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Pollination methods for maintaining carrot germplasm collections

R.L. Wilson¹, M.P. Widrlechner¹ and K.R. Reitsma¹

Summary
Field cage tests were run to determine if honey bees, *Apis mellifera* L., flies, *Musca domestica* L., or a combination of the two would provide the best pollination for carrot germplasm seed increase. In 1985, honey bees alone were significantly better pollinators than flies alone, but in 1988, a combination of honey bees and flies produced more carrot seed per cage than did honey bees or flies alone. Seed obtained by using the two pollinators did not differ significantly in 100 seed weight or germination percentage. Considerable time savings resulted from using insect pollinators rather than hand pollination. In the future, the North Central Regional Plant Introduction Station will use a combination of honey bees and flies to pollinate carrots for germplasm seed increase.

Introduction
The US national carrot (*Daucus* spp. L.) germplasm collection is maintained at the North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa. In 1985, there were eight species and 486 accessions in the collection (Wilson et al., 1985). By 1989, the collection had increased to 12 species and 625 accessions.

Periodically, seed stocks of this collection need to be replenished. The regeneration of an accession is done when germination of distribution lots drops below 50% or if the number of seeds drops below 5000.

At the NCRPIS, cage pollination using honey bees was started in 1949 to maintain the genetic integrity of the carrot collection. Hand pollination was used in the greenhouse when not enough seed was available to do a cage increase. Hand pollination was done by rubbing the umbels together or with a soft brush when the stigmas became receptive. Pollination was usually carried out three times per week (every other day). Hand pollination was very time consuming and usually resulted in poor seed set.

Although honey bees have often been used to produce control pollinated seed (Hawthorn et al., 1956, 1960; Alam et al., 1987), other researchers have successfully used flies for this purpose (Borthwick and Emsweller, 1933; Simon and Peterson, 1984; Peterson and Simon, 1986). Since flies have also been shown to be an effective pollinator of carrot, experiments were begun in 1985 to determine if honey bees, house flies, or a combination of both insects would be the best pollinators to use in cages under Ames, Iowa field conditions. Hand pollination was included in this study as a comparison with insect pollination.

Materials and Methods
The NCRPIS germplasm collection includes many landrace accessions. One of these, PI 222794, was used for our experiments. This accession of *Daucus carota* L. was collected at Rezaiyeh (now Orumiyeh), Azerbaijan, Iran, as a local market carrot and placed in the US germplasm collection in 1954.

The 1985 test
Seeds of PI 222794 were sown in potting soil (six parts field soil: three parts Sunshine mix: one part Perlite) in the greenhouse in 12.7-cm square pots in the first week of November 1984. Each pot was thinned to eight plants. The plants remained in the greenhouse until February 1985, at which time about five or more true leaves had developed. The plants were fertilized weekly with Peter's fertilizer while in the greenhouse. During the last week of February, the plants were not watered and the foliage was trimmed to 2.5-3.8 cm above the crown. The pots were then placed in a cool room maintained at 7-10 °C and 80-85% RH for 40 to 60 days. The plants were not watered during this time. The plants were transplanted to the field in late April or early May and covered with a saran mesh cage 1.5 x 1.5 x 6.1 m. There were 75 plants per cage. No herbicides or pesticides were applied.

The test was established as a randomized complete-block design of three treatments replicated three times. The treatments were: (1) a nucleus hive containing about 5000 honey bees (Ellis et al., 1981) placed inside each of three cages on 14 June, (2) about 200 house fly (Musca domestica L.) pupae per week placed in three cages beginning 20 June, and (3) hand pollinations in three cages beginning 20 June. The treatments were terminated on 8 August. All plants in each cage were harvested weekly for a period of one month beginning

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the last week in August. The seeds obtained per cage were the total accumulation of the weekly harvests. The primary umbel was harvested separately from the secondary umbels (see Gray and Steckel, 1983, for a discussion of primary and secondary umbels).

The honey bee nucleus hives were supplied with 5% sugar solution. The bees were shifted out of the cages every other day, by placing the hive half inside the cage and by moving a bottom plate on the hive to allow the bees to fly either inside or outside the cage.

The house flies used in this study were reared at the NCRPIS. The initial colony was started by collecting flies near a swine barn by using a standard insect sweep net. The flies were then brought into the laboratory and from then on reared by using a Purina rearing medium for flies. The rearing room was kept in continuous light at 23-25°C and 70-80% RH.

Hand pollinations were performed daily, from Monday to Friday, when the stigmas were receptive. Soft brushes were used to rub the umbels and thus effect pollination.

Data recorded were seed index (weight in grams of 100 seeds), total seed weight and germination percentage. A log was kept of the time involved per cage to accomplish the fly- and the hand-pollination treatments (e.g. travel time to and from the field plots, time spent placing fly pupae in cages, and the time involved hand pollinating).

Data were analyzed by using the analysis of variance procedure in the Michigan State University statistical program, MSTAT (Nissen et al., 1982). A correlation analysis was run to compare harvest date with seed index and percentage germination.

### The 1986 test

Carrots were planted the first week of November 1985, and plants were handled in the same manner as in the 1985 test. We replaced the hand pollination treatment with a treatment consisting of both honey bees and house flies, and the number of fly pupae per cage per week was increased to about 1000. The number of honey bees remained the same.

Data were recorded and analyzed as in 1985. Two replications per treatment were analyzed. The third replication was lost because insufficient numbers of plants survived the vernalization.

### The 1988 test

The test was repeated as in 1986. Primary and secondary umbels were not separated at harvest. Three replications per treatment were analyzed.

### Results

#### 1985

There were no significant differences among the treatments for seed index (100 seed weight), but there was a significant difference ($P < 0.01$) for seed index between the primary (0.28 g) and the secondary (0.24 g) umbels. There were no significant differences among treatments or between primary vs. secondary umbels for percentage germination. There were significant differences among treatments for total seed weight in the cage (Table 1). Compared with hand pollination, honey bees produced more than 14 times as much seed. Although there was no statistical difference between the hand and the fly treatments, the amount of seed obtained from the fly treatments was more than four times that of hand pollination.

There was a significant ($P < 0.01$) negative correlation between harvest date and seed index ($r = -0.338$) and between harvest date and total percent germination ($r = -0.438$). Thus, later harvest dates reduced the quality of seed produced.

Total time per cage per year involved to accomplish pollination was 153 minutes for placing fly pupae in the cages and 594 minutes for hand pollinations. Cages with honey bees required 20 minutes to place the nucleus hives into the cages and 315 minutes to shift the bees and provide supplemental feeding.

#### 1986

Seed harvested from the primary umbels produced significantly ($P < 0.01$) more seed per plant (9.54 g) when house flies were used as compared with both honey bees and flies (8.94 g) and honey bees alone (8.60 g). There were no significant differences among the treatments for seed index, percentage germination, or for seed harvested from secondary branches. An analysis of the total seed weight per cage indicated that there were no significant differences among treatments for seed weight, seed index, or seed germination. However, as reported in Table 1, the mean seed weight obtained by using honey bees and house flies together was considerably higher than the other treatments. There was a significant ($P < 0.01$) positive correlation ($r = -0.399$) of seed index with total germination. Seed collected from secondary branches had a significantly ($P < 0.01$) lower seed index (0.112 g) than did seed harvested from the primary umbel (0.146 g).

#### 1988

Honey bees and flies together produced significantly ($P < 0.05$) heavier seed weight per cage than did flies alone. Seed weight per cage was intermediate when

### Table 1. Comparison of different pollination methods for total carrot seed weight obtained in cages. Ames, Iowa, 1985, 1986 and 1988

<table>
<thead>
<tr>
<th>Pollination method</th>
<th>Average total seed weight per cage (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1985</td>
</tr>
<tr>
<td>Honey bees</td>
<td>384.5 a</td>
</tr>
<tr>
<td>Flies</td>
<td>113.2 b</td>
</tr>
<tr>
<td>Both</td>
<td>—</td>
</tr>
<tr>
<td>Hand</td>
<td>26.2 b</td>
</tr>
</tbody>
</table>

*Means separated in columns by Duncan's (1955) multiple range test, $P < 0.05$.

*Significant differences among primary vs. secondary umbels for total seed weight. There were no significant differences among primary vs. secondary umbels for percentage germination. There were significant differences among treatments for total seed weight in the cage (Table 1). Compared with hand pollination, honey bees produced more than 14 times as much seed. Although there was no statistical difference between the hand and the fly treatments, the amount of seed obtained from the fly treatments was more than four times that of hand pollination.

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produced by honey bees (Table 1). There were no significant differences between treatments for seed index or germination.

**Discussion**

Considerable variation occurred between years for total seed weight obtained in the cages. Below average rainfall and extremely high temperatures (to 40°C) were recorded in 1986 and 1988.

Variation can occur within honey bee treatments due to the relative strength of the hives. During the dry seasons of 1986 and 1988, nectar sources outside the cages were not readily available to the bees. A possible reason why hand pollination does not produce good seed set is that the higher temperatures inside the cages might adversely affect the pollen. Iapichino and Loy (1987) found that temperatures of 38°C for 7 hours reduced pollen germination in bottle gourd, Lagenaria siceraria (Mol.) Standl. Perhaps time of day influences pollination success. Most hand pollination was done between 9:00 am and 3:00 pm. Insects do better as pollinators because they are persistent and work throughout the day. Honey bees are interested in providing the hive with pollen and nectar while flies spend considerable time walking across the umbels. Their activities may be complimentary.

Our data from 1985, 1986 and 1988 suggest that a combination of honey bees and house flies will produce more carrot seed per cage than if only bees or flies are used. The increase can be obtained without a large increase in labour costs. We intend to use a combination of both insects to pollinate the seed increase plots of carrot germplasm in cages.

**Acknowledgements**

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**Résumé**

**Méthodes de pollinisation pour conserver les collections de matériel génétique de carotte**

Des essais en cage sur le terrain on été effectués pour déterminer si les abeilles *Apis mellifera* L., les mouches *Musca domestica* L., ou bien une association des deux, fourniraient la meilleure pollinisation pour une augmentation du matériel végétal de carotte conservé sous forme de semences. En 1985, les abeilles seules ont été des pollinisateur nettement meilleurs que les mouches seules, mais en 1988, une association d'abeilles et de mouches a produit davantage de semences de carotte par cage que les abeilles ou les mouches seules. Les semences obtenues en utilisant les deux pollinisateur n'ont pas été très différentes du point de vue du poids de 100 grains ou du taux de germination. On a gagné beaucoup de temps grâce aux insectes pollinisateurs par rapport à la pollinisation à la main. A l'avenir, la Station régionale d'introduction des plantes pour le centre-nord utilisera une association d'abeilles et de mouches pour poliniser les carottes destinées à l'accroissement du matériel végétal conservé sous forme de semences.

**Resumen**

**Métodos de polinización para el mantenimiento de colecciones de germoplasma de zanahoria**

Se han realizado experimentos con cajas en el campo para determinar si el mejor polinizador para aumentar la producción de semillas de zanahoria que han de conservarse como germoplasma lo constituyen las abejas, *Apis mellifera* L., las moscas, *Musca domestica* L. o una combinación de ambas. En 1985, las abejas solas dieron unos resultados significativamente mejores que las moscas solas como polinizadoras, pero en 1988 se obtuvieron más semillas por caja con una combinación de ambas que con los dos insectos por separado. Las semillas obtenidas mediante el uso de los dos polinizadores no presentaron diferencias significativas en cuanto al peso de 100 semillas o el porcentaje de germinación. La utilización de los insectos en lugar de la polinización a mano permitió ahorrar bastante tiempo. En el futuro, en el Centro Regional Centro-Septentrional de Introducción de Plantas se empleará una combinación de abejas y moscas para polinizar las zanahorias, a fin de aumentar la producción de semillas destinadas a germoplasma.

**References**


