A method was devised to use honey bees and cages for controlled interpollination of numerous plant introduction accessions grown for seed increase. Honey bees (Apis mellifera L.) were maintained for 4 months (June-September) in small nuclei (minihive) for use in pollinating various crops grown under cages for seed increase. The nuclei remained as effective pollinating units without dwindling, swarming, overcrowding, starvation, or any of the other problems associated with extended maintenance of small pollinating nuclei. Cages used were easily assembled and stored, and were practical for caging large numbers of plantings for controlled pollination. Seed yields were excellent and of good quality.

(c) pollination by honey bees in cages. Controlled pollination is essential for maintaining the original genotype of the germplasm received. Under open pollination, outcrossing would dilute the germplasm, and valuable traits might be lost or masked. Crops used in this study were carrots (Daucus carota L.), parsley (Petroselinum crispum (Mill.) Nym., ex A.W. Hill), cucumbers (Cucumis sativus L.), chicory (Cichorium intybus L.), endive (Cicorium endivia L.), alfalfa (Medicago sativa L.), and white sweetclover (Medicago alba Medik).

Pearson (16) used small colonies of about 3,000 honey bees to pollinate Brassica oleracea L. in cheesecloth cages. Butler and Haigh (1) recognized that colonies confined without much forage inevitably became weak and ineffective. They allowed bees to fly into the open and into cages on alternate days by means of a gated tunnel leading from the beehive to the cage. They found that Brassica oleracea (cabbage, kale, and brussel sprouts) and Raphanus L. (radish) in cages produced pure seed even though other cultivars of both species were growing just outside the cage.

Free and Durrant (5) reported that any pollen on bees when they enter the hive probably becomes inviable during the night or other long periods in the hive. Krait (10) caged honeybees for 18 to 72 hr with vegetable and ornamental crops cultivars that had easily recognizable marker genes. He concluded that there is generally no risk of cross-pollination if bees that have visited plants of a certain species visit other plants of the same species after an overnight enclosure in the beehouse.

Kehr and Laberge (8) studied cross-pollination of alfalfa in cages with honey bees by using purple- and white-flowered clones. The purple-flowered clone always dominates in crosses with the white-flowered clone. Using a hive and connector of the type designated by Butler and Haigh (1), they reported 10% contamination of the white-flowered clone when it was grown alone in a cage. However, they were skeptical of this result. They do not mention whether bees were directed to the cages before or after foraging had begun on a given day. If bees had begun foraging when they were directed to the cages, this could have been a factor in their results.

Pankiw and Goplen (15) recommended a 2-day confinement for honey bees to prevent contamination of sweetclover.
with foreign pollen. They made their test for contamination at 2-day intervals from day 0 to day 20, which meant that no trials were made to indicate that over-night confinement was not adequate.

Scriven et al. (19) reported that colonies occupying 3 or 4 combs (deep broodcomb size) were ideal for pollination in cages. Weaver (20) found that it could be disadvantageous to use colonies that are too large for the area needing pollination, when he put colonies of about 10,000 bees into cages with *Vicia villosa* Roth, the anthers, stigmas, and corollas were damaged by such an excess of insects. He found this surprising, inasmuch as the presence of a queen stimulates foraging. Goplen and Pankiw (7) reported that presence of a queen stimulates foraging. Minderhoud (13) found no difference in pollination of *Brassica oleracea* by colonies with or without queens. Free (4) found this surprising, inasmuch as the presence of a queen stimulates foraging. They emphasized the importance of using a nucleus of young bees and a laying queen when they left their hives, and nearly all of bees' return in the evening. Rothenbuhler et al. (18) found that colonies composed mostly of young bees without previous flight experience oriented when they left their hives, and nearly all of them returned. Scriven et al. (19) also emphasized the importance of using a nucleus of young bees and a laying queen with brood.

Collison and Martin (3) showed a highly significant correlation between the number of bee visits and cucumber seed counts for up to 20 bee visits. They concluded that each pistillate flower on the day of anthesis should receive 15-20 bee visits for optimum high-quality fruit set.

**Cages**

The cages, nucleus design, and bee-handling techniques described here were developed and used at the North Central Regional Plant Introduction Station, Ames, Iowa. They were adaptations of Pederson et al. (17) portable field cage and Butler and Haigh's (1) beehive with tunnel and sleeve entrance. The simple cages used could easily be installed or taken down in 10 minutes by two people. The frames for the cages were made from 1.27-cm thin-walled conduit. Conduit was bent and drilled as required and fastened by 0.48-cm by 5.08-cm stove bolts with wing nuts. The assembled frame was then pushed into the ground to a depth of 7.62 cm. Rectangular pieces of nylon netting 4.7 m by 3.23 m were then draped over the frame, and soil was mounded over the edges. As the soil settled, the netting was pulled down firmly over the frame. The cage size most frequently used was 4.57 m long by 1.52 m high by 1.52 m wide; however, the frames could be placed end to end in the field to make cages of any desired length.

**Pollinating nucleus**

The pollinating nucleus (herein referred to as nuc) used was a queenrite 6-frame nuc (16.8 cm deep). Nucs were made up in mid-May by supplying each nuc with 2 frames of brood with adhering bees, 2 frames of pollen and honey, 2 empty combs, and a sealed queen cell produced by standard queen-rearing techniques (2). Two weeks later, the nucs were checked to determine whether the queens were hatched, mated, and had begun to lay eggs. When young brood was found in the nucs, they were allowed 10 to 14 days to build up in strength before being moved to the caged area. A parent apiary of 15 colonies was maintained, from which all brood was obtained and queen rearing was done. Care was taken to ensure that drone comb was not culled from all colonies so that adequate drones for mating would be present.

The nuc box illustrated in Fig. 1, 2, 3, 4, and 7, was 16.8 cm by 50.8 cm by 27.0 cm. All equipment was designed to be interchangeable and frames used were the same size as in the parent apiary. The bottom board for these nucs provided a 1.9 cm entrance opening at both ends (Fig. 3). Two L-shaped entrance blocks connected by two 61 cm by 1.9 cm by 0.3 cm pieces of strap iron were provided so that bees could be directed to either entrance by sliding one of the blocks in or out. Thus, opening one entrance closed the other, and vice versa. The blocks were grooved at the bottom to accommodate the strap iron, which were attached by wood screws and epoxy glue. This design was used to direct bees in and out on alternate days. Bees pupolizing the strap iron to the bottom board and restricting free movement was not a problem. A 2.2-cm² by 27.9-cm block of wood was used to seal or reduce the entrance as desired (Fig. 1).

The nucs were positioned half in and half out of the cages. The netting was placed over the nucs and secured by mounding soil on the netting (Fig. 4 and 7). The settling soil drew the net secure so that it was windproof and bee-tight. Bees were directed into and out of the nucs on alternate days (Fig. 4). The entrance block position was always changed in the early morning before any bees began flight. Bees were confined overnight to their nucs by darkness.

Caged nucs were supplied with water in 9.5-liter plastic buckets with wooden floats. No feeding of sugar syrup or pol-
Fig. 4. Letting bees in and out of cages. This operation was done before dawn and before bee flight had begun.

Fig. 5. Partly and completely erected cages.

Fig. 6. Cage pollination area.

Fig. 7. Nuc positioned in cage. Netting held around cage by mounding soil over netting. Carrot umbels are visible in the cage.

Bee handling technique

One Plant Introduction (PI) or several incompatible species of PIs were planted in each cage. Most cages were 4.57 m long, but one cage 22.86 m long was used. The caged area was treated with trifluralin to control weeds. Materials were direct-seeded or transplanted to the caged area before anthesis. Any flowers that blossomed before caging, were removed.

In maintaining the plant germplasm collections in Ames, 100 or more cages are used each year. Flowering is spread over a 4-month period because of the diversity of the material handled. Pollination, therefore, is a continuing process rather than a brief concentrated effort as in commercial pollination of large fields and orchards of a single crop.

In 1978, nucs were maintained throughout the summer without any loss in pollinating ability by the bees and with minimal labor. Alternate confinement to cages and free foraging kept brood rearing at a constant level rather than increasing to a peak(s) and then diminishing as in normal colony development (12). We presume this was due to the limited availability of pollen and nectar caused by alternate confinement and free foraging.
Under these conditions, bees readily foraged when directed to the cages. A 22.86-m-long cage was tried, and bees foraged the entire length.

The cages had little head space over the canopy of most crops, which allowed very little free-flying room. We believe that this greatly discouraged unproductive flying into the cage walls and encouraged foraging. When taller cages, with more open space above the plants were used, the number of confused bees, which flew into the walls of the cages, seemed to increase. This point has not been stressed in previous studies, but we believe that it has a very important effect on the behavior of honey bees in cages. Trials were made by using queenless and adequate pollination for good seed set. Queenless bees, allowed to forage in and out of cages alternately, quickly dwindled and lost interest in foraging. Likewise, queens, bees spent more time clustering at the entrance and flying into the cage walls than foraging. Likewise, queenless bees, allowed to forage in and out of cages, finished the summer with laying queens and brood. A loss of 13% under these conditions is tolerable, and we were able to meet our pollination requirements by making up 15% more nucs than we had anticipated needing.

Evidence for the effectiveness of this program is the excellent seed harvest obtained with this method. The program has been particularly effective with some cucumber PI's that are difficult to increase. Five of them had been grown as many as 12 times and hand-pollinated in an effort to obtain sufficient seed for storage and distribution. By using honey bees in cages, sufficient seed was obtained for storage and distribution of all five PI's, and in one case, excess seed was discarded. Caging had the added advantage of excluding harmful insects. This was particularly noticeable in the 1978 cucumber planting when bacterial wilt *)*) transmitted by the cucumber beetle (Acalympa spp.) devastated the uncaged lines but did not affect the caged materials.

**Pollination procedures**

Pollination every other day provided adequate pollination for good seed set with our materials. A few bees often remained in the cage when the entrance blocks were moved to let bees outside for free foraging. This often was the case on hot nights, and these bees probably provided some pollination on "out" days.

The safety of the technique for interpollinating cucumbers was tested by caging two PI lines known to have a double recessive character of short internodes (308915, 308916). They were treated the same as all other accesses. Numerous uncaged cucumber plants were growing closely. The seed obtained from these 2 caged were planted the following year (250 plants each) and checked for segregation. No segregation was observed indicating that no crossing with the long internode varieties growing near-by had occurred. We feel that the technique will be a useful tool in maintaining plant germplasm collections but agree with Nye (14) that more research should be done using many crops before intermittent confinement of colonies of honey bees could be recommended for all pollination work in which cages are used for genetic isolation.

Several factors probably are responsible for overnight confinement leaving bees free of contaminating pollen. Lehman and Purci (11) suggested that this might be caused by an accumulation of sugar and possibly changes in pH, insufficient much as the bee uses saliva and honey to stick the pollen to its leg.

Golubinski (6) observed that pollen carried by bees for 50 or more on their bodies was highly subject to desiccation and probably lost its viability. Other factors are the thorough brushing and cleaning that honey bees give their bodies and the warm, moist conditions of the brood nest (35°C) during the night (4). Using the method described, it is not certain that foraging bees would visit the same species grown in the cage on their out days. Furthermore, the first flower that a bee visits in the cage covers the bee with its pollen. If a few viable grains of other pollen were on its body, they would be diluted by a factor of several hundred or even thousand, and the probability of their being the ones to fertilize the ovules would be very small.

Information currently available in the literature indicates that pollen contamination is unlikely with the use of the method described. Preliminary tests found no contamination in 2 Cucumis sativus PI lines. Cage design and provisions for letting bees work in and out of the cage are simple enough to be practical on a very large scale. All equipment for 100 cages and pollinating nucs can be stored in a single garage stall. If proved safe from contamination, the method described here will greatly facilitate handling of large germplasm collections and improve the quality and quantity of seed obtained at the Plant Introduction Station.

**Literature Cited**

10. Kraii, A. 1970. How long do honey bees carry germinable pollen on their bodies and the warm, moist conditions of the brood nest (35°C) during the night (4). Using the method described, it is not certain that foraging bees would visit the same species grown in the cage on their out days. Furthermore, the first flower that a bee visits in the cage covers the bee with its pollen. If a few viable grains of other pollen were on its body, they would be diluted by a factor of several hundred or even thousand, and the probability of their being the ones to fertilize the ovules would be very small.