The Influence of Seed Quality, Dormancy Breaking, and Water Stress on Germination of Pollinator Species for Biodiversity Enhancement

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Article

The Influence of Seed Quality, Dormancy Breaking, and Water Stress on Germination of Pollinator Species for Biodiversity Enhancement

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Abstract

The lack of protocols for breaking seed dormancy, inconsistent seed quality, and abiotic stress factors such as drought impede large-scale restoration efforts of pollinator seed species. This research explores the germination response, dormancy-breaking techniques, and water stress tolerance in selected pollinator-friendly plant species with characteristics facilitating mechanized rehabilitation protocols and biodiversity enhancement. Furthermore, this study supports utilization of Multiple Seed Pellets (MSP), to facilitate mechanical sowing of pollinator seeds. Forty-two commercial seed lots representing seven plant families with 28 species were evaluated under two alternating temperature regimes (15/25°C and 20/30°C) with and without gibberellic acid (GA_3) pre-treatment. GA_3 significantly enhanced germination percentage, and reduced T_{50} (time to 50% germination) across most seed lots. Overall, germination was higher and faster at 20/30°C than 15/25°C. Six species were further examined for dormancy-breaking responses to GA_3 and kinetin applied in a hydrogen peroxide (H_2O_2), soak. GA_3 + H_2O_2 had the greatest germination compared to other treatments. The effect of water stress on seed germination was assessed in controlled chambers at soil water potentials of −1.08, −0.75, −0.13, and 0 MPa. Milkweed species (*A. incarnata*, *A. syriaca*, and *A. tuberosa*) exhibited consistently high germination across a broad moisture range of -0.75 to 0 MPa. In contrast, *Echinacea purpurea* required high moisture levels (-0.13 to 0 MPa) for optimal germination. *Monarda fistulosa* and *Rudbeckia hirta* showed their best performance under moderate moisture conditions (-0.13 MPa). The use of GA_3 to break physiological seed dormancy offers a promising approach to enhance germination. With the utilization of MSP technology, these strategies provide scalable, practical tools to improve native seed performance and advance pollinator habitat restoration in agroecosystems.

Keywords: gibberellic acid; physiological seed dormancy; MSZP; hydrogen peroxide; drought stress

1. Introduction

Pollination is a fundamental process essential for maintaining biodiversity and sustaining life on Earth. Pollination is a vital life cycle event for most plants and benefits many animal species. Many plant species are unable to complete the pollination process and produce seeds without the help of animal pollinators. Consequently, the stability of natural ecosystems relies heavily on the enduring relationship between plants and pollinators. In this regard, pollinators are recognized as a vital component of global biodiversity [1]. It is estimated that 75% of cultivated plants and 90% of wild flowering plants depend on insects for pollination. Various studies indicate that pollinators provide these services to more than 180,000 plant species and over three-quarters of the crops that feed humanity and are either necessary or play a significant role in enhancing the yield and quality of

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agricultural products [2–5]. Globally, the economic value of pollinator-dependent agriculture is estimated at approximately $577 billion [6].

The rapid decline of pollinator populations worldwide has raised significant concerns [7,8]. Approximately 16% of vertebrate pollinators, such as birds and bats and 40% of invertebrate pollinators, including bees and butterflies are at risk of extinction [9]. Specific declines can be observed clearly in North America, where the eastern population of the monarch butterfly (*Danaus plexippus* L.) has decreased by 80% [10] and several wild bee species have experienced a dramatic decline of 96% since the early 2000s [11]. Despite increasing global recognition of the importance of pollinators, threats such as habitat loss, climate change, and pesticide use continue to endanger these populations. Habitat loss can be attributed to the conversion of grassland and pasture areas into cropland for agricultural production and/or rezoning of land for urban development [12]. Lark *et al.* (2015) estimated that between 2008 and 2012, 5.7 million acres of pastureland in the United States were converted into cropland, accounting for 77% of total agricultural land conversion during that period [13]. Additionally, the shift towards glyphosate-tolerant (Roundup Ready) and other herbicide resistance traits since the late 1990s has accelerated the decline of pollinator food and nectar plants in fields and is one of the primary causes of the decline in monarch butterfly populations. These combined factors have diminished most breeding grounds of monarch butterflies in the eastern part of North America [14].

Seed coating technologies are utilized to improve seed germination and seedling emergence, facilitate sowing of irregular shaped seeds, serve as a delivery of plant protectants, and provide species identification with the addition of colorants to the coated seeds [15]. Of particular interest to pollinator biodiversity is multi-seed pellets that involves combining multiple seeds into a single unit propagule. Early studies in the Taylor lab, demonstrated agglomeration seed technology as an effective delivery system for multiple tomato and lettuce seeds [16]. The multiple seed delivery system was developed using a molding, pelleting technology that used conventional seed coating fillers and binders [15]. Compression forces used to produce the agglomerated seed pellets were notably important, those exceeding 1 kg for lettuce or 3 kg for tomato were detrimental to the percent and rate of germination [16]. Similar findings were reported by Amirkhani *et al*. (2019), who demonstrated that increasing the binder proportion enhanced coating strength but reduced the percent germination, delayed germination and reduced uniformity [17]. A new pelleting technology was patented by Loos *et al,* (2024) and further reported by Westbrook *et al*. (2023) on the development of Multi-Seed Zea Pellets (MSZP) [18,19]. This research introduced a novel pelleting technique intended to produce MSZPs that resemble the size, shape, and density of *Zea mays* (corn) seeds. Unlike other conventional seed pelleting technologies, this method combined multiple seeds of a single species into a pellet that visually resembled a field crop seed. With the evolution of MSZP technology, now termed Multiple Seed Pellets, MSP (https://kannargroup.com/products/msp/) were designed to be sown using conventional corn or other field planting equipment, making them suitable for mechanized sowing. However, to exploit the full potential of a multi-seed pellet technology requires starting with high quality seed lots with good germination. These pollinator seed species need to germinate in the field under a wide range of soil moisture conditions.

Direct seeded, large-scale habitat restoration requires knowledge of both seed dormancy and seed quality. Present research indicates that protocols for breaking seed dormancy and the germination of plant species used by pollinators have not been fully developed, existing protocols may be inconsistent or contradictory, and there are limited number of studies on this topic [20]. In addition, significant gaps remain in current efforts to scale up pollinator habitat restoration using direct seeding. First, many native pollinator-friendly species exhibit physiological dormancy [21], yet standardized, species-specific dormancy-breaking protocols are lacking. Second, the interactions between plant hormones (e.g., GA_3 and kinetin), oxidative cues (H_2O_2), and seed enhancement additives (surfactants) have not been systematically evaluated across diverse species. Third, species-specific responses to soil moisture stress during germination are poorly understood, limiting predictive capacity for field establishment success.

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This study was designed to address these three critical gaps:

1- The dormancy-breaking potential of GA_3 and kinetin across multiple pollinator-relevant species.

2- The role of oxidative signals (via H_2O_2) and surfactants in enhancing germination uniformity and speed.

3- The influence of water availability on germination behavior to guide restoration strategies under variable field conditions.

By combining hormonal treatments, oxidative signaling, and surfactants, this study introduces an improved seed enhancement strategy specifically designed to support the propagation and establishment of native pollinator-friendly plant species. An understanding of drought stress on germination of selected pollinator seed species establishes limiting factors on successful stand establishment.

2. Methods and Materials

2.1. Selection of Pollinator Plant Species and Acquisition of Materials

The species selected for this study were chosen from pollinator wildflowers identified by the Natural Resources Conservation Service (NRCS) of the United States Department of Agriculture (USDA) [22]. Seed samples were acquired from Shooting Star Native Seeds, Spring Grove, MN and seed lots were donated by Ernst Conservation Seeds**,** Meadville, PA. Seeds of all lots were stored at 4°C and 30% relative humidity until germination tests were performed described in section 2.2. The selected plant species are particularly vital for the monarch butterfly (*Danaus plexippus* L.), honeybees, and other pollinator insects.

Forty-two commercial seed lots were obtained from the two seed companies representing 28 different species and 7 plant families, each with its inherent germination and dormancy. Information provided on the seed label of each seed lot was presented on germination percentage, dormancy percentage, total viable seeds, PLS (pure live seed), and TSW (1000 seed weight), (Table A1).

2.2. Effect of GA_3 Application and Two Test Regimes on Germination and Dormancy of 42 Seed Lots

Two laboratory germination test regimes were conducted to determine the germination potential of 42 seed lots. Germination test methods were the same for all seed lots, utilizing two temperature cycles. Germination chambers (Percival Scientific Inc., model I-36LL, Perry, IA, USA) were set to 20/30°C (16 hours cool/dark, 8 hours warm/light), and 15/25°C (10 hours cool/dark, 14 hours warm/light), light provided at 3600 lux. Tests were conducted for 14 days and counts were taken daily. All germination tests were conducted in accordance with the International Rules for Seed Testing (ISTA) [23]. The two temperature regimes (15/25°C and 20/30°C) were selected: the alternating 15/25°C was adopted to simulate soil temperatures in spring sowing in the temperate region [24], while the alternating 20/30˚C is commonly used in seed testing (ISTA) [23].

Radicle emergence of 2 mm was the criterion for a positive germination score and was expressed in percentages (%). Six selected seed lots, marked with (•) in Table A1, were identified for further study. For each temperature regime, seeds were tested with and without a gibberellic acid (GA_3) pretreatment. GA_3, CAS # 77-06-05 was purchased from Gold Biotechnology Inc., St. Louis, MO, USA. This GA_3 formulation was termed Quick-Dissolve™ and mixed readily in deionized water. A 1.3 mM (500 ppm) solution was prepared and the pH of the gibberellic acid solution was adjusted to pH 6 using 50 mM KOH (Mallinckrodt Inc, Paris, KY, USA). The pH adjustment of the GA_3 solution to 6.0 using 50 mM KOH ensured chemical stability and treatment consistency, thereby facilitating a standardized assessment of GA_3 efficacy across different temperature regimes. To apply GA_3, seed samples from each lot were placed on blue blotters (Anchor Paper Co., St. Paul, Minnesota, USA) saturated with solution, placed inside a germination chamber (Percival Scientific Inc., model I-30BL, Perry, IA, USA) set at 15°C constant and with light at 4500 lux for 24 hours, then thoroughly rinsed

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with deionized water, and dried overnight in ambient environment. For each lot, a minimum of two replicates of 25 seeds were planted with nontreated seeds and GA_3 pretreated seeds.

The effect of GA_3 was analyzed for paired comparisons at 4, 7, and 14 days for germination studies at 20/30 and 15/25 °C for the 42 lots. For statistical analysis of data, variance analysis (ANOVA) was performed using SPSS 21 software, and grouped using the t-test. Comparisons where GA_3 treatment was effective are marked with an asterisk * and indicate significantly higher germination compared to the control group.

Using daily counts, the T_{50} (time required for 50% of the seed lots to germinate) was determined using the following formula [25,26].

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)}$$

T_{50}: Time required for 50% germination (days)
t_i: Observation Day before 50% germination
N: Total number of germinated seeds
n_i and n_j: Total number of germinated seeds at times t_i and t_j, respectively

T_{50} values were only calculated for non-GA_3 treated seed lots with positive and increasing counts at least on days 7 and 14. T_{50} values were shown as N/A (nonapplicable) for both -GA_3 and +GA_3 comparison for those seed lots not satisfying the positive and increasing count criteria.

2.3. Effect of GA_3 and Kinetin Seed Soaks on Breaking Dormancy of 5 Pollinator Species

The effects of GA_3 and kinetin (K) applied as a seed soak were tested singly and in combination on breaking seed dormancy on *Asclepias incarnata* (lot JG040418), *Asclepias syriaca* (lot ASCSYR602A), *Asclepias tuberosa* (lot ASCTUB670B), *Echinacea purpurea* (lot ECHPUR503A), *Monarda fistulosa* (lot MONFIS463A), and *Rudbeckia hirta* (lot RUDHIR463B). The concentration of GA_3 and K was 1 mM and 0.05 mM, respectively. The GA_3 material and method was described in 2.2. Kinetin, CAS # 525-70-1, was obtained from Gold Biotechnology Inc., St. Louis, MO, USA. K was dissolved in 50 mM KOH to prepare an aqueous solution, and this same solution was adjusted to pH 6.0. Similarly, the pH of the GA_3 solution was adjusted to 6.0 using 50 mM KOH to ensure chemical consistency across treatments. Each solution contained 0.3% hydrogen peroxide (H_2O_2). A water soak check and non-soaked control was included. Seeds were soaked in solutions of 20 times their seed weight of each species for 24 hours under temperature conditions described in section 2.2. After the 24-hour soak, treated seeds were dried overnight. Treated seeds were placed on moistened 10 x 10 cm blue blotter paper and subjected to germination tests at alternating temperatures of 20/30ºC as described in 2.2. Each treatment had four replicates, with 25 seeds per replicate. Daily counts were recorded, and results were reported for days 4, 7, and 14. At the end of day 14, moldy seeds in each seed lot were also counted. All results were reported as percentages. The study was conducted with a randomized complete block design. Percent values were subjected to arc-sine transformation prior to statistical analysis. Results between treatments on days 4, 7, and 14 were analyzed using ANOVA and groupings were established according to Duncan's test.

2.4. Effect of Non-Ionic Surfactants Seed Soaks Applied with Two GA_3 Concentrations on Breaking Dormancy of Asclepias Syriaca

Treatments were applied to *Asclepias syriaca* (common milkweed, lot ASCSYR602A) seeds to determine the interaction of gibberellic acid with non-ionic surfactants and to evaluate their effect on seed germination/dormancy. The concentrations of components of the seed soak: 0.3 mM and 1 mM GA_3, and 0.1% Tween 20, Tween 80, and K-wet 20, and 0.01% for Silwet 408. Tween 20 and 80 were purchased from Sigma-Aldrich, St. Louis, MO, US, K-wet 20 and Silwet 408 were donated by Kannar Earth Science, Lawrenceville, GA, and Momentive Performance Materials Inc., Niskayuna, NY, US, respectively. Each solution contained 0.3% hydrogen peroxide (H_2O_2). A water soak check and non-

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soaked control was included. To each solution, 50 mM potassium hydroxide (KOH) was used to adjust the pH of all solutions to 6. Seed soaks were conducted as described in section 2.2.

Germination tests were conducted using four replicates of 25 seeds. The seeds were sown on 10x10 cm blue blotter paper moistened with each solution, placed in a germination chamber set at 20/30°C with 3600 lux lighting and an 8-hour photoperiod. Counts were taken daily, and blotters were re-moistened with distilled water as needed. Results were evaluated on days 4, 7, and 14, and the effect of non-ionic surfactants on preventing mold formation on seeds was also examined at the end of the experiment. Data obtained were adjusted using arc-sine transformation before statistical analysis. To determine differences between treatments, germination percentages on days 4, 7, and 14 were analyzed using ANOVA. Group differences were evaluated using Duncan's test.

2.5. The Effect of Water Stress on Germination of 6 Pollinator Seed Species

This study investigated the effects of water stress on seed germination of the 6 seed lots described in section 2.3 and all seed lots pretreated with 1.0 mM GA_3 only as described in the same section. To achieve a known and constant soil media moisture content, a particulate sized, proprietary montmorillonite clay was donated by Oil-Dri Corporation of America, Chicago, IL, USA and used as the germination media in enclosed plastic containers with lids (2.5 x 15 x 24 cm). The media moisture contents were adjusted to 32%, 35%, 42%, 90% and 92% with resulting soil water potential of -1.08, -0.75, -0.13 and 0 MPa, respectively as measured by a WP4 Dewpoint, PotentiaMeter (MeterGroup, Pullman, WA, USA). The germination containers with media and seeds were maintained at alternating 20/30 ˚C, with germination counts recorded daily for 14 days. There were 4 replicates of 25 seeds for each species at each moisture level. This experimental design allowed for the assessment of a controlled water potential on germination rates across multiple pollinator-friendly plant species, providing valuable data for conservation.

3. Results

3.1. Effect of GA_3 Application and Two Test Regimes on Germination and Dormancy of 42 Seed Lots

A wide range of germination values were recorded from the two test regimes with data recorded at 4, 7 and 14 days from the 42 lots (Table B1). Differences in germination between seed lots may be attributed to differences in seed quality and dormancy among lots. Each seed lot may carry distinct genetic characteristics that affect germination [27,28]. Previous studies have similarly noted that GA_3 can have inconsistent effects across species and even within genera [29,30].

The T_{50} values presented in Table B1 offer important insights into the speed of germination. As expected, lower T_{50} values indicate faster germination. In this study, GA_3 pretreatment consistently reduced the T_{50} values compared to nontreated seeds, demonstrating that GA_3 not only enhanced final germination by overcoming dormancy, but also accelerated the germination process. For instance, in *Echinacea purpurea* (lot PCF311210), the T_{50} at 20/30°C decreased from 3.7 to 2.8 days with GA_3 treatment, while final germination increased from 82% to 98%. At 15/25°C, the T_{50} dropped from 4.6 to 3.2 days, with germination improving from 86% to 90% (Table B1).

Utilizing the combined data, results indicate that GA_3 significantly increased the rates and totals of germination. For most species, seeds treated with GA_3 had the highest germination rate at the 20/30°C temperature regime, resulting in 59% on the 14th day, versus the control at 49%. The cooler 15/25°C temperatures yielded germination of 57% for seeds treated with GA_3 and 47% for those without GA_3 (Figure 1a). Evidence from the T_{50} values supports that GA_3 pretreatment can also accelerated germination (Figure 1b). Under the 20/30°C temperature regime, the T_{50} was reduced from 4.7 to 3.9 days with GA_3-treated seeds. Similarly, under the 15/25°C regime, GA_3 reduced the T_{50} from 5.1 days to 4.4 days. These findings clearly indicate that GA_3 pretreatment increases germination totals and enables seeds to germinate in less time. In conclusion, the combined use of GA_3 pretreatment and appropriate temperature regimes can be considered an important strategy for optimizing the germination processes of pollinator plant species.

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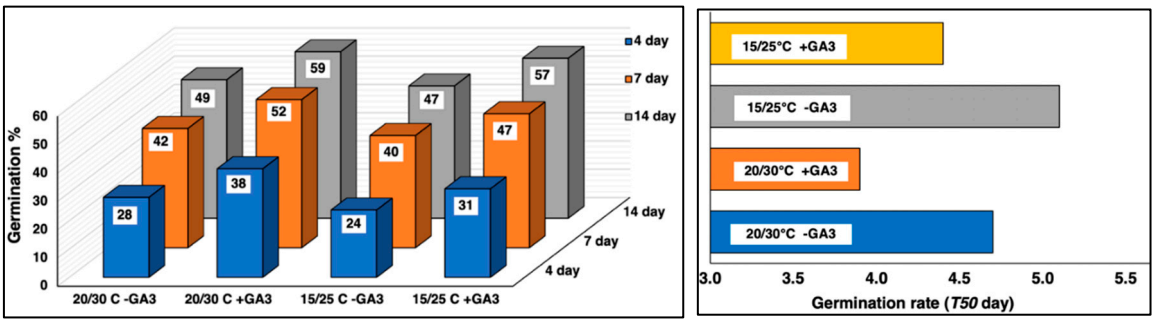

(a) **(b)**

Figure 1. (a) Mean germination and **(b)** T50 of 42 seed lots at two temperatures (20/30C and 15/25C), without and with GA_3.

The associations between seed lots were determined by making a correlation analysis among the obtained values. Table 2 shows the correlation coefficients between the germination percentages of the samples in different conditions, germination rate (T_{50}), labeled germination and laboratory germination. The coefficients of correlation in the germination percentages after 4 and 14-day germination tests varied from 0.83 to 0.87 with significance at a probability level of $p<0.001$. This shows a significant positive relationship between the germination percentages from the early count at 4-day with the final count at 14-day. The negative coefficients of correlation, in 4-day germination percentage versus T_{50}, ranged from -0.52 to -0.68 all at the same level of probability, $p<0.001$, showing higher percentage germination was associated with faster germination rate. Similar trends were measured with 14-day germination percentages and T_{50} The positive values of the correlation coefficient between labeled germination and laboratory germination ranged from 0.44 to 0.61 at $p<0.01$ or $p<0.001$, showing that there was a moderate positive relationship between information on the seed tag and experimental germination data either with or without GA_3.

Table 2. Correlation coefficients of germination percentage with germination rate (T_{50}), and Label and Lab germination.

Correlation coefficients	20/30°C -GA_3	20/30°C +GA_3	15/25°C -GA_3	15/25°C +GA_3
4-day vs 14-day	0.86***	0.87***	0.83***	0.85***
4-day vs T50	-0.52***	-0.68***	-0.53***	-0.63***
14-day vs T50	-0.35*	-0.54***	-0.38*	-0.50***
Label germ vs Lab germ (14-day)	0.61***	0.57***	0.58***	0.44**

Significance: * 0.05, ** 0.01, ***0.001.

Figure 2 presents the germination progress of six pollinator species over a 14-day period under two temperature regimes (20/30°C and 15/25°C), with and without GA_3 pretreatment. In general, seeds treated with GA_3 germinated faster and reached higher final germination percentages than nontreated seeds. At the warmer temperature (20/30°C), this effect was especially clear. For example, *Asclepias incarnata* started germinating just three days after sowing when treated with GA_3 and reached over 85% germination by day 10, while the nontreated seeds germinated more slowly and only reached about 78% by day 14. Similar trends were seen in *Asclepias tuberosa*, *Monarda fistulosa*, *Echinacea purpurea* and *Rudbeckia hirta*, where GA_3 helped seeds germinate more quickly and more evenly. Even under the cooler 15/25°C temperature, GA_3 still had a positive effect, although the germination was slower compared to 20/30°C. For example, *Monarda fistulosa* seeds treated with GA_3 reached about 85% germination, compared to less than 65% in untreated seeds at the same temperature (Figure 2). These results clearly show that GA_3 helps break dormancy and speeds up germination across different species. Especially under warmer conditions, GA_3 treatment leads to

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earlier and more uniform germination, which is important for successful seedling establishment in the field.

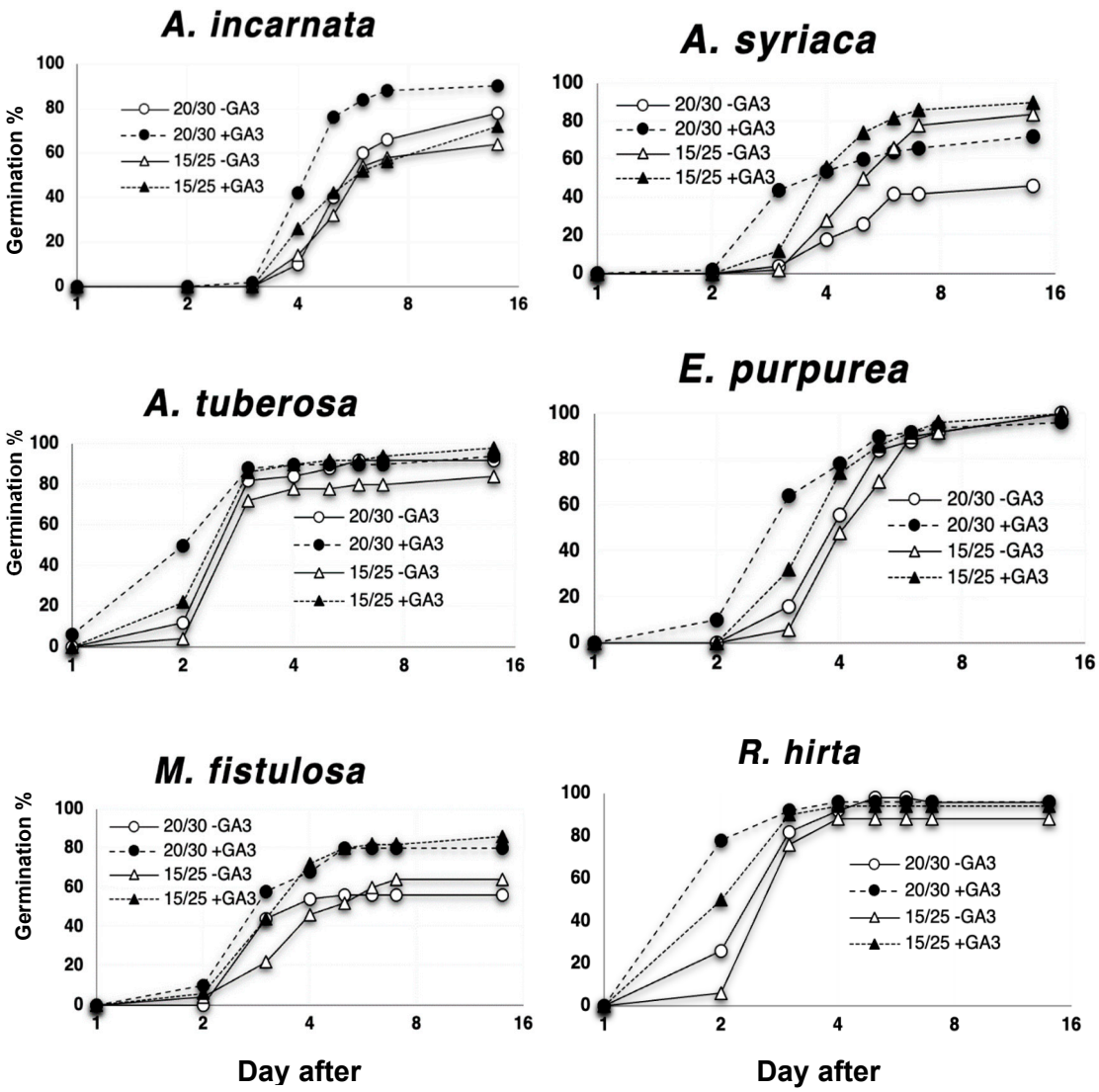

Figure 2. Germination time course of six pollinator species at two temperatures (20/30°C and 15/25°C both with light) and pretreated without or with GA_3 at 1.3 mM (500 ppm) for 24 hours at 15°C with light.

3.2. Effect of GA_3 and Kinetin Seed Soaks on Breaking Dormancy of 5 Pollinator Species

The effects of GA_3 and kinetin applications on germination and mold formation during the study were evaluated on days 4, 7, and 14 for the five selected species (*Asclepias incarnata, Asclepias tuberosa, Echinacea purpurea, Rudbeckia hirta,* and *Monarda fistulosa*) with results presented in Table 3a. In *Asclepias incarnata*, the combination of GA_3 and H_2O_2 resulted in the highest germination rate (96%) by day 14, while the lowest mold formation (4%) was obtained from the hydrogen peroxide treatment. Similarly, in *Asclepias tuberosa*, GA_3 and H_2O_2 applications increased germination percentage (93%) with reduced mold formation compared to the control. In *Echinacea purpurea*, GA_3, H_2O_2, and the K+H_2O_2 combination treatments increased germination rates to 98% and 99%, respectively. No mold formation was observed in any of the *Echinacea* treatments, including the control group.

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Table 3. a. Effects of GA_3 and Kinetin treatments on germination percentage and mold growth at 4, 7, and 14 days for five pollinator species.

Treatments	4	7	14	Mold
	***Asclepias incarnata* (Swamp Milkweed)**			
Control	42 ± 3.5 b	59 ± 3.4 c	73 ± 4.7 c	12 ± 2.8 c
H_2O	58 ± 12.1 ab	74 ± 5.3 b	90 ± 1.2 b	4 ± 2.8 ab
H_2O_2	61 ± 3.0 ab	77 ± 1.0 ab	88 ± 3.7 b	1 ± 1.0 a
GA_3+H_2O_2	67 ± 7.0 a	85 ± 3.4 a	96 ± 1.6 a	9 ± 3.4 bc
K+H_2O_2	66 ± 2.6 a	80 ± 1.6 ab	91 ± 2.5 ab	10 ± 3.5 bc
GA_3+K+H_2O_2	69 ± 2.5 a	81 ± 3.0 ab	88 ± 1.6 b	7 ± 1.9 bc
	Asclepias tuberosa (Butterfly Milkweed)			
Control	53 ± 3.4 b	79 ± 2.5 a	80 ± 1.6 b	14 ± 3.8 bc
H_2O	78 ± 3.5 a	80 ± 4.3 a	81 ± 3.4 b	6 ± 2.6 a
H_2O_2	78 ± 3.5 a	80 ± 4.3 a	87 ± 1.9 ab	9 ± 1.0 abc
GA_3+H_2O_2	86 ± 3.5 a	89 ± 3.0 a	93 ± 1.9 a	12 ± 1.6 bc
K+H_2O_2	83 ± 5.3 a	84 ± 5.6 a	89 ± 1.9 ab	6 ± 1.2 a
GA_3+K+H_2O_2	86 ± 5.0 a	88 ± 4.3 a	88 ± 4.3 ab	15 ± 1.0c
	Echinacea purpurea (Purple Coneflower)			
Control	61 ± 1.9 a	84 ± 1.6 b	88 ± 1.6 c	0
H_2O	75 ± 4.4 a	86 ± 4.8 ab	89 ± 4.4 bc	0
H_2O_2	67 ± 6.4 a	94 ± 1.2 ab	96 ± 2.3 ab	0
GA_3+H_2O_2	78 ± 5.3 a	95 ± 1.9 a	98 ± 2.0 a	0
K+H_2O_2	78 ± 5.3 a	92 ± 4.3 ab	99 ± 1.0 a	0
GA_3+K+H_2O_2	77 ± 8.0 a	95 ± 2.5 a	95 ± 2.5 abc	0
	Rudbeckia hirta (Black-eyed Susan)			
Control	86 ± 2.0 ab	91 ± 1.9 a	92 ± 1.6 b	7 ± 3.0 a
H_2O	90 ± 2.0 ab	93 ± 1.0 a	93 ± 1.0 ab	6 ± 1.2 a
H_2O_2	90 ± 2.6 ab	93 ± 3.4 a	95 ± 1.9 ab	10 ± 1.2 a
GA_3+H_2O_2	94 ± 2.6 a	95 ± 1.9 a	98 ± 1.2 a	7 ± 1.9 a
K+H_2O_2	94 ± 2.6 a	94 ± 3.8 a	96 ± 2.3 ab	5 ± 3.0 a
GA_3+K+H_2O_2	82 ± 3.8 b	86 ± 2.6 a	94 ± 1.2 ab	10 ± 1.2 a
	Monarda fistulosa (Wild Bergamot)			
Control	68 ± 2.8 a	69 ± 1.9 b	70 ± 1.2 c	12 ± 1.6 a
H_2O	69 ± 4.4 a	75 ± 1.9 ab	78 ± 2.0 bc	10 ± 1.2 a
H_2O_2	80 ± 5.7 a	82 ± 4.8 a	84 ± 4.3 ab	7 ± 3.0 a
GA_3+H_2O_2	69 ± 5.3 a	71 ± 5.0 ab	88 ± 2.8 a	13 ± 3.8 a
K+H_2O_2	70 ± 5.3 a	76 ± 4.9 ab	80 ± 4.6 abc	12 ± 1.6 a
GA_3+K+H_2O_2	78 ± 3.8 a	83 ± 1.0 a	85 ± 1.9 ab	12 ± 1.6 a

Table 3b presents the main effects of species and treatment type on germination percentages at days 4, 7, and 14. Across species, *Rudbeckia hirta* exhibited the highest germination percentages at day 4 compared to other species, while *Rudbeckia hirta* and *Echinacea purpurea* both had high percentage germination (>90%) at days 7 and 14. In contrast, the lowest percentage germination was determined for *Asclepias incarnata* at day 4 and *Monarda fistulosa* at day14. Among the treatment groups, GA_3 + H_2O_2 consistently resulted in the highest germination percentages (79% on day 4, 87% on day 7, and 95% on day 14), significantly outperforming the control group. Other combination treatments (e.g., K + H_2O_2 and GA_3 + K + H_2O_2) also improved germination compared to control, though to a lesser extent. These findings confirm the statistically significant ($p < 0.01$) contribution of combined growth regulator and hydrogen peroxide treatments in enhancing germination performance across multiple species and time points.

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Table 3. b. Species (5 levels) and Seed treatment (6 levels) effect on 4, 7 and 14 DAP germination %.

Species	% Germination			Seed treatment	% Germination		
	4	7	14		4	7	14
Asclepias incarnata	61 c	76 c	88 b	Control	62 b	76 b	81 c
Asclepias tuberosa	77 b	83 b	86 bc	H_2O	74 ab	81 ab	86 bc
Echinacea purpurea	73 b	91 a	94 a	H_2O_2	75 a	85 ab	90 ab
Rudbeckia hirta	89 a	92 a	95 a	GA_3+H_2O_2	79 a	87 a	95 a
Monarda fistulosa	72 b	76 c	81 c	K+H_2O_2	78 a	85 ab	91 ab
				GA_3+K+H_2O_2	78 a	87 a	90 ab
P-value	< 0.01	< 0.01	< 0.01		< 0.01	< 0.01	< 0.01

3.3. Effect of Non-Ionic Surfactants Seed Soaks Applied with Two GA_3 Concentrations on Breaking Dormancy of Asclepias syriaca

The effects of two GA_3 concentrations with H_2O_2 in combination non-ionic surfactants on the germination percentage and mold formation of *Asclepias syriaca* seeds were evaluated on days 4, 7, and 14 (Table 4a). In the nontreated control group, final germination was 42% by day 14. Treatments combining GA_3 and H_2O_2, with or without surfactants, significantly enhanced germination compared to the control ($p<0.01$). Only 0.3 mM GA_3 + H_2O_2 with Silwet 408 had greater germination than 0.3 mM GA_3 + H_2O_2 + Tween 20 or water, but only at 4 days. On day 14, most surfactant treatments with 1.0 mM GA_3 resulted in >90% germination. However, no improvements were measured at any day for any surfactant treatment at 1.0 mM GA_3. All surfactants were observed to improve the initial wetting of the soak treatments.

Table 4. a. Effects of GA_3 Treatments with Non-Ionic Surfactants on percent germination at 4, 7, and 14 days (d) and mold growth for *Asclepias syriaca* (lot ASCSYR602A).

PGR Treatment	4 d	7 d	14 d	Mold
Control (nonsoaked)	14 ± 1.2 e	40 ± 3.6 e	42 ± 2.6 e	5 ± 1.9 ab
Water	46 ± 2.8 d	66 ± 4.4 d	68 ± 3.5 d	8 ± 2.6 ab
H_2O_2+ Water	56 ± 10.1 cd	73 ± 5.3 cd	74 ± 4.9 cd	6 ± 1.6 ab
0.3 mM GA_3+H_2O_2+Tween 20	61 ± 6.4 bc	84 ± 2.8 abc	88 ± 1.6 ab	6 ± 1.16 ab
0.3 mM GA_3+H_2O_2+Tween 80	65 ± 4.1 abc	84 ± 2.8 abc	87 ± 3.4 ab	3 ± 1.0 a
0.3 mM GA_3+H_2O_2+Silwet 408	80 ± 1.6 a	85 ± 2.5 ab	88 ± 0.6 ab	5 ± 1.9 ab
0.3 mM GA_3+H_2O_2+Kwet 20	65 ± 5.3 abc	78 ± 2.5 bc	81± 5.7 bc	2 ± 2.0 a
0.3 mM GA_3+H_2O_2+ Water	58 ± 3.4 bcd	87± 5.0 ab	89 ± 5.0 ab	9 ± 3.0 ab
1 mM GA_3+H_2O_2+Tween 20	79 ± 4.4 a	94 ± 2.6 a	95 ± 1.9 a	5 ± 1.0 ab
1 mM GA_3+H_2O_2+Tween 80	72 ± 4.0 ab	89 ± 3.4 ab	93 ± 3.0 a	14 ± 3.8 b
1 mM GA_3+H_2O_2+ Silwet 408	78 ± 4.7 a	86 ± 2.0 ab	87 ± 1.0 ab	10 ± 6.0 ab
1 mM GA_3+H_2O_2+Kwet 20	80 ± 2.8 a	90 ± 3.8 a	92 ± 2.8 a	14 ± 5.3 b
1 mM GA_3+H_2O_2+ Water	73 ± 4.1 ab	91 ± 1.9 a	93± 1.0 a	10 ± 4.2 ab

The mold appearance scores indicated that certain combinations of GA_3 and surfactants may increase the incidence of mold (Table 4a). While the control group exhibited 5% mold, this value rose to 14% in treatments combining 1 mM GA_3 with either Tween 80 or K-wet 20. Therefore, caution should be exercised regarding surfactant role in promoting microbial growth. This increase may stem from interactions between surfactant and soak incubation, and further detailed studies are warranted to elucidate the underlying mechanisms.

The main effect of GA_3 concentration, regardless of surfactant type, is presented in Table 4b. Seeds treated with 1 mM GA_3 consistently exhibited higher germination percentages at all observation times compared to those treated with 0.3 mM GA_3. On day 4, germination was significantly higher with 1 mM GA_3 (76%) than with 0.3 mM GA_3 (66%). This difference continued

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over time, with 1 mM treatments reaching 90% on day 7 and 92% by day 14, while 0.3 mM treatments reached 84% and 87%, respectively.

Table 4. b. GA_3 main effect on percent germination.

Factor II: GA_3	4 d	7 d	14 d
0.3 mM GA_3	66 B	84 B	87 B
1 mM GA_3	76 A	90 A	92 A

3.4. The Effect of Water Stress on Germination of 6 Pollinator Seed Species

Germination study was conducted on six pollinator plant species under five distinct media moisture contents (32%, 35%, 42%, 90%, and 92%) with resulting soil water potential of -1.08, -0.75, -0.13, 0 and 0 MPa, respectively. All seeds were pretreated with GA_3 as described in section 2.2 to mitigate physiological dormancy, allowing the study to isolate the impact of soil moisture levels on germination performance. The impact of water stress on germination was better illustrated with a log transformation of time (x-axis) in this time-course germination investigation (Figure 3).

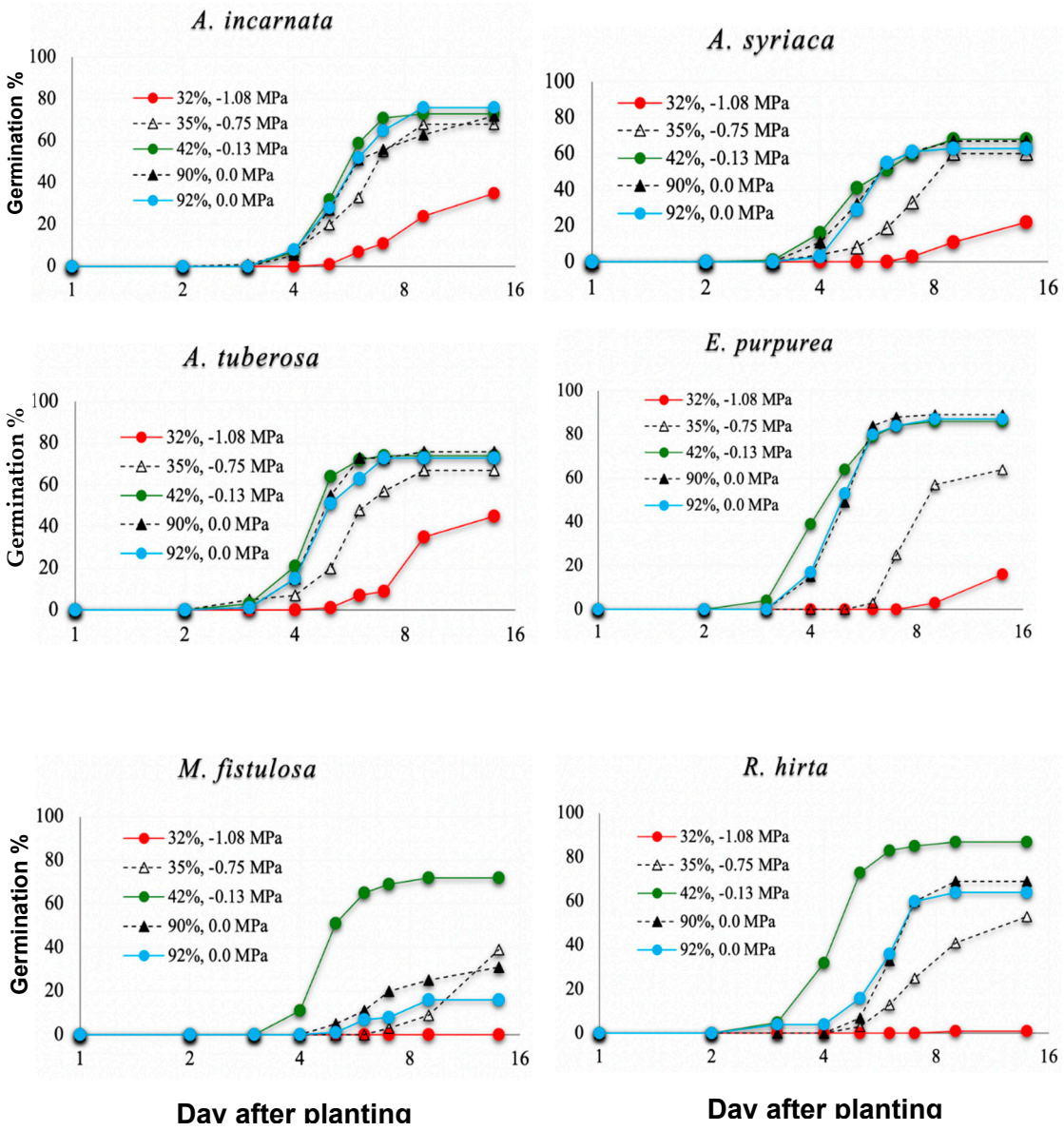

Figure 3. Germination time course of six pollinator species at five distinct media moisture content (MC) level at temperatures 20/30 °C and pretreated with GA_3. 32% MC = -1.08 MPa, 35% MC = -0.75 MPa, 42% MC = -0.13

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MPa, 90% MC = 0.0 MPa, 92% MC = 0.0 MPa. WP4 Dewpoint PotentiaMeter is utilized to measure the water potential of substrate.

The three *Asclepias* species (*A. incarnata*, *A. syriaca*, and *A. tuberosa*) exhibited similar trends: maximum germination was recorded under moisture levels ranging from 35% to 92%, with minimal variation across these conditions. However, a pronounced decline in germination occurred at 32% moisture, indicating that water stress below this level severely impaired germination. *Echinacea purpurea* showed a strong preference for higher moisture content, with peak germination observed at 90–92%, with limited germination below 42%. In contrast, *Monarda fistulosa* and *Rudbeckia hirta* achieved highest germination percentages at intermediate moisture levels (42%), while both lower (32%) and supra-optimal (92%) conditions led to suppressed germination, suggesting sensitivity to both drought and oversaturation.

These observations were further validated by the germination outcomes summarized in Figure 4, which depicts final germination percentages on day 14 under three distinct moisture regimes—drought (32%), optimal (42%), and supra-optimal (92%). Across all six pollinator species, seeds exposed to the optimal moisture level (42%) exhibited the highest germination performance, particularly in *Echinacea purpurea* and *Rudbeckia hirta*, where germination exceeded 90%. In contrast, germination significantly declined under drought conditions, most notably in *Monarda fistulosa* and *Asclepias incarnata*. Statistical comparisons revealed significant differences ($p < 0.05$) among treatments within each species, as denoted by different letters atop the bars. These findings reinforce the critical role of moderate moisture availability in supporting robust germination across diverse native pollinator-friendly species and underscore the species-specific sensitivity to soil water potential during early germination stages.

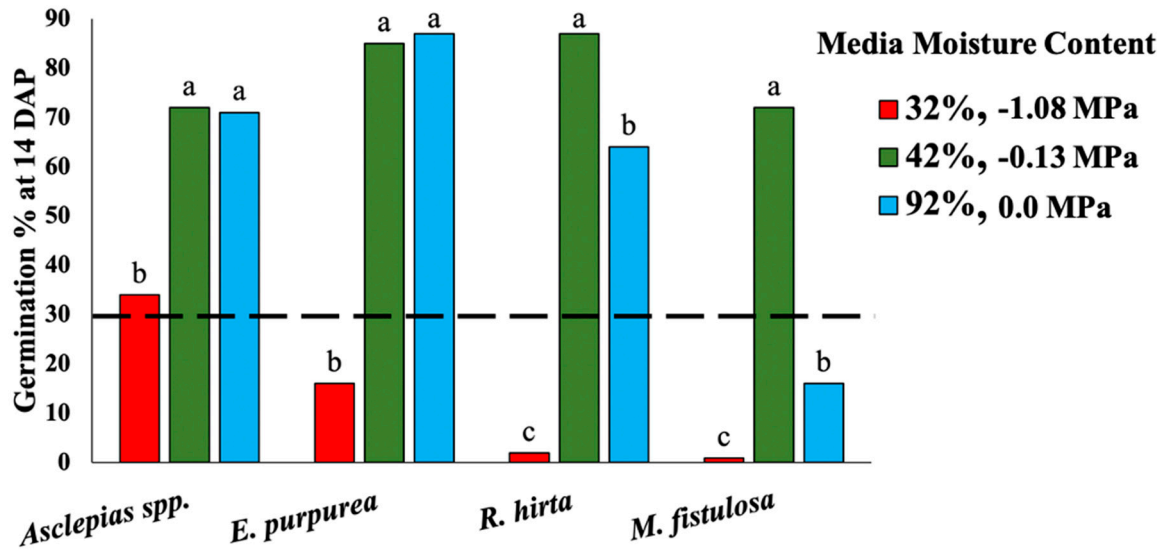

Figure 4. Total germination % of six pollinator species at three different media moisture content level 14 days after planting (DAP) at temperatures 20/30 °C. Different letters (a, b or c) above the columns indicate significant difference between the three moisture levels within each species ($P<0.05$).

4. Discussion

Seed dormancy and germination represent critical transitions in the plant life cycle, tightly regulated by a complex network of hormonal and environmental cues [31]. Dormancy mechanisms in wild plant species are largely shaped by natural selection, allowing seeds to avoid germination under unfavorable environmental conditions and synchronize emergence with periods conducive to seedling establishment [32]. As an adaptive trait, dormancy is essential for survival in natural ecosystems, yet it poses a significant obstacle in the propagation of native species for restoration purposes. Overcoming seed dormancy remains a key challenge in ecological restoration, seed

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technology, and biodiversity conservation [33]. Classical studies, especially those by Anwar Khan, have shown how important gibberellins and cytokinins are for breaking seed dormancy, with gibberellins helping the embryo grow and activate enzymes, while cytokinins encourage cell division and growth [34,35]. Recent studies have also highlighted the role of hydrogen peroxide (H_2O_2) not just as a byproduct of oxidative stress but as a signaling molecule in seed physiology. H_2O_2 has been shown to stimulate germination across various species [36–38], although its exact mechanism of action remains under investigation. It accumulates during the early imbibition stages and is now understood to function as a key regulatory signal in the transition from dormancy to germination, by modulating endosperm weakening and activating metabolic pathways [39–44]. This study integrates classical hormonal insights and novel oxidative signaling perspectives to establish refined seed enhancement protocols aimed at improving germination performance in ecologically significant pollinator plant species.

The results from GA_3 pretreatment across 42 seed lots confirmed that gibberellic acid application significantly enhanced germination percentages and reduced time to germination (T_{50}). These findings align with earlier research indicating that GA_3 promotes dormancy release by weakening the endosperm and enhancing embryo expansion [45–47]. Seed lot differences in variability of germination could be due to differences in physiological seed quality, storage history, or genetic differences between lots, as noted previously by Smith-Jochum and Albrecht (1987) [27]. Moreover, the strong inverse correlations observed between T_{50} values and final germination percentages highlight GA_3's dual role in both accelerating and increasing germination. This dual effect is particularly important in restoration contexts, where both rapid and uniform emergence are desirable traits for field establishment.

Thus, in our experimental design, the pH of the GA_3 solution was consistently adjusted to pH 6.0 using 50 mM KOH to ensure solution stability and treatment consistency. This practice of pH standardization aligns with other studies that have adjusted the pH of GA_3 solutions during preparation to enhance reproducibility and hormone stability [48]. Although pH 6.0 is close to neutral and not fully acidic, previous studies have suggested that the undissociated (protonated) form of GA_3, which predominates under more acidic conditions, may represent the biologically active form responsible for dormancy release and germination stimulation [49–51]. Indeed, Vieira *et al*. (1999) [50] demonstrated that GA_3 solutions prepared at both pH 2.0 and pH 6.0 were effective at partially overcoming seed dormancy, although the effect was more pronounced at lower pH due to increased membrane permeability of the undissociated GA_3 molecules. Thus, pH remains a critical factor influencing GA_3 uptake and its physiological activity in seed tissues.

Hydrogen peroxide (H_2O_2) solutions have been used as a seed soak to germinate sugar beet (*Beta vulgaris*) seeds [52]. As seeds imbibe the solution, catalase present in seeds converts the H_2O_2 to water and O_2, released as a gas. Hydrogen peroxide is the most stable form of reactive oxygen species (ROS) and it plays an essential role in cellular signaling. The present findings align well with prior reports demonstrating H_2O_2's synergistic interaction with growth regulators in promoting seed germination [37,38,53,54]. As a signaling molecule, H_2O_2 participates in a range of physiological processes, including dormancy release and germination, particularly by modifying redox status and enhancing sensitivity to hormones [55]. Its dual function improving seed coat permeability and acting as a metabolic cue makes it an attractive agent in seed enhancement protocols targeting ecologically critical species.

Apart from gibberellins, a kinetin (a cytokinin) and hydrogen peroxide (H_2O_2) combination showed synergy in the promotion of seed germination in several different species. Kinetin has been previously proven to be responsible for cell division and cell growth, while H_2O_2 has been recognized as a major signaling agent in seed germination processes. In this study, the GA_3 + H_2O_2 combination consistently yielded the highest germination rates in *Asclepias incarnata* (96%) and *Asclepias tuberosa* (93%) by the 14th day, while the addition of kinetin in combination with H_2O_2 further improved germination in *Echinacea purpurea*, reaching up to 99%.

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These results agree with previous studies indicating that H_2O_2 accumulation during early imbibition can serve as a biochemical cue for dormancy release [39,40,56]. According to the "oxidative window" hypothesis, only optimal concentrations of reactive oxygen species (ROS) such as H_2O_2 can effectively stimulate germination [41]. Therefore, kinetin's role in stimulating metabolic activity may have potentiated H_2O_2's signaling function, thereby promoting more synchronized germination across treatments. While no mold formation was observed in *Echinacea purpurea* treatments, slight increases were seen in other species, particularly when H_2O_2 was used alone. This suggests that species-specific responses and seed coat permeability may influence the balance between beneficial and potentially harmful oxidative effects. Further enhancement was achieved through the application of kinetin and hydrogen peroxide, alone and in combination. The GA_3 + H_2O_2 combination proved particularly effective in several species, such as *Asclepias incarnata* and *Echinacea purpurea*, suggesting a synergistic mechanism where ROS signaling complements hormone-induced metabolic activation. These results are consistent with findings by Sarath *et al*. (2007) and Li *et al*. (2018), who demonstrated a mutual induction between GA_3 and H_2O_2 that regulates ABA/GA homeostasis and promotes reserve mobilization, ultimately enhancing germination efficiency [57,58].

The integration of non-ionic surfactants into GA_3-based seed enhancement protocols demonstrated an improvement in germination, particularly in *Asclepias syriaca*. Treatments combining GA_3 with Tween 20, Tween 80, or K-wet 20 achieved final germination rates exceeding 90%, significantly outperforming nontreated controls. These results suggest that surfactants may facilitate greater uptake of growth regulators by improving seed coat permeability, thereby enhancing hormonal penetration and action. This is consistent with earlier studies indicating that surfactants reduce surface tension, increasing contact and absorption efficiency [59]. Low doses of non-ionic surfactants were shown to increase germination in wheat [60,61], onion, and lettuce seeds [62]. The results herein further demonstrate that pretreatment of seeds with non-ionic surfactants facilitates the absorption of GA_3 and accelerates the germination process, likely by enhancing water uptake and growth regulator translocation across the seed coat.

However, while surfactants enhanced germination, certain combinations also elevated mold formation, especially with Tween 80 and K-wet 20 at the higher 1.0 mM GA_3 concentration. This outcome implies that non-ionic surfactants, while biologically beneficial in promoting germination, may also modify the microenvironment around the seed—potentially fostering microbial proliferation under the soak condition. As a result, the application of surfactants in field-scale treatments should consider both the physiological benefits and the potential phytopathological risks. Further research is needed to optimize concentrations and combinations that balance enhanced germination with minimal microbial risks.

Water availability is a major environmental factor influencing seed germination, particularly in direct-seeded habitat restoration projects. The time-course data (Figure 3) and final germination rates (Figure 4) demonstrated that seed responses to moisture conditions are species-specific and strongly dependent on the physiological adaptability of each species. For instance, *Asclepias* species such as *A. incarnata* and *A. tuberosa* exhibited broad tolerance across a wide range of soil moisture conditions in our study, suggesting an evolutionary adaptation to variable field environments. This observation aligns with ecological descriptions of these species, where *A. tuberosa* is known to thrive in dry, sunny habitats, and *A. incarnata* is typically found in moist areas such as marsh edges but demonstrates adaptability to sunnier and drier conditions if adequate moisture is available [63]. In contrast, *Echinacea purpurea* demonstrated significantly higher germination only under elevated moisture levels (≥ 42%), consistent with its ecological preference for more humid environments. This observation aligns with previous studies showing that *Echinacea* species are more responsive to higher water availability, which supports successful seedling establishment [64]. This niche-specific germination response should be considered when selecting species and sowing times for conservation and restoration efforts. *Monarda fistulosa* and *Rudbeckia hirta*, on the other hand, exhibited peak germination at medium moisture conditions (42%), suggesting that they may be more suited to regions with moderate, but stable soil moisture. These findings emphasize the importance

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of species-specific germination ecology when designing seed enhancement protocols and restoration strategies. The differential responses to water availability not only reflect inherent ecological adaptations but also highlight the necessity of tailoring germination treatments and site preparation methods according to target species.

These findings align with earlier studies that emphasize the role of osmotic potential and water uptake kinetics in regulating dormancy release and radicle emergence [65,66]. The significant decrease in germination under drought conditions (32% moisture) across all species underscores the physiological limitations imposed by low water potential (−1.08 MPa), which likely delays metabolic reactivation and cellular expansion. This highlights the importance of identifying species-specific soil moisture thresholds when planning restoration in drought-prone regions.

5. Conclusions

This research provided new insights to enhance seed germination potential of selected plant pollinator species. Furthermore, a laboratory testing protocol was developed to break dormancy in species exhibiting physiological dormancy. Using soaking method to apply GA_3 combined with non-ionic surfactants, significantly increased germination. By selecting the most compatible coating formulation, seedling growth can be enhanced, thereby improving stand performance.

Multi-Seed Zea Pellets (MSZP) have shown promising results that will contribute to the germination and growth processes of pollinator plants. By combining multiple seeds of a single species into a pellet of a specific size and shape, they become suitable for agricultural mechanization and seeding. Studies indicate that MSZP technology, now commercially termed MSP (https://kannargroup.com/products/msp/) can be used in the field and will significantly increase efficiency by saving labor and time in agricultural production while allowing for uniform distribution of seeds during the sowing process. Compared to traditional methods, this technology can achieve higher germination and seedling emergence with less seed, reducing seed waste and enhancing cost-efficiency.

These studies offer valuable insights into enhancing seed germination and the overall performance of pollinator plant seeds. Helping to design best management practices, the findings are essential for agricultural sustainability and safeguarding both pollinators and their associated plant populations. The methods employed and data obtained from this project will shape future research directions and support the development of innovative strategies to boost agricultural productivity while promoting biodiversity conservation.

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

Appendix A Table A1. Taxonomic description of 42 seed lots, including seed source, USDA species code, seed lot number and seed test results from the label. The six selected seed lots (•) were designated for further investigation in subsequent experiments.

Latin name	Plant Family	Common name	Company name	USDA species	Cornell Lab code	Seed Lot	% Germ	% Dorm	Total viable	PLS	TSW (g)
Asclepias incarnata L.	Apocynaceae	Swamp milkweed	Ernst Seed	ASIN	OR-22	•JG040418	85	10	95	92.48	5.38
Asclepias incarnata L.	Apocynaceae	Swamp milkweed	Shooting Star	ASIN	SS-3	ASCINC463B	77	22	99	93.94	4.70
Asclepias incarnata L.	Apocynaceae	Swamp milkweed	Shooting Star	ASIN	SS-4	ASCINC263B	14	85	99	94.95	5.04
Asclepias syriaca L.	Apocynaceae	Common milkweed	Shooting Star	ASSY	SS-5	ASCSYR553A	6	92	98	97.58	5.49
Asclepias syriaca L.	Apocynaceae	Common milkweed	Shooting Star	ASSY	SS-6	•ASCSYR602A	11	88	99	98.66	5.32
Asclepias syriaca L.	Apocynaceae	Common milkweed	Ernst Seed	ASSY	AS-KER1	ASCSYR01-22PA	30	66	96	93.31	4.28
Asclepias syriaca L.	Apocynaceae	Common milkweed	Ernst Seed	ASSY	AS-E4	ASCSYR01-23PA	9	73	82	74.56	3.93
Asclepias tuberosa L.	Apocynaceae	Butterfly milkweed	Shooting Star	ASTU	SS-1	•ASCTUB670B	90	6	96	93.75	4.36
Asclepias tuberosa L.	Apocynaceae	Butterfly milkweed	Shooting Star	ASTU	SS-2	ASCTUB463A	18	75	93	92.96	5.31
Asclepias tuberosa L.	Apocynaceae	Butterfly milkweed	Ernst Seed	ASTU	AT-KER2	ASCTUB01-21PA	8	71	79	78.72	4.94
Bidens aristosa (Michx.) Britt.	Asteraceae	Burr marigold	Shooting Star	BIAR	BA-E3	BIDARI-2223A	82	8	90	88.67	2.43
Chamaecrista fasciculata (Michx.) Greene	Fabaceae	Partridge pea	Shooting Star	CHFA2	SS-7	CHAFAS653A	82	4	86	85.87	8.31
Dalea candida Willd.	Fabaceae	White prairie clover	Shooting Star	DACA7	SS-19	DALCAN053B	93	4	97	94.15	1.20
Doellingeria umbellata (Mill.) Nees	Asteraceae	Flat-topped aster	Shooting Star	DOUM2	SS-18	DOEUMB103A	N/A	N/A	99	97.60	0.51
Echinacea purpurea (L.) Moench	Asteraceae	Purple coneflower	Ernst Seed	ECPU	PCF	PCF311210	78	15	93	90.53	3.87
Echinacea purpurea (L.) Moench	Asteraceae	Purple coneflower	Shooting Star	ECPU	SS-9	ECHPUR503A	98	0	98	95.69	3.75
Echinacea purpurea (L.) Moench	Asteraceae	Purple coneflower	Ernst Seed	ECPU	EP-KER3	PCF221547	61	30	91	88.52	3.87
Echinacea purpurea (L.) Moench	Asteraceae	Purple coneflower	Shooting Star	ECPU	EP-KSS1	•ECHPUR504A	92	0	92	87.42	3.67
Eryngium yuccifolium Michx.	Apiaceae	Rattlesnake master	Ernst Seed	ERYU	ERYYUC	ERYYUC01-190H	95	0	95	92.60	3.79
Eutrochium maculatum (L.) E.E. Lamont	Asteraceae	Spotted Joe-pye weed	Ernst Seed	EUMA9	EUMA9	EUPMAC01-21PA	19	52	71	56.75	0.27
Eutrochium purpureum (L.) E.E. Lamont	Asteraceae	Sweetscented Joe-pye weed	Ernst Seed	EUPU21	EUTPUR	EUPPUR462A	35	60	95	89.91	0.45
Eutrochium purpureum (L.) E.E. Lamont	Asteraceae	Sweetscented Joe-pye weed	Shooting Star	EUPU21	SS-14	EUTPUR463C	51	45	96	60.77	0.44
Gaillardia pulchella Foug.	Asteraceae	Indian blanket	Shooting Star	GAPU	GP-E2	KB011723-14	68	22	90	88.35	2.23
Helianthus petiolaris Nutt.	Asteraceae	Prairie sunflower	Shooting Star	HEPE	HP-A1	QQ034123-31	19	71	90	88.84	4.09
Heliopsis helianthoides (L.) Sweet	Asteraceae	Oxeye sunflower	Shooting Star	HEHE5	SS-8	HELHEL463A	54	42	96	95.37	3.63
Liatris ligulistylis (A.Nelson) K.Schum.	Asteraceae	Meadow blazing star	Shooting Star	LILI	SS-16	LIALIG032A	88	10	98	78.29	1.33
Monarda fistulosa L.	Lamiaceaee	Wild bergamot	Ernst Seed	MOFI	WB	MONFIS03-19-2	57	22	79	78.58	0.49
Monarda fistulosa L.	Lamiaceaee	Wild bergamot	Shooting Star	MOFI	SS-13	•MONFIS463A	99	0	99	98.24	0.42
Monarda punctata L.	Lamiaceaee	Spotted beebalm	Shooting Star	MOPU	SS-20	MONPUN253B	88	2	90	89.13	0.31
Oligoneuron rigidum (L.) Small	Asteraceae	Stiff goldenrod	Shooting Star	OLRIR	SS-17	SOLRIG463B	N/A	N/A	99	98.70	0.72
Rudbeckia fulgida Aiton	Asteraceae	Orange coneflower	Ernst Seed	RUFUF	RUDFUL	RUDFUL01-22VA	1	96	97	95.00	0.90
Rudbeckia hirta L.	Asteraceae	Black-eyed Susan	Ernst Seed	RUHI2	BES	RUDHIR05-20VT	74	21	95	94.76	0.29
Rudbeckia hirta L.	Asteraceae	Black-eyed Susan	Shooting Star	RUHI2	SS-12	•RUDHIR463B	99	0	99	98.93	0.29
Solidago nemoralis Aiton	Asteraceae	Gray goldenrod	Ernst Seed	SONE	GG	SOLNEM01-20PA	56	32	88	84.66	0.12
Solidago rugosa Mill.	Asteraceae	Wrinkleleaf goldenrod	Ernst Seed	SORU2	SOLRUG	SOLRUG01-20PA	N/A	N/A	41	30.90	0.08
Symphyotrichum novae-angliae (L.) G.L.Nesom	Asteraceae	New England aster	Ernst Seed	SYNO2	SYMNOV	ASTNOV01-22PA	63	21	84	67.15	0.27
Symphyotrichum pilosum (Willd.) G.L.Nesom	Asteraceae	Hairy white oldfield aster	Ernst Seed	SYPI2	SYMPIL	ASTPIL01-20EK2	54	8	62	61.88	0.34
Symphyotrichum pilosum (Willd.) G.L.Nesom	Asteraceae	Hairy white oldfield aster	Shooting Star	SYPI2	SS-15	SYMPIL463A	77	13	90	89.98	0.13
Verbena stricta Vent.	Verbenaceae	Hoary vervain	Ernst Seed	VEST	VERSTR	QQ4722-60	31	34	95	94.33	0.98
Verbesina alternifolia (L.) Britton ex Kearney	Asteraceae	Wingstem	Shooting Star	VEAL	SS-10	ACTALT403A	21	78	99	93.37	3.18
Veronicastrum virginicum (L.) Farw.	Plantaginaceae	Culver's root	Ernst Seed	VEVI4	VERVIR	VERVIR02-21PA	13	71	84	79.56	0.05
Zizia aurea (L.) W.D.J. Koch	Apiaceae	Golden alexanders	Shooting Star	ZIAU	SS-11	ZIZIAUR463A	2	96	98	97.29	2.53
						Mean	**54**	**37**	**91**	**86.73**	**2.47**

Appendix B Table B1. Forty-two seed lots described in Table A1 Laboratory germination test results conducted at alternating 20/30C with 8-hour photoperiod or 15/25°C with 14-hour photoperiod. Samples of each lot were treated with 1.3 mM (500 ppm) GA_3 solution for 24 hours at 15°C with light, followed by drying overnight before the germination test. Germination data shown for 4, 7 and 14 days, and T50 (days) calculated from daily counts. T_{50} values were only calculated for non-GA_3 treated seed lots with positive and increasing counts at least on days 7 and 14. T_{50} values were shown as N/A (nonapplicable) for both GA_3 ands +GA_3 comparison for those seed lots not satisfying the positive and increasing count criteria. Those GA_3 treatments with * had significantly higher percentage germination than non-GA3 treatment of each comparison. The six selected seed lots (•) were designated for further investigation in subsequent experiments.

		20/30 C								15/25 C							
		4 d		7 d		14 d		T50		4 d		7 d		14 d		T50	
Latin name	Seed Lot	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3	-GA3	+GA3
Asclepias incarnata	•JG040418	10	42*	66	88*	78	90*	5.0	4.1	14	26*	58	56	64	72*	5.0	4.6
Asclepias incarnata	ASCINC463B	6	36*	26	66*	26	68*	4.9	3.9	0	0	2	8*	4	12*	7.0	6.5
Asclepias incarnata	ASCINC263B	20	34*	54	54	60	54	4.4	3.4	0	0	20	32*	24	38*	5.0	5.9
Asclepias syriaca	ASCSYR553A	2	32*	10	42*	18	48*	6.8	3.3	2	10*	26	54*	38	74*	5.7	5.8
Asclepias syriaca	•ASCSYR602A	18	54*	42	66*	46	72*	4.6	2.8	28	56*	78	86*	84	90*	4.6	3.8
Asclepias syriaca	ASCSYR01-22PA	12	34*	20	66*	26	74*	4.5	4.2	6	30*	24	56*	34	78*	6.1	4.7
Asclepias syriaca	ASCSYR01-23PA	0	6	10	12	12	14	6.3	5.5	0	0	0	10*	6	30*	N/A	N/A
Asclepias tuberosa	•ASCTUB670B	84	90*	92	90	92	94	2.5	1.9	78	90*	80	94*	84	98*	2.6	2.4
Asclepias tuberosa	ASCTUB463A	22	34*	42	40	44	46	4.0	2.5	14	36*	32	46*	32	50*	5.2	2.9
Asclepias tuberosa	ASCTUB01-21PA	2	2	2	8*	2	8*	N/A	N/A	0	0	0	2	0	12	N/A	N/A
Bidens aristosa	BIDARI-2223A	0	2	6	10	16	30*	8.0	7.8	0	0	0	0	10	14	N/A	N/A
Chamaecrista fasciculata	CHAFAS653A	18	30*	22	38*	26	38*	1.9	2.0	26	14	30	20	32	24	3.4	2.0
Dalea candida	DALCAN053B	96	94	98	98	100	98	1.9	1.6	94	84	94	88	96	90	1.7	1.9
Doellingeria umbellata	DOEUMB103A	0	6	4	12*	18	20	7.8	6.0	0	2	2	10*	10	16*	7.8	6.5
Echinacea purpurea	PCF311210	52	80*	80	94*	82	98*	3.7	2.8	30	72*	70	88*	86	90	4.6	3.2
Echinacea purpurea	•ECHPUR503A	56	78*	92	94	100	96	3.9	2.7	48	74*	92	96	100	100	4.1	3.4
Echinacea purpurea	PCF221547	86	92*	86	94*	88	94*	2.3	1.7	74	86*	96	96	96	98	2.9	2.7
Echinacea purpurea	ECHPUR504A	64	76*	92	100*	98	100	3.5	2.9	36	58*	78	94*	90	96*	4.8	3.4
Eryngium yuccafolium	ERYYUC01-190H	0	0	0	0	0	8	N/A	N/A	0	0	0	0	0	0	N/A	N/A
Eutrochium maculatum	EUPMAC01-21PA	0	4	4	12	10	22*	7.5	6.8	0	0	6	6	14	28*	9.3	8.0
Eutrochium purpureum	EUPPUR462A	12	20*	20	36*	32	48*	5.0	4.5	0	8	8	48*	16	48*	8.0	4.7
Eutrochium purpureum	EUTPUR463C	2	16*	20	38*	30	50*	6.0	4.9	0	8	8	26*	16	30*	7.0	4.4
Gaillardia pulchella	KB011723-14	84	76	90	76*	90	80	3.3	2.8	46	64*	82	80	88	86	3.9	3.6
Helianthus petiolaris	QQ034123-31	10	30*	12	38*	12	38*	3.0	2.6	14	20*	18	20	18	28*	3.5	3.3
Heliopsis helianthoides	HELHEL463A	36	48*	48	66*	62	72	3.7	3.3	22	14	52	46*	68	62	5.0	3.8
Liatris ligulistylis	LIALIG032A	28	54*	52	62*	58	62	4.3	2.6	26	52*	46	74*	60	82*	4.5	3.1
Monarda fistulosa	MONFIS03-19-2	56	54	66	60	74	64*	3.3	3.0	44	56*	60	74*	70	82*	3.6	3.1
Monarda fistulosa	•MONFIS463A	54	68*	56	80*	56	80*	2.6	2.6	46	72*	64	82*	64	86*	3.4	3.0
Monarda punctata	MONPUN253B	92	94	92	96	92	98*	2.6	2.2	86	100*	88	100*	88	100*	2.5	2.3
Oligoneuron rigidum	SOLRIG463B	18	4	40	22	42	40	5.5	6.8	8	6	26	14	46	40	6.0	8.4
Rudbeckia fulgida	RUDFUL01-22VA	0	0	0	0	0	0	N/A	N/A	0	2	2	2	2	2	N/A	N/A
Rudbeckia hirta	RUDHIR05-20VT	62	74*	74	80	86	88	3.3	2.7	78	80	86	90	86	94*	2.9	2.8
Rudbeckia hirta	•RUDHIR463B	92	96	96	96	96	98	2.4	1.6	88	94*	88	94*	88	94*	2.5	1.9
Solidago nemoralis	SOLNEM01-20PA	38	52*	64	88*	80	90*	4.1	3.8	26	42*	80	66*	82	72*	4.5	3.8
Solidago rugosa	SOLRUG01-20PA	16	24*	56	44	60	64	5.2	4.7	20	12	52	44	60	72*	4.6	5.3
Symphyotrichum novae-angliae	ASTNOV01-22PA	32	38	50	56	60	62	3.9	3.7	40	40	50	64*	56	70*	3.5	3.7
Symphyotrichum pilosum	ASTPIL01-20EK2	2	18*	38	64*	64	70*	5.9	4.7	0	6*	44	44	58	66*	5.9	5.7
Symphyotrichum pilosum	SYMPIL463A	0	6	4	18*	22	26	9.2	5.5	0	2	10	10	26	18	7.3	6.8
Verbena stricta	QQ4722-60	4	0	16	18	24	40*	7.0	7.5	0	0	4	8	24	28	10.0	8.5
Verbesina alternifolia	ACTALT403A	0	14*	6	50*	36	86*	9.5	6.4	0	0	2	42*	22	66*	10.2	6.1
Veronicastrum virginicum	VERVIR02-21PA	0	2	14	28*	26	32*	5.9	5.0	0	0	4	20*	18	34*	6.0	5.8
Zizia aurea	ZIZIAUR463A	0	0	0	2	6	14*	N/A	N/A	0	0	0	0	0	6	N/A	N/A

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