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USE OF HONEY BEES FOR CONTROLLED INTERPOLLINATION OF WILD *Helianthus annus* L. AND *Helianthus petiolaris ssp. petiolaris* Nuttall.

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SUMMARY

The research evaluates four methods of pollination: 1) bees, 2) open pollination, 3) hand polination using mixted pollen, 4) self-pollination. The test included 11 populations of wild *H. annuus* and six populations of *H. petiolaris ssp. petiolaris*. The method of controlled pollination with bees produced significantly higher numbers of achenes than the other three methods in all but two *H. annuus* populations. With *H. petiolaris*, the controlled bee pollination produced a lower numbers of achenes than the open pollination. After 50 days in cage, the bee swarms stopped multiplying, i.e., the queens stopped ovipositing and the workers threw eggs out of the cells. After the end of pollination, the swarms were fewer in number than at the beginning of pollination.

Key words: Sunflower, pollination, bees, isolation cages

INTRODUCTION

Sunflower is predominantly an open-pollinated plant. According to Heiser (1954), wild sunflower species have a high degree of self-sterility and under natural open pollinated conditions the number of achenes produced may be low (Seiler et al., 1990). After repeated tests, we concluded that manual pollination does not produce sufficient number of achenes for further testing. Thus we tried to produce more achenes and to reduce labor input by using domesticated bees (*Apis mellifera* L.). We conducted a comparative test with four methods of pollination: 1) with bees, 2) open pollination, 3) manual pollination, using pollen mixture, and 4) self-pollination.

MATERIAL AND METHOD

Experiments were conducted at the Institute of Field and Vegetable Crops in Novi Sad in 1990 and 1991. Fifty isolation cages 3.5 x 1 x 2.5 m were used. Their sides were covered with PVC foil with a UV protectant to prolong durability. The fronts were covered with PVC foil without the protectant, but with 0.3 mm perforations. The test included 11 populations of wild *H. annuus* and six populations of *H. petiolaris ssp. petiolaris* which had been collected in different parts of the USA (Table 1.). Experimental plants were initially grown in the open field. The cages with bee hives were installed at

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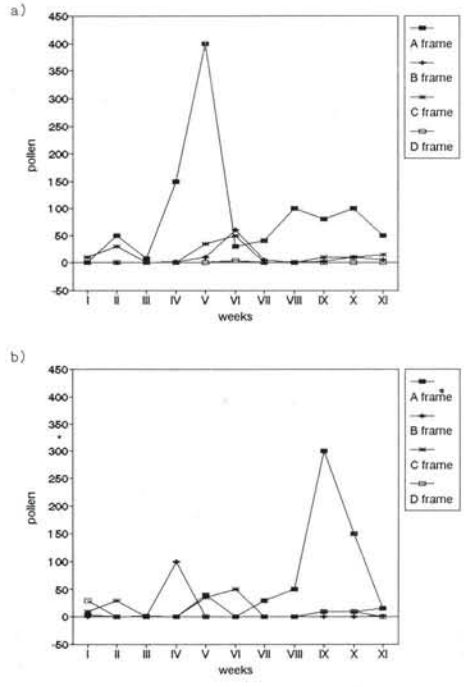

Figure 1.
Number of cells with pollen per frame in the free beehive (a) and the hive inside the isolation cage (b)

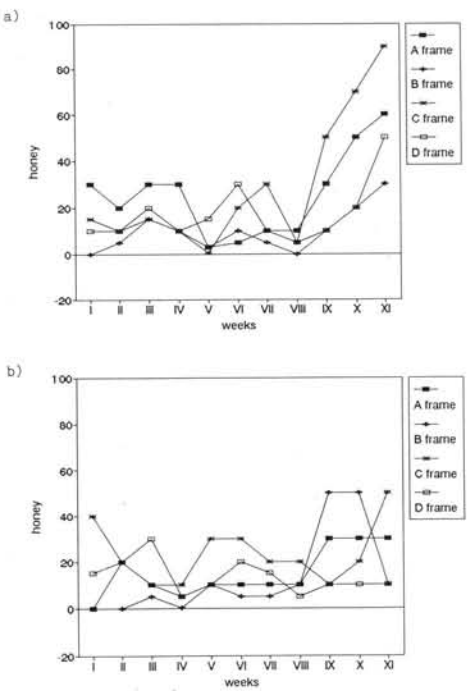

Figure 2.
Amount of honey (in dkg) per cage in the free beehive (a) and the hive inside the isolation cage (b)

the beginning of flowering and kept till maturity. The parameter under observation was the rate of achene formation per disk.

Having no experience with the number of bees needed for successful pollination, we chose about 1,000 foraging bees per cage. Each beehive had three frames with foraging bees and a single queen. Two frames were added on the top which held reserve honey and pollen. Because the forage was restricted to a small number of flowers, mortality was high and new frames with bees had to be added. The queens were first fed with a syrup made of sugar and water in the ratio (1:1). Later on, the ratio was increased to 2:1. Ellis et al. (1981) obtained very good results using a system of several beehives per cage with two exits: one into the restricted area and another outside. The exits were closed over night and the collected pollen became non-functional by morning. Despite the obvious advantages of the system, it could not be used in our case because of the confounding effects it would have on other experiments conducted at the same experimental field.

RESULTS

Bee visitation increased the rate of achene formation in all *H.annuus* except populations 1963 and 1970 (Figure 1). Seed set variability was higher using bees than in any other methods. It was difficult to establish a relationship between population origin and pollination rate. Using sibbing method, the number of achenes ranged from 0.0 to 50 per disc. In general, the rates of achene formation were significantly lower for sibbing and selfing than for bees or open pollination (Table 1). The lowest rate was observed with selfing. Again it was difficult to find any pattern since the population with the lowest number of achenes per disk using sibbing had the largest number of achenes using bees.

H.petiolaris, unlike *H.annuus*, produced a smaller number of achenes using bees than with open pollination (Table 2). In population 2090, even sibbing produced a larger number of achenes than bees. As in *H. annuus*, selfing produced a small number of achenes (Table 2). Nevertheless, the populations of *H. petiolaris* were more uniform than the *H. annuus* population, especially using bees.

Table 1. Number of achenes/disk in the populations of wild *Helianthus annuus*

Collection No.	1963	1964	1970	1975	1983	1985	1990	1991	1992	2002	2006	Average
PI NUMBER	531009	531010	531015	531016	531022	531024	531025	531026	531027	531032	531035	
STATE	ILL	ID	OR	WA	MT	ID	ID	ID	ID	MT	MT,	
CAGE	17.00	60.27	5.00	152.00	66.90	51.68	78.95	166.00	198.60	55.00	163.36	92.25
OP	86.50	49.95	49.65	103.73	63.09	62.48	55.25	63.42	40.12	46.09	56.68	61.54
SIB	29.30	5.40	6.22	0.16	20.29	3.83	0.33	50.06	0.00	32.23	37.44	16.84
SELF	1.58	0.12	0.72	0.08	11.67	5.43	0.06	2.60	0.33	4.15	0.07	2.44
LSD 0.05 = 30.55		LSD 0.01 = 40.88										

Table 2. Number of achenes/disk in the populations of wild *Helianthus petiolaris* ssp. *petiolaris*

Collection No.	1264	1910	1982	2004	2009	2090	AVERAGE
PI NUMBER	468831	503232	531055	531056	531057	547210	
STATE	TX	NJ	MT	MT	MT	IL	
CAGE	16.31	48.94	31.79	31.75	29.34	2.88	26.83
OP	57.91	59.65	32.00	39.54	24.56	58.54	45.37
SIB	6.47	18.14	5.25	10.70	24.12	16.16	13.47
SELF	0.52	0.92	0.12	0.00	4.23	2.08	1.31
LSD 0.05 = 13.96	LSD 0.01 = 19.04						

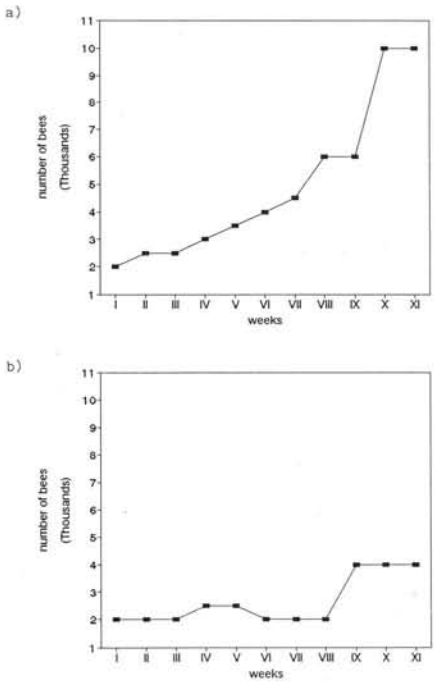

Figure 3.
Total number of bees in the free hive (a) and the hive inside the isolation cage (b)

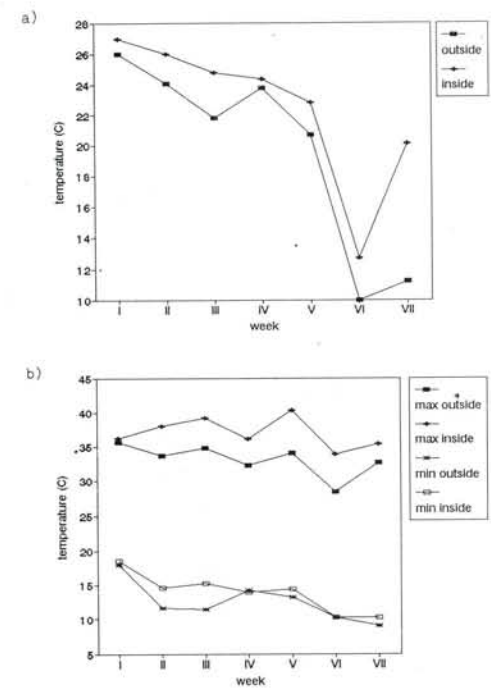

Figure.4.
Mean daily temperatures calculated on the weekly basis in the free beehive and the hive inside the isolation cage (a). Maximum and minimum temperatures in the free beehive and the hive inside the isolation cage (b).

It was tried to determine the effect of microclimate inside the isolation cage on the content of pollen, honey, and the total number of bees. It was found that the number of cells with pollen was higher in the beehive outside the isolation cage than in the beehive inside the isolation cage (Figures 1a and 1b). In the free beehive, a significant increase in the first frame was observed in the 5th week of the experiment, which decreased in the 6th week and 50 to 100 cells with pollen maintained at that level to the end of the experiment. In the beehive inside the isolation cage, the number of cells with pollen was small till the 8th week, when the beehive was taken out of the cage (from the 8th to the 9th week) for beehive resting. The number of cells fell under 50 after the beehive was returned into the cage. A small increase of cell pollen on C and D frame occurred in the period of beehive respite, while all pollen was spent after it was returned into the cage.

Significant differences were observed with respect to the amount of honey. The free beehive was slightly variable in all frames up to the 8th week, while from the 8th to the 11th week the amount of honey increased (Figure 2a). The beehive inside the cage did not differ significantly from the free beehive to the 8th week; an increase was registered in the period of resting. When the beehive was returned to the cage, the accumulation of honey slowed down again, but at a much slower rate than it was the case with the free beehive.

Large differences were recorded for the total number of bees per beehive. In the free hive, the increase in the number of bees was more or less linear (Figure 3a), the number reaching the figure of 10,000 bees at the end of the experiment. In the hive in the cage, the total number of bees to the 8th week stayed between 2,000 and 2,500, it increased to 4,000 during the resting period and it remained at that level to the end of the experiment (Figure 3b).

The temperature readings were comparable inside and outside the cage. The mean daily temperature, calculated on the weekly basis, was steadily higher within the cage but the difference never exceeded 4°C (Figure 4a). Identical relationships were observed for the maximum and minimum temperatures (Figure 4b).

During the period when the experiment was irrigated (from the 1st to the 5th week), air humidity was higher outside the cage. After the fifth week, the situation was reversed (Figure 5). However, the observed differences were negligible.

DISCUSSION

Considerable varability was observed in the *H.annuus* populations regarding the number of achenes formed, especially using bees. The difference in the bees' predilection may be due to the differences in the amounts of pollen, nectar, and aromatic substances between the populations. Vear et al., (1990) reported significant differences in the nectar content per inflorescence but constant contents of fructose and glucose. Although the attractiveness of sunflower evidently depends on the content of aromatic substances in disk flowers, it is hard to believe that this particular component played a significant role since the bees were forced to use pollen from a single population inside the cage. It should be mentioned for *H.annuus* that the number of achenes was significantly larger using bees than any other method. Pham-Delague and Piquemal (1986) report that in France the domestic bee, which makes up 60 to 90 percent of the total population of pollinators, has a choice to visit other more attractive species, as well as cultivated sunflower. In our case,

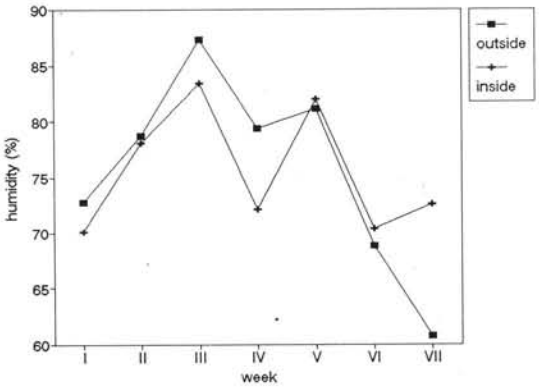

Figure.5.
Air humidity outside and inside the isolation cage (%)

a difference was observed for *H. petiolaris* between using bees and open pollination, with open pollination having a higher number of achenes per disk.

It has been shown that the 'S' allele affects the controlled pollination. Inbreeding coefficient may also play a partial role. When a natural population is collected, we do not know whether it was a natural spacial isolation or the expression of its 'S' allele was affected by open pollination. It is obvious that the differences between the populations and the species in the number of achenes may be interpreted only if all the factors are carefully considered because each factor may be the dominant one in an individual case.

Our doubt with respect to the effect of microclimatic conditions on bees work and the differences in the amount of pollen, honey, and the number of bees was justified. It is evident that there are no optimum conditions for the bees inside the isolation cage, which was proved by peaks, which occurred in the period of hive rest. Measuring temperature and air humidity, we found that the differences were small and are not the limiting factor. Considerable attention should be paid to the content of pollen. Pollen is indispensable feed for bees and for hatching the litter, thus the differences occurring between the hives inside and outside the isolation cage depend on the amount of the pollen collected. Wild sunflower species inside the limited space of 3.5 m^2 form a low number of flowers which is insufficient for normal feeding of a bee society. Consequently, adding the frames with pollen and the strengthening of bee swarms is essential when using bees for wild sunflower species pollination, since after 60 days there is no oviposition in the hives inside the isolation cage.

CONCLUSION

The number of achenes formed in the controlled pollination using domestic bees was higher that those obtained with the other three methods, with the exception of two populations of wild *H. annuus*. The variability in the number of achenes was largest using bees, while selfing produced the lowest number of achenes.

In the case of *H. petiolaris*, a significant difference was observed between using bees and open pollination, unlike for *H. annuus*. *Helianthus petiolaris* population 2090 formed almost the same number of achenes using bees and selfing. The variability among the populations indicates the complexity of factors which may affect the rate of pollination achieved in the cage.

It was found that the number of cells with pollen was higher in the beehive outside the isolation cage than in the beehive inside the isolation cage. Significant differences were observed with respect to the amount of honey. Large differences were recorded for the total number of bees per beehive. The temperature readings were comparable inside and outside the cage. During the period when the experiment was irrigated, air humidity was higher outside the cage. After the fifth week, the situation was reversed.

The use of bees for interpollination between wild populations allows the production of a sufficient number of achenes for research purposes. The cost of the method is about 100 USD per cage.

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USO DE AREJAS PARA INTERPOLINIZACION CONTROLADAS DE LAS ESPECIES SILVESTRES *Helianthus annuus* L. Y *H. Petiolaris* SSP *Petiolaris nuttall*

RESUMEN

La presente investigación evalua cuatro metodos de polinización: 1) abejas, 2) polinización libre, 3) polinización manual utilizando mezela de polen, 4) autopolinización. El ensayo incluyó once poblaciones de *H. annuus* silvestre y seis poblaciones de *H. petiolaris* ssp. petiolaris. El método de polinización controlada con abejas produjo un número de aquerios significativemente más alto que los otros tres mètodos en todas las poblaciones de *H. annuus* menos dos. Con *H. petiolaris*, la polinización contrastadas por abejas produjo un número más bajo de aquemion que la polinización libre. Despues de cincuenta días en jaulones los enjambres de abejas dejaron de multipliearse, las reinas pararon la ovoposición y las obreras sacaron los huevos fuera de las celdillas. Despues del final de la polinización los enjambres fueron menores en nùmero que al principio de la polinización.

UTILISATION D'ABEILLES POUR LA POLLINISATION CONTRÔLÉE D'HELIANTHUS SAUVAGES

RÉSUMÉ

Cette étude a eu pour but de comparer quatre méthodes de pollinisation: 1. par les abeilles, 2. pollinisation ouverte, 3. pollinisation manuelle utilisant du pollen mélangé, 4. auto pollinisation. Le test a été réalisé sur onze populations sauvages de *H. annuus* et six populations d'*H. petiolaris ssp. petiolaris*. Experté deux populations d'*Heliantus annus*, la méthode utilisant une pollinisation contrôlée par des abeilles produit un nombre d'achènes significativement supérieur par rapport aux trois autres méthodes. Avec *H. petiolaris*, ce type de pollinisation produit un nombre inférieur d'achénes comparé à une pollinisation ouverte. Après cinquante jours en cage, la multiplication de l'essaim stoppe (la reine arrête l'oviposition) et les ouvrières jettent les oeufs des alvéoles. Aprés la fin de la pollinisation les essaims avaient réduit en nombre par rapport au début de la pollinisation.