



# Producing Pollen, Pollination, and Assessment of Seed Yields and Cannabinoid Levels in Industrial Hemp (*Cannabis sativa*)



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## Abstract

Research was focused on creating *Cannabis* pollen from feminized plants in order to breed viable high CBD varieties and assess seed yields. Feminized *Cannabis* refers to plants that have been specifically bred to produce only female plants (3). The five specific varieties that are being used are Variety A, Variety B, Variety C, and Variety D\*. These varieties have no base-line data with respect to seed yields and cannabinoids produced. This knowledge is crucial to future research in several different fields, such as, medicine and agriculture, by determining the best breed to maximize CBD levels. Feminized pollen is being created through several treatments of silver thiosulfate. Silver thiosulfate inhibits ethylene sensitivity in the plant. Ethylene inhibition is vital to this project because it will extend the plant’s flowering time so that male flowers can develop (2). Once the male flowers develop, they expel feminized pollen. In this research the plants are chemically manipulated for several weeks, pollinated, buds and seeds are harvested, and seed production of each plant is being analyzed.

## Introduction

Hemp (*Cannabis sativa*) is said to have originated from regions of Central Asia. Early civilizations discovered that *Cannabis* has many useful properties. The flowers and leaves have been used in medicines and in recreational drugs. The stems have been used for fiber to make paper or clothes, and the seeds have been used for oils and foods (1). *Cannabis* contains several cannabinoids; two of the most important being Cannabidiol (CBD), which is found in the female buds, and Tetrahydrocannabinol (THC) (Civilized 2017). Tetrahydrocannabinol is the psychoactive component of *Cannabis* that is predominantly used in recreational drugs and medicines. The other major cannabinoid, CBD, is a non-psychoactive component used mainly in medicines and topical items, such as, lotions and lip balms (4). If the total production of THC in the plant is more than 0.3%, it is considered marijuana. If the total production of THC in the plant is less than 0.3%, it is considered hemp. Due to the larger production of CBD within hemp, there is a substantial interest in *Cannabis sativa*. This project solely uses hemp.

## Materials and Methods

### Overview:

Five varieties of *Cannabis sativa* are being used, Variety A, Variety B, Variety C, and Variety D\*. Due to limited space, only two varieties can be used at a time. Ten plants of each variety are used for each experiment. Five of the plants are left to produce female flowers, and the other five plants produced pollen after treatments of silver thiosulfate. Each variety is kept in a 1.2-meter x 2.4-meter x 1.8-meter-tall growth chamber. The chamber has LED lighting with a light/dark cycle, a ventilation system, temperatures from 66°F-72°F, and humidity from 29%-36%.

### Treatment:

For a female *Cannabis* plant to produce pollen, the plant is manipulated using breeding techniques and chemicals. First, “solution A” was made using 15 mM (2.55 grams) of silver nitrate per liter of water. “Solution B” was made using 15 mM (3.72 grams) of sodium thiosulfate pentahydrate per liter of water. On the day of treatment, 150 mL of “solution A” is mixed with 450 mL of “solution B” to create a 600 mL solution of silver thiosulfate. The final concentration is 0.015 M of silver thiosulfate (5). Each treated plant is sprayed using a spray bottle after being placed in a large plastic tub with a tray to catch any dripping silver thiosulfate. The plants are sprayed with a circular plexiglass covering the plant’s root area because silver thiosulfate can be detrimental to plant roots. After one spray (five days), the lighting is changed from a vegetative schedule (seventeen hours of light and seven hours of dark) to a flowering schedule (eight hours of light and sixteen hours of dark). The spraying continues on a regular five-day schedule until about ten percent of the flowers are open and expelling pollen. This is approximately thirty days after the first spray (5). After approximately two weeks in the flowering schedule, the male flowers and the white pistils of the female flowers are visible. At this point, pollination occurs. There are two fans within each chamber that spread the pollen, allowing the female plants to be pollinated.



Figure 1: Treatment of hemp with silver thiosulfate.

### Harvest:

Two or three weeks after pollination, the seeds are ready for harvest. The seeds are harvested by cutting each bud from the plant and placing the buds on a drying rack for at least a week. Once the buds are dry, the seeds are manually extracted from each bud and counted to see how many are produced per plant. The buds are then prepped for HPLC.



Figure 2: Variety A test plant is pictured. White stamens of male buds can be seen.



Figure 3: Variety A control plant is pictured. Female buds can be seen.

## Results

Variety A\* is the only variety to-date that has produced pollen and seeds (Tables 1 and 2). This variety produced an average of 483 seeds per plant. Variety B\* produced pollen, but no seeds. Variety C produced pollen and seeds, but due to most of the plants dying as a result of insects, no seeds were able to be harvested. Variety D produced pollen, but no seeds. Additional treatments are ongoing.

Table 1: Variety, treatment time, and pollination status . Total of plants varied.

Variety*	Treatment Time	Produced Pollen
A	34 days	Yes
B	34 days	Yes
C	45 days	Yes
D	80 days	No

Table 2: Variety, if plants produced seeds, and average seeds produced per plant.

Variety*	Produced Seeds	Average Seeds Produced Per Plant
A	Yes	483 seeds
B	No	0 seeds
C	Yes	Unable to collect data
D	No	0 seeds

## Conclusion

To-date, Variety A\* is the only one that responded fully to silver thiosulfate treatments. As a result, Variety A is the optimal variety for this treatment. Production of pollen by Varieties B & C\* show response to treatment. Variety B was unable to produce seeds, further research is being done to determine the reason. Variety C was able to produce seeds and shows potential for project success. Treatment time should take an average of forty days.

## Future Research

Current research in the DuBois lab, involves continuing the current project with Variety E\* and repeating Varieties B, C, and D\*. Plants will be treated with silver thiosulfate until pollination, then seeds and cannabinoids production will be analyzed. Cannabinoid production will be analyzed using high performance liquid chromatography (HPLC). Seeds will be checked for viability by germinating them and growing them to flower. This will be done in the Biology Department’s greenhouse. The seeds produced by this protocol should be 99.9% female (5).

## References

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\* Due to proprietary intellectual property, the actual variety names have been omitted.