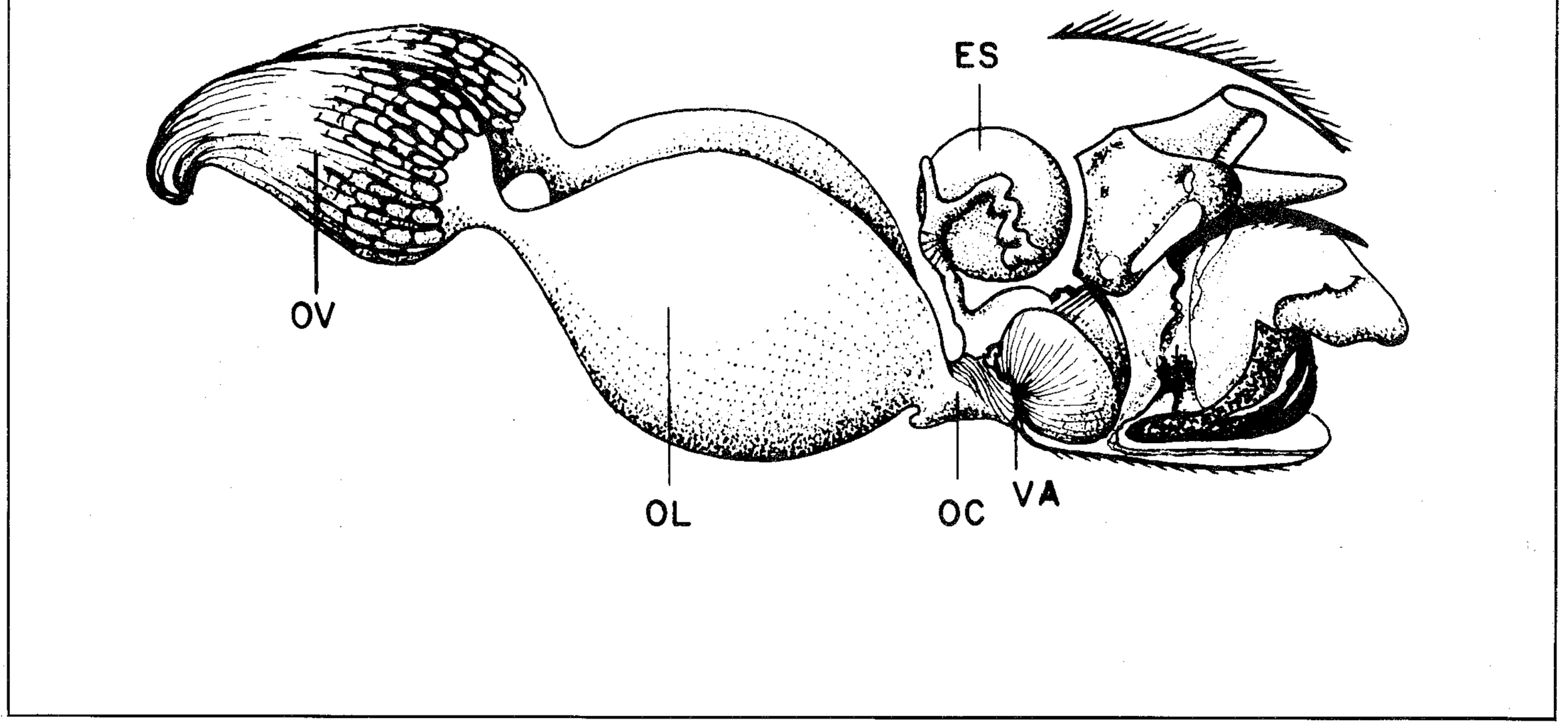

Figure 1. *Reproductive organs of a honey-bee queen immediately after returning from a mating flight.* OV, *ovary;* OL,*lateral oviduct;* OC, *common median oviduct;* VA, *vagina;* ES, *spermatheca*

INSTRUMENTAL INSEMINATION OF HONEY-BEE QUEENS IN THE DEVELOPMENT OF BEEKEEPING

• **J. Woyke**

One of the first steps to be taken in developing beekeeping is the improvement of the honey-bee. Since queens mate in the open air, the only way to control mating is to use artificial insemination, which this article describes.

Considerable potential exists in many countries for the development of beekeeping. This can be of great benefit not only to individuals but to the countries concerned as a whole.

Generally speaking, developing countries have greater potentialities for developing beekeeping than the developed countries, where agriculture has already been developed to a high level. There are many honey- and pollen-yielding plants in developing countries of which honey-bees do not take advantage.

Beekeeping needs very little investment. The most important factors are a knowledge of modern management of bee colonies and assistance in acquiring the skill to practise it.

During recent years a number of international organizations have helped to develop apiculture in developing countries.

The first step in countries in which there already exists some rural beekeeping is to learn how to transfer bee colonies from the crude forms of box hive now in use to modern bee-hives. In the rural box hive, the combs are fixed to the top and walls of the hive. Consequently, the bee colonies cannot be inspected and modern management cannot be applied. In modern hives, the combs are fixed into movable frames, which allow operations to be undertaken within the hive without destroying it.

The second step in developing beekeeping is to learn how honey-bees in those modern hives should be managed and to attract new recruits to beekeeping.

The third step is to improve the honey-bee itself. Unselected bees swarm continuously and escape from the apiary, sting a great deal and produce little honey. Progress in improving bees that have not previously been selected is very rapid at the beginning.

Certain difficulties do exist in improving honey-bees that do not arise in

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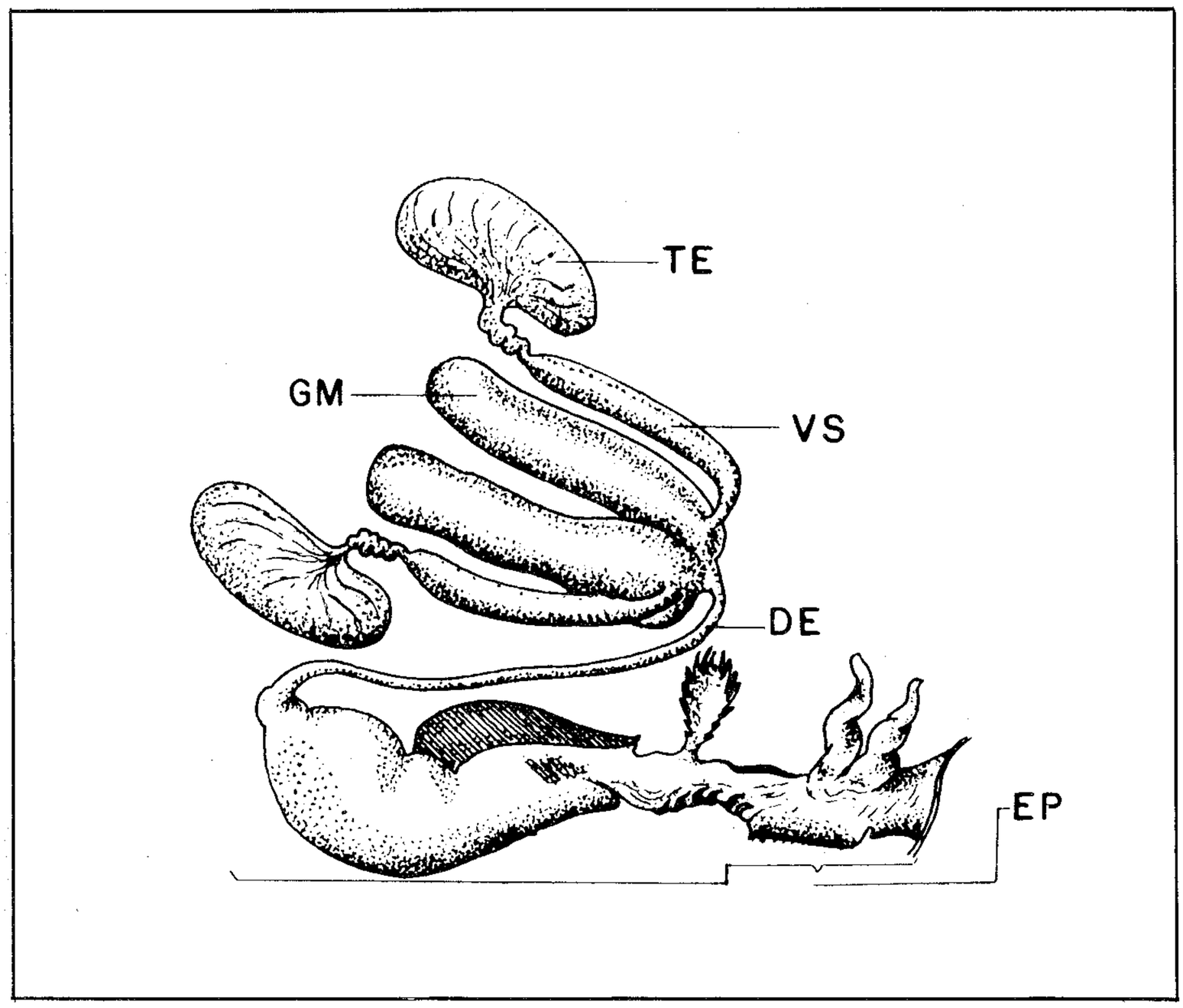


Figure 2.
Reproductive organs of a drone.
VS, *seminal vesicle;*
DE, *ejaculatory duct;*
EP, *endophallus copulatory organ;*
GM, *mucous glands;*
TE, *testes*

improving other domestic animals. The honey-bee queen mates only in the open air, where the crossing of parents cannot be controlled. Many attempts have been made to mate the queen in closed spaces but all have failed. Therefore, the only way to control mating of honey-bees is by instrumental insemination. The significant advantage in instrumental insemination of the honey-bee as compared with other domestic animals is that the queen only needs to be inseminated once.

Anatomy and physiology of reproductive organs

Figure 1 shows the reproductive organ of a queen immediately after returning from a mating flight. Development of eggs occurs in two ovaries (OV), each composed of 180 ovarioles. The mature egg passes through the lateral oviduct (OL) into the median oviduct (OC) and later on into the vagina (VA). A duct leads from the top of the vagina to the spermatheca (ES), where the queen stores the spermatozoa. A fold acting as a valve extends from the bottom of the vagina and can close the entrance to the median oviduct as well as the reproductive opening to the sting chamber. During mating, the semen is injected into both lateral oviducts (Woyke and Ruttner, 1958). Out of the 80 million spermatozoa injected, only 5 million (less than 10 percent) enter the spermatheca (Woyke, 1962). The rest of the spermatozoa are rejected from the abdomen. The queen stores the spermatozoa in her spermatheca during her whole life of several years.

The spermatozoa develop in two testes (TE) in the abdomen of a drone (see Fig. 2). All the spermatozoa are produced during the pupal stage of development. One drone produces some 10 million spermatozoa. All the spermatozoa pass from the testes into seminal vesicles (VS) a few days after the drone emerges from the comb cell. They are stored there until the mating act. During that act, the spermatozoa pass through the ejaculatory duct (DE) into the copulatory organ, the endophallus (EP). The drone then everts the endophallus and the semen, being pressed by mucus derived from the mucous glands (GM), is injected into the reproductive organs of the queen. The drone leaves part of its copulatory organ in the tip of the abdomen of the queen and dies as a result of the mating act.

Biology of fertilization

It has been known since the eighteenth century (Huber, 1792), that the honey-bee queen mates in open air outside the hive. It was believed that the queen mated with only one drone and it was because of this that at the beginning semen from only one drone was used for artificial insemination of a queen. But Triasko (1951), Taber (1954) and Woyke (1955, 1956) showed that the queen mates with several drones, in fact about eight, during one mating flight. Alber *et al.* (1955) showed that some queens are also mated during two or even three mating flights. Woyke (1964) showed that those queens mated during a second mating flight had stored less than 3.5 million spermatozoa in their spermatheca from the previous mating flight. Queens that store about 5 million spermatozoa in their spermatheca do not leave the hives for another flight. Queens with a large spermatheca carry more spermatozoa and can inseminate more eggs than those with smaller spermatheca (Woyke, 1966). The queen herself may or may not fertilize the eggs she lays. Workers and queens develop from fertilized eggs and drones from non-fertilized eggs.

Instrumental insemination procedure

Several manuals on the instrumental insemination of queen bees have been written (Watson, 1927; Mackensen and Roberts, 1948; Woyke, Ruttner and Vesely, 1966; and Ruttner *et al.*, 1978). However, considerable progress has been achieved in recent years in developing the practice.

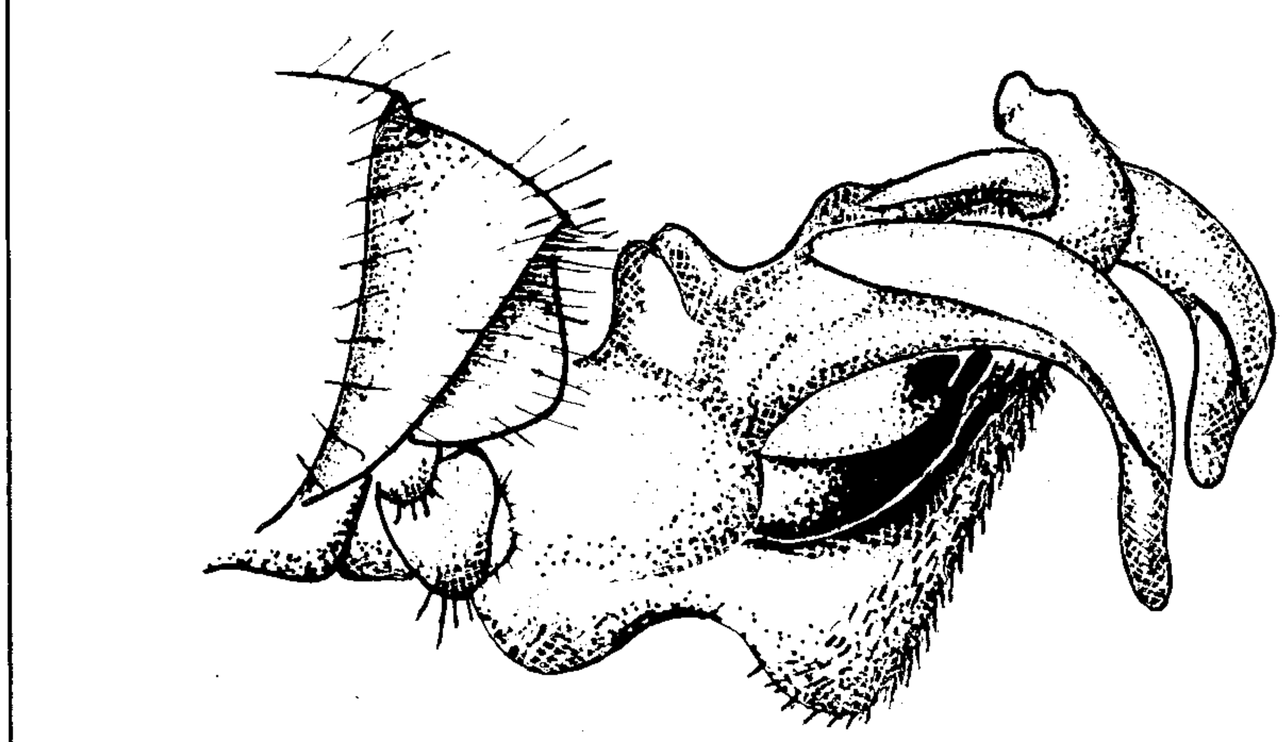
Drones ten to 20 days old should be used for instrumental insemination (Woyke and Jasiński, 1978). They

should originate from colonies with high production levels of honey. Squeezing the thorax of a sexually mature drone with the thumb and forefinger results in the partial eversion of the copulatory organ (see Fig. 3). The drone dies during this procedure. Further pressure on the abdomen results in a more advanced eversion so that the semen (SE) appears on the end of the endophallus (see Fig. 4). The semen can now be sucked into the plastic tip of the syringe. About 1.0-1.25 mm^3 of semen can be collected from one drone. Woyke (1962) showed that 8 mm^3 of semen should be used for artificial insemination of a queen. This amount of semen contains about 60 million spermatozoa.

The drones of Indian bees (*Apis cerana*) produce only 0.2 mm^3 of semen. Woyke (1973a and b, 1975) showed that 3-4 mm^3 of semen collected from 15-20 drones should be used for instrumental insemination of Indian honey-bee queens.

Queens five to 15 days old should be used for instrumental insemination (Woyke and Jasiński, 1976). It is important that queens reared from the youngest brood be used. Woyke (1971) showed that queens reared from eggs, or from one-day-old larvae, had, after instrumental insemination, 30-50 percent more spermatozoa in their spermatheca than did queens reared from three-day-old larvae.

The queens are inseminated under a stereoscopic microscope with the aid of a special apparatus (see Fig. 5). The basic part of this apparatus is a stand made of an iron plate with two vertical rods. Two holders are attached to these rods, supporting two hooks that open the tip of the queen's abdomen. A third holder supports the syringe for artificial insemination. The syringe consists of a metallic tube with a screw plunger inside and a plastic tip. A plastic-tube "queen holder" is located at the bottom, between the two rods, and is connected by a rubber tube to a metallic bottle containing carbon dioxide (CO_2) gas. The queen is placed in the plastic tube holder in such a way that the two last segments of the abdomen protrude from the holder. The holder, with the queen, is fastened to the apparatus for



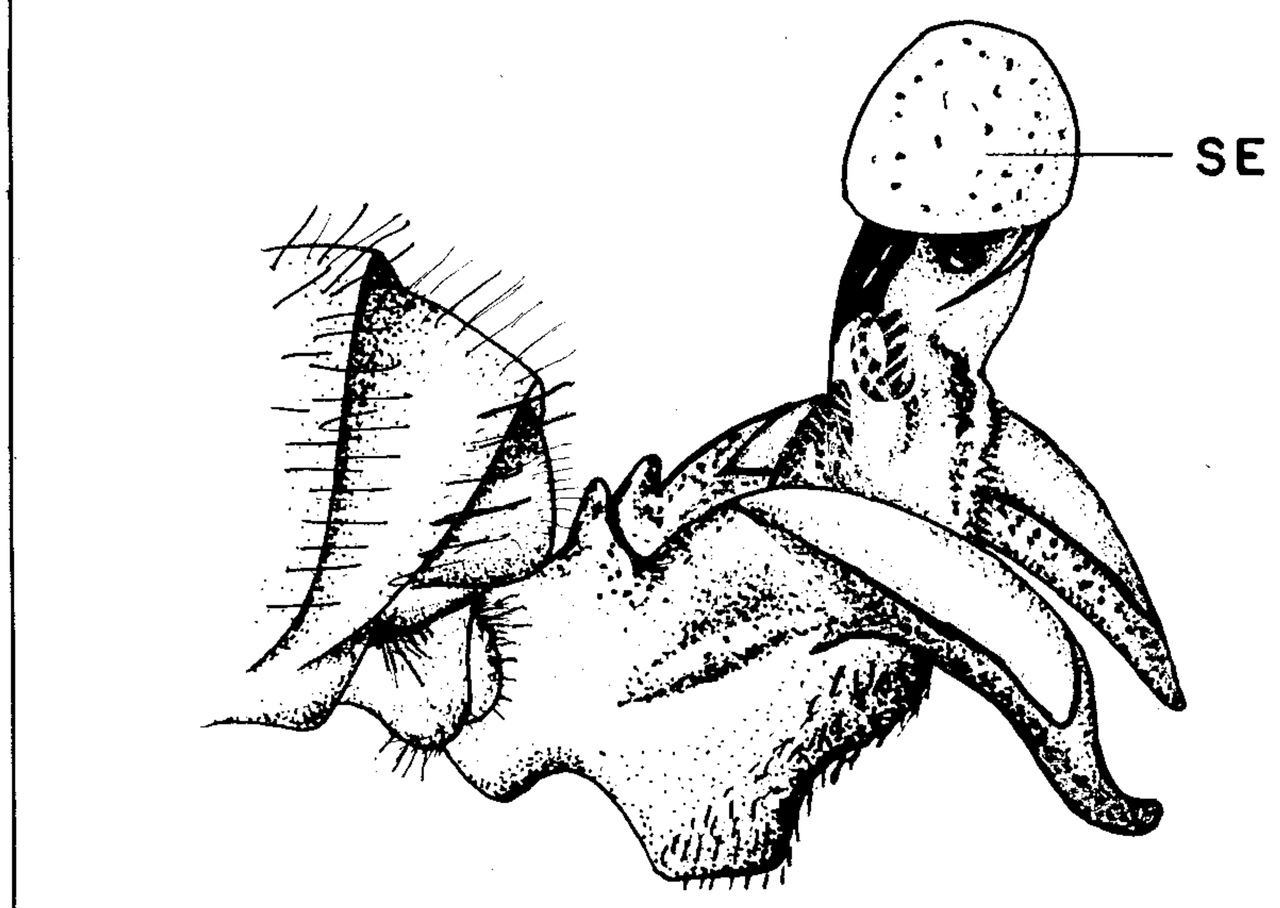


Figure 3. Top *Copulatory organ partially everted*

Figure 4. Bottom *Copulatory organ everted until the semen* (SE) *appears at its end*

Figure 5. Opposite page, top *Apparatus used in instrumental insemination of queen bees*

Figure 6. Opposite page, bottom *Injecting semen into the oviducts of a queen.* A, *tip of the syringe used for artificial insemination;* G, *hooks for opening the tip of the abdomen;* T, *queen holder*

artificial insemination and the flow of carbon dioxide is opened. The gas flows during the whole procedure of insemination.

The carbon dioxide treatment acts on the queen in two ways. First, it causes the queen to sleep, facilitating the whole procedure of insemination, and secondly, it stimulates the beginning of oviposition after insemination.

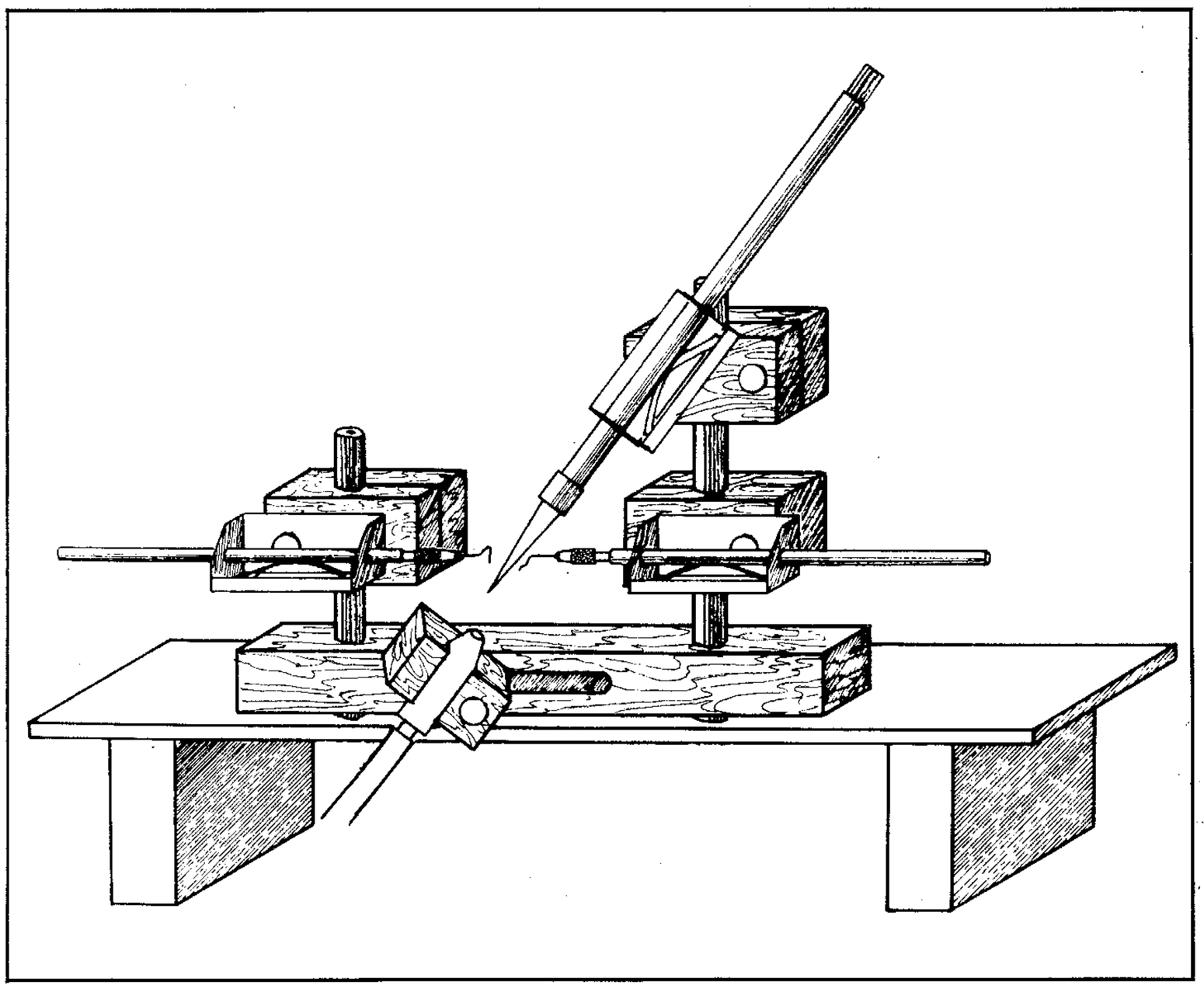
When the queen is quiet, the tip of the abdomen is opened with the aid of the two hooks. The valve fold of the vagina is pushed down with a vaginal probe (Laidlaw, 1944). The syringe tip filled with the semen is introduced into the median (common) oviduct and the probe is removed (see Fig. 6). The semen is then injected into the common and lateral oviducts and the syringe is withdrawn. The inseminated queen should be introduced into a group of worker bees (Woyke, 1979). At least 250 workers should take care of inseminated queens kept at room temperature (Woyke and Jasiński, 1980) and 350 workers of queens kept outdoors (Woyke and Jasiński, 1982).

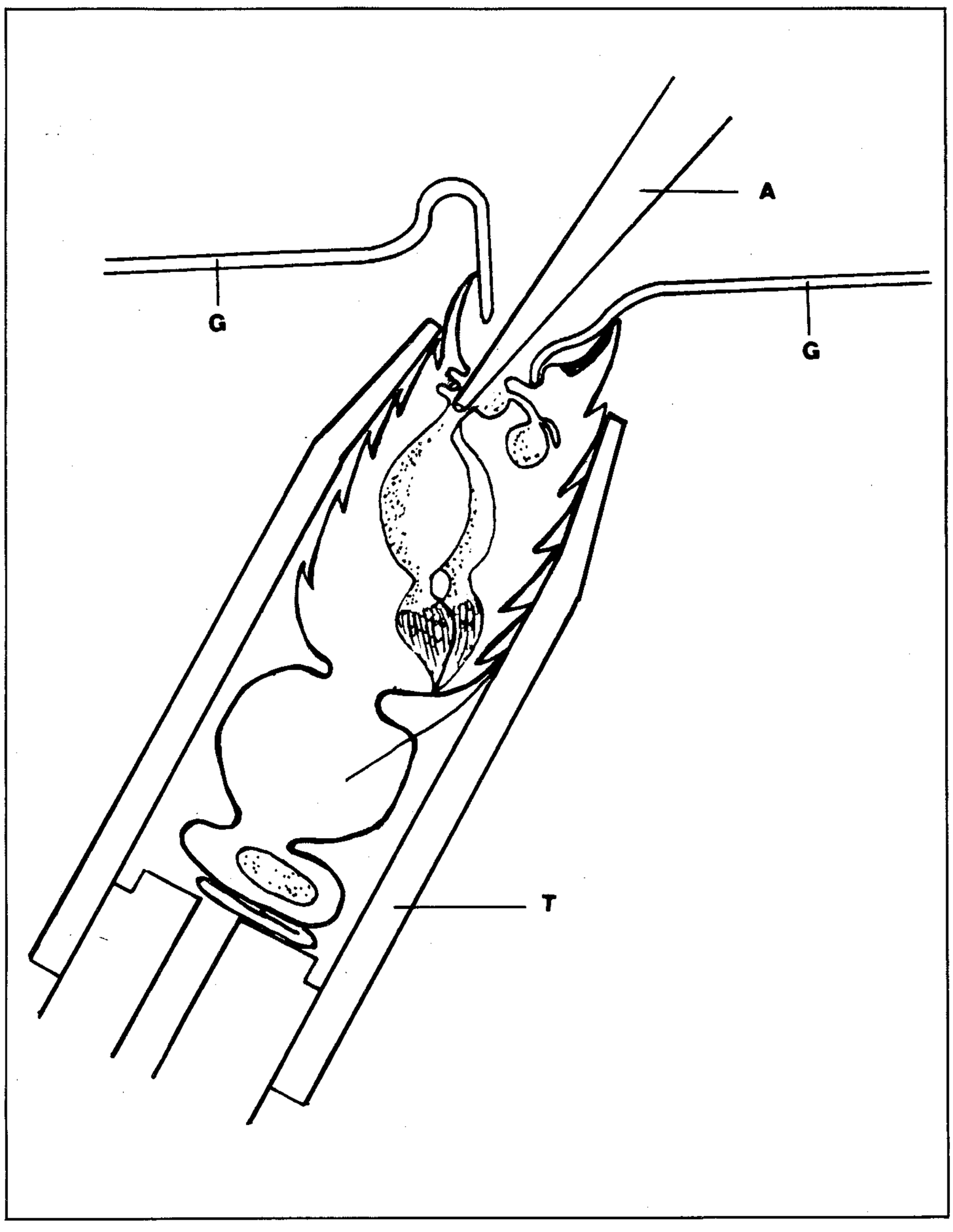
In the 24 hours after instrumental insemination, about 4.5 million spermatozoa enter the spermatheca, where they are stored for the queen's whole life of three to five years. The rest of the spermatozoa is rejected from the spermatheca in the same way as after natural mating.

If it is desirable to have more spermatozoa in a queen's spermatheca, then the queen should be inseminated twice. The first time she should be inseminated with 4-6 mm^3 of semen and after two days inseminated again with the same amount (Woyke, 1962). The Indian bee queens should be inseminated each time with 3 mm^3 of semen (Woyke, 1973a and b, 1975). One or two inseminations suffice for the whole life of the queen. As mentioned previously, the queen herself may or may not fertilize the eggs as occurs in natural mating.

Treatment of queens after instrumental insemination

In spite of the continuing improvement of the instrumental insemination of queens over the 30 years since its intro-





duction, the results obtained have not been wholly satisfactory. Many queens died after the operation and others had few spermatozoa in their spermatheca. The usual treatment was to separate instrumentally inseminated queens in small screened cages and to place them in queenless colonies.

Vesely (1970) showed that not all the semen was removed from the oviducts under these conditions, thus resulting in the death of some of the queens. Woyke and Jasiński (1973) demonstrated that the temperature inside these cages was lower than the necessary 34°C, thus inhibiting spermatozoa from entering the spermatheca. Woyke (1978, 1979) showed that the number of spermatozoa entering the spermatheca increased up to 170 percent when the queen had direct contact with the workers. It was shown that only 1.8 million spermatozoa entered the spermatheca when the queens were attended by 20 workers but as many as 4.1 million when they were attended by 350 workers (Woyke and Jasiński, 1979).

Today, instrumental insemination of honey-bee queens is not only applied in scientific laboratories. In Poland, at stations for artificial insemination of cattle, there are also divisions for instrumental insemination of honey-bee queens which provide services for practical beekeepers.

(It is of interest to record that Dr Woyke, the author of this article, with his colleague Dr Z. Jasiński, have recently received an award, First Class, from the Minister of Agriculture, Poland, for the introduction and improvement of instrumental insemination of queen bees in that country.) □

References

ALBER, M., JORDAN, R., RUTTNER, F. & RUTTNER, H. 1955. Von der Paarung der Honigbiene. *Z. Bienenforsch.*, 3(1): 1-28.

HUBER, F. 1792. *Nouvelles observations sur les abeilles.*

LAIDLAW, H.H. JR. 1944. Artificial insemination of the queen bees (*Apis mellifera* L.): morphological basis and results. *J. Morph.*, 74: 426-465.

MACKENSEN, O. & ROBERTS, W.C. 1948. A manual for the artificial insemination of queen bees. *USDA Bur. Ent. Plant Q. Circ.*, ET 250: 2-33.

RUTTNER, F., WOYKE, J., TRIASKO, V., MAKENSEN, O. & DRESCHER, W. 1978. The instrumental insemination of the queen bee. *Apimondia* (Bucharest).

TABER, S. 1954. The frequency of multiple mating of queen honeybees. *J. Econ. Entomol.*, 47(6): 995-998.

TRIASKO, V. 1951. Priznaki osemienosti pchelinyh matok. *Pchelovodstvo*, 28(11): 25-31.

VESELY, V. 1970. Retention of semen in the lateral oviducts of artificially inseminated honeybee queens (*Apis mellifera* L.). *Acta Entomol. Bohemoslov.*, 67(2): 83-92.

WATSON, L.R. 1927. Controlled mating of queen bees. *Am. Bee J.*

WOYKE, J. 1955. Multiple mating of honeybee queen (*Apis mellifera* L.) in one nuptial flight. *Bull. Acad. Pol. Sci. Cl. 2,* 3(5): 175-180.

WOYKE, J. 1956. Anatomo-physiological changes in queen bees returning from mating flight. *Bull. Acad. Pol. Sci. Cl. 2,* 4(3): 81-87.

WOYKE, J. 1962. Natural and artificial insemination of honeybees. *Bee W.,* 43(1): 21-25.

WOYKE, J. 1964. Causes of repeated mating flights by queen honeybees. *J. Apic. Res.*, 3(2): 91-98.

WOYKE, J. 1966. Wovon hängt die Zahl der Spermien in der Semenblase der auf natürlichem Wege begatteten Königinnen ab? *Z. Bienenforsch.*, 8(3): 236-247.

WOYKE, J. 1971. Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *J. Apic. Res.,* 10(1): 45-55.

WOYKE, J. 1973a. Artificial insemination of *Apis cerena indica* queens. *J. Apic. Res.,* 12(3): 151-158.

WOYKE, J. 1973b. Reproduction and artificial insemination of *Apis cerena indica. Indian Bee J.*, 35(3-4): 10-20.

WOYKE, J. 1975. Natural and instrumental insemination of *Apis cerena indica* in India. *J. Apic. Res.,* 14(3-4): 153-159.

WOYKE, J. 1978. *Biology of reproduction and genetics of honeybee.* Final technical report for USDA. Warsaw, Bee Cult. Div., Agric. Univ., pp. 1-381.

WOYKE, J. 1979. Effect of the access of worker honeybees to the queen on the result of instrumental insemination. *J. Apic. Res.,* 19(2): 136-149.

WOYKE, J. & JASIŃSKI, Z. 1973. Influence of external conditions on the number of spermatozoa entering the spermatheca of instrumentally inseminated honeybee queens. *J. Apic. Res.,* 12(3): 145-151.

WOYKE, J. & JASIŃSKI, Z. 1976. The influence of age on the results of instrumental insemination of honeybee queens. *Apidologie,* 7(4): 301-306.

WOYKE, J. & JASIŃSKI, Z. 1978. Influence of age of drones on the results of instrumental insemination of honeybee queens. *Apidologie*, 9(3): 203-212.

WOYKE, J. & JASIŃSKI, Z. 1979. Number of worker bees necessary to attend instrumentally inseminated queens kept in an incubator. *Apidologie,* 10(2): 149-155.

WOYKE, J. & JASIŃSKI, Z. 1980. Influence of the number of attendant workers on the results of instrumental insemination of honeybee queens kept at room temperature. *Apidologie,* 11(2): 173-180.

WOYKE, J. & JASIŃSKI, Z. 1982. Influence of the number of attendant workers on the number of spermatozoa entering the spermatheca of instrumentally inseminated queens kept outdoors in mating nuclei. *J. Apic. Res.*, 21(3): 129-133.

WOYKE, J. & RUTTNER, F. 1958. An anatomical study of the mating process in the honeybee. *Bee W.*, 39(1): 3-18.

WOYKE, J. RUTTNER, F. & VESELY, V. 1966. *Manual on artificial insemination of the queen bees.*

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