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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*)

Running title (80 characters): Methyl jasmonate as seed treatment to combat seed corn maggot herbivory in corn

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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 lab 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 0.2mM 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.

Keywords: Seed corn maggot, methyl jasmonate, seed treatment, corn, induced plant resistance.

1. Introduction

While the use of the plant hormone jasmonic acid and its derivative methyl jasmonate in foliar induction of host plant resistance to insect herbivory has been studied extensively[1], there is less known about its potential as a seed treatment. Exogenous foliar applications of jasmonic acid and its derivative methyl jasmonate have been shown to increase endogenous levels of jasmonic acid in plants and subsequently increase plant resistance to insect herbivores feeding on leaves, roots, stems and flowers. In the last decade methyl jasmonate and jasmonic acid have also 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 jasmonic acid 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 in terms of 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.

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, while 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 low enough level that is not a 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 prior to 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 prior to 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 lab-scale[11].

While these commercial coatings are used extensively for pesticides application, it has 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 daltons[12]. Corn seeds were demonstrated to be permeable only to nonionic compounds, while ionic compounds were restricted by the pericarp-testa[13]. Many species have this differential permeability to nonionic vs 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. In contrast, methyl jasmonate is a nonionic compound and based on its molecular charge it 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), while the optimum log K_{ow} for uptake for the pericarp-testa of corn is 2.2 to 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 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.

While 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]. While little research has been conducted on induction at cool temperatures, it may be lower due to an overall lower rate of plant metabolism[16,17]. For example, foliar treatment with jasmonic acid has been shown to be temperature-dependent in soybean where soybean aphids performed better on jasmonic acid treated plants at 25°C compared to 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 below-ground pest with a diverse host range of more than 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 non-target 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 United States. The mean soil temperatures in early spring in such regions can be as low as 15°C -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 1) 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 lab. 2) Using doses of both types of seed treatment methods that does not limit germination, we conducted lab and field-based choice and lab-based no-choice oviposition preference assays with adult flies and measured the performance of the seed corn maggot larvae. 3) 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 that were obtained from Prairie Seeds (Illinois, USA) and this seed lot was not treated by the seed company. Adult flies and larva of seed corn maggots (*Delia platura*) were collected from the corn fields in Tompkins County, New York and were brought back to the lab. 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 at 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 lab-scale seed treatment equipment and a commercial film coating polymer, L-650. For the wet soaking method, two hundred corn seeds were soaked in 150ml of 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM methyl jasmonate solution overnight (for 14hrs). 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. Since soaking the corn seeds in 0.2mM 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, Iowa, USA). For 100 grams of corn seeds, 1ml of coating suspension was used which was composed of 100ul of L650 and 900ul of water or water+ methyl jasmonate. Seeds were treated in a Hege 11, seed treater (Wintersteiger, Salt Lake City, UT) for 0.5 mins, 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 0.2mM, 0.4mM or 0.8mM methyl jasmonate solution, respectively, overnight. For the corn variety we used, 1g of corn seed absorbed 0.2895 gram of water overnight. Therefore, in a 0.2mM methyl jasmonate solution, the corn seeds would absorb 12.98 micrograms of methyl jasmonate. Based on the density of methyl jasmonate

(0.998g/mL), we used 13.00nl of methyl jasmonate per gram of corn seed to treat the seeds. For our experiments, we treated 1000 seeds (~220 grams of corn seeds) with a subset of the concentration of methyl jasmonate from the aqueous treatments to save on costs. We used 0, 0.2mM, 0.4mM and 0.8mM 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:

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 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM concentration and in water as control. Seeds were soaked in a shaker incubator at 24˚C for 14hrs with constant shaking at 200 rpm. Seed germination cups were set up as shown in Figure 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 14hr:10hrs – Light:Dark cycle and 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 mM, 0.4 mM 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 237ml (8oz) cups. The sand was kept moist by threading a cotton wick into the cup with sand that was wetted with water from a cup below it as demonstrated in the supplemental Figure S1. For two-choice oviposition assays in the lab, thirty male and female flies were selected from the lab colony that were the same age and were at least two weeks post-eclosion. The flies were then released in 30X30X30cm 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 X 28 cm mesh cage was placed above the two cups with seeds and thirty flies in each cage. A total of 16 replicates were set up for the two-choice assays in the lab in a walk-in growth chamber. The growth chamber was set at 25˚C with a 14hr:10hr dark cycle for the lab 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, ten 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 day time temperature at the research farm during the course of the experiment was 28˚C in both July and September while the average nighttime 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 mins. Thereafter, the clear glycerol solution containing the eggs was decanted and sieved through a 1um sieve and the number of eggs were 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 (14hr:10hrs – Light:Dark cycle).

For the no-choice oviposition assays in the lab, a similar set-up was used as the two-choice assays, except the flies were offered only seeds soaked in 0.2mM 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 thirty 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 0.2mM methyl jasmonate two days prior to 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 thirty 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 first instar (two days old) seed corn maggot larva were placed in 8-ounce cups with ten seeds soaked in 0.2mM aqueous methyl jasmonate or in water. The number pupa emerging was counted after two 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 the Tukey 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 fit 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 version 4.3.1 (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 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM of aqueous methyl jasmonate to control seeds. Seeds treated with 0.2mM, 0.4mM, 0.8mM and 1mM methyl jasmonate had no difference in total germination 7 days post treatment compared to controls while seeds treated with 10mM methyl jasmonate showed less than 20% germination (Fig 1a). However, there was a two-day delay in germination post methyl jasmonate treatment with concentrations of 0.2mM, 0.4mM, 0.8mM and 1mM compared to control seeds (Fig 1a). Since we did not observe any difference in germination 7 days after sowing with seeds soaked in aqueous methyl jasmonate for any concentration other than 10mM 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 concentration of 0.2mM, 0.4mM and 0.8mM. The seeds treated with methyl jasmonate using conventional polymer-based seed treatment did not show differences in seed germination two weeks post treatment compared to seeds with a control coating without methyl jasmonate (Fig 1c).

The delay in the germination of seeds soaked in aqueous methyl jasmonate of 0.2mM, 0.4mM, 0.8mM and 1mM was reflected in reduced seedling height after 14 days ($F_{3,20}$= 27.34, p<0.001; Fig 1b). Seedlings emerging from seeds treated with 0.2mM and 0.4mM methyl jasmonate showed the least amount of growth reduction (~12-15%) when compared to control water-soaked seeds, so we used 0.2mM 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 1d).

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% less eggs on 0.2mM methyl jasmonate treated seeds compared to water-soaked seeds ($F_{1,14}$= 14.64, p<0.001; Fig 2a). 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 to water-soaked seeds ($F_{1,18}$ = 12.046, p=0.0027; Fig 2b). Similarly, 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 0.2mM or 0.4mM levels of methyl jasmonate had ~20% fewer eggs deposited on them compared to control-treated seeds (Fig 2 c,d).

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

In our germination and growth bioassay, the seeds soaked in 0.2mM aqueous methyl jasmonate germinated marginally slower than control water-soaked seeds at days 3 and 4 (Fig 1a). 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 to methyl jasmonate treated seeds in our subsequent two-choice assays (Fig 2a,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 3b), indicating that the increased oviposition on the control seeds is not due to 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 0.2mM aqueous methyl jasmonate solution to freshly hatched first 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 3c).

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

The percent germination of seeds soaked in 0.2mM methyl jasmonate was 5% lower compared to water-soaked seeds at 15 °C (Fig 4a; $F_{1,17}$ = 4.516, p-value = 0.0485). Three-week-old seedlings that emerged from seeds soaked in 0.2mM methyl jasmonate were also ~25% shorter than the water-soaked seeds (Fig 4b; $F_{1,17}$ = 16.357, p-value < 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 0.2mM methyl jasmonate had nearly 15% fewer eggs deposited on them compared to the number on water-soaked seeds (Fig 4c; $F_{1,14}$ = 6.041, p-value = 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 lab-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 0.2mM 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. Second, we show that seeds that are soaked with 0.2mM 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. Third, 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 since 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 2.5mM methyl jasmonate induce resistance against rice weevil while maintaining growth, and tomato seeds treated with a 0.05 mM - 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 3mM 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 3mM methyl jasmonate[28]. In our system, corn seeds treated with any concentration of methyl jasmonate between 0.2 mM to 1 mM using an aqueous methyl jasmonate solution or 0.2 mM to 0.8 mM methyl jasmonate using conventional polymer-based seed treatment method showed no difference in percent germination 5 days after sowing. When we look at the daily germination of seeds treated with aqueous methyl jasmonate, we see some delays, but they converged by day 5. 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 mM and 0.4 mM methyl jasmonate treatment, the low costs of 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 0.2mM and 0.8mM in both the delivery methods we used, there was a temporal delay in germination of methyl jasmonate treated seeds. Notably, we see that the germination delay due to 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 after germination is not the determinant of where eggs are laid.[29]

Weston and Miller (1989) found that the *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 to other chemical stimulus 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 to our room temperature assays, although these experiments were conducted at separate times and so not directly comparable. In addition, 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]. While methyl jasmonate seed treatment is a promising tool for pest management, 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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Figure Legends

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 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 after 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 test. Error bars indicate standard error around the mean.

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 0.2mM methyl jasmonate soaked corn seeds were performed in the laboratory (a) and in the field (b). Two-choice oviposition assays were also performed with corn seeds treated with methyl jasmonate using conventional seed treatment and the coating matrix only (control) or 0.2mM methyl jasmonate equivalent (c) and 0.4mM MeJA equivalent (d). 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 0.2mM methyl jasmonate soaked corn seeds measured in no-choice bioassays. The number of eggs deposited on each seed treatment was fit into a generalized linear mixed model at p=0.05. (b) The percentage of eggs deposited on seeds that were soaked in water or 0.2mM methyl jasmonate and were matched for growth stage was also measured in a two-choice oviposition assay. (c) Larval performance of *Delia platura* on seeds treated with methyl jasmonate. The percentage of first instar seed corn maggot larva that matured to becoming pupa when fed on corn seeds soaked in water versus 0.2mM methyl jasmonate 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 0.2mM methyl jasmonate solution was measured 21 days after sowing in growth chambers at 15 °C daytime and 5 °C night temperature. Two-choice oviposition choice assays on seeds soaked in 0.2mM methyl jasmonate or water was also 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.

Figure S1. (a) Schematic diagram of set-up showing two-choice oviposition assay in the lab, (b) picture of oviposition cups set-up with corn seeds used for oviposition assays.

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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*)

Running title (80 characters): Methyl jasmonate as seed treatment to combat seed corn maggot herbivory in corn

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Abstract

BACKGROUND: Eliciting host plant resistance using plant hormones such as jasmonates ~~is a promising technique that~~ has the potential to protect seeds and seedlings against ~~multiple life-stages of herbivorous~~ insect pests, however, ~~. While methyl jasmonate seed treatment has been shown to reduce insect preference and performance,~~ several hurdles exist for adapting it for pest management. This includes determining a dose that promotes resistance while not 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 ~~this series of~~ lab and field assays, ~~our goal was to~~we tested if treating corn seeds with ~~the plant hormone~~multiple concentrations of methyl jasmonate would reduce the preference of ovipositing seed corn maggot adults ~~(*Delia platura*)~~ and the performance of ~~seed corn maggot~~ larvae feeding on seeds.

RESULTS: ~~We tested two methyl jasmonate application methods: soaking seeds in aqueous methyl jasmonate and treating seeds using a conventional film coating polymer mixed with methyl jasmonate. We conducted dose response experiments to determine a concentration that induces resistance to the adult and larval flies with minimal reductions in seed germination and seedling growth.~~ We found that corn seeds soaked ~~with~~ in aqueous 0.2mM ~~aqueous~~ methyl jasmonate solution showed ~~had the same germination rates and~~ marginally lower ~~early~~ 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.~~compared to water-treated controls; whereas 0.2 mM conventional polymer-based seed treatment with methyl jasmonate did not affect seed germination or growth. In choice and no-choice bioassays with adult flies in the laboratory and field, we found reduced oviposition on both seeds soaked with aqueous methyl jasmonate and on seeds treated with methyl jasmonate in conventional polymer-based seed treatments compared to controls.~~ Larval performance measured as 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.

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

Introduction

While the use of the plant hormone jasmonic acid and its derivative methyl jasmonate in foliar induction of host plant resistance to insect herbivory has been studied extensively[1], there is less known about its potential as a seed treatment. Exogenous foliar applications of jasmonic acid and its derivative methyl jasmonate have been shown to increase endogenous levels of jasmonic acid in plants and subsequently increase plant resistance to insect herbivores feeding on leaves, roots, stems and flowers. In the last decade methyl jasmonate and jasmonic acid have also 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 jasmonic acid 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 in terms of 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.

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, while 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 low enough level that is not a 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 prior to 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 prior to 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 lab-scale[11].

While these commercial coatings are used extensively for pesticides application, it has 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 daltons[12]. Corn seeds were demonstrated to be permeable only to nonionic compounds, while ionic compounds were restricted by the pericarp-testa[13]. Many species have this differential permeability to nonionic vs 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. In contrast, methyl jasmonate is a nonionic compound and based on its molecular charge it 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), while the optimum log K_{ow} for uptake for the pericarp-testa of corn is 2.2 to 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 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 ~~right~~ 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 ~~in this study~~.

While 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]. While little research has been conducted on induction at cool temperatures, it may be lower due to an overall lower rate of plant metabolism[16,17]. For example, foliar treatment with jasmonic acid has been shown to be temperature-dependent in soybean where soybean aphids performed better on jasmonic acid treated plants at 25°C compared to 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 below-ground pest with a diverse host range of more than 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 non-target 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 United States. The mean soil temperatures in early spring in such regions can be as low as 15°C -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 1) 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 lab. 2) Using doses of both types of seed treatment methods that does not limit germination, we conducted lab and field-based choice and lab-based no-choice oviposition preference assays with adult flies and measured the performance of the seed corn maggot larvae. 3) We tested the effect of methyl jasmonate seed treatment on germination, seedling growth, and fly oviposition preference at cool temperatures.

Results

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 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM of aqueous methyl jasmonate to control seeds. Seeds treated with 0.2mM, 0.4mM, 0.8mM and 1mM methyl jasmonate had no difference in total germination 7 days post treatment compared to controls while seeds treated with 10mM methyl jasmonate showed less than 20% germination (Fig 1a). However, there was a two-day delay in germination post methyl jasmonate treatment with concentrations of 0.2mM, 0.4mM, 0.8mM and 1mM compared to control seeds (Fig 1a). Since we did not observe any difference in germination 7 days after sowing with seeds soaked in aqueous methyl jasmonate for any concentration other than 10mM 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 concentration of 0.2mM, 0.4mM and 0.8mM. The seeds treated with

methyl jasmonate using conventional polymer-based seed treatment did not show in differences in seed germination two weeks post treatment compared to seeds with a control coating without methyl jasmonate (Fig 1c).

The delay in the germination of seeds soaked in aqueous methyl jasmonate of 0.2mM, 0.4mM, 0.8mM and 1mM was reflected in reduced seedling height after 14 days ($F_{3,20}$= 27.34, p<0.001; Fig 1b). Seedlings emerging from seeds treated with 0.2mM and 0.4mM methyl jasmonate showed the least amount of growth reduction (~12-15%) when compared to control water-soaked seeds, so we used 0.2mM 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 1d).

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% less eggs on 0.2mM methyl jasmonate treated seeds compared to water-soaked seeds ($F_{1,14}$= 14.64, p<0.001; Fig 2a). 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 to water-soaked seeds ($F_{1,18}$ = 12.046, p=0.0027; Fig 2b). Similarly, 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 0.2mM or 0.4mM levels of methyl jasmonate had ~20% fewer eggs deposited on them compared to control-treated seeds (Fig 2 c,d).

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

In our germination and growth bioassay, the seeds soaked in 0.2mM aqueous methyl jasmonate germinated marginally slower than control water-soaked seeds at days 3 and 4 (Fig 1a). 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 to methyl jasmonate treated seeds in our subsequent two-choice assays (Fig 2a,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 3b), indicating that the increased oviposition on the control seeds is not due to 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 0.2mM aqueous methyl jasmonate solution

to freshly hatched first 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 3c).

Methyl jasmonate also reduced adult oviposition preference at cool temperatures:

The percent germination of seeds soaked in 0.2mM methyl jasmonate was 5% lower compared to water-soaked seeds at 15 °C (Fig 4a; $F_{1,17}$ = 4.516, p-value = 0.0485). Three-week-old seedlings that emerged from seeds soaked in 0.2mM methyl jasmonate were also ~25% shorter than the water-soaked seeds (Fig 4b; $F_{1,17}$ = 16.357, p-value < 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 0.2mM methyl jasmonate had nearly 15% fewer eggs deposited on them compared to the number on water-soaked seeds (Fig 4c; $F_{1,14}$ = 6.041, p-value = 0.027).

Materials and Methods

Plant material and insects

We used the corn hybrid variety 410 with a maturity time of 91-days that were obtained from Prairie Seeds (Illinois, USA) and this seed lot was not treated by the seed company. Adult flies and larva of seed corn maggots (*Delia platura*) were collected from the corn fields in Tompkins County, New York and were brought back to the lab. 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 at al., in review). Organic lima bean seeds were used to feed the larvae.

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 lab--scale seed treatment equipment and a commercial film coating polymer, L-650. For the wet soaking method, two hundred corn seeds were soaked in 150ml of 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM methyl jasmonate solution overnight (for 14hrs). 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. Since soaking the corn seeds in 0.2mM 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, Iowa, USA). For 100 grams of corn seeds, 1ml of coating suspension was used which was composed of 100ul of L650 and 900ul of water or water+ methyl jasmonate. Seeds were treated in a Hege 11, seed treater (Wintersteiger, Salt Lake City, UT) for 0.5 mins, 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 0.2mM, 0.4mM or 0.8mM methyl jasmonate solution, respectively, overnight. For the corn variety we used, 1g of corn seed absorbed 0.2895 gram of water overnight. Therefore, in a 0.2mM methyl jasmonate solution, the corn seeds would absorb 12.98 micrograms of methyl jasmonate. Based on the density of methyl jasmonate (0.998g/mL), we used 13.00nl of methyl jasmonate per gram of corn seed to treat the seeds. For our

experiments, we treated 1000 seeds (~220 grams of corn seeds) with 0.2mM, 0.4mM and 0.8mM equivalent amounts of methyl jasmonate and the amount of methyl jasmonate, L650 and water used to coat the seeds are summarized in Table 1.

Seed germination assays:

To determine the effect of methyl jasmonate seed treatment on germination of seeds and seedling growth, we soaked 200 corn seeds in ~~each of~~ 150 ml ~~of~~ aqueous methyl jasmonate solutions of 0.2mM, 0.4mM, 0.8mM, 1mM and 10mM concentration and in water as control. Seeds were soaked in a~~n~~ shaker incubator at 24˚C for 14hrs with constant shaking at 200 rpm. Seed germination cups were set up as shown in F~~f~~igure 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 14hr:10hrs – Light:Dark cycle and a temperature of 24˚C during the light cycle and 16˚C for the dark cycle. Placement of the germination cups ~~with~~ 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, methyl jasmonate concentrations used were 0.2 mM, 0.4 mM 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.

Adult oviposition assays

For oviposition bioassays, 20 corn seeds that were treated with methyl jasmonate (overnight soaked or in polymer-based seed treatment) or ~~untreated~~ control seeds (soaked in water only or coated with polymer only) were placed on sand in 237~~6.59~~ml (8oz) cups. The sand was kept moist by threading a cotton wick into the cup with sand that was wetted with water from a cup below it as demonstrated in the supplemental Figure S1. For two-choice oviposition assays in the lab, thirty male and female flies were selected from the lab colony that were the same age and were at least two weeks post eclosion. The flies were then released in 30X30X30cm 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 X 28 cm mesh cage was placed above the two cups with seeds and thirty flies in each cage. A total of 16 replicates were set up for the two-choice assays in the lab in a walk-in growth chamber. The growth chamber was set at 25˚C with a 14hr:10hr dark cycle for the lab 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, ten 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 day time temperature at the research farm during the course of the experiment was 28˚C in both July and September while 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 was thoroughly mixed in 30% glycerol

solution and then set aside at room temperature for 30 mins. Thereafter, the clear glycerol solution containing the eggs were decanted and sieved through a 1um sieve and the number of eggs were counted. ~~The growth chamber was set at 25˚C with a 14hr:10hr dark cycle for the lab oviposition assays.~~ 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 (14hr:10hrs – Light:Dark cycle).

For the no-choice oviposition assays in the lab, a similar set-up was used as the two-choice assays, except the flies were offered only seeds soaked in 0.2mM 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 thirty 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.

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 0.2mM methyl jasmonate two days prior to 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 thirty flies in each cage. A total of 12 replicates were set up for this bioassay.

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 first instar (two days old) seed corn maggot larva were placed in 8-ounce cups with ten seeds soaked in 0.2mM aqueous methyl jasmonate or in water. The number pupa emerging was counted after two weeks.

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 the Tukey post-hoc test ($\alpha = 0.05$) to compare 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 fit 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 version 4.3.1 (R Core Team 2023).

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 lab-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 0.2mM methyl jasmonate can be ~~an optimal~~used ~~dose~~ 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. Second, we show that seeds that are soaked with 0.2mM 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. Third, 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 since 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 2.5mM methyl jasmonate induce resistance against rice weevil while maintaining growth, and tomato seeds treated with a 0.05 mM - 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 3mM 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 3mM methyl jasmonate[28]. In our system, corn seeds treated with any concentration of methyl jasmonate between 0.2 mM to ~~0.8~~1 mM using an aqueous methyl jasmonate solution or 0.2 mM to 0.8 mM methyl jasmonate using conventional polymer-based seed treatment method showed no difference in percent germination 5 days after sowing. When we look at the daily germination of seeds treated with aqueous methyl jasmonate, we see some delays, but they converged by day 5. 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 the 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 appropriate~~an optimal~~ 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 mM and 0.4 mM methyl jasmonate treatment, the low costs of 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 that 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 by the use of methyl jasmonate concentrations between 0.2mM and 0.8mM in both the delivery methods we used, there was a temporal delay in germination of methyl jasmonate treated seeds. Notably, we see that the germination delay due to methyl jasmonate seed treatment ~~is limited to~~occurs in the first 5 days after seed treatment, which falls within the time frame of our adult oviposition assays. Therefore, we thought it wa~~i~~s possible that the lower oviposition preference of adult flies wa~~i~~s because the flies wea~~r~~e unable to detect any germinating seeds in cups containing methyl jasmonate seeds in the first few days. Prior research has shown that cues from germinating seeds are known to affect adult preference of seed corn maggot flies. Weston and Miller (1989) found that the *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 to other chemical stimulus from the germinating seeds[29]. Likewise, in corn most of the oviposition by *D. platura* happens before the seedlings have emerged from the soil. However~~Moreover~~, o~~O~~ur results with stage matched seeds show that the precise developmental stage in the days immediately after germination is not the determinant of where eggs are laid~~., consistent with Weston and Miller (1989) where they show that volatile~~ cues that are not ~~visual determines~~ from the methyl jasmonate treated corn seeds may be causing reduced oviposition ~~preference in adult flies~~~~not physical or visual cues~~[29]. Additionally, our no-choice oviposition assay also shows that adult oviposition is lower in seeds treated with methyl jasmonate compared to control water-soaked seeds. This further indicates that deterrence of adult flies to oviposit on methyl jasmonate treated seeds may be based on several cues emanating from the germinating seeds.

Methyl jasmonate may alter plant volatiles released from germinating seedlings that contribute to resistance to seed corn maggot. ~~Prior research has shown that cues from germinating seeds are known to affect adult preference of seed corn maggot flies. Weston and Miller (1989) found that the *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 to other chemical stimulus from the germinating seeds[29]. Likewise, in corn most of the oviposition by *D. platura* happens before the seedlings have emerged from the soil.~~ 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. ~~Our results with stage matched seeds show that the precise developmental stage in the days immediately after germination is not the determinant of where eggs are laid, consistent with Weston and Miller (1989) where they show that volatile cues from the methyl jasmonate treated corn seeds may be causing reduced oviposition not physical or visual cues[29].~~ 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 to our room temperature assays, although these experiments were conducted at separate times and so not directly comparable. In addition, 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]. While methyl jasmonate seed treatment is a promising tool for pest management, 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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Figure Legends

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 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 after 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 test. Error bars indicate standard error around the mean.

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 0.2mM methyl jasmonate soaked corn seeds were performed in the laboratory (a) and in the field (b). Two-choice oviposition assays were also performed with corn seeds treated with methyl jasmonate using conventional seed treatment and the coating matrix only (control) or 0.2mM methyl jasmonate equivalent (c) and 0.4mM MeJA equivalent (d). 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 0.2mM methyl jasmonate soaked corn seeds measured in no-choice bioassays. The number of eggs deposited on each seed treatment was fit into a generalized linear mixed model at p=0.05. (b) The percentage of eggs deposited on seeds that were soaked in water or 0.2mM methyl jasmonate and were matched for

growth stage was also measured in a two-choice oviposition assay. (c) Larval performance of *Delia platura* on seeds treated with methyl jasmonate. The percentage of first instar seed corn maggot larva that matured to becoming pupa when fed on corn seeds soaked in water versus 0.2mM methyl jasmonate 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 0.2mM methyl jasmonate solution was measured 21 days after sowing in growth chambers at 15 °C daytime and 5 °C night temperature. Two-choice oviposition choice assays on seeds soaked in 0.2mM methyl jasmonate or water was also 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.

Figure S1. (a) Schematic diagram of set-up showing two-choice oviposition assay in the lab, (b) picture of oviposition cups set-up with corn seeds used for oviposition assays.

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

Concentration of methyl jasmonate	Wt. of methyl jasmonate (mg)	Vol. of methyl jasmonate (µL)	Vol. of L-650 (µL)	Vol. of Water (µL)
0mM (Ffilm coat control)	0	0	220	1980
0.2mM	220*12.98 = 2855.6µg=2.855	2.86	220	1977.14
0.4mM	220*12.98*2 = 5.71	5.72	220	1974.28
0.8mM	220*12.98*4= 11.42	11.44	220	1968.56

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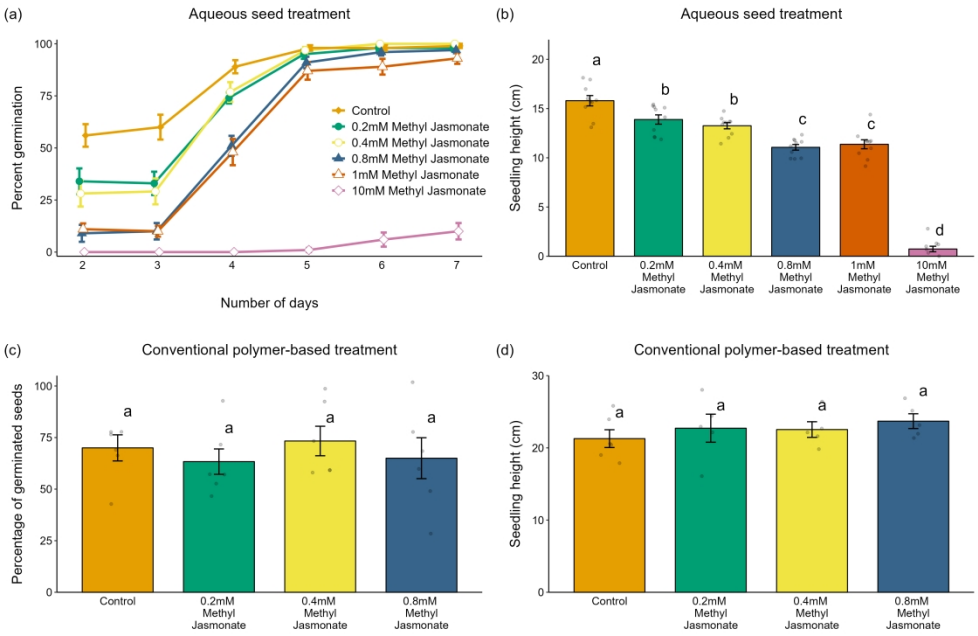

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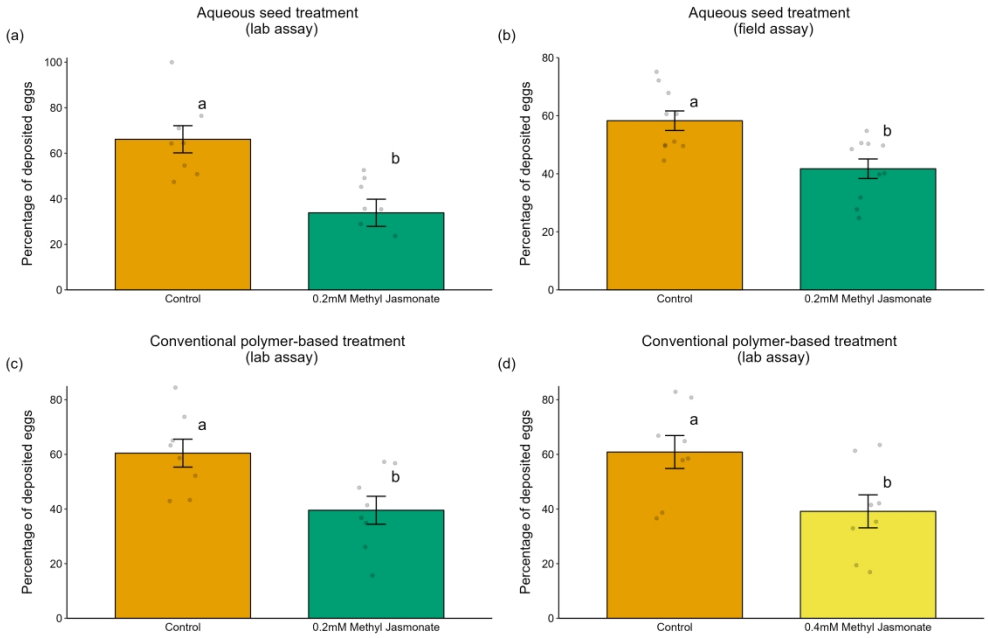

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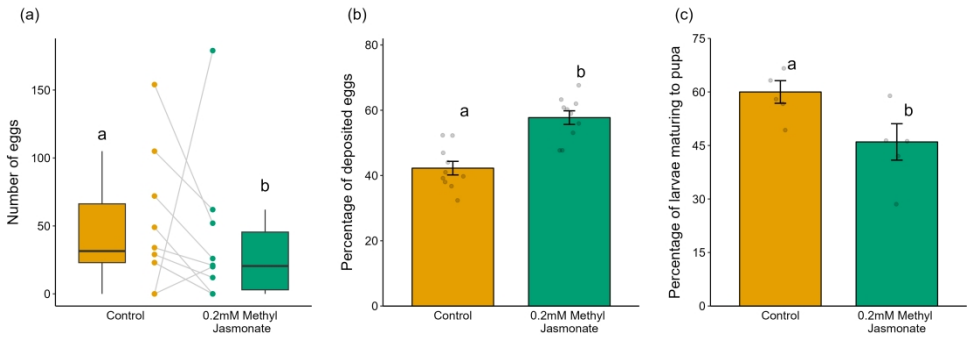

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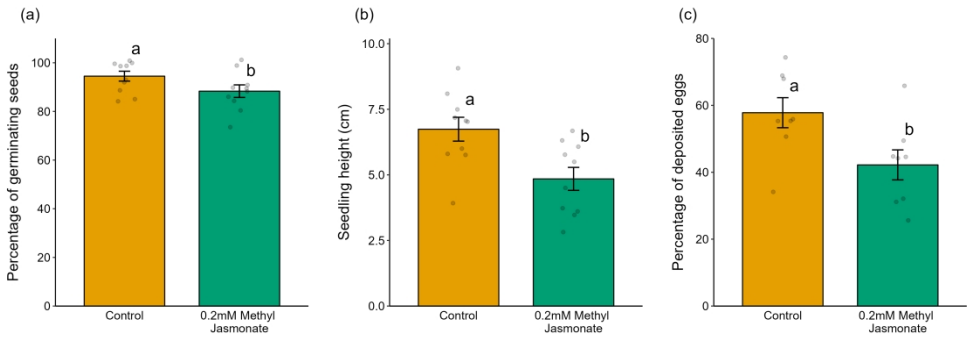

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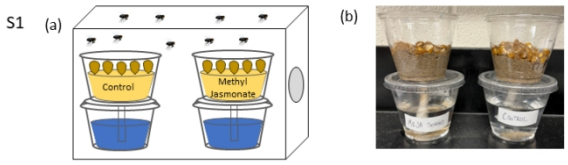
Concentration of methyl jasmonate	Wt. of methyl jasmonate (mg)	Vol. of methyl jasmonate (µL)	Vol. of L-650 (µL)	Vol. of Water (µL)
0mM (Film coat control)	0	0	220	1980
0.2mM	220*12.98 = 2855.6µg=2.855	2.86	220	1977.14
0.4mM	220*12.98*2 = 5.71	5.72	220	1974.28
0.8mM	220*12.98*4= 11.42	11.44	220	1968.56

S1



338x190mm (96 x 96 DPI)