

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

Journal:	<i>Pest Management Science</i>
Manuscript ID	PM-24-0742
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	15-May-2024
Complete List of Authors:	Ray, Swayamjit; Cornell University Department of Entomology Hsu, Gen-Chang; Cornell University Department of Entomology Pappous, Noelle T.; Cornell University Department of Entomology Rooney, Olivia; Cornell University Department of Entomology A.G., Taylor; Cornell University Horticulture Section Thaler, Jennifer; Cornell University Department of Entomology
Key Words:	seed corn maggot, methyl jasmonate, seed treatment, plant resistance, corn
Suggested Editor:	Steve Duke (Editor-in-Chief)

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Swayamjit Ray¹, Gen-Chang Hsu¹, Noelle T. Pappous¹, Olivia Rooney¹, Alan G. Taylor² and Jennifer S. Thaler¹

Author affiliations:

¹Department of Entomology, Cornell University, Ithaca, NY 14850

²School of Integrative Plant Science, Horticulture Section, Cornell AgriTech, Geneva, NY 14456

Abstract

Eliciting host plant resistance using jasmonates is a promising technique that has the potential to protect seeds and seedlings against multiple life-stages of herbivorous insect pests. 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 test if treating corn seeds with the plant hormone 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. 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 seeds soaked with 0.2mM aqueous methyl jasmonate had the same germination rates and marginally lower early seedling growth 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, methyl 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 extensively¹, 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 rice²⁻⁴. 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 seeds⁵. In addition, rice seeds that were soaked in methyl jasmonate or jasmonic acid were resistant to rice weevil damage⁴. 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 pathways⁸. 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 growth⁵.

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 technology¹⁰. The same commercial film coating polymer formulations applied with rotary pan technology can be performed on a lab-scale¹¹.

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 daltons¹². Corn seeds were demonstrated to be permeable only to nonionic compounds, while ionic compounds were restricted by the pericarp-testa¹³. Many species have this differential permeability to nonionic vs ionic compounds termed selective seed coat permeability¹⁴. Jasmonic acid is an ionic compound and therefore would not be able to be taken up into corn seeds. 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 3.8¹². Collectively, the uptake of methyl jasmonate is in the optimal range for diffusion into corn seeds.

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 temperatures¹⁵. 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°C¹⁸. 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 species¹⁹. The larvae of seed corn maggots feed on the cotyledons of the seeds during germination and the roots of emerging seedlings²⁰. 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

1
2
3 methyl jasmonate on corn seed germination and seedling growth after soaking seeds in aqueous methyl
4 jasmonate and by using a conventional polymer-based seed treatment method in the lab. 2) Using doses
5 of both types of seed treatment methods that does not limit germination, we conducted lab and field-
6 based choice and lab-based no-choice oviposition preference assays with adult flies and measured the
7 performance of the seed corn maggot larvae. 3) We tested the effect of methyl jasmonate seed
8 treatment on germination, seedling growth, and fly oviposition preference at cool temperatures.
9
10

11 **Results**

12
13 **Seeds soaked in aqueous methyl jasmonate slowed germination and early seedling growth, but it was**
14 **not affected in seeds treated with methyl jasmonate using conventional polymer-based treatment:**
15

16 We compared the rate of germination in corn seeds treated with 0.2mM, 0.4mM, 0.8mM, 1mM
17 and 10mM of aqueous methyl jasmonate to control seeds. Seeds treated with 0.2mM, 0.4mM, 0.8mM
18 and 1mM methyl jasmonate had no difference in total germination 7 days post treatment compared to
19 controls while seeds treated with 10mM methyl jasmonate showed less than 20% germination (Fig 1a).
20 However, there was a two-day delay in germination post methyl jasmonate treatment with
21 concentrations of 0.2mM, 0.4mM, 0.8mM and 1mM compared to control seeds (Fig 1a). Since we did
22 not observe any difference in germination 7 days after sowing with seeds soaked in aqueous methyl
23 jasmonate for any concentration other than 10mM methyl jasmonate, we measured germination in an
24 endpoint assay at 7 days post sowing with seeds treated with methyl jasmonate using conventional
25 polymer-based seed treatment at concentration of 0.2mM, 0.4mM and 0.8mM. The seeds treated with
26 methyl jasmonate using conventional polymer-based seed treatment did not show in differences in seed
27 germination two weeks post treatment compared to seeds with a control coating without methyl
28 jasmonate (Fig 1c).
29
30
31

32 The delay in the germination of seeds soaked in aqueous methyl jasmonate of 0.2mM, 0.4mM,
33 0.8mM and 1mM was reflected in reduced seedling height after 14 days ($F_{3,20} = 27.34$, $p < 0.001$; Fig 1b).
34 Seedlings emerging from seeds treated with 0.2mM and 0.4mM methyl jasmonate showed the least
35 amount of growth reduction (~12-15%) when compared to control water-soaked seeds, so we used
36 0.2mM methyl jasmonate treated corn seeds to perform our subsequent bioassays. There was no
37 difference in seedling growth when treated with methyl jasmonate using the conventional polymer-
38 based seed treatment method (Fig 1d).
39
40

41 **Methyl jasmonate treated seeds were less preferred by adult flies and had lower larval performance**
42 **compared to controls:**
43

44 When given the choice to oviposit between water-treated and corn seeds soaked in methyl
45 jasmonate in two-choice oviposition assays, the adult flies of seed corn maggots laid ~60% less eggs on
46 0.2mM methyl jasmonate treated seeds compared to water-soaked seeds ($F_{1,14} = 14.64$, $p < 0.001$; Fig 2a).
47 We also performed this two-choice oviposition assay in the field setting where methyl jasmonate
48 treated seeds had ~20% lower oviposition by adult flies compared to water-soaked seeds ($F_{1,18} = 12.046$,
49 $p = 0.0027$; Fig 2b). Similarly, in a two-choice assay with seeds treated with methyl jasmonate using
50 conventional polymer-based seed treatment, we found that seeds that were treated with 0.2mM or
51 0.4mM levels of methyl jasmonate had ~20% fewer eggs deposited on them compared to control-
52 treated seeds (Fig 2 c,d).
53
54
55
56
57
58
59
60

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 ($\chi^2 = 90.5$, $df = 1$, $P < 0.001$; 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; $F_{1,17} = 4.516$, $p\text{-value} = 0.0485$). 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; $F_{1,17} = 16.357$, $p\text{-value} < 0.0001$). 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; $F_{1,14} = 6.041$, $p\text{-value} = 0.027$).

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 source²⁵ (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. The number of seeds that germinated each day was measured daily for 7 days for seeds that were soaked in methyl jasmonate solution. 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 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 were placed on sand in 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. 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. 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. 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” package²⁶. 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” package²⁷. 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 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 germination². Worrall 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* on tomato plants was grown from seeds that were treated with 3mM methyl jasmonate²⁸. In our system, corn seeds treated with any concentration of methyl jasmonate between 0.2 mM to 0.8 mM using an aqueous methyl jasmonate solution or 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 lines¹². Therefore, 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.

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 seeds²⁹. 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 cues²⁹. 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 axis⁵. Subsequent work needs to be done to pinpoint traits involved in resistance in germinating seedlings.

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 temperatures³¹. Tomato seedlings did not respond to wounding at temperatures below 20°C³². However, we could find little other work looking at the ability of plants to induce responses at cool temperatures¹⁸, 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 wheat¹⁷ 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.

Acknowledgements:

We would like to acknowledge Masoume Amirkhani and Michael Loos from Cornell AgriTech for their help in treating the corn seeds with methyl jasmonate using the conventional polymer-based seed treatment method. We would also like to thank Avni Bhalla, Michaela Fitzgerald, Natalie Brennan, Emily Cavanaugh, Laura Martinez, Jules Davis, Matt Barrett and Moshe Zaghoury for reviewing the manuscript. We would like to thank Alexa Stratton and Lilly Elliott-Vidauri help setting up the experiments and insect colony care and Drs. Katja Poveda and Michael Stout for helping us troubleshoot our experiments. This work was funded by New York Ag & Markets.

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 (film coat control)	0	0	220	1980
0.2mM	$220 \times 12.98 = 2855.6 \mu\text{g} = 2.855$	2.86	220	1977.14
0.4mM	$220 \times 12.98 \times 2 = 5.71$	5.72	220	1974.28
0.8mM	$220 \times 12.98 \times 4 = 11.42$	11.44	220	1968.56

References:

1 Howe G and Jander G, Plant immunity to insect herbivores, *Annu Rev Plant Biol* **59**:41–66 (2008).

2 Paudel S, Rajotte EG, and Felton GW, Benefits and costs of tomato seed treatment with plant

3 defense elicitors for insect resistance, *Arthropod Plant Interact* **8**:539–545 (2014).

4 Strapasson P, Pinto-Zevallos DM, Paudel S, Rajotte EG, Felton GW, and Zarbin PHG, Enhancing

5 Plant Resistance at the Seed Stage: Low Concentrations of Methyl Jasmonate Reduce the

6 Performance of the Leaf Miner *Tuta absoluta* but do not Alter the Behavior of its Predator

7 *Chrysoperla externa*, *J Chem Ecol* **40**:1090–1098 (2014).

8 Bhavanam S and Stout M, Seed treatment with jasmonic acid and methyl jasmonate induces

9 resistance to insects but reduces plant growth and yield in rice, *Oryza sativa*, *Front Plant Sci* **12**

10 (2021).

11 Erazo-Garcia MP, Sotelo-Proañño AR, Ramirez-Villacis DX, Garcés-Carrera S, and Leon-Reyes A,

12 Methyl jasmonate-induced resistance to *Delia platura* (Diptera: Anthomyiidae) in *Lupinus*

13 *mutabilis*, *Pest Manag Sci* **77**:5382–5395, John Wiley & Sons, Ltd (2021).

14 Painter RH, Insect resistance in crop plants, *Soil Sci* **72** (1951).

15 Stout MJ, Reevaluating the conceptual framework for applied research on host-plant resistance,

16 *Insect Sci* **20**:263–272, John Wiley & Sons, Ltd (2013).

17 Züst T and Agrawal AA, Mechanisms and evolution of plant resistance to aphids, *Nat Plants*

18 (2016).

19 Kraus EC and Stout MJ, Seed treatment using methyl jasmonate induces resistance to rice water

20 weevil but reduces plant growth in rice, *PLoS One* **14**:e0222800-, Public Library of Science (2019).

21 Afzal I, Javed T, Amirkhani M, and Taylor AG, Modern seed technology: seed coating delivery

22 systems for enhancing seed and crop performance, *Agriculture* **10** (2020).

23 Wilson RG, Orloff SB, and Taylor AG, Evaluation of insecticides and application methods to

24 protect onions from onion maggot, *Delia antiqua*, and seedcorn maggot, *Delia platura*, damage,

25 *Crop Prot* **67**:102–108 (2015).

26 Yang D, Avelar SAG, and Taylor AG, Systemic seed treatment uptake during imbibition by corn

27 and soybean, *Crop Sci* **58**:2063–2070, John Wiley & Sons, Ltd (2018).

28 Dias M, Taylor AG, and Cicero S, Uptake of systemic seed treatments by maize evaluated with

29 fluorescent tracers, *Seed Sci Technol* **42**:101–107 (2014).

30 Salanenka YA and Taylor AG, Seedcoat permeability: uptake and post-germination transport of

31 applied model tracer compounds, *HortScience horts* **46**:622–626, American Society for

32 Horticultural Science, Washington, DC (2011).

33 Havko NE, Das MR, McClain AM, Kapali G, Sharkey TD, and Howe GA, Insect herbivory

34 antagonizes leaf cooling responses to elevated temperature in tomato, *Proc Natl Acad Sci*

- 117:2211–2217, Proceedings of the National Academy of Sciences (2020).
- 16 Tayyab N, Naz R, Yasmin H, Nosheen A, Keyani R, Sajjad M, *et al.*, Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize, *PLoS One* **15**:e0232269-, Public Library of Science (2020).
 - 17 Repkina N, Ignatenko A, Holoptseva E, Misalski Z, Kaszycki P, and Talanova V, Exogenous methyl jasmonate improves cold tolerance with parallel induction of two cold-regulated (COR) genes expression in *Triticum aestivum* L., *Plants* **10** (2021).
 - 18 Whalen R and Harmon JP, Temperature alters the interaction between a herbivore and a resistant host plant, *Arthropod Plant Interact* **9**:233–240 (2015).
 - 19 Gill HK, Goyal G, and Gillett-Kaufman JL, (2013).
 - 20 Guerra PC, Keil CB, Stevenson PC, Mina D, Samaniego S, Peralta E, *et al.*, Larval Performance and Adult Attraction of *Delia platura* (Diptera: Anthomyiidae) in a Native and an Introduced Crop, *J Econ Entomol* **110**:186–191 (2017).
 - 21 van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, and Belzunces LP, Neonicotinoids, bee disorders and the sustainability of pollinator services, *Curr Opin Environ Sustain* **5**:293–305 (2013).
 - 22 Main AR, Webb EB, Goynne KW, and Mengel D, Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis, *Ecol Appl* **28**:1232–1244, John Wiley & Sons, Ltd (2018).
 - 23 Li Y, Miao R, and Khanna M, Neonicotinoids and decline in bird biodiversity in the United States, *Nat Sustain* **3**:1027–1035 (2020).
 - 24 Grout TA, Koenig PA, Kapuvvari JK, and McArt SH, Neonicotinoid insecticides in New York state: economic benefits and risk to pollinators., 18–22, Renewable Natural Resources Foundation (RNRf), Bethesda (2021).
 - 25 Kim TH and Eckenrode CJ, Establishment of a laboratory colony and mass rearing of *Delia florilega* (Diptera: Anthomyiidae), *J Econ Entomol* **76**:1467–1469 (1983).
 - 26 Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, *et al.*, glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling, *R J* **9**:400, Technische Universitaet Wien (2017).
 - 27 Fox J and Weisberg S, *An R Companion to Applied Regression*, SAGE Publications (2019).
 - 28 Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, Taylor JE, *et al.*, Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens, *New Phytol* **193**:770–778, John Wiley & Sons, Ltd (2012).
 - 29 Weston PA and Miller JR, Ovipositional Responses of Seedcorn Maggot, *Delia platura* (Diptera: Anthomyiidae), to Developmental Stages of Lima Bean, *Ann Entomol Soc Am* **82**:387–392 (1989).
 - 30 Smart LE, Martin JL, Limpalaër M, Bruce TJA, and Pickett JA, Responses of Herbivore and Predatory Mites to Tomato Plants Exposed to Jasmonic Acid Seed Treatment, *J Chem Ecol* **39**:1297–1300 (2013).

31 Havko NE, Kapali G, Das MR, and Howe GA, Stimulation of Insect Herbivory by Elevated
Temperature Outweighs Protection by the Jasmonate Pathway, *Plants* **9** (2020).

32 Green TR and Ryan CA, Wound-induced Proteinase Inhibitor in Tomato Leaves: Some Effects of
Light and Temperature on the Wound Response 1, *Plant Physiol* **51**:19–21 (1973).

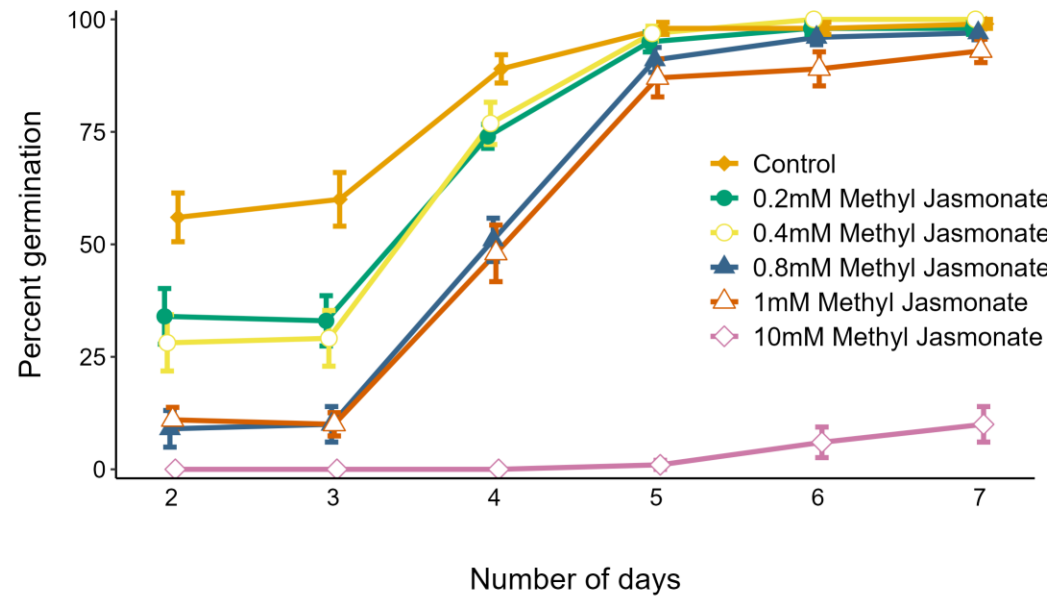
33 Sheteiwy MS, Gong D, Gao Y, Pan R, Hu J, and Guan Y, Priming with methyl jasmonate alleviates
polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile,
Environ Exp Bot **153**:236–248 (2018).

For Peer Review

1

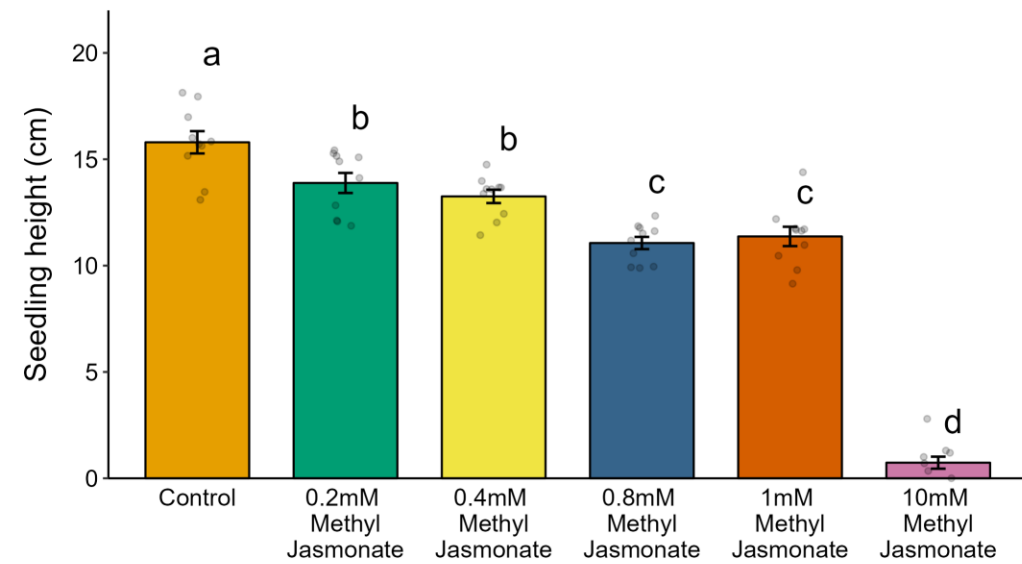
(a)

Aqueous seed treatment



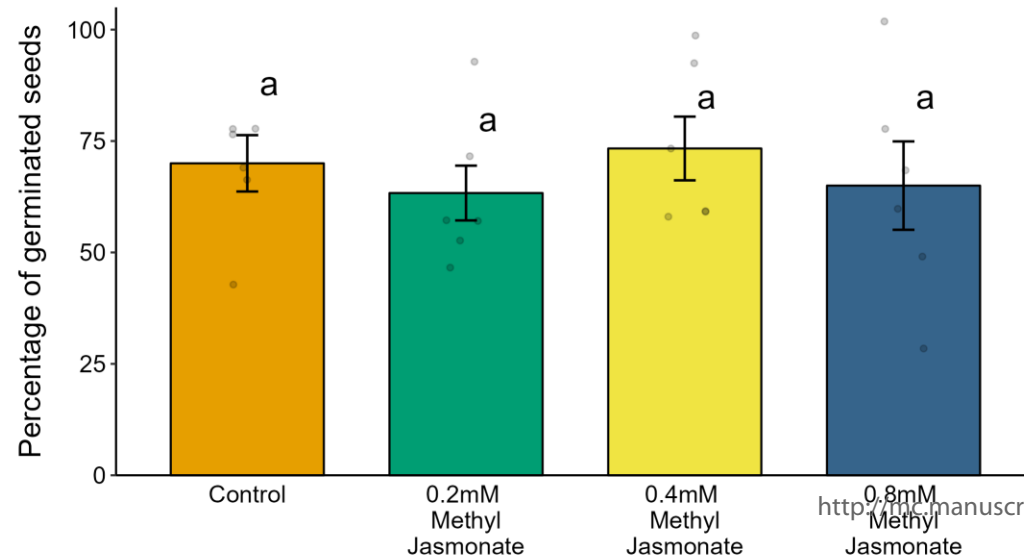
(b)

Aqueous seed treatment



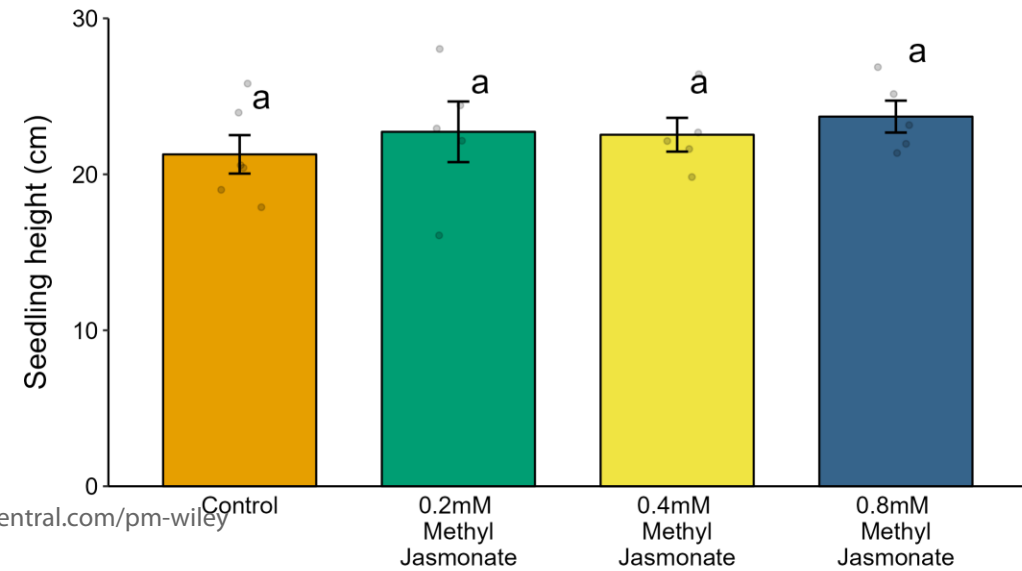
(c)

Conventional polymer-based treatment



(d)

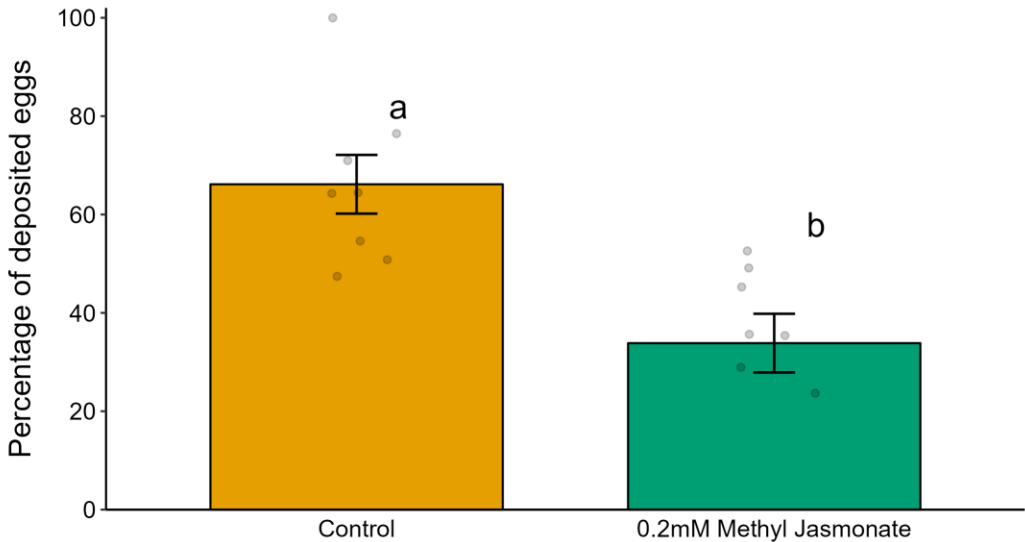
Conventional polymer-based treatment



2

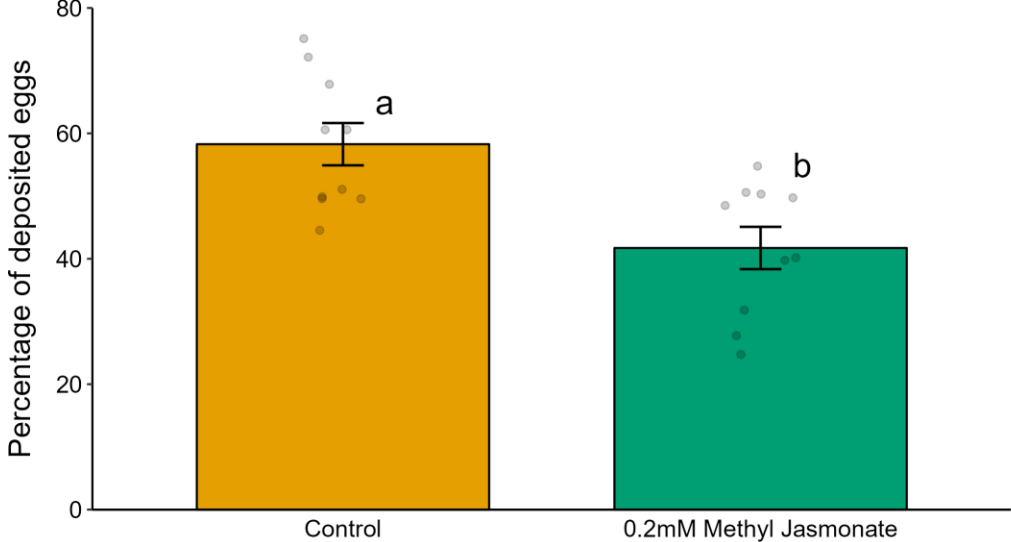
(a)

Aqueous seed treatment
(lab assay)



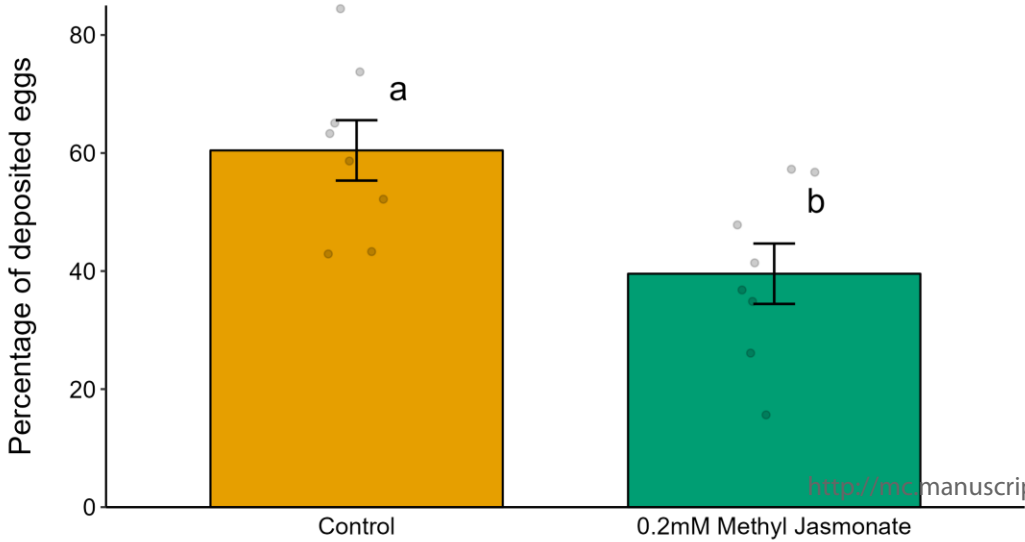
(b)

Aqueous seed treatment
(field assay)



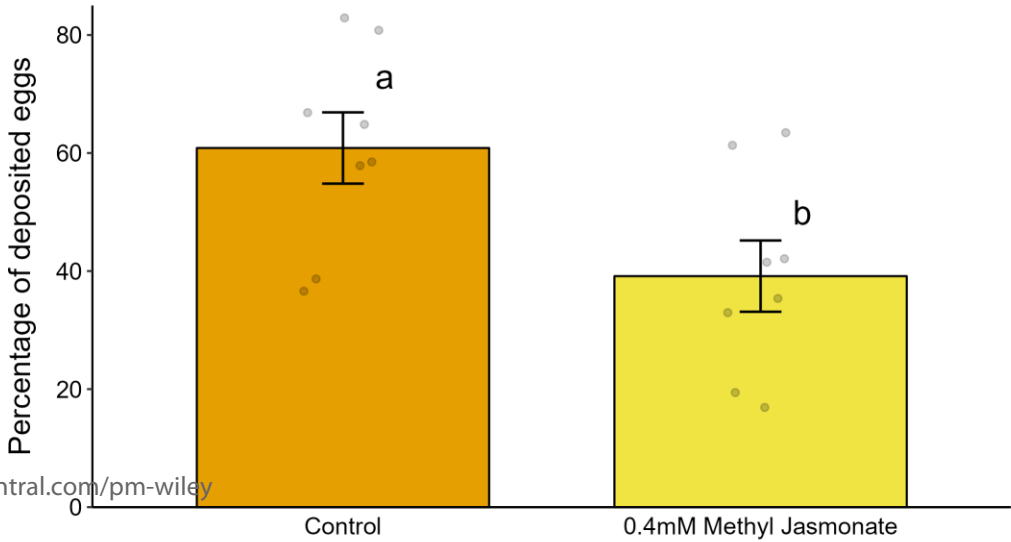
(c)

Conventional polymer-based treatment
(lab assay)

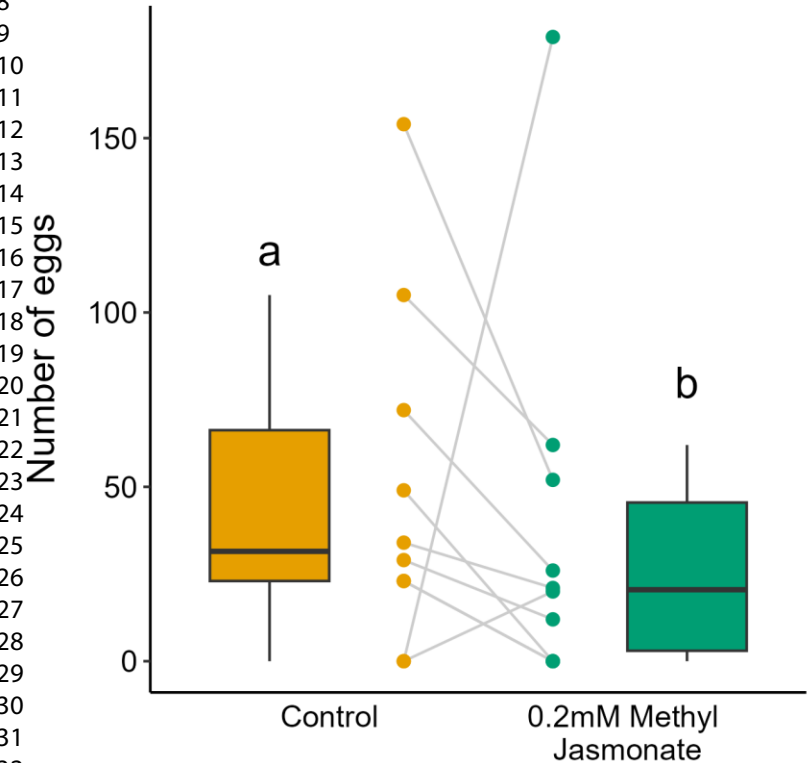


(d)

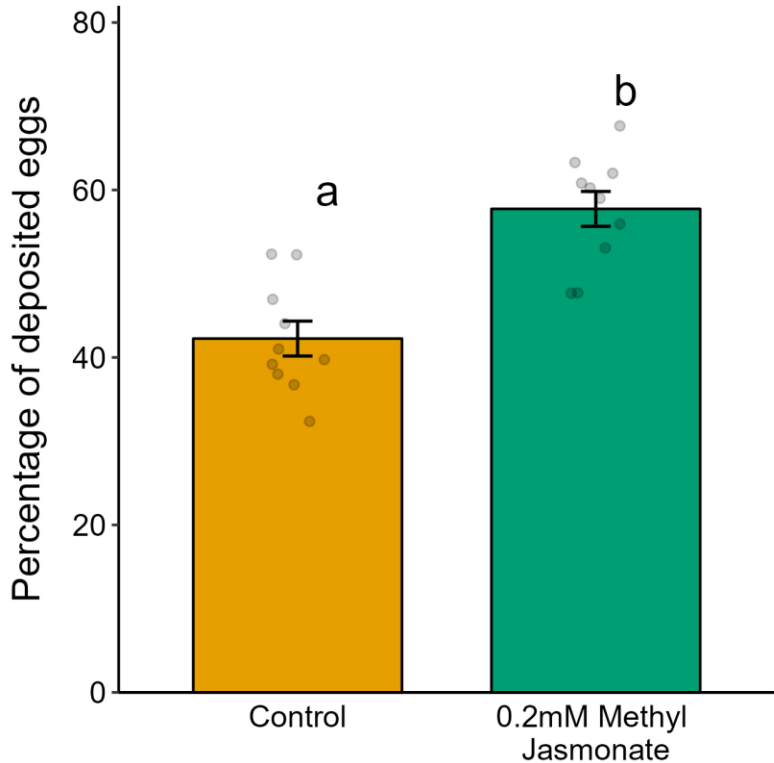
Conventional polymer-based treatment
(lab assay)



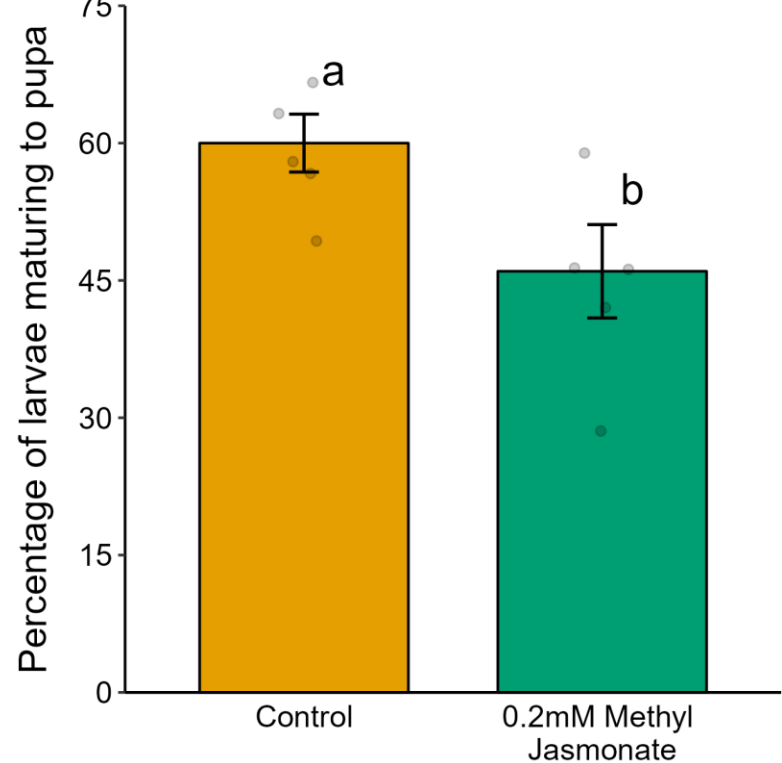
3 (a)



(b)

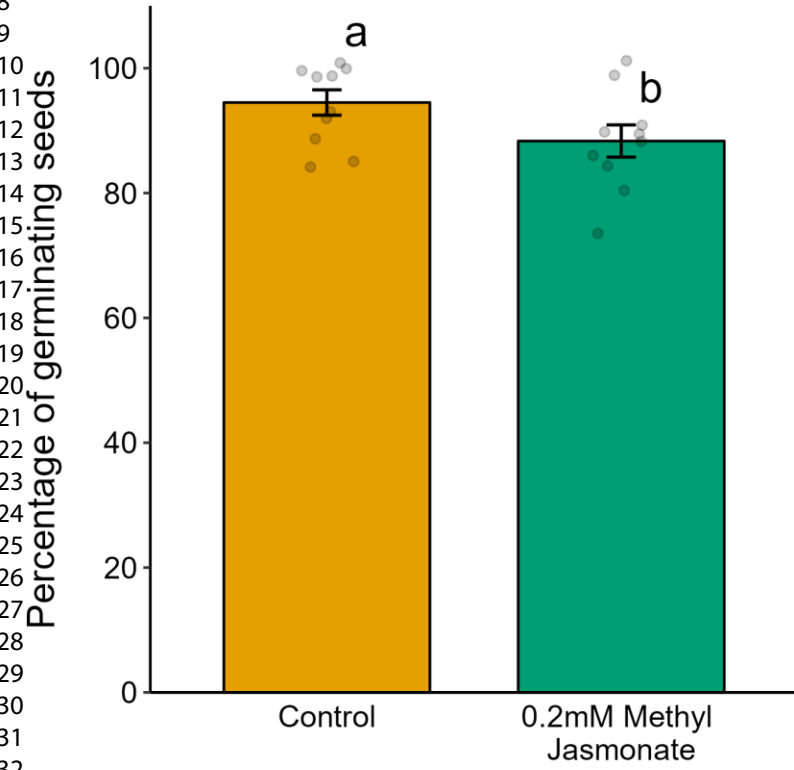


(c)

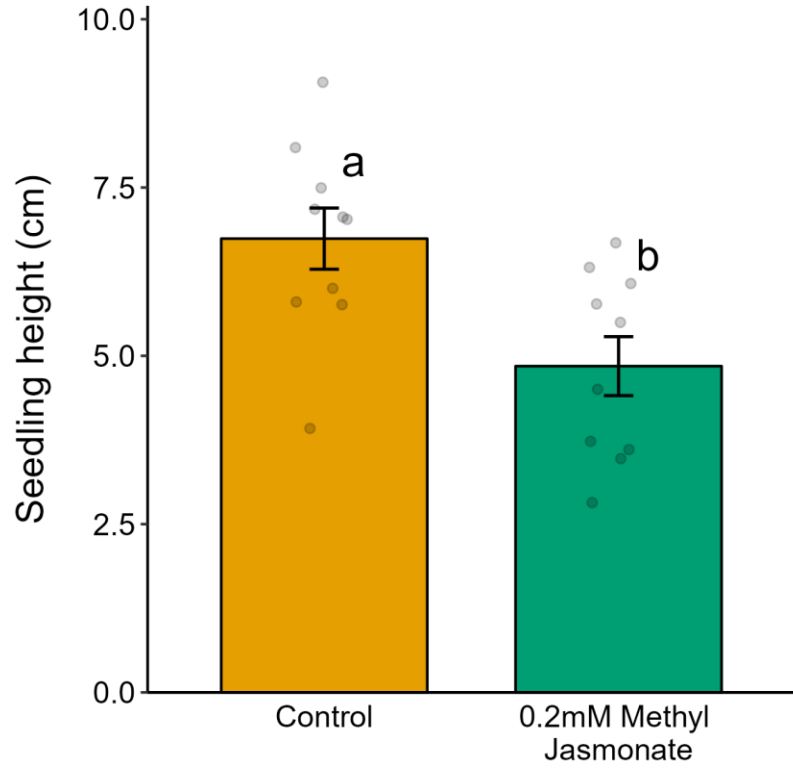


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

4 (a)



(b)



(c)

