)].

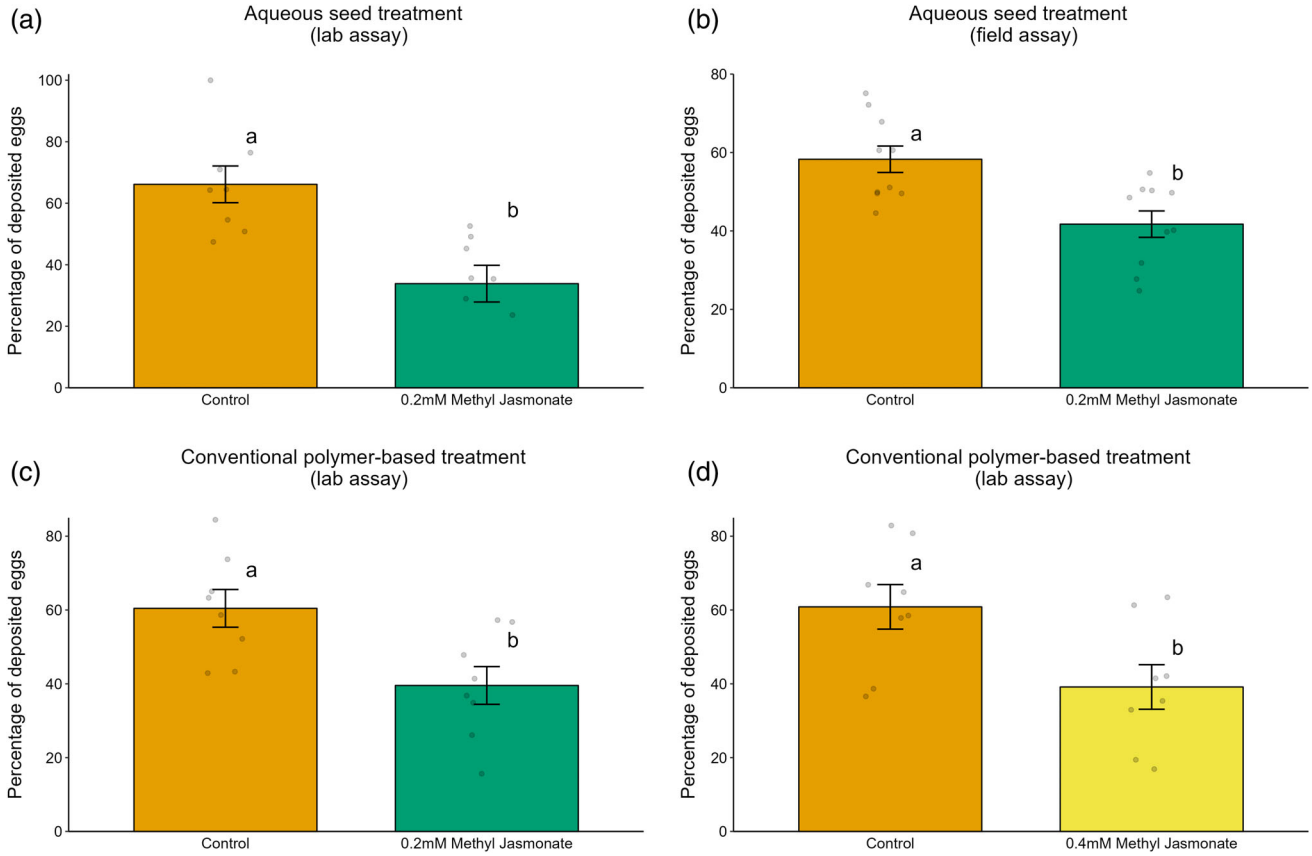


**Figure 2.** Oviposition choice assays of adult flies of *Delia platura* on seeds treated with methyl jasmonate. The percentage of eggs deposited by adult flies of seed corn maggots in two-choice assays with water-soaked (control) and 0.2 mM methyl jasmonate (MeJa)-soaked corn seeds were performed in the laboratory (a) and in the field (b). Two-choice oviposition assays also were performed with corn seeds treated with methyl jasmonate using conventional seed treatment and the coating matrix only (control), or (c) 0.2 mM and (d) 0.4 mM MeJA equivalent. One-way ANOVA was performed to measure significant difference among treatments for the two-choice bioassays. Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.

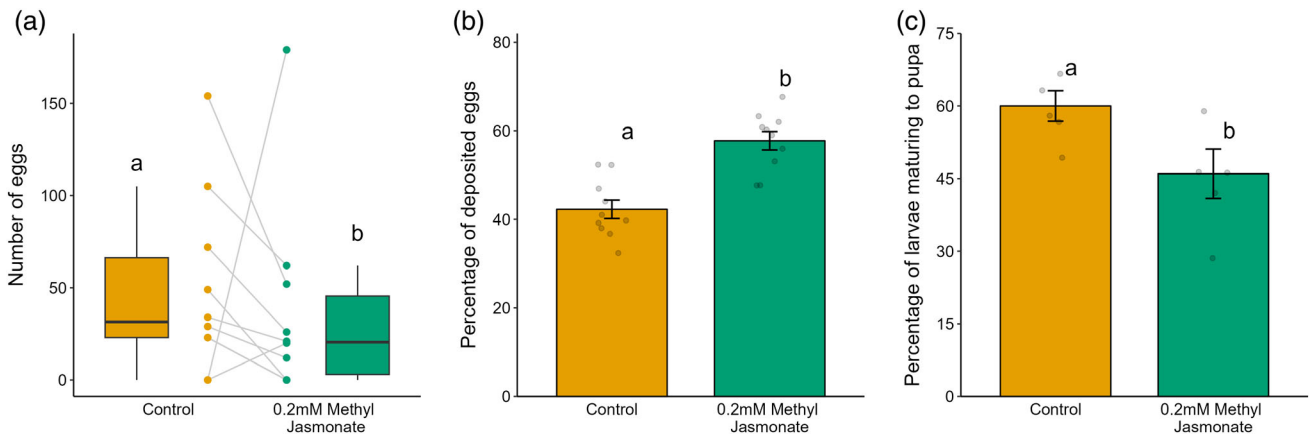


**Figure 3.** (a) Total number of eggs deposited by adult flies on water-soaked (control) and 0.2 mM methyl jasmonate (MeJa)-soaked corn seeds measured in no-choice bioassays. The number of eggs deposited on each seed treatment was fitted into a generalized linear mixed model at $P = 0.05$. (b) The percentage of eggs deposited on seeds that were soaked in water or 0.2 mM MeJa and were matched for growth stage also was measured in a two-choice oviposition assay. (c) Larval performance of *Delia platura* on seeds treated with methyl jasmonate. The percentage of 1$^{st}$-instar seed corn maggot larva that matured to becoming pupa when fed on corn seeds soaked in water *versus* 0.2 mM MeJa was measured after 14 days. One-way ANOVA was performed to measure significant difference among treatments in the two-choice oviposition assay (b) and larval performance assay (c). Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.



  

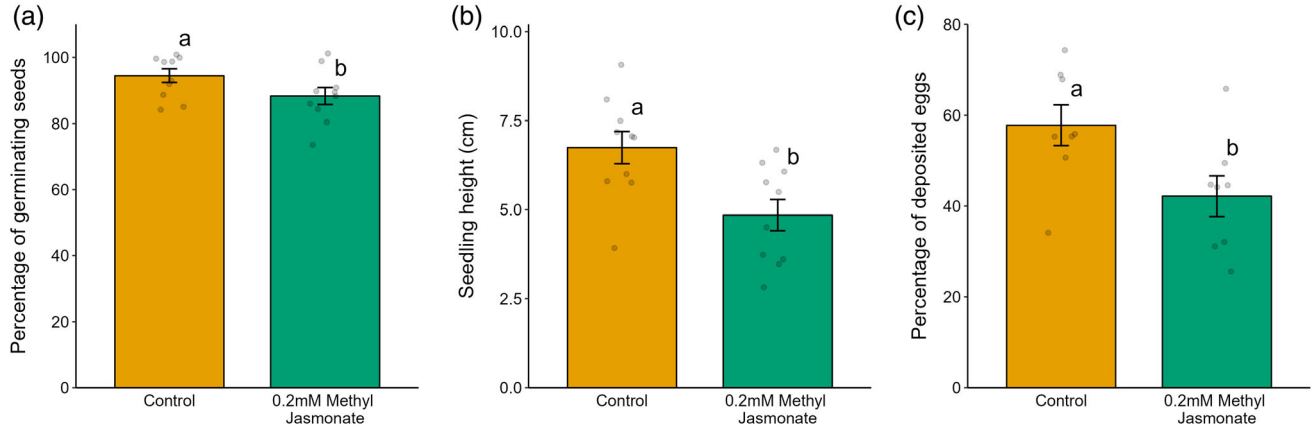


**Figure 4.** Germination rate, seedling growth of corn and oviposition preference of *Delia platura* on corn seeds treated with methyl jasmonate at low temperature. The percentage germination of seeds (a) and height of seedlings (b) emerging from seeds soaked in water (control) or 0.2 mM methyl jasmonate (MeJa) solution was measured 21 days postsowing in growth chambers at 15 °C daytime and 5 °C night-time temperature. Two-choice oviposition choice assays on seeds soaked in 0.2 mM MeJa or water also was measured at these low temperatures (c). One-way ANOVA was performed to measure significant difference among treatments for the two-choice bioassays, seed germination rate and seedling height at $P = 0.05$. Means that are different from each other are denoted by different letters. Error bars indicate standard error around the mean.

### 3.3 Methyl jasmonate also reduced adult oviposition preference at cool temperatures

The percentage germination of seeds soaked in 0.2 mM methyl jasmonate was 5% lower compared with water-soaked seeds at 15 °C [Fig. 4(a); $F_{1,17} = 4.516$, $P = 0.0485$]. Three-week-old seedlings that emerged from seeds soaked in 0.2 mM methyl jasmonate were also ~25% shorter than the water-soaked seeds [Fig. 4(b); $F_{1,17} = 16.357$, $P$- <0.0001]. The deterrent effect of methyl jasmonate treatment on oviposition preference of the adult flies was maintained at the cool temperature. Seeds that were soaked in 0.2 mM methyl jasmonate had ≈15% fewer eggs deposited on them compared with the number on water-soaked seeds [Fig. 4(c); $F_{1,14} = 6.041$, $P = 0.027$].

## 4 DISCUSSION

The efficacy of any elicitor-based strategy to control an insect pest depends on developing the key parameters that are contextually relevant for a specific plant species and the insect pest. As the use of jasmonates as elicitor-based seed treatment to manage insect pests gain momentum, our laboratory-based work provides foundational knowledge towards adapting it for use in the field. Our work brings clarity on three key considerations for using methyl jasmonate as seed treatment to combat seed corn maggot herbivory. First, we show that a concentration as low as 0.2 mM methyl jasmonate can be used to treat corn seeds by either soaking seeds overnight or when used in a conventional polymer-based seed treatment to induce host plant resistance without significantly affecting seed germination. Secondly, we show that seeds that are soaked with 0.2 mM methyl jasmonate solution or treated with methyl jasmonate in a conventional polymer-based seed treatment of an equivalent amount are both equally effective in deterring adult flies from ovipositing on treated seeds. Thirdly, the induction of host plant resistance can deter adult flies from oviposition at temperatures as low as 5–15 °C. This is especially significant in this system because adult flies of seed corn maggots emerge in late spring when the temperatures in temperate corn-growing regions tend to be cool. Collectively, the use of methyl jasmonate as a seed treatment has the potential to be a viable method for corn growers.

In different plant species, the dose of methyl jasmonate seed treatment affects the trade-off between growth and resistance. For example, rice seeds treated with 2.5 mM methyl jasmonate induce resistance against rice weevil while maintaining growth, and tomato seeds treated with a 0.05–1 mM dose of methyl jasmonate suppress tomato fruit worm larval performance while maintaining growth and germination.[2] Worral *et al.* (2012) showed that tomato plants emerging from seeds treated with 3 mM methyl jasmonate had shorter roots compared to untreated controls, but there was no long-term effect on plant height and fruit growth. Meanwhile, the performance of spider mites, *Manduca sexta* and *Myzus persicae* was shown to be lower on tomato plants was grown from seeds that were treated with 3 mM methyl jasmonate.[28] In our system, corn seeds treated with any concentration of methyl jasmonate between 0.2 and 1 mM using an aqueous methyl jasmonate solution or 0.2 to 0.8 mM methyl jasmonate using conventional polymer-based seed treatment method showed no difference in percentage germination 5 days postsowing. When we looked at the daily germination of seeds treated with aqueous methyl jasmonate, we saw some delays, but they converged by D5. This delay in germination may have caused the reduction in plant height seen at 14 days; however, when seeds were treated with methyl jasmonate using in the conventional polymer-based seed treatment method we do not see any differences in plant height. Therefore, the costs of treating seeds with methyl jasmonate using conventional polymer-based seed treatment method appeared less than the aqueous method. It has been reported, however, that there may be varietal differences in dosage response to uptake of active ingredients using the conventional polymer-based seed treatment. For example, a four-fold difference in seed uptake of a model nonionic compound was measured between two inbred lines.[12] Therefore, the dosage of methyl jasmonate seed treatment may need to be established for commercial varieties using conventional seed treatment application. Additionally, the bioassays in this study focused on the 0.2 and 0.4 mM methyl jasmonate treatments, yet the low costs on growth even at higher concentrations may allow for higher doses in the field. Taken together, the dose of methyl jasmonate needed to treat seeds is unique to each plant species but it is possible to find doses of methyl jasmonate that can induce resistance without incurring a high growth cost.

Although the endpoint germination rate was not hampered using methyl jasmonate concentrations between 0.2 and 0.8 mM in both of the delivery methods we used, there was a temporal delay in germination of methyl jasmonate-treated seeds. Notably, we see that the germination delay resulting from methyl jasmonate seed treatment occurs in the first 5 days after seed treatment, which falls within the time frame of our adult oviposition assays. Prior research has shown that cues from germinating seeds are known to affect adult preference of seed corn maggot flies.[29] Therefore, we thought it was possible that the lower oviposition preference of adult flies was because the flies were unable to detect any germinating seeds in cups containing methyl jasmonate seeds in the first few days. However, our results with stage-matched seeds show that the precise developmental stage in the days immediately postgermination is not the determinant of where eggs are laid.[29]

Weston and Miller (1989) found that *D. platura* adults preferred to lay eggs on germinating lima bean seedlings over surrogate artificial seedlings, suggesting that the flies do not need visual cues from germinating seeds but are attracted instead to chemical stimuli from the germinating seeds.[29] Methyl jasmonate may alter plant volatiles released from germinating seedlings that contribute to resistance to seed corn maggot. Methyl jasmonate seed treatment is known to affect volatile emissions from plants emerging from treated seeds.[3,30] Volatile compounds from methyl jasmonate-treated lupin seeds deter oviposition by *D. platura*.[5] Volatiles could be playing a role in oviposition decisions on corn seeds. Although larvae performance could have been affected by host volatiles, methyl jasmonate could also affect other seed and seedling traits. For example, in lupin, methyl jasmonate seed treatment induces expression of genes involved in jasmonate biosynthesis, including lipoxygenase and allene oxide synthase, as well as terpene synthesis and the antioxidant pathway in the embryonic axis.[5] Subsequent work needs to be done to pinpoint traits involved in resistance in germinating seedlings emerging from seeds treated with methyl jasmonate.

We expected the effects of methyl jasmonate treatment on plant growth and induction to be temperature-dependent. Foliar induction of the jasmonate pathway can be enhanced at warmer temperatures.[31] Tomato seedlings did not respond to wounding at temperatures below 20 °C.[32] However, we could find little other work looking at the ability of plants to induce responses at cool temperatures,[18] and nothing with seed induction. In our study, the seeds were held at a cool temperature for the duration of the assay. The effects of methyl jasmonate treatment on seed germination and seedling growth appeared to be stronger at the low temperature compared with our room temperature assays, although these experiments were conducted at separate times and so were not directly comparable. Additionally, recent studies show potential additional benefits of treating seeds with methyl jasmonate such as increased cold tolerance in wheat,[17] and drought tolerance in corn and rice.[16,33] Methyl jasmonate seed treatment is a promising tool for pest management, yet the costs and benefits of methyl jasmonate treatment are multifaceted and need to be assessed in field environmental conditions with respect to insect performance, longevity of treatment, and long-term effect on plant growth and crop yield.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for the article.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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