Laidlaw-Goss Queen Bee Pre-Set Artificial Insemination Instrument

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INSTRUMENTAL insemination (artificial insemination) of *Apis mellifera* queen bees was developed into a practical apicultural procedure in the late 1920's and the 1930's (Laidlaw 1987a) and will no doubt be a useful apicultural tool as long as bees are kept. The utilization of this tool is curtailed because of beekeepers' hesitancy to attempt what is perceived to be beyond their ability, and particularly by the high cost of instruments needed for the insemination operation. It has been demonstrated repeatedly that learning to artificially inseminate queens and to quickly attain reasonable skill is within the reach of virtually anyone willing to make the necessary effort. The cost of insemination equipment is the more serious impediment. This, however, need not be the problem it may seem to be. Binocular dissecting microscopes with 15X to 20X magnifying power, suitable for instrumental insemination, and lamps for them are commercially available at moderate prices. Used microscopes and lamps can be purchased for even less. Carbon dioxide, other supplies, and small items can be purchased locally.

Instrumental insemination of queen bees is essentially the introduction of drone semen into the reproductive tract of queens. For the operation to be successful, however, it must be performed in a manner that safeguards the semen from contamination with disease organisms or other deleterious substances. It is a precise operation though not a difficult one, and it can be performed swiftly with low cost, simple, smoothly functioning instruments. The minimum special insemination equipment needed for semen injection into the queens is a tilted holder for anesthetized queens, apparatus to open the sting chamber and vaginal orifice of the queens, a syringe to inject semen into the female reproductive tract, and a syringe manipulator.

The new instrument described in this paper was designed to meet these criteria and to be made with a minimum of machining in beekeepers' own shops from parts and materials available commercially. Most of the materials used in the construction of this instrument were purchased from Small Parts, Inc., P.O. Box 381966, Miami, Florida 33238.

The new instrument strongly resembles the Nolan-Mackensen pattern, but differs in that the queen micromanipulator and the insemination syringe micromanipulator are mounted on separate bases, Fig. 1, to allow independent movement of the queen as well as of the syringe to align them for syringe tip insertion into the vagina. This instrument incorporates some features from instruments of Laidlaw (1939, 1944, 1977, 1987b, 1988), Mackensen and Roberts (1948), Mackensen and Tucker (1970), Nolan (1932), Ruttner (1976), Ruttner, Schneider, and Fresnaye (1974), and Schley (1990a). It is not meant to be superior to its donors, but to be efficient and generally affordable.

Any suitable syringe can be used with this instrument. The Mackensen syringe is excellent and can be fitted with an adapter for glass tips. The insemination syringe devised by Harbo

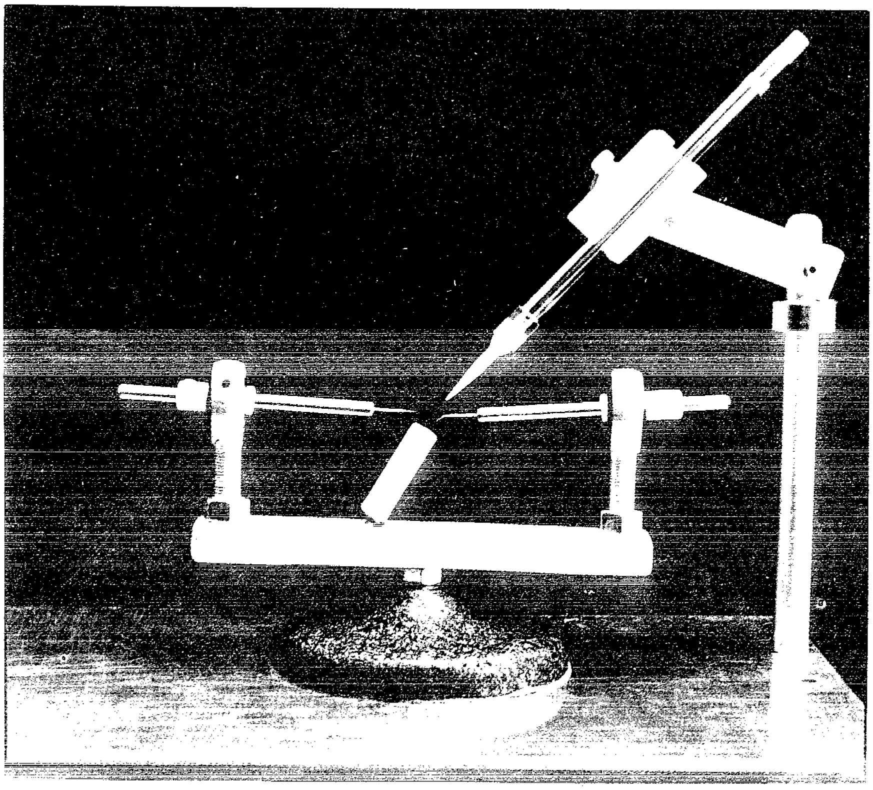

Fig. 1 — Laidlaw Goss queen bee pre-set insemination instrument Near side

(1985), which is patterned after a semen collecting apparatus that was used by Pool and Taber (1969) for collection of bee semen for storage, is recommended because its actuator, the Gilmont micropipette, is easily combined with a glass point to make a good insemination syringe. It is recommended that a polished steel syringe barrel be substituted for the usual glass one of the Harbo syringe because polished steel has less friction than glass with the plastic syringe holder. The Schley syringe includes a glass tip and is also efficient.

Queen Micromanipulator

The elements of queen micromanipulators are now more or less standardized. Hence, it is possible to discard some less useful features and to pre-set some of those that are retained and are usually adjustable, as has been done with the instrument described in this paper. This is an innovation of considerable importance because improper adjustment of micromanipulators is a major cause of difficulties and failure encountered by inexperienced inseminators.

The Laidlaw-Goss queen micromanipulator, Figs. 1 and 2, is a simplified device to hold the virgin queen steady under anesthetization with her sting chamber opened by ventral and dorsal hooks and the sting drawn dorsally by the dorsal hook, or by forceps, from over the vaginal orifice to facilitate insertion of the syringe tip into the queen's reproductive tract. It is freely movable horizontally on the microscope stage or on a metal plate overlying the stage.

The central part of the Laidlaw-Goss queen micromanipulator is a "central" metal bar with a slanted queen holder that is screwed into a hole offset 3/4" from the middle of the bar, Fig. 1, and with the free end of the holder directly over the middle of the bar. A 3/8" rod-end ball joint at each end of the central bar carries in the hole of its movable ball a 3/8" x 1" Delrin rod with the handle of a sting chamber opening hook inserted in a 3/16" longitudinal clearance hole drilled through the center of the rod. The central bar is attached at its middle to a heavy circular movable base that is 3 3/4" in diameter and 3/8" thick. The operator can slide the micromanipulator horizontally in any direction on the microscope stage or on a metal plate overlying the stage to bring the queen into the microscope field and to position the vaginal orifice for insertion of the syringe tip and to bypass the valvefold. The lower surface of the central bar is 1 1/2" from the lower surface of the heavy base to provide clearance for the operator's fingers between the bar and the supporting base.

The central bar, Fig. 1, is aluminum 6" long, 3/4" wide, and 1/2" thick. For convenience, the end of the bar toward which the queen holder slants is called the "right" end and the other end is the "left" end. To position the queen holder, a hole for a 1/4" aluminum rod is made on the central bar upper centerline 2 1/4" from the left end. It is drilled toward the left end of the bar at an angle of 31° ± 1° from perpendicular, Fig. 1, to a depth of 3/8" to form a blind hole which is tapped with a 1/4" National fine tap. One end of a 1 3/8" section of 1/4" copper or aluminum rod with a 9/64" blind center hole drilled to 1/16" from the blind end, is threaded to screw into the tapped blind hole. One-half inch of the other, or blind end, of the section of the metal rod is filed on opposite sides sloping toward the closed end to a thickness of 1/8". A 3/64" hole is drilled through the sloping sides 1/4" from the blind end. An "O" ring is slipped down the metal rod and a 1 3/8" section of 1/4" ID plastic, glass, metal, or rubber tube, fully open at both ends, is slipped down over the metal rod leaving a space at the top to form a chamber to hold the queen. The length of the queen-holding chamber above the end of the metal rod can be varied by moving the queen holding tube up

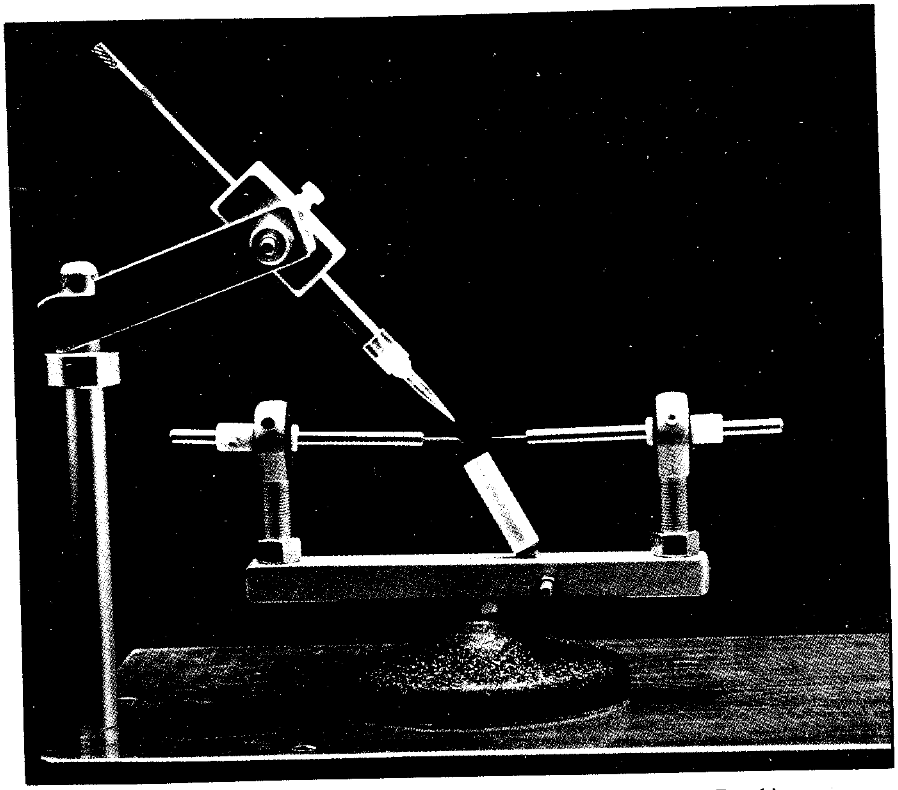

Fig. 2 — Laidlaw-Goss queen bee pre-set insemination instrument. Far side.

or down on the rod. The upper end of the queen holding tube need not be constricted because the queen is anesthetized before she is put into the queen holding tube at the upper end (Laidlaw 1988), where she will remain quietly.

On the far side of the central bar a hole is drilled into the bar 2¹⁄₁₆" from the left end and ⁵⁄₃₂" from the bottom surface of the bar to intercept the blind hole, Fig. 2. This is tapped to take a ⅛" brass tube fitting for the attachment of Latex tubing that conducts CO_2 to the queen holder from the gas source.

The sting chamber opening hooks should be bought initially. Replacements can be made later, if needed. Durable hooks can be made from 0.65 mm (0.025") diameter phosphor bronze spring temper wire or from other spring temper wire. Guidance in making the hooks is given in Laidlaw (1977).

The small hooks are fastened into handles which are two ³⁄₁₆" diameter 3¼" long polished cold rolled steel rods. A slightly oversize hole is drilled into one end of a hook handle and the shank of a finished hook is dipped in liquid solder, glue, or other fastening substance and is inserted into the drilled hole. A slight burr left at the end of the hook slant will usually hold the hook securely in the handle without adhesive.

The polished steel hook handles are mounted in plastic rods that are lightly pressed into the opening of the ball of the rod-end ball joints of the two lateral ⅜" rod-ends and can be moved in a wide circular area. Each polished hook handle extends through a ³⁄₁₆" clearance hole drilled in the center of a 1" section of a ⅜" diameter Delrin or Nylon molybdenum-filled round stock rod in which it will slide smoothly. Light friction on the hook handle is controlled with a 10-32 Nylon set screw installed near the outer end of the plastic rod. Equally good control of friction on the hook handles can be obtained by threading the outer ends of the plastic rods in the ball joints with a ⅜-24 die and capping them with the cap nuts from a brass compression connector for ¼" copper tubing. The cap nuts have a ³⁄₁₆" center hole which guides the steel hook handle. A section of string packing long enough to partly encircle the hook handle is inserted into each cap to give adjustable control of the sliding movement of the hook handle.

Each lateral ⅜" male rod-end ball joint is screwed into a ⅜" tapped hole drilled ⅜" from the end of the central bar, Fig. 1. When a rod-end ball joint is received from the supplier, the ball may bind slightly at its upper and lower contacts. This can be remedied by lightly tapping the sides of the rod-end ball joint between the binding points until the ball moves freely in any direction.

To control and ensure smooth ball movement, two 8-32 Nylon Allen-head set screws are used with each rod-end ball joint to form a 3-point suspension of the ball. The set screws are positioned by rotating the rod-end ball so the ball hole is facing a rod-end edge 45° from the vertical, drilling a hole through the outer wall of the rod-end ball joint, and tapping the hole for the Nylon set screw. The second Nylon set screw is installed in the same manner on the opposite edge of the rod-end.

The heavy base of the instrument may be bought or it can be cut from a ⁵⁄₁₆" or ⅜" brass sheet. It should be approximately 3¾" in diameter with a ⅛" to ¼" wide, and ¹⁄₃₂" to ¹⁄₁₆" deep, marginal ring on the lower side to prevent wobble, and should be heavy enough to give stability to the instrument. Felt covering the bottom of the base greatly improves the sliding maneuverability of the queen micromanipulator on some surfaces.

A base can be made from molten lead poured into a suitable mold such as a tuna fish can (Coby Lorenzen personal communication).

Syringe Micromanipulator Assembly

The syringe is movable up and down firmly and smoothly in a holder and it can be tilted and moved sideways. The syringe is fastened in a narrow cradle-channel made in a ¾" x ⅝" x ½" low-friction Delrin block that is supported on an arm that extends from a ½" x 7" aluminum or steel post bolted to a ⅛" x 8" x 11" aluminum plate which rests on or under the microscope stage, Fig. 1. The syringe-holding block is prepared to hold the syringe by drilling a hole with a ³⁄₁₆" drill bit lengthwise through the block ³⁄₁₆" from one ½" side and ⅜" from an adjacent ⅝" side (which side becomes the back of the syringe holder). A channel ⅜" wide and ⅜" deep is milled from the front side of the block toward the back and cuts through the middle of the longitudinal hole to make a u-shaped cradle for the syringe. A hole directly opposite the cradle is drilled and tapped for a 8-32 Nylon thumb screw for adjusting the friction on the up and down movement of the syringe in the holder, Fig. 1.

To attach the syringe holder to its supporting arm a ¹⁄₁₆"-thick steel plate is bolted to the back of the Delrin syringe holding block and a ³⁄₁₆" hole is drilled through the center of the plate. A ³⁄₁₆" x 1½" cold rolled, steel rod is silver soldered into the ³⁄₁₆" hole. The rod, with its free end threaded, is the shaft that extends through a ³⁄₁₆" ID x ⅜" OD x ¾" long sintered bronze bearing that is pressed into a ⅜" clearance hole drilled horizontally ¼" from the end of a 4¼" x ¾" x ¾" aluminum bar, Fig. 1. Washers or collars on the shaft on each side of the supporting bar make the syringe tilting movement smooth, and a 8-32 stop nut on the end of the shaft adjusts the amount of torque. The syringe holder support bar is tilted upward 20° from its post center, and a ½" diameter vertical hole through the base of the support bar receives the vertical support post, Fig 1. A 8-32 Nylon set screw in each side of the base of the support bar permits controlled sideways movement. A ½" ID set screw shaft collar placed beneath the syringe support bar holds the bar at the selected height on the rod when the support bar is swiveled.

A metal syringe holder can be made from a ¾" x ½" x ½" section of steel or aluminum channel. A "V"-shaped longitudinal groove is filed in the middle of one leg of the channel, and a Nylon tension adjusting screw is set in the other leg directly opposite the groove. The bottom of the groove should be 1/16" plus the radius of the syringe barrel from the rear channel wall. For smoothest syringe slippage a metal syringe holder can be lined with felt or with other low-friction material.

To prepare this instrument for use, it is suggested that the microscope be placed on or under the syringe post base plate and the upper end of the queen holding tube of the queen micromanipulator be brought to the center of the microscope field and in focus. Then the microscope and queen micromanipulator are moved together until the queen-holding tube and the syringe are in line. The microscope is fixed in this position. The inseminator can slide the queen micromanipulator horizontally to position the queen for insertion of the syringe tip into the queen's reproductive tract or, instead, the syringe can be tilted. It is easier to move the queen for positioning than to tilt the syringe.

Sources for purchase of honey bee artificial insemination syringes and sting chamber opening hooks

Sue Cobey. Honey Bee Insemination Service, 7417 Hayden Run Rd., Amlin, Ohio 43002.
- Harbo syringe
- Glass tips
- Sting chamber opening hooks

Dr. Otto Mackensen. P.O. Box 1557, Buena Vista, Colorado 86211.
- Mackensen syringe
- Adapter for glass tips
- Sting chamber opening hooks

Dr. Peter Schley. Heinestrasse 6, D6301 Polheim 2, Germany.
- Schley syringe
- Glass tips
- Sting chamber opening hooks

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