A Preliminary Study of Pollen Dispersal in *Cannabis sativa* in Relation to Wind Direction

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ABSTRACT. Pollen of *Cannabis sativa* is disseminated by wind in large amounts and for long distances, and regulations concerning the production of pedigreed seed of industrial hemp, therefore, often call for extremely large isolation distances to prevent unwanted pollination. In Europe and Canada, a standard distance of 5 km is required for the highest classes of hemp seed. This study examines the relative distribution of pollen from an isolated field over the 3-week maximum flowering period, with particular reference to wind direction. The amount of pollen distributed downwind was about six times the amount distributed upwind. In effect, this means that an isolation distance of 5 km on the downwind side is about equivalent to an isolation distance of 0.9 km on the upwind side. In theory, at the experimental site examined, the required isolation area could be reduced by about 58% while still achieving the equivalent of 5 km isolation in all directions. Given that weedy

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and illegally cultivated plants are widespread, making it difficult to ensure their absence over a distance of 5 km, it seems advisable, when possible, to take advantage of the considerably reduced isolation distance that is necessary on the upwind side. Pollen distribution appeared to follow the expected leptokurtic curve, reducing rapidly with initial distance from the source, but much more slowly with increasing distance. This makes it impossible to guarantee complete absence of potentially contaminating pollen in the field and, for practical purposes, a very low amount of undesired gene flow needs to be tolerated. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www. HaworthPress.com>]*

KEYWORDS. *Cannabis sativa*, hemp, marijuana, pollen, isolation distance

INTRODUCTION

In 1694 Rudolf Jacob Camerer (known in formal Latin as Joachim Camerarius) wrote a scientific letter concerning the first experimental evidence of sex in plants (this was published in 1700 and cited in Morton, 1981). Camerer noted that careful removal of male plants from a field of dioecious hemp did not completely deter production of fertile seed, and he commented that he was "quite upset" at the observation, obviously caused by hemp pollen from distant sources. In modern times, long-distance pollination is of great concern because of the possibility of genetic contamination. Hemp is exclusively a wind-pollinated plant. Although bees will collect the pollen, they are not attracted to the female flowers. Hemp pollen can be carried long distances by the wind. Cabezudo et al. (1997) noted that *C. sativa* pollen, apparently from marijuana cultivated in North Africa, was transported by wind currents to southwestern Europe. Stokes et al. (2000) recorded that in the Midwestern United States (where cultivation of hemp is not permitted, but weedy hemp is common) in mid-August, hemp pollen represented up to 36% of total airborne pollen counts! Hemp pollen is an allergen for some people (Lindemayr and Jager, 1980), although this is not the concern of this paper.

Hemp produces prodigious amounts of pollen. A single flower can generate about 350,000 pollen grains (Faegri et al., 1989), and there are hundreds of flowers on larger plants. However, monoecious strains have far fewer flowers and pollen production is much less than in dioecious varieties (Soroka, 1974c). Migalj (1969) found that acetolyzed pollen grains of dioecious strains tended to have a diameter averaging about 33 μm, while the grains of monoecious strains were smaller, with a diameter averaging about 27 μm. Pollen of dioecious plants was also more uniform, while that of monoecious plants was more variable, both in size and in number of pores. Also, pollen viability in monoecious strains tends to be much lower than in dioecious strains.

Pollen grains typically dehydrate before dispersion and rehydrate by acquiring water from the stigma on which they land (compare C and D in Figure 1). Bassani et al. (1994) noted that in their study of *C. sativa* pollen, viability fell from about 70% at anthesis (opening of the anthers) to less than 5% after 72 hours, although lower humidity somewhat prolonged viability. Demkin and Astachova (1952) similarly reported that viability fell from about 70% to less than 10% after 72 hours. Zhatov (1983) reported higher figures: he found that hemp pollen viability may reach 90% under favorable weather conditions, but usually ranges from 60 to 80%; and that after 3 days of storage pollen viability was 50%, dropping to about 16% after 7 days.

Hybridization of hemp with illicit marijuana, which is widespread, can be disastrous, both for the breeding of new industrial hemp varieties and the production of pure seed for planting. In particular, genes that promote high levels of tetrahydrocannabinol (THC), if transferred to a seed source, could result in subsequent plants that test over the permitted THC limit. That crop would have to be destroyed, which could result in large investment losses, as well as possible legal consequences. Marijuana growers normally eliminate male plants, or grow female clones, and the extent to which pollen from marijuana cultivation represents a threat to the genetic purity of hemp has not been established. As a rule, inspections required to ensure that pure seed is being generated rely on external plant appearance to verify that "off-types" have been rogued out of a field (Table 1). However, appearance cannot be used to judge the presence of THC potential in the developing seed, and so industrial hemp breeders are faced with a perennial problem of ensuring that their plants are adequately protected from contaminating pollen.

Canadian regulations for production of pedigreed hemp seed require up to a 5 km isolation distance (Table 1), a length which exceeds the requirements for all other Canadian crops. These regulations were based on a survey of European regulations, which differ somewhat among countries but are generally comparable. In Russia, an isolation distance of 3-4 km was used for seed increases of some germplasm collections of the Vavilov Institute, the world's most important collection of hemp

FIGURE 1. Photographs of living, uncoated reproductive portions of *Cannabis sativa*, taken with a Philips XL30 environmental scanning electron microscope. A, copious release of pollen from the apex of an anther (note row of sessile secretory glands). B, Germinating pollen grain on a stigmatic branch (note irregular pollen germination tube). C, Hydrated pollen grain. D, Dehydrated pollen grain. E, Portion of style, showing numerous pollen grains on stigmatic branches.

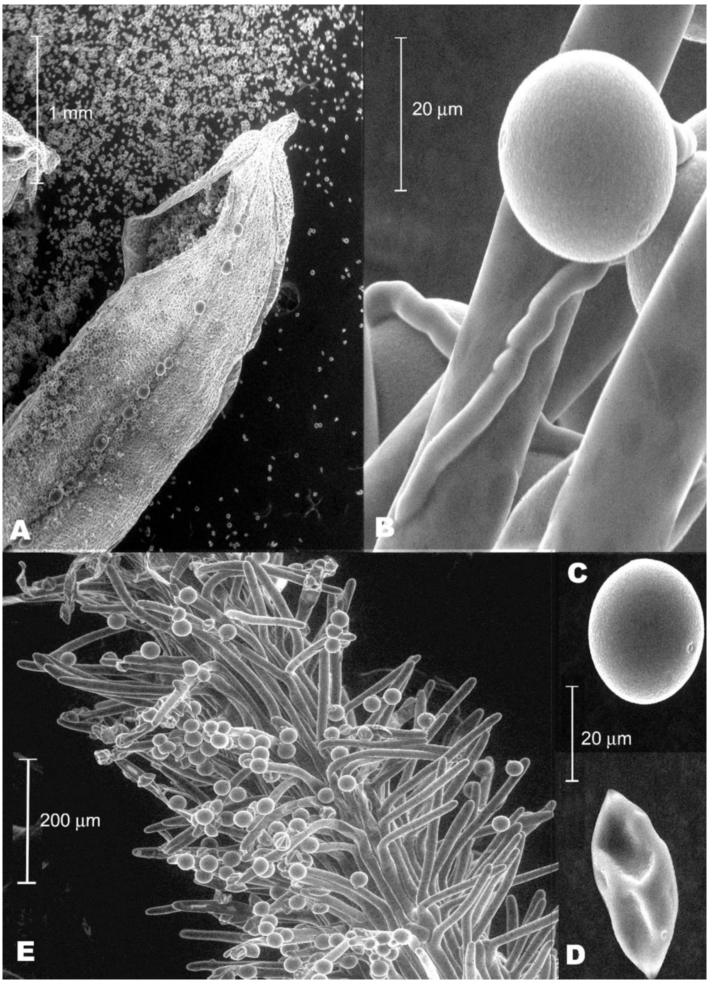


TABLE 1. Required isolation distances for production of pedigreed hemp seed in Canada[1]

Sexual system	Type of seed	Isolation from	Minimum isolation distance (m)
Dioecious	Foundation[2]	Different varieties and non-pedigreed plants	5,000
		Same variety but of lower pedigree	2,000
	Registered[2]	Different varieties and non-pedigreed plants	5,000
		Same variety but of lower pedigree	2,000
	Certified[2]	Different varieties and non-pedigreed plants	1,000
		Same variety but of lower pedigree	200
Monoecious or unisexual	Foundation	Dioecious varieties and non-pedigreed plants	5,000
		Different monoecious or unisexual varieties	3,000
		Same variety but of lower pedigree	3,000
	Registered	Dioecious varieties and non-pedigreed plants	5,000
		Different monoecious or unisexual varieties	2,000
		Same variety but of lower pedigree	1,000
	Certified	Dioecious varieties and non-pedigreed plants	1,000
		Different monoecious or unisexual varieties	200
		Same variety but of lower pedigree	200

[1] Note that information is available on the Canadian Seed Growers Association web site at www.seedgrowers.ca/regulations/sections/08a.html. The above table of information was not yet available at the web site at the time of preparation of this paper.

[2] *Foundation seed* is the highest class of seed of commerce, reproduced from seed from the breeder (*breeder seed*), managed according to strict regulations to maintain its specific genetic identity and purity. *Registered seed* is defined, depending on crop, as the approved progeny of a high-ranking class of seed (e.g., breeder or foundation seed), managed to maintain a relatively high genetic identity and purity (but not so stringent as required for foundation seed). It is typically the class of seed recommended to be used for commercial crop production. *Certified seed*, the lowest class of pedigreed seed, is the seed which for most crops is normally recommended for use by the commercial farmer. Breeder seed isolation requirements are the same as for foundation seed. Breeder seed field plot areas must be 0.4 to 0.5 ha and foundation plots must be 0.4 to 1.0 ha. There are no size restrictions for generating other classes of seed. In plants used to generate foundation seed, a maximum of 3 off-types in 10,000 are permitted, while for registered and certified seed 10 off-types in 10,000 are allowed.

seeds (Lemeshev et al., 1995). The 5 km distance can be very difficult to effect in practice and, in any event, was never validated experimentally and appears to be the result of collective empirical observations. It is impractical in many circumstances to guarantee such long isolation distances because of the frequency of clandestine marijuana growth and weedy hemp. Indeed, with increasing popularity of industrial hemp in the New World, weedy hemp will become more common as plants escape from cultivation. Organic farmers often double or triple official requirements (Moyes and Dale, 1999), but in the case of *C. sativa* it is quite unrealistic to consider 10 km or 15 km isolation distances. However, as this paper addresses, absolute distance is not the only consideration to be taken into account.

It is known that in wind-pollinated plants, pollen is carried upwind far less than downwind. For example, Jo et al. (1984), in a study of larch (*Larix kaempferi* (Lamb.) Car., identified as *L. leptolepis*) pollen distribution in Korea, found about one tenth as much pollen was distributed upwind compared to downwind. In a study of hybrids resulting from a transgenic sugar beet (*Beta vulgaris* L.) pollen source, Saeglitz et al. (2000) found that the pollen fertilized plants downwind about five times as much as upwind. By establishing the relationships between density of pollen, wind direction, and seed set, a more realistic and practical basis for isolating hemp may result. Toward this goal, this study examines the influence of wind direction on hemp pollen dissemination.

MATERIALS AND METHODS

The Canadian Food Inspection Agency is responsible for conducting varietal purity assessments of hemp grown in Canada. Their 2001 trial, conducted at an experimental plot in Ottawa, Canada (GPS coordinates of the field: Zone 18, East 438664 and North 5018089 employing UTM), was used as the experimental pollen source. The 0.4 ha experimental plot was within a large (ca. 4 km × 4 km) highly secure fenced area, used for agricultural studies and production of various crops. The area is substantially kept clear of weeds and it was ascertained that no weedy or unauthorized plants of *Cannabis sativa* were within 2 km of the experimental plot. Equal-sized blocks of 36 accessions were seeded in the first week of June, all of European cultivars, including Finola (Fin-314), Fedrina 74, two stocks of Zolo 11, Zolo 15, Fasamo, 18 stocks of USO 14, ten stocks of USO 31, Uniko B, and an experimental line. Density was approximately 12 plants/m^2. About 90% of the plants were monoecious, 10% dioecious.

Pollen was collected in 24 hour intervals over a 21-day period (8-22 Aug.), which represented the time of peak pollen production. It rained significantly for periods of 3 days during this time, reducing the pollen collection by about half. Pollen traps consisted of standard microscopic slides smeared with Dow Corning stopcock grease, and were positioned 1 m above the ground. Duplicate sample sites were placed in the center of the field, and N, E, W, and S of the field at various distances (1, 10, 30, 100, 200, 300, and 400 m) from the edge of the plot. Because of the presence of a very wide and deep gully on the W side, it was not possible to collect farther than 30 m in that direction. Calberla's solution (5 ml glycerol, 10 ml 95% ethanol, 15 ml distilled water, 2 drops of saturated, aqueous solution of basic fuchsin) was used to stain the grains. Ten microscopic fields of view on each slide were examined for a total area of 15 mm^2 for each slide (and two slides were collected at every sampling location). Pollen counts reported here are based on the means of the duplicate slides, averaged over the 21-day collection period. Pollen grains were identified morphologically (Migalj, 1969 provides a competent description). Pollen identification was facilitated considerably by the fact that the flora in the area was very limited, and indeed about 90% of the land within 2 km of the hemp field was planted with maize.

RESULTS AND DISCUSSION

As illustrated in Figure 2, pollen density fell off very rapidly initially with distance from the source, but subsequently decreased very slowly, tending to become asymptotic in relation to distance, typical of an inverse square relationship. It is known that pollen dispersal over distance tends to form such a leptokurtic curve (Moyes and Dale, 1999), with a tail containing long distance dispersal events. Leptokurtic decrease of pollen with distance from a source is characteristic of both wind and insect pollinated species (Moyes and Dale, 1999). From the point of view of pollen distribution, *C. sativa* is unusual only in that it spreads larger amounts of its pollen over longer distances than most other crops. At 100 m from our hemp field, pollen density fell to less than 1% of the density within the field, but subsequent decrease was proportionately less (Table 2). While it is well known that pollen grains such as those of *C. sativa* can be carried by wind for 1,000 km (Erdtman, 1937; Sack, 1949), the extremely low concentrations and possibly also very low viability of such pollen also need to be considered. However, it is not possible to guarantee the absence of potential contaminating pollen outdoors.

The only other study of hemp pollen dispersal relative to distance that we are aware of is by Demkin and Astachova (1952), who examined pollen distribution for distances up to 10 km from large hemp fields (100-150 ha). Their data show no indication of regular decrease of pollen concentration in relation to distance. The relative concentra-

FIGURE 2. Mean relative percentage of pollen collected over the 21 day period of the study (based on N, E and S directions for which complete data were available) plotted against $\log_{10}$ of distance from edge of hemp field.

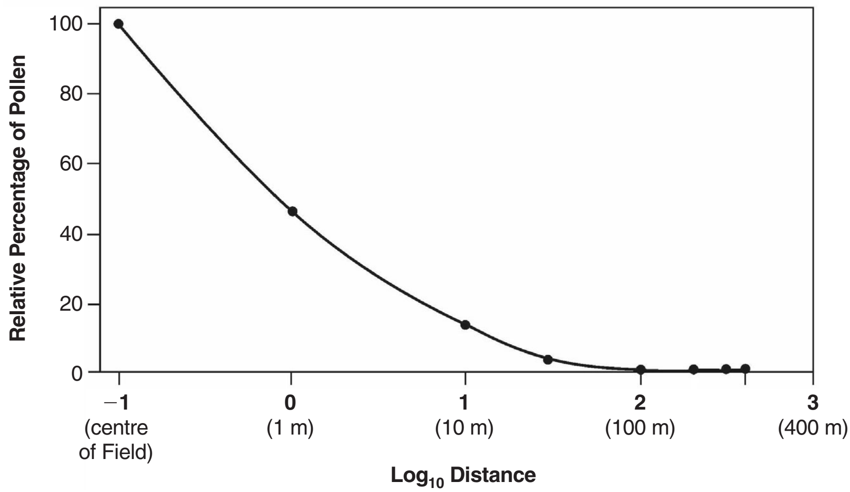


TABLE 2. Mean density of pollen grains/cm^2/day over the 3 week main pollen period with respect to field position

Distance from edge of field (m)	N[1]	S	E	W	Mean (excluding W)	Mean relative percentage (excluding W)
0 (centre of field)					866.5	100.0
1	293.3	383.3	534.5	48.9	403.7	46.6
10	79.0	148.7	132.9	12.6	120.2	13.9
30	21.8	40.3	46.6	13.6	36.2	4.2
100	8.2	3.5	5.4		5.7	0.7
200	1.6	1.6	6.1		3.1	0.4
300	6.0	0.8	5.3		4.0	0.5
400	3.3	0.0	1.8		1.7	0.2

[1] In this table N means north of the field

tions they reported at various distances (about 1 to 10 km) from their study fields were much higher than would be predicted from our study; for example, in one experiment a density of pollen at a distance of about 10 km from a source field was 38% as high as at the field itself. These considerations suggest to us either that the pollen these researchers collected was coming from additional sources, or that pollen from other plants was misidentified as *Cannabis sativa*.

At 400 m from our field, the mean pollen rain (excluding the W direction which could not be examined at our site) was 1.7 grains/cm^2/day, or 357,000 grains/m^2 over the 3-week period of measurement. Although in theory only one pollen grain on the stigma is required to fertilize the ovule of the flower of hemp, Soroka (1974a, 1974b) noted that the percentage of ovule fertilization in hemp increases with the number of pollen grains on the stigma. As can be seen in Figure 1E, the style is highly branched and capable of receiving numerous pollen grains. While data are unavailable relating pollen density and degree of pollination, the substantial deposition of pollen even 400 m from the field would seem adequate to achieve excellent seed set. Our 0.4 ha plot was quite small (commercial hemp fields, by Canadian law, cannot be less than 4 ha), and was mainly occupied by monoecious plants, which as noted earlier, are known to be far less productive of pollen than dioecious plants. Larger fields and more dioecious plants would considerably increase the absolute concentration of pollen at various distances, although the relative pollen concentrations would not be expected to differ from the relationship shown in Figure 2.

Table 3 summarizes wind direction data at the meteorological station nearest to our study site. Wind was most frequent toward the east and least toward the west and, at the experimental site, pollen deposition was correspondingly largest toward the east and smallest toward the west (Table 4). The duration of wind was about double toward the east compared to the west, while the pollen density was approximately six times as large over the period of the study (Table 4). Pollen deposition (Table 4) did not correspond well with wind records (Table 3) for the north and south directions, perhaps reflecting local wind patterns due to topography at the experimental site (as noted, the airport from which wind records were obtained is 8 km away from the experimental site). In any event, the dramatic lowering of pollen density on the predominantly upwind side of the pollen source (the west side in our location) suggests that this factor deserves major consideration.

Figure 3 shows the pattern of relative distribution of pollen at our site, with the pollen source at the centre. Figure 4, the inverse of Figure 3,

TABLE 3. Mean percentage wind frequency[1] and mean wind speed at Ottawa International Airport for the month of August for the period 1955-1980. The airport is 8 km southeast of the experimental site. Overall mean wind speed was 11.5 km/hour, and a mean 8.2% of the time the wind was calm.

Direction	Percentage Frequency[1]	Speed (km/hour)[2]
N[3]	5.0	13.0
NNE	2.7	11.4
NE	3.0	10.3
ENE	3.4	12.2
E	5.1	12.2
ESE	2.2	11.1
SE	2.1	10.0
SSE	2.9	9.6
S	7.4	10.1
SSW	9.3	11.7
SW	10.7	12.6
WSW	8.1	12.3
W	10.3	12.5
WNW	8.9	15.4
NW	6.4	15.0
NNW	4.3	15.1

[1] Wind frequency refers to the time that the wind is blowing.
[2] Wind measurements were made by anemometers installed at 10 m above the ground. For additional information, see http://www.msc-smc.ec.gc.ca/climate/climate_normals/climate_info_e.cfm
[3] In this table N means a north wind, i.e., one blowing *from* the north.

TABLE 4. Relative percentage pollen distribution with respect to geography

Distance from edge of field	Relative percentage of pollen (relative within each distance group)			
	E[1]	S	N	W
1	100.0	71.7	54.9	9.2
10	89.3	100.0	53.1	11.8
30	100.0	86.4	46.8	29.1
Mean of relative % pollen	96.4	86.0	51.6	16.7

[1] In this table, E means east of the field.

treats the centre of the figure as a target to be protected from surrounding pollen sources. Based on our data, the (Canadian) recommended isolation distance for production of pure hemp seed of 5 km, as applied to a site west of a pollen source (i.e., where the wind maximises pollen spread), is equivalent in effectiveness to the following distances in the other directions: N = 4.46 km, S = 2.68 km, and E = 0.87 km. This means that an area of about 33 km^2 around the site needs to be clear of *C. sativa* in order to achieve the equivalent of 5 km in all directions, compared to present regulations in Europe and Canada that require an area of about 79 km^2. In effect, the area presently required to ensure 5 km of isolation could be reduced by 58% (note the shaded area in Figure 4 compared to the area of the circle). While it may be awkward to enforce different isolation distances in different directions, certainly plant breeders and inspectors responsible for enforcing regulations regarding the purity of hemp seed should be acutely aware of the importance of

FIGURE 3. Geographical pattern of pollen dispersion at the experimental study site. The pollen source is located at the intersection of the axes. The shaded inner figure showing the pattern of dispersion has been placed within a theoretical isolation radius. (The inner figure is based on the means of equally weighted pollen counts at 1, 10 and 30 m from edge of pollen source, expressed as proportions of the direction of maximum count, i.e., east.)

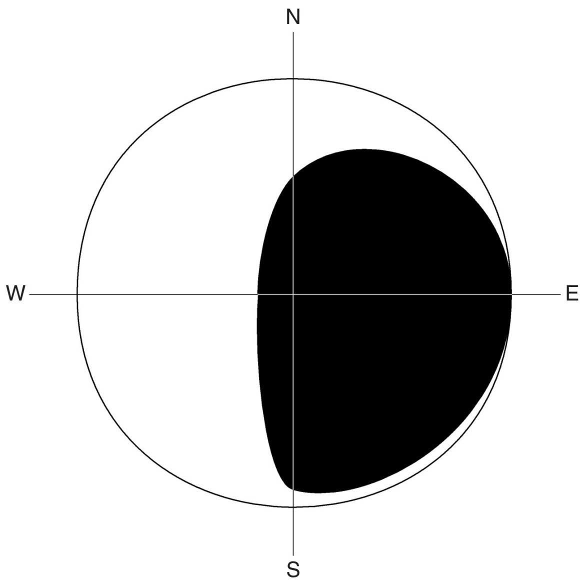
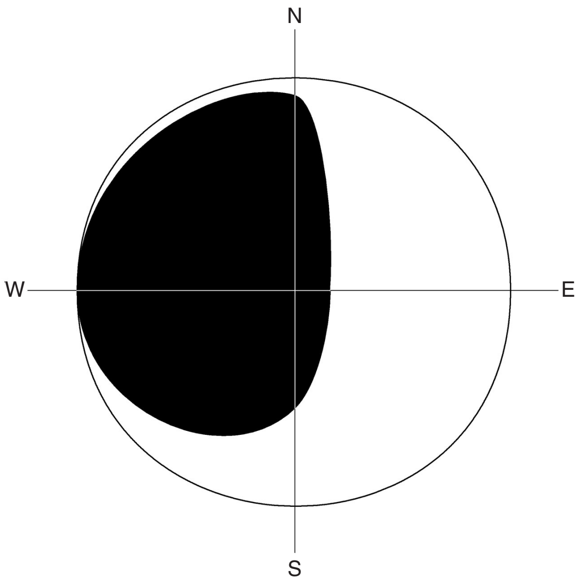


FIGURE 4. Pattern of protection required to achieve a minimum equivalent isolation from possible contaminating sources of pollen in all directions, at the experimental study site, taking wind distribution into account. This is the exact reverse of Figure 3. The seed generation source requiring protection is positioned at the intersection of the axes. The shaded figure now provides equivalent isolation from potential pollen sources in all directions. The shaded area requiring assurance of absence of contaminating sources is about 42% of the corresponding area necessitated if one defines the exclusion zone without taking account of wind pattern.



wind direction, taking special precautions against the possibility of weedy or illegal plants growing upwind of the seed generation site. Indeed, wind direction should be considered with respect to isolation distances for all plants that are fertilized by wind-disseminated pollen carried for moderate to long distances in areas where it is difficult or impossible to reliably ensure the absence of potentially contaminating pollen sources.

We have included the phrase "preliminary study" in our title because it remains to be determined precisely how amount of pollen supplied to *C. sativa* is correlated with seed set, and indeed very likely this will vary with cultivar and environment. Dempkin and Astachova (1952) conducted some studies in this regard, but reported only means, and it isn't possible to judge the significance of their data. Ideally, a study of the relationship of seed set and pollen availability would utilize cloned female plants. Another consideration is that while our "passive" pollen sampling method, greased laboratory slides, is sufficient for comparative purposes, it is known to often produce lower measures of pollen concentration compared to "active" volumetric samplers that are widely preferred for research purposes (Smith, 1990). However, our results suggest that the passive slide collection method may be useful for hemp pure seed growers to ascertain the distribution pattern of pollen at their site, as the procedure is simple, cheap and easy, while the use of volumetric samplers is probably prohibitively expensive for most growers.

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