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Optimizing Ethephon Concentrations for Male Plant Feminization and Enhanced Seed Yield in Dioecious Thai Hemp (*Cannabis sativa* L. cv. RPF3)

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Abstract

Dioecious hemp cultivation faces significant productivity challenges, as approximately 50% of plants (males) produce no seeds whatsoever, functioning exclusively as pollen donors, creating substantial resource inefficiency in commercial production. This study evaluated ethephon (2-chloroethylphosphonic acid) concentrations from 250 to 1000 ppm for inducing feminization in male plants of the Thai hemp cultivar RPF3 under controlled greenhouse conditions. Complete morphological sex conversion (100%) was achieved across all tested concentrations, successfully transforming male plants into seed-producing individuals. Male plants exhibited a linear, concentration-dependent increase in seed production (R^2 = 0.76–0.91), with the highest concentration (1000 ppm) producing sevenfold more seeds than the lowest effective concentration (250 ppm). Female plants showed optimal yields at intermediate concentrations (500–750 ppm), with a significant decline in yield at 1000 ppm, indicating hormone toxicity. For commercial optimization, concentration selection depends on production objectives. 500 ppm provides optimal cost-effectiveness for mixed dioecious systems, delivering a 2.2-fold increase in total yield, while 1000 ppm maximizes productivity for male-only cultivation systems. Seeds from feminized males remained consistently 62–73% lighter than those from natural females across all treatments, indicating genetic control over seed morphology, despite hormonal sex conversion. This ethephon-based approach preserves the genetic diversity advantages of dioecious systems while doubling land-use efficiency, providing a sustainable intensification strategy for commercial hemp seed production.

Keywords: ethephon; seed production; male plant; dioecious hemp; concentration response

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1. Introduction

Hemp (*Cannabis sativa* L.) is one of agriculture's most multipurpose and economically important crops, with applications across industrial, medical, nutritional, and environmental sectors [1]. The economic sustainability of hemp cultivation depends heavily on seed production, as hemp seeds are considered a superfood containing 20–25% high-quality protein with all essential amino acids, 25–35% essential fatty acids in optimal omega-3–omega-6 ratios, and various bioactive compounds such as fatty acids, phytosterols, and minerals [2,3]. The pharmaceutical and cosmetic industries further increase demand by utilizing hemp seed oil for its anti-inflammatory properties and balanced fatty acid profile in high-value products [4,5].

The dioecious nature of hemp presents significant challenges for commercial seed production. While monoecious varieties allow harvests from all plants, dioecious hemp populations naturally divide into equal male and female ratios controlled by an XY chromosome system, where males are heterogametic (XY) and females are homogametic (XX) [6,7]. Only female plants produce seeds; males primarily serve as pollen donors [8]. As a result, roughly 50% of the plants contribute no harvestable yield, leading to resource inefficiency.

Current management strategies, including early removal of male plants and selective breeding, are insufficient for large-scale commercial production. Removing male plants shortly after pollination can reduce resource competition but also decrease plant density and land-use efficiency. Plant density significantly affects growth and production, with important implications for cannabis plant development and structure [9]. Breeding for monoecious varieties has shown limited success due to genetic instability, environmental sensitivity, and a tendency to revert to dioecious expression under stress [10,11]. Mechanical sex identification and removal systems require significant labor and precise timing, often making them impractical for small-scale farmers, especially in developing regions.

Plant growth regulators, particularly ethylene-releasing chemicals, have become valuable biotechnological tools for manipulating sexual expression in dioecious plants. Ethylene signaling pathways play key roles in sex determination across various plant families, including cucurbits, where ethylene biosynthesis genes regulate the transition between male and female flowers through complex hormonal interactions that can override genetic sex determination systems [12,13]. Recent advances reveal that sex-determining genes converge on floral developmental pathways, with cytokinin, auxin, and gibberellin signaling genes working cooperatively during reproductive organ formation [14,15]. These mechanisms involve ethylene receptor coordination in controlling sex determination, where mutations in ethylene receptors influence the shift from female to male flower development by modulating ethylene sensitivity [16,17].

Ethylene, itself a gaseous plant hormone, is crucial in regulating growth, stress responses, and reproductive development across many species [18,19]. In hemp, ethylene functions as a central regulator of sex expression, capable of overriding genetic sex determination linked to the XY system and inducing morphological sex conversion in genetically male plants to develop female reproductive structures [20,21]. Ethephon (2-chloroethylphosphonic acid) is the most widely studied and commercially available ethylene-releasing compound used in agriculture. When absorbed, ethephon decomposes in a pH-dependent manner, releasing ethylene directly within plant tissues and providing controlled, sustained hormone delivery, an advantage over gaseous ethylene application [22,23]. Its stability, ease of use, and regulatory approval in many countries make it an attractive option for commercial sex manipulation protocols [24].

Recent studies indicate that ethephon is effective for male feminization in hemp, but optimization protocols for commercial applications are still underdeveloped. Moon et al. [25] achieved complete morphological feminization in male plants, with an average seed yield of 960 seeds per plant, compared to 1404 from natural females. Although these seeds were smaller and had lower germination rates, they remained viable and could significantly boost overall production. Garcia-de Heer et al. [26] reported through transcriptomic analysis that ethephon-induced feminization involves significant alterations in class B and E floral homeotic gene expression, revealing the molecular mechanisms underlying sex conversion while maintaining distinct secondary metabolite profiles compared to natural females. Ruzgas et al. [27] attained nearly total feminization in monoecious hemp varieties using ethephon concentrations ranging from 420 to 1660 μM, showing the compound's potential for extensive sex control. However, knowledge gaps remain regarding the optimal application procedures for commercial dioecious systems. Most current studies employ binary treatments or limited concentration ranges without thorough concentration–response evaluations.

Research suggests that suitable ethephon concentrations for male feminization typically fall between 60 and 500 ppm [25,27]. Nonetheless, significant uncertainty persists about the exact optimal concentration. The timing of application is equally critical, as efficacy is limited to specific phenological windows. Moon et al. [25] found that applying ethephon immediately after primordia development resulted in a 100% feminization success rate; delaying application by seven days reduced effectiveness to 40%, and a fourteen-day delay led to complete failure. This temporal sensitivity highlights the importance of precise phenological timing combined with optimal concentration.

Variability in sex expression among hemp cultivars further complicates ethephon optimization. Recent research by Thongplew et al. [28] revealed significant genotype-by-environment interactions affecting natural sex ratios in Thai hemp cultivars under different seasonal conditions. Among the evaluated cultivars, RPF3 reported exceptional stability in sex expression, maintaining consistent female-to-male ratios of approximately 44:56 across diverse environments. This stability indicates superior adaptability compared to other Thai cultivars (RPF1, RPF2, and RPF4), which showed variable responses to changes in day length and temperature [28]. Such inherent stability makes RPF3 an ideal candidate for developing standardized ethephon application protocols, as it reduces confounding environmental variables that could interfere with hormonal treatments. Environmental factors, including temperature and photoperiod, significantly influence sex expression and plant development in cannabis cultivation [10,28].

From a practical standpoint, reliable and cost-effective male feminization techniques could revolutionize hemp seed production, especially benefiting small-scale farmers in developing regions [29,30]. This approach could double land-use efficiency with minimal additional inputs beyond hormone application, thereby improving food security and economic returns without requiring expensive infrastructure. Developing optimized ethephon protocols offers a sustainable means of intensification that preserves the genetic diversity advantages of dioecious systems while addressing their productivity limitations [31,32].

Therefore, this study aims to optimize ethephon concentrations (250–1000 ppm) for male feminization and seed production in the stable RPF3 cultivar, addressing critical gaps in concentration–response knowledge for dioecious hemp systems and examining differential responses between sexes to establish evidence-based application protocols. The research systematically evaluates the effects of different concentrations on both male and female plants to establish evidence-based application methods that maximize seed yield while maintaining the genetic integrity of dioecious breeding systems. By examining the differential responses between sexes across a broad concentration range, this study provides practical guidelines for commercial implementation, balancing feminization efficiency in males with potential adverse effects in females and ultimately contributing to sustainable intensification strategies for hemp seed production that can be adopted across diverse agricultural systems without substantial infrastructure investments.

2. Materials and Methods

2.1. Experimental Design and Plant Materials

The pot experiment was conducted in a greenhouse at the Faculty of Agricultural Production, Maejo University (18°53′35.2″ N 99°00′58.4″ E; 320 MASL) from November 2024 to March 2025. Greenhouse environmental conditions were maintained at 31.2 ± 8.1 °C during the day and 20.5 ± 2.9 °C at night, with relative humidity of 60–80% and natural photoperiod without supplemental lighting. The experiment followed a randomized complete block design (RCBD) using the RPF3 cultivar with five ethephon concentrations: 0, 250, 500, 750, and 1000 ppm. The greenhouse was divided into 5 spatial blocks to control environmental gradients. Individual plants (n = 5 per sex per treatment) were randomly assigned within blocks and served as experimental units. Plants maintained natural dioecious spacing for cross-pollination within treatment groups. RPF3 is a photoperiod-sensitive dioecious Thai hemp cultivar that exhibits consistent performance across different growing seasons, making it suitable for standardized hormone treatment protocols [28]. Individual plants were spaced 1 × 1 m apart to ensure uniform growth conditions. Seeds were sown in germination pots and transplanted into pots (9 inches in height × 12 inches in diameter) after 14 days when the first true leaves appeared. The growing medium consisted of 50% loam soil, 20% coconut husk, 10% bat guano mixed fertilizer, 10% chicken manure, 5% perlite, and 5% vermiculite to provide optimal drainage and nutrition for hemp cultivation.

2.2. Ethephon Preparation

Ethephon (2-chloroethylphosphonic acid, 48% *w/v* SL, Ethrel® 48 PGR, Bayer Thai Co., Ltd., Bangkok, Thailand) was prepared at concentrations of 250, 500, 750, and 1000 ppm by diluting the commercial formulation with distilled water. The treatment solutions were prepared fresh on the day of application to ensure their chemical stability and effectiveness. The control treatment consisted of distilled water only.

2.3. Ethephon Application

Application timing was determined by male flower bud emergence stage (Figure 1A), following the timing protocol of Moon et al. [25]. Treatments were applied when male reproductive structures (approximately 2–3 mm diameter) first became visible at node positions, representing the critical developmental window before irreversible flower differentiation. Applications were made using handheld spray bottles between 6:00 and 7:00 PM to minimize evaporation and maximize chemical uptake. Each plant received uniform coverage of all aerial parts with approximately 50 mL of solution per plant, with particular focus on developing inflorescences and young shoots where sex expression occurs. Control plants (0 ppm) received an equivalent volume of distilled water at the same time to maintain experimental consistency. All ethephon applications were conducted following safety protocols with appropriate protective equipment. Cross-pollination occurred naturally within treatment groups, with treated males retaining viable staminate tissues alongside induced pistillate structures. The greenhouse was isolated from external cannabis pollen sources, and natural airflow facilitated pollen distribution. Pollination success was confirmed through seed set measurements. Hemp cultivation was conducted under institutional oversight at Maejo University in accordance with local regulations, and chemical waste was disposed of according to institutional guidelines. Natural cross-pollination occurred within treatment groups under isolated conditions, with pollination success measured by seed set per plant.

(**A**) (**B**)

Figure 1. Morphological identification of male reproductive development in RPF3 hemp under greenhouse conditions.: (**A**) male flower bud emergence stage used for ethephon application timing, following Moon et al. [24] protocol; (**B**) ethephon spray application methodology.

2.4. Data Collection

Data collection was organized into three main categories: sex expression, ethephon-induced feminization, and yield characteristics. Sexual expression was monitored daily until all plants expressed their sex. Sex expression timing was recorded as days to first sex expression (DAG), representing the duration from germination to the appearance of distinguishable male or female reproductive structures. Female plants were identified by the emergence of white stigmas and paired styles at leaf axils, while male plants were identified by the appearance of clustered pollen sacs (anthers) within protective sepals at node positions, following the methodology of [28]. Sex ratios were calculated as percentages of male and female plants within each treatment group.

Following ethephon application, detailed monitoring focused on sex conversion in male plants. The parameters assessed included (1) the total number of inflorescences per plant, (2) the number of male inflorescences exhibiting female flower induction (feminization) per plant, and (3) the seed number per plant. The progression of feminization was documented by noting the development of pistillate structures (stigmas, styles, and bracts) on initially male inflorescences.

Plants were harvested when approximately 70% of the seeds reached maturity, indicated by a brown coloration and firm texture. Yield components measured included the total number of seeds per plant (counted manually), seed yield per plant (measured with a precision balance with ±0.01 g accuracy), and hundred-seed weight (determined by randomly selecting and weighing 100 well-developed seeds from each plant in triplicate). Seeds were dried to 12% moisture content before final weight measurements. Seed yield per hectare was calculated based on individual plant yields, sex ratio proportions, and a theoretical planting density of 10,000 plants per hectare (1 × 1 m spacing), following the calculation methodology of [28]. Calculations included seed production from both female plants and feminized males at each treatment level.

2.5. Statistical Analysis

Statistical analysis was performed using R software (version 4.5.1). Data were analyzed using ANOVA with ethephon concentration as a fixed effect and spatial block as a random effect. Residuals were examined for normality and homoscedasticity; when assumptions were met, Fisher's least significant difference (LSD) test at $\alpha = 0.05$ was applied for pairwise comparisons. Linear regression models were used to evaluate concentration–

response relationships between ethephon treatments and plant parameters. The coefficient of determination (R^2) was calculated to indicate the proportion of variance explained by ethephon concentration. Data are presented as means ± standard error.

3. Results

3.1. Sex Expression Timing and Ratios

Ethephon application at all tested concentrations (250, 500, 750, and 1000 ppm) did not significantly affect the timing of initial sex expression in either male or female RPF3 hemp plants. Male plants consistently required 45 days after germination to reach sex expression, which was approximately one week earlier than female plants. No significant differences were observed among the treatments or when compared to untreated controls (Table 1). The sex ratio remained stable at approximately 44:56 (male–female) across all ethephon concentrations. These findings suggest that ethephon application at 45 DAG (when male reproductive structures first became visible) allowed hormone treatments to influence phenotypic expression during early reproductive development without altering the fundamental genetic determination of sex within the population.

Table 1. Effect of ethephon concentrations on initial sex expression timing and population sex ratio in dioecious RPF3 hemp.

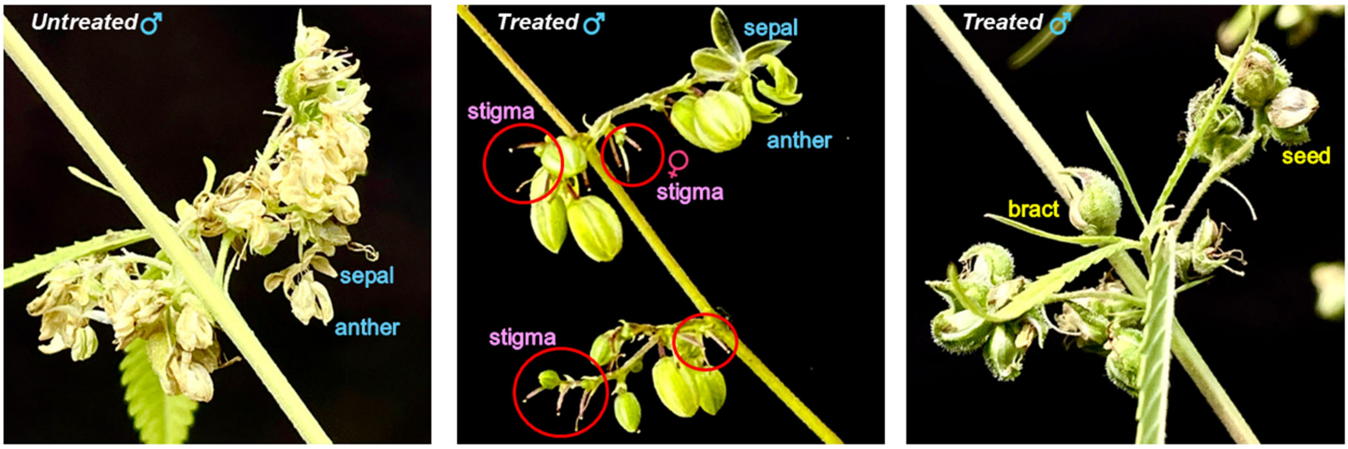
Ethephon Concentration (ppm)	Days to First Sex Expression (DAG)		Sex Ratio (Male–Female, %)
	Male	Female	
0	45.3 ± 0.6	53.7 ± 3.7	44:56
250	46.7 ± 0.6	54.6 ± 3.3	44:56
500	45.4 ± 0.5	54.3 ± 3.1	44:56
750	45.2 ± 0.4	54.3 ± 2.9	44:56
1000	45.7 ± 1.2	54.3 ± 2.0	44:56
F-test	ns	ns	ns

Note: Each concentration treatment contained 5 plants per sex from the total population.

3.2. Ethephon-Induced Feminization and Reproductive Conversion in Males

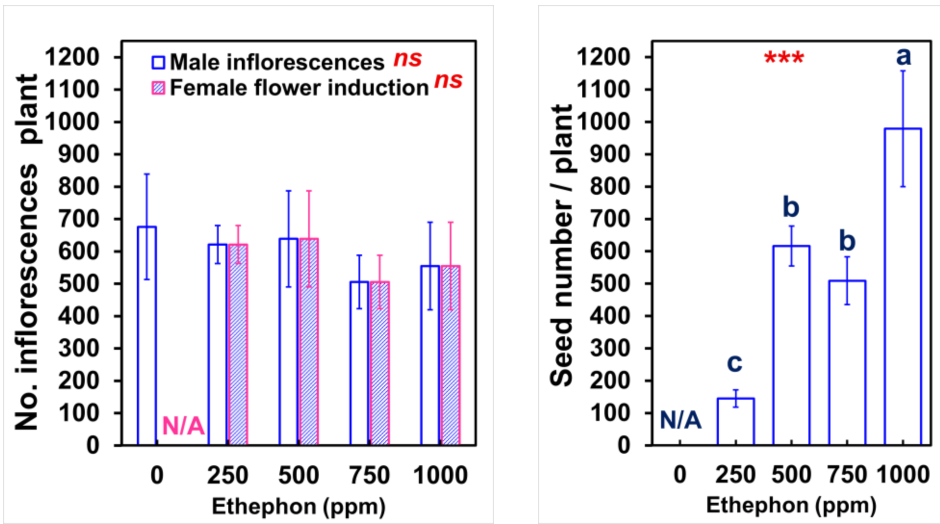
Ethephon treatment applied at the male flower bud emergence stage (45 DAG) successfully induced morphological sex conversion in male RPF3 plants, characterized by the development of pistillate reproductive structures on genetically male individuals (Figure 2). Untreated male plants exhibited typical staminate flowers with prominent anthers and sepals (Figure 2A), whereas ethephon-treated males displayed induced stigmas alongside residual male structures (Figure 2B). Over time, these structures progressed to functional seed-bearing inflorescences with developed bracts and mature seeds (Figure 2C).

Concentration-dependent analysis showed that ethephon application did not significantly affect the total number of inflorescences per plant across treatments (Figure 3A). Ethephon treatment induced complete feminization (100%) in male plants across all tested concentrations (250–1000 ppm), resulting in functional seed-producing inflorescences. However, hormone treatment exhibited a pronounced concentration-dependent effect on reproductive functionality. Control plants (0 ppm) maintained exclusive male reproductive function with no seed production, while all ethephon treatments (250–1000 ppm) successfully induced seed-bearing capacity in male plants. The 1000 ppm treatment produced significantly higher seed numbers compared to all lower concentrations, yielding approximately seven times more seeds than the 250 ppm treatment. Intermediate concentrations (500 and 750 ppm) resulted in 4-fold and 3.5-fold increases, respectively, compared to the lowest effective concentration (Figure 3B). All concentrations achieved complete morphological feminization (100%), with seed production increasing 4–7 fold compared to the lowest effective concentration.



(A) (B) (C)

Figure 2. Morphological progression of ethephon-induced sex conversion in genetically male RPF3 hemp plants showing (**A**) untreated male flowers, (**B**) early feminization with induced stigmas, and (**C**) mature converted flowers with viable seeds.



(A) (B)

Figure 3. Concentration-dependent effects of ethephon on reproductive conversion efficiency and seed production capacity in male RPF3 hemp plants: (**A**) total inflorescence number per plant (blue bars) and percentage of successful female flower induction (pink striped bars); (**B**) seed production per plant (blue checkered bars) measured 28 days post-application. Error bars represent standard error ($n = 5$ per treatment). Different letters indicate significant differences ($p < 0.001$); ns = non-significant; N/A = no seed production in control; *** = $p < 0.001$ or confident 99.9%.

3.3. *Effects of Ethephon on Seed Yield and Weight Parameters*

Ethephon application produced distinct, sex-specific responses in both seed yield and seed weight. In males converted by ethephon, yield increased approximately linearly with concentration across the tested range (Figure 4A). Specifically, the highest concentration (1000 ppm) produced significantly greater yields than all lower treatments, surpassing the 750 and 500 ppm applications by 42–54% and exceeding the 250 ppm treatment by approximately 90% (Figure 4A).

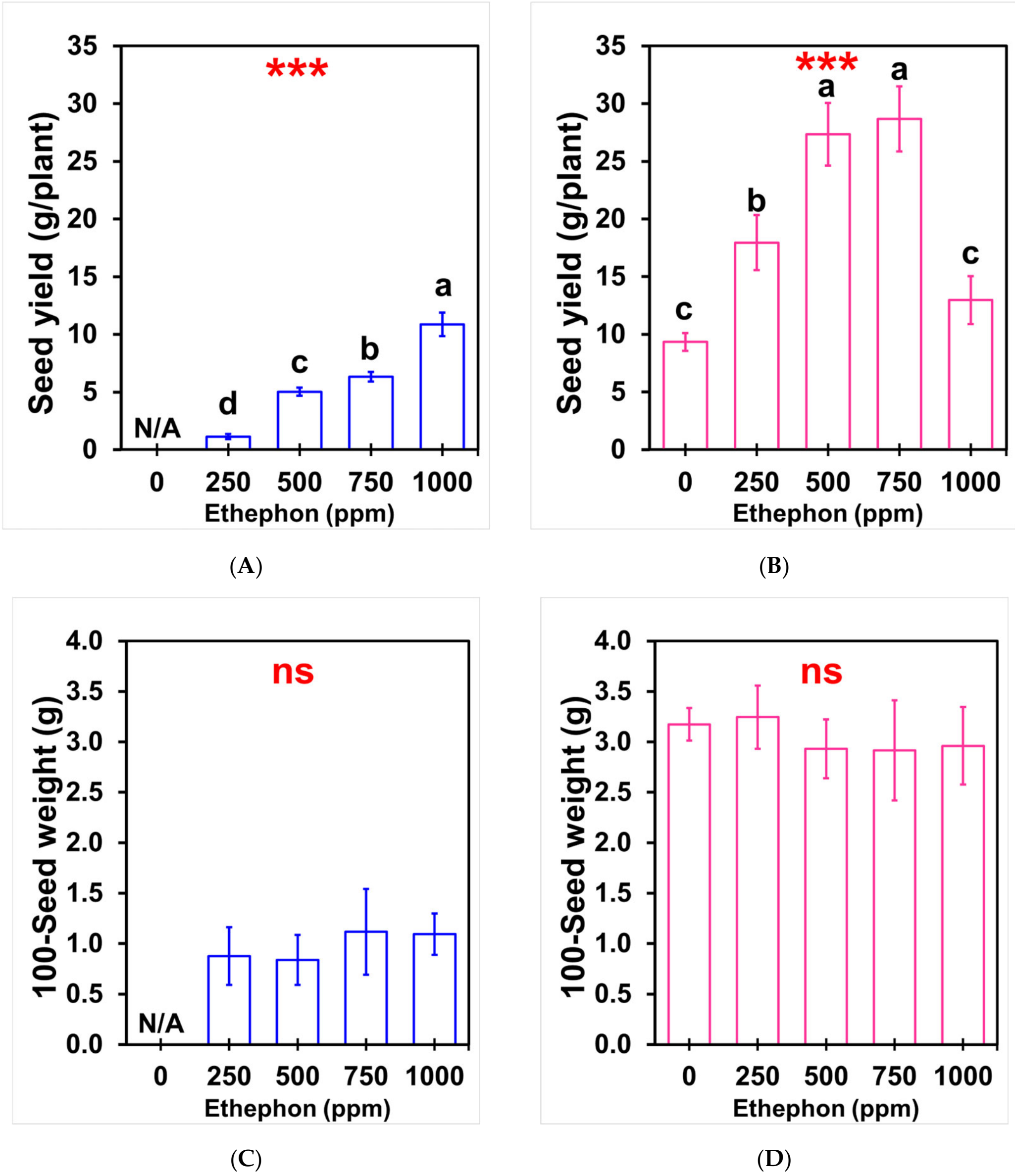


Figure 4. Comparative analysis of seed yield per plant and hundred-seed weight between ethephon-treated male and female RPF3 hemp plants: (**A**) male plant seed yield (g/plant); (**B**) female plant seed yield (g/plant); (**C**) male plant hundred-seed weight (g); (**D**) female plant hundred-seed weight (g). Error bars represent standard error (n = 5 per treatment). *** = $p < 0.001$; different letters = significant differences between treatments; ns = non-significant; N/A = no seed production.

Conversely, in females, yield increased from 250 to 500–750 ppm and declined at 1000 ppm (Figure 4B), indicating a biphasic response. Remarkably, at this highest concentration, ethephon-induced male plants approached the productivity of genetically female plants, indicating that sufficiently high hormone concentrations can enable male plants to match the reproductive capacity of natural females.

Despite these differences in seed yield, ethephon application across all concentrations did not affect seed weight parameters. Analysis revealed consistent sex-specific differences in hundred-seed weight, with seeds from male plants being 62–73% lighter than those from female plants at all treatment levels (Figure 4C,D). The stability of seed weight within each sex across different ethephon concentrations suggests that seed mass is primarily determined by genetic sex rather than hormonal influence. This morphological distinction is further supported by the comparative seed analysis (Figure 5), which shows consistent size differences between male-produced and female-produced seeds across all ethephon treatments.

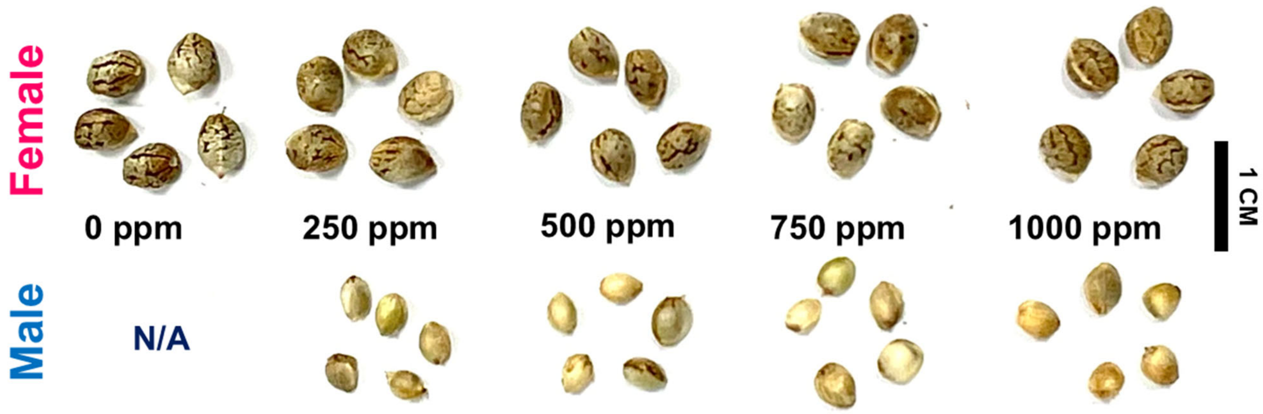


Figure 5. Morphological seed size comparison between female-produced (**upper** row) and ethephon-induced male-produced seeds (**lower** row) across ethephon concentration treatments (0, 250, 500, 750, and 1000 ppm). Scale bar = 1 cm. Note the consistent size differential between sex types; N/A = no seed production.

3.4. Population Productivity and Concentration–Response Relationships

All ethephon treatments (250–1000 ppm) significantly increased total population productivity compared to controls (0 ppm), achieving yields of 110–120 kg/ha compared to 50 kg/ha in untreated systems (Figure 6), representing a 2.2–2.4-fold increase in seed yield per hectare. The seed yield increase occurred through different concentration–response patterns between sexes, as displayed in Figures 3 and 4.

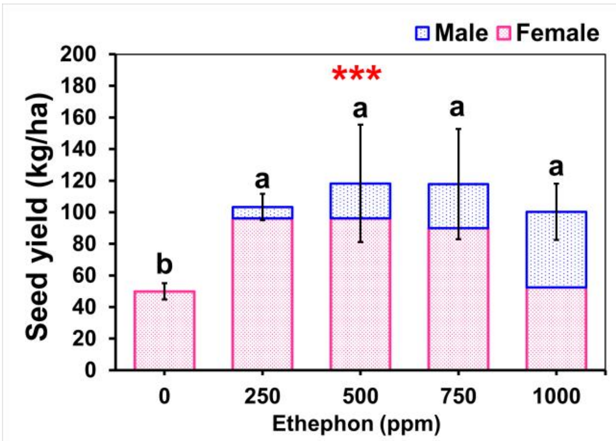


Figure 6. Total seed yield per hectare comparing male and female contributions across ethephon concentration treatments in the RPF3 dioecious hemp cultivation system. Error bars represent standard error. *** = $p < 0.001$; different letters indicate significant differences between total productivity levels. Combined bars represent total field productivity relevant to commercial applications.

Concentration–response analysis revealed distinct patterns across sex types (Figure 7). In male plants, ethephon treatments exhibited strong positive correlations with seed production parameters, with seed yield per plant showing concentration-dependent responses (R^2 = 0.76–0.91) (Figure 7B). Hundred-seed weight in males exhibited weaker correlations (R^2 = 0.12–0.21). Female plants displayed moderate to strong correlations for seed yield parameters (R^2 = 0.31–0.88), with peak responses at intermediate concentrations (500–750 ppm) (Figure 7A). Female hundred-seed weight showed minimal correlation with treatment concentration (R^2 = 0.04–0.08).

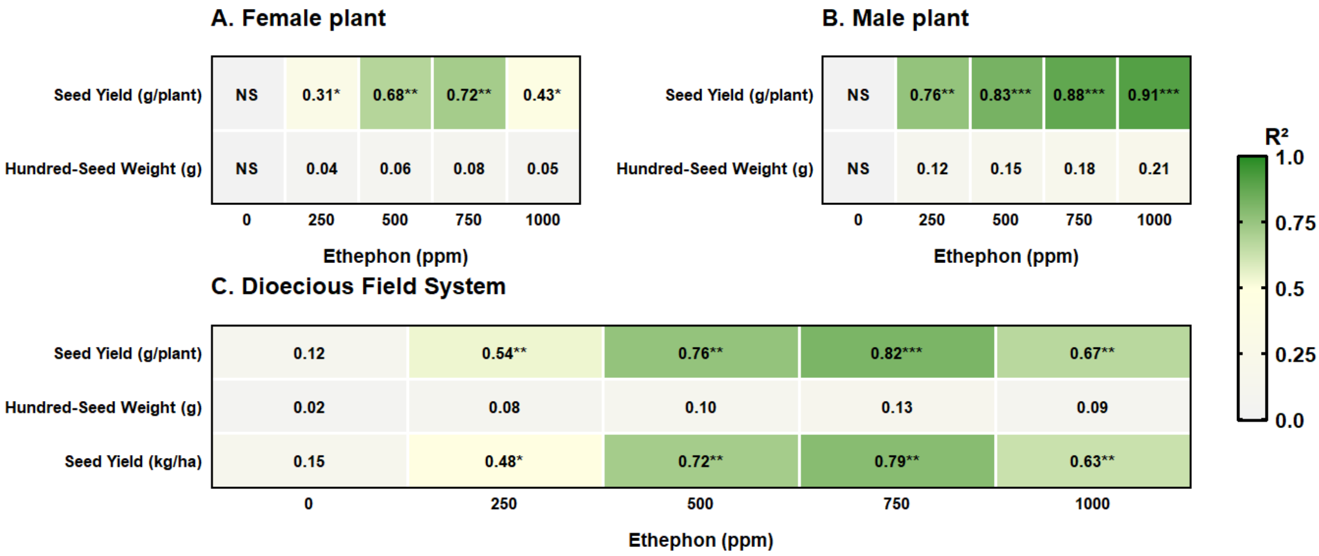


Figure 7. Correlation matrix displaying concentration–response relationships (R^2) for ethephon effects on seed yield and weight parameters in RPF3 hemp across different plant types and cultivation systems. (**A**) Female plants, (**B**) Male plants, (**C**) Combined dioecious field system performance. Color gradient represents correlation strength. Asterisks indicate significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The population exhibited strong concentration–response associations for seed yield characteristics (R^2 = 0.54–0.82) when evaluated as a unified dioecious production system (Figure 7C). The maximum total field productivity occurred within the concentration range of 500–750 ppm. The hundred-seed weight parameters throughout the population exhibited weak correlations (R^2 = 0.02–0.13).

4. Discussion

This research indicates that ethephon-induced feminization of male dioecious hemp plants represents an important advance in seed production technology, enabling complete morphological sex conversion and revealing distinct concentration-dependent responses between sexes that enhance overall population yield. This study indicates that ethephon application does not significantly influence the timing of initial sex expression or the population sex ratios in RPF3 hemp, with male–female ratios remaining stable at approximately 44:56 across all treatments. The consistent timing of the required 45 DAG for sex expression for males and 53–54 days for females indicates that ethephon does not affect the fundamental genetic mechanisms of sex determination. Instead, it influences phenotypic expression after sexual differentiation has occurred [18,19]. This stability aligns with previous findings that RPF3 maintains consistent sex ratios despite environmental fluctuations [28]. Preserving natural sex ratios is essential in commercial production, ensuring predictable population dynamics and enabling targeted hormonal manipulation of reproductive traits.

Ethephon treatment results in complete morphological feminization (100%) across all tested concentrations (250–1000 ppm) in male RPF3 plants, reflecting the compound's strong capacity to induce sex conversion. The findings align with previous studies. Moon et al. [25] achieved full feminization in male hemp, and Ruzgas et al. [27] reported nearly complete feminization in monoecious varieties using ethephon concentrations ranging from 61 to 240 ppm. The transition from typical staminate flowers with prominent anthers to functional seed-producing inflorescences with developed bracts signifies a fundamental reprogramming of reproductive development. Applying ethephon at the male flower bud emergence stage (45 DAG) maximizes effectiveness, when reproductive structures measuring approximately 2–3 mm in diameter are visible at node positions as small, clustered buds consisting of protective sepals surrounding developing staminal structures. Delayed application significantly reduces feminization success, decreasing from 100% to 40% after seven days and leading to complete failure after fourteen days [25].

Male plants show a clear, concentration-dependent linear increase in seed production. The highest concentration tested (1000 ppm) yields approximately seven times more seeds than the lowest effective concentration (250 ppm), corresponding to increases of 42–54% over intermediate treatments (500–750 ppm). This concentration–response relationship suggests the physiological mechanism where ethephon, upon absorption and pH-dependent breakdown, may release sustained ethylene levels that could potentially override the gibberellin-driven male development pathway encoded by XY chromosomes [19,22,23]. Ethylene-dependent activation of specific transcription factors directs male floral meristems toward pistillate development by disrupting the hormonal balance that normally promotes gibberellin-induced male flowers [8]. Higher ethephon concentrations provide more prolonged ethylene release, enabling more complete reprogramming of reproductive tissues and increasing seed production capacity without evident phytotoxic effects on male plants.

Female plants exhibited a biphasic concentration–response pattern, with optimal seed yields observed at intermediate concentrations (500–750 ppm), nearly double the yields of untreated controls. At 1000 ppm, seed production declined markedly to levels similar to those in untreated plants. This pattern suggests that natural female reproductive success depends on maintaining endogenous ethylene levels within an optimal range, while excessive concentrations induce hormonal stress. The substantial decrease at high concentrations is hypothesized to result from ethylene-induced premature senescence, interference with pollination processes, disruption of hormonal balance required for ovule development, or inhibition of photosynthetic activity and essential auxin–cytokinin pathways necessary for normal reproductive development [33,34]. This biphasic response contrasts with the linear response observed in males, indicating that females have stricter regulatory mechanisms limiting ethylene tolerance. Seeds from ethephon-treated male plants consistently weighed 62–73% less than those from female plants across all concentrations. This indicates that genetic sex primarily influences seed morphology rather than hormonal effects. The persistent weight disparity suggests that while ethephon effectively induces functional female structures in males, it cannot override the intrinsic developmental constraints dictated by chromosomal composition. The size difference likely results from the delayed feminization process in males compared to natural females, along with incomplete reproductive organ development and reduced resource allocation in hormonally induced structures [8,35]. Natural female plants possess evolutionary adaptations for efficient nutrient transport and seed development that are lacking in males, even after hormonal sex conversion [6,26,36].

An analysis of overall population productivity indicates that all ethephon treatments significantly increased total seed yield by 2.2 to 2.4 times compared to controls, with optimal results at 500–750 ppm. This range balances maximum feminization efficiency in males and avoids the yield decline observed in females at higher concentrations. The concentration–response relationships showed strong correlations for seed yield parameters within a dioecious production system, supporting ethephon's suitability for commercial applications. The 500–750 ppm range offers practical guidelines for field use, providing flexibility based on economic factors while ensuring consistent results across different scales of production.

The hormone interaction networks relevant to ethylene-gibberellin interactions are crucial for understanding sex conversion mechanisms. Ethylene signaling can override gibberellin-driven male development pathways, with higher concentrations providing more sustained ethylene release that enables more complete reprogramming of reproductive tissues [17,37]. Recent transcriptomic evidence from Garcia-de Heer et al. [26] supports this mechanism, showing that ethephon treatment significantly alters class B and E floral homeotic gene expression patterns, with differential expression of SEPALLATA homologues potentially contributing to sex-specific subfunctionalization in cannabis. The biphasic response in female plants at high ethephon concentrations may result from disruption of the hormonal balance required for normal auxin and cytokinin pathways necessary for optimal reproductive development [14].

The concentration-dependent responses observed align with the established understanding of plant growth regulator interactions. Environmental factors such as temperature, photoperiod, and water stress can significantly influence both natural sex expression and ethephon effectiveness, as shown in comparative studies across different cultivation environments [30,32]. The consistent seed weight differences between male-produced and female-produced seeds across all ethephon treatments suggest that genetic sex determination maintains primary control over certain developmental characteristics, even after hormonal sex conversion [38]. This finding aligns with Garcia-de Heer et al. [26], who reported that while ethephon effectively induces morphological sex conversion, induced female flowers (IFFs) maintain distinct trichome morphology with preferential development of sessile trichomes and altered secondary metabolite profiles compared to natural females, indicating persistent genetic influences on reproductive organ characteristics.

Alternative sex manipulation approaches using gibberellic acid or silver-based ethylene inhibitors exist, though systematic comparative studies remain limited [18,39]. The consistent dose–response patterns and commercial availability observed in this study suggest ethephon's practical advantages for large-scale implementation. From a sustainable agriculture perspective, this ethephon-based approach offers significant advantages over traditional breeding approaches for monoecious varieties, which often suffer from genetic instability and environmental sensitivity [29,31]. The preservation of genetic diversity inherent in dioecious systems, combined with the ability to maximize productivity from both sexes, represents a significant advancement in hemp cultivation technology that can be readily adopted across diverse agricultural systems without requiring substantial infrastructure investments [40,41]. While this study focused on seed yield and morphological parameters, additional quality metrics warrant future investigation for commercial applications. These include seed viability and germination rates, with previous research indicating that ethephon-feminized seeds remain viable despite size differences [25]. Furthermore, a comprehensive evaluation of nutritional composition, including protein content and fatty acid profiles essential for hemp seed food applications, requires study to ensure consumer product quality. This approach doubles land-use efficiency with minimal inputs, benefiting small-scale farmers without requiring costly infrastructure [1,29,30]. From a breeding perspective, the consistent seed weight differences between male-produced and female-produced seeds across all treatments [38] raise questions about inheritance patterns and progeny characteristics when using feminized males as seed parents, areas requiring future investigation for commercial breeding applications. However, limitations include controlled greenhouse pot trials with low plant density (1×1 m) optimized for seed production, single cultivar testing, and potential effects of field conditions on ethephon stability. Practical implementation should consider potential phytotoxicity at high concentrations, local regulatory compliance, and that 1000 ppm reduces female yield in mixed stands. Therefore, 500–750 ppm is recommended for mixed systems, while 1000 ppm suits male-only cultivation. Future research should validate protocols across diverse cultivars and environments while assessing nutritional quality and long-term genetic stability for commercial applications. While these concentration–response patterns provide practical guidance for commercial applications, the underlying physiological mechanisms require further investigation to establish definitive causal relationships.

5. Conclusions

Ethephon treatment (250–1000 ppm) successfully achieved complete feminization (100%) in male RPF3 hemp plants, transforming reproductively inactive males into functional seed producers with distinct concentration-dependent responses: males exhibited linear increases in seed production with higher concentrations, while females showed optimal performance at intermediate levels (500–750 ppm) before declining at 1000 ppm due to hormonal toxicity. Commercial implementation requires concentration-specific strategies, with 500 ppm providing optimal cost-effectiveness for mixed dioecious systems through 2.2-fold productivity increases, whereas 1000 ppm maximizes individual plant productivity for specialized male-only cultivation despite higher input costs. The persistent 62–73% weight differential between male-produced and female-produced seeds across all treatments reveals that genetic sex determination maintains primary control over seed morphology despite successful hormonal conversion, indicating fundamental developmental constraints limiting complete phenotypic transformation. This approach offers substantial sustainability advantages over the 50% productivity loss inherent in traditional dioecious systems, requiring minimal infrastructure investment and enabling adoption across diverse agricultural settings, particularly benefiting small-scale hemp producers. Future research should validate these protocols across multiple cultivars and field conditions, investigate long-term genetic stability, and conduct comprehensive economic analyses to optimize commercial implementation strategies. For practical application, apply ethephon at male flower bud emergence (2–3 mm primordia) using 500–750 ppm for mixed systems or 1000 ppm for male-only cultivation, with single applications and standard safety protocols.

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