Killing and Preserving Insects

Insects may be collected and preserved dry or in ethanol and different methods of killing and preservation are used for different orders (see Appendix). As a general rule, soft bodied insects (including immature stages of most orders) should be preserved in ethanol and hard bodied insects should be pinned, although many hard bodied insects will also preserve well in ethanol.

Killing insects

The killing process is determined by the way in which the material will be preserved and what it will be used for. Therefore it helps to know what the orders being collected are beforehand. In general, any insect with scales on the wings (moths, butterflies) is unidentifiable if collected or preserved in liquid, unless it is going to be identified from DNA only. Otherwise wet collecting is by far the easiest way to manage bulk sampling of insects and is the normal method for immatures. Dry collecting is the only safe method for butterflies and moths and is the preferred method for some Hemiptera (plant bugs, scales) and Diptera (flies).

Dry killing

Since dry killing is trickier to deal with than wet, it is discussed first.

For general purpose dry killing, one method is to **place the samples of specimens in the freezer**. Freezing is not recommended if the samples include mixed insects that may have been damaging or even eating each other. Time required depends on the size and type of insect but several hours (e.g. overnight) is enough. Take care when removing specimens from the freezer as they are brittle when frozen and allow time to thaw before handling.

Dry killing is more done often with an evaporative poison, the safest and most frequently used and easily accessible being ethyl acetate. This is highly flammable, and should not be directly inhaled. Follow safe working procedures and follow recommended guidelines for handling contained in the SDS (safety data sheet) for the chemical. Ethyl acetate has the additional advantage of leaving

specimens relaxed for mounting for several hours after death. It has the disadvantage of dissolving some plastics, so vials should be tested first before use. Use polyethylene (PE) or polypropylene (PP) and avoid polycarbonate plastics. Death rates of specimens vary according to size and type of insect but ideally specimens should be left in the killing bottle for an hour or so after immobility.

2 to 3 of drops of ethyl acetate onto tissue in a sealed collection tube is enough to kill most small insects. It's important not to add too much ethyl acetate to avoid wetting small delicate specimens (it doesn't matter with beetles) and tubes should be kept out of light and away from heat as much as possible.

Larger insects may require a larger wide mouthed killing bottle with a larger amount of ethyl acetate to kill the insects as quickly as possible. A small ball of cotton wool is a good absorbent material for the liquid but the fibres get tangled up in legs and mouthparts of some insects (e.g. beetles). Tissue or paper towel would be fine. Keep Lepidoptera separate where possible as their scales will get on other insects, or clean the jar first before using for other insect groups. Lepidoptera and other fragile insects should be placed in separate jars to larger and more robust insects such as grasshoppers and beetles, to prevent damage to the specimens.

Butterflies, some moths and some dragonflies can be rendered immobile by pinching the thorax under the wings between the thumb and a finger, breaking the propulsion system for the wings. This is a quick method for collecting butterflies in particular but requires carrying a sealed box of paper envelopes in the field. **Paper envelopes** are rectangles of paper folded so that they are reduced to a triangle with flaps over each edge to prevent the specimen from falling out. The pinched immobile specimen is placed inside, making sure wings are upright and collection data written on the envelope. This is placed in a sealed box (e.g. plastic lunch box). The box needs to be kept out of the sun to avoid condensation damaging specimens and the sealed lid prevents ants from entering.

Temporary storage of field collections

Specimens should only be left as long as necessary in killing bottles. If specimens are not going to be immediately pinned then they should be transferred from the killing jars to temporary storage containers such as pill boxes (see below) or paper envelopes (as above). This storage in the field will protect specimens from damage, especially in transportation and is a way to keep the specimens safe and in good condition indefinitely. Pill boxes or paper envelopes can then be placed in a larger waterproof container, however it should be noted that specimens should be allowed to dry out first before sealing in a plastic bag or airtight container. Silica gel may be added to the larger container and checked and changed regularly to remove humidity and assists in drying out specimens too. 'Dettol' and ethyl acetate can also act as antifungals and could be added to a ball of cotton wool and sealed inside the larger container.

'Pill boxes' are small cardboard boxes that are useful temporary storage for most small to medium dry collected specimens. Pill boxes can be made using any small cardboard boxes, such as matchboxes, and filling with layers of some kind of soft tissue, tissue paper or toilet paper, so that specimens do not move around inside the box. Specimens are laid between the tissue layers. Transfer temporary field labels to the top of tissue layers inside the box and also write the field

collection data or codes on the pill box lid. Groups of these small sample boxes should be stored in airtight containers to prevent damage from ants.

Specimens may be kept for long periods in paper envelopes or pill boxes as long as they are in a pest and moisture free environment i.e. an airtight plastic container, a wooden insect box or drawer with naphthalene and desiccant (silica gel) that is regularly inspected and replaced as required.

Wet killing

Wet killing directly into **ethanol (preferably 100% concentration for most insects)** in vials is a convenient and fast method in the field for most insects (See Appendix), such as beetles, ants, wasps, bees, flies. Prepare different diameter vials of ethanol prior to fieldwork and small paper labels and a pencil. Specimens may be kept in ethanol indefinitely and when removed from ethanol for pinning their structure will be preserved. However 100% ethanol makes some specimens too brittle to prepare, e.g. scale insects, so these should be collected or at least preserved in 70-80% ethanol. Another small problem is that killing directly in ethanol can make larval insects turn black. This can be avoided by killing them in a mixture of ethanol (10 parts) kerosene (1 part) and acetic acid (2 parts) then transferring them after 24 hours to 100% ethanol. Specimens collected for DNA analysis should always be collected into 100% ethanol.

Wet preservation

Wet killed insects can generally be maintained in ethanol for years. They need to be checked regularly to monitor evaporation of the ethanol. If kept in a dark place they will only fade slightly.

When removed from ethanol storage for pinning or mounting they will be brittle. Specimens will be less brittle if passed through more dilute ethanols to water, before drying on tissue. Small Diptera (flies) and other soft bodied insects may shrivel badly when removed from 100% ethanol for drying and mounting. This can be prevented by placing then directly in ethyl acetate for a few hours then drying them.

Also, some hard-bodied insects can be stored in ethanol temporarily before drying and pinning, such as beetles, ants and some hemipterans and thus can be collected in the field as such. However some insects such as plant bugs (Hemiptera: Heteroptera: Miridae) once in alcohol cannot easily be dried for pinning without shrivelling up.

For long term wet preservation specimens should generally be placed in **100% ethanol**, which is also required for DNA preservation. Eggs, larvae, nymphs and pupae should all be stored in ethanol. Soft bodied adults such as bristletails, silverfish, stoneflies and caddis flies should be preserved in ethanol as well. Ethanol is the most commonly used wet preservative although, some groups may benefit from or require a slightly different liquid preservative for better long term storage, e.g. aphids and scale insects are recommended to be preserved in lactic-alcohol, a mixture of ethanol and lactic acid. Therefore, it is important to read up on specific techniques for a particular group you may be working on.

Dry preservation

The longer material is kept dry in temporary storage, the more brittle or exposed to damage it becomes. So pinning or other mounting for long term maintenance is best done as soon as possible.

Specimens kept in a freezer will remain relaxed for some time as well as being protected from pest attack and mould. Once thawed, these specimens will be soft and easy to pin without damage.

Relaxing dried specimens before pinning

Dry stored specimens in pillboxes or paper envelopes will become very brittle if stored for some time. These dry specimens will need to be relaxed before pinning or mounting and wing setting. A **relaxing chamber** is used for this purpose and can be made easily from any airtight container such as a wide mouthed glass jar or any kind of plastic crate or food storage container, sized to your needs. The bottom of the relaxing chamber is filled with either water, wet sand or wet paper towel. Specimens should then be set inside the container on a platform above the wet substrate, such as on the lid of another smaller container placed inside the relaxing chamber. One to two or up to three days will be required to relax insects depending on the size and body type. Specimens should be checked daily and **mould growth is the biggest concern**. Larger specimens such as butterflies can take 2 full days and it can help to turn them over after a day. Most traditional antifungal agents are highly toxic and should not be used. 'Dettol" with antifungal properties could be added to the relaxing chamber.

Robust insects like beetles and ants can be immersed in water to relax them rather than needing to go into a relaxing chamber and **quick relaxing** of some hard bodied insects such as robust beetles can be done by dropping them in near boiling water for up to a minute or so.

Note: Hot or warm water will also speed up the relaxing process if added to the relaxing chamber.

Note: smaller insects in pill boxes, especially more robust specimens like beetles, may sometimes be point mounted without needing to be relaxed first.

Preservation methods of different insect orders

For immature stages, mostly being soft bodied, wet preservation is required for the most part. However some nymphs of hemimetabolous groups such as Hemiptera and Orthoptera have a hard exoskeleton and may be dry preserved and pinned or point mounted.

For adults the following killing/preservation techniques are used:

Archaeognatha [bristletails]	Ethanol
Blattodea [roaches, termites]	Dry or Ethanol
Coleoptera [beetles]	Dry or Ethanol
Dermaptera [earwigs]	Ethanol
Diptera [flies]	Dry or Ethanol
Embioptera [footspinners]	Ethanol
Ephemeroptera [mayflies]	Ethanol
Hemiptera [aphids, psyllids, scales, cicadas, true bugs]	Dry or Ethanol
Hymenoptera [bees, sawflies, wasps and ants]	Dry or Ethanol
Lepidoptera [butterflies and moths]	Dry
Mantodea [mantids]	Dry or Ethanol
Neuroptera [lacewings]	Ethanol
Odonata [dragonflies and damselflies]	Dry
Orthoptera [crickets, grasshoppers]	Dry
Phasmatodea [walking sticks]	Dry
Plecoptera [stoneflies]	Ethanol
Psocodea [book lice, lice]	Ethanol
Siphonaptera [fleas]	Ethanol
Strepsiptera	Ethanol
Thysanoptera [thrips]	Ethanol
Trichoptera [caddisflies]	Ethanol
Zoraptera [zorapterans]	Ethanol
Zygentoma (= Thysanura) [silverfish]	Ethanol

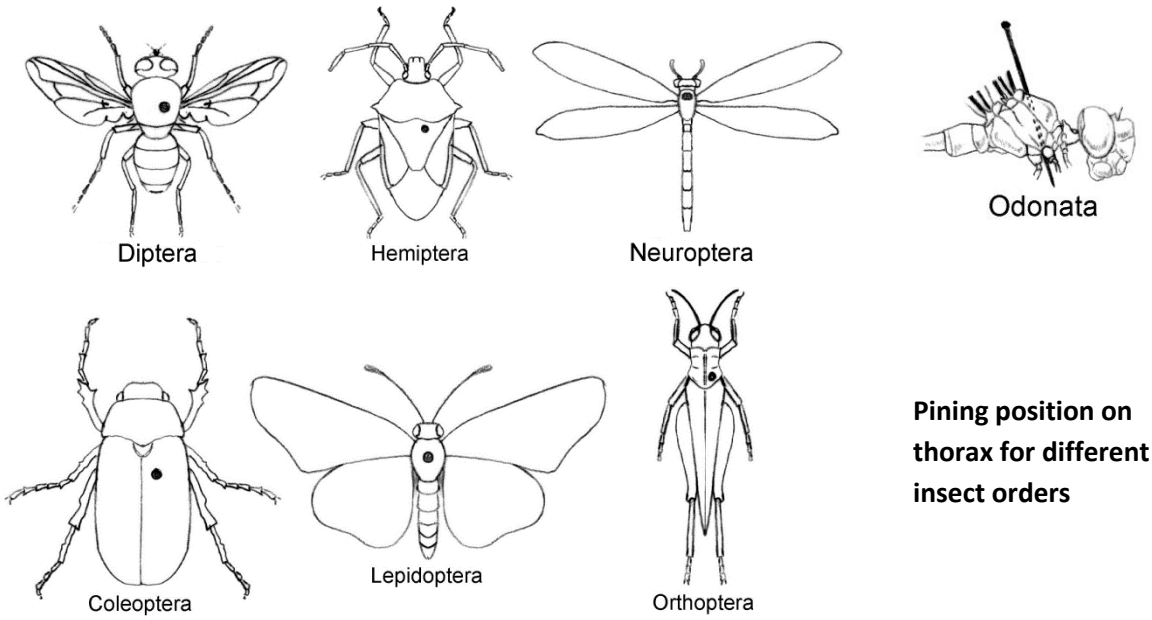
Insect pinning

To pin or not to pin?

Specimens that are about 8mm or larger if elongate-oval, or 10mm or longer if thin and narrow, may be pinned without damaging the body. Pinning insects involves the insertion of an insect pin through the thorax, right of centre, aside for butterflies, moths, dragonflies and lacewings which are pinned through the centre of the thorax.

Exact pin positioning for different groups of insects is as follows and illustrated in the figure below:

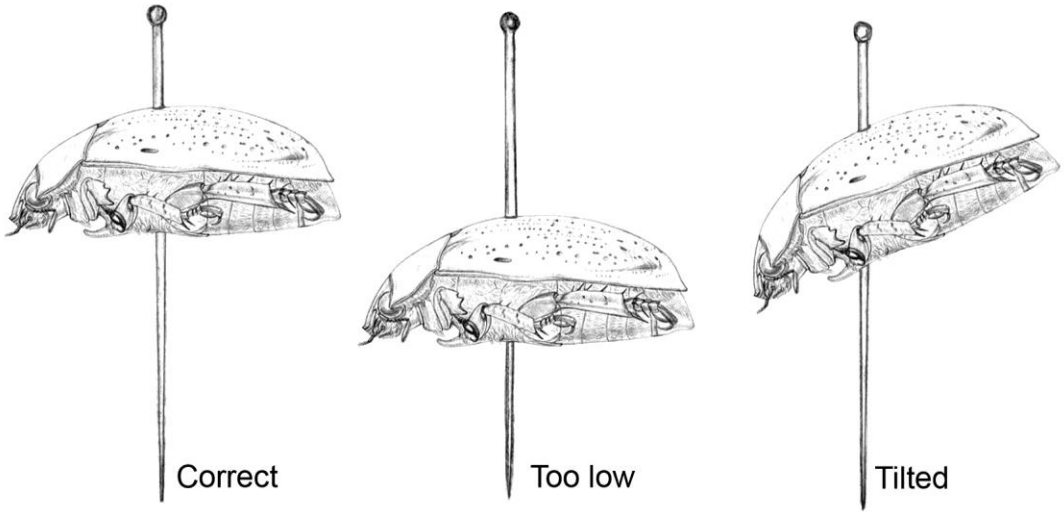
- **Bees, wasps, flies** — Pin through the thorax between bases of fore wings and slightly to right of midline.
- **True bugs** — Pin through the scutellum, which is the triangular area between the bases of the wings.
- **Grasshoppers, crickets** — Pin through the prothorax or "saddle" slightly to the right of the midline
- **Beetles** — Pin through the forepart of the right wing cover (elytra) near the midline.
- **Butterflies, moths, dragonflies, damselflies and lacewings** — Pin through centre of thorax between the bases of forewings.



Pining position on thorax for different insect orders

Place your insect on a styrofoam sheet with its ventral surface closest to the styrofoam. Place your pin through the thorax in the above positions and ease the pin through the body until you push through the styrofoam. Once the specimen is secured push the pin further to about ¾ pin length or use forceps to move the specimen up the pin. Make sure the specimen is straight on the pin as shown in preceding illustration.

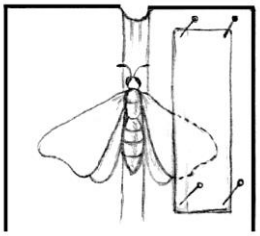
Correct orientation and height of specimen on the pin – straight, ¼ from top of pin



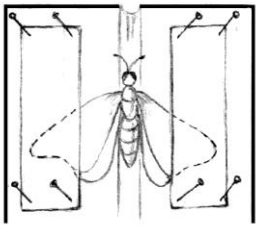
Wing setting

Setting out wings of specimens is commonly done for a number of orders as follows and generally for larger specimens or particular subgroups within the order, depending on the group. A setting board, either purchased readymade or constructed from styrofoam sheets with a medial groove, is used. The position for setting wings varies slightly for different orders as follows:

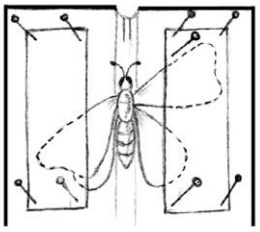
- **Bees, wasps (Hymenoptera)** - both pairs of wings set, with front edges at right angles to the body. Note that the forewings and hindwings are often linked by a series of tiny hooks, and stay together when set.



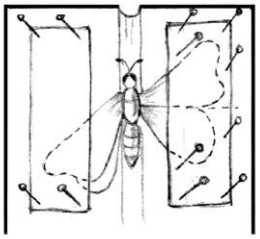
- **Dragonflies, damselflies (Odonata)** - both pairs of wings set, with front edges of hindwings at right angles to the body and forewings then set just a little in front of the hindwings.



- **Flies (Diptera)** - one pair of wings, set with the front edges around 60° to body.



- **Butterflies, moths (Lepidoptera)** - both pairs of wings set, with hind margin of forewings set at right angles to the body, and front edges of hind wings tucked under forewings.

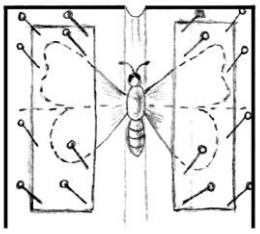


- **Grasshoppers, crickets (Orthoptera), cockroaches (Blattodea), mantids (Mantodea), stick insects (Phasmatodea)** – setting both pairs of wings is optional. One pair is sufficient, with hindwing at right angle to the body, then forewing set a little in front of the hindwing. Legs may also be positioned and held in place by pins to set.

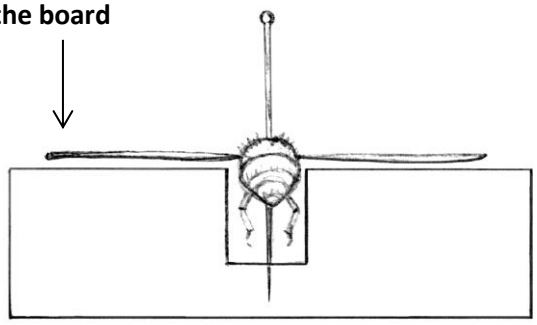
The abdomen of large specimens can be supported by cross pinning.

One to three weeks is needed for drying depending on the size of the specimen.

Strips of tissue paper or tracing paper pinned over wings to hold in place as they are spread out →



Pinned to height where underside of wings rests on the top of the board

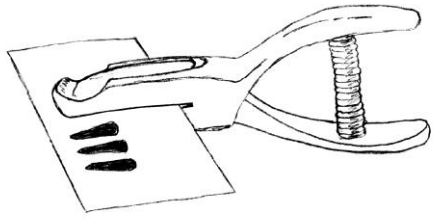


Point mounting insects

Insects that are smaller than about 8mm if elongate-oval or larger if thin, are usually mounted on a point.

Although there are other mounting methods, the most common and straightforward method that can be used for small specimens across most pinned insect orders, is to point mount. Consult other suggested references at the end of the chapter for further information on other mounting (e.g. card) and micro-pinning techniques.

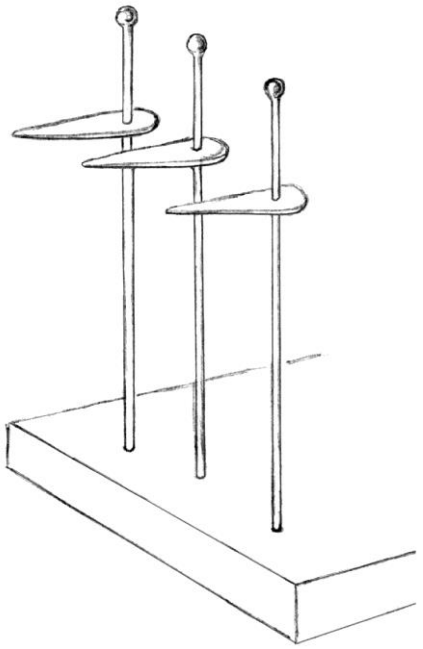
A point is a triangular card through which a pin is inserted. The **free end of the point is dabbed in wood glue** (only a very small amount) before then touching onto the right side of thorax. The insect is pointed always on the **right hand side of the thorax between the middle and hind legs**. The point needs to be **attached to the body and not the legs**. For most insects **bend the tip of the point downwards with forceps** before gluing. For some beetles with flat venters (e.g. lady beetles) and very small insects the tip of the point does not need to be bent.

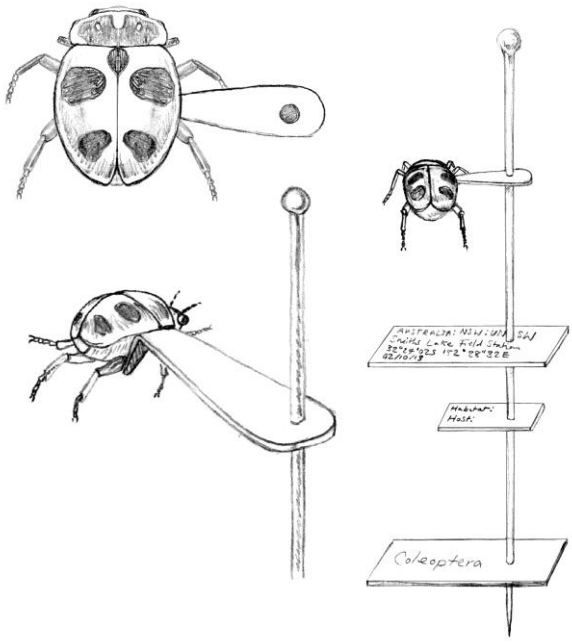


Points are made with a point punch or by cutting small triangles from acid-free archival card

Points are placed through the pin using a foam board on pinning block

The tip is bent (usually) and glued to the right side of the thorax between the mid- and hind legs

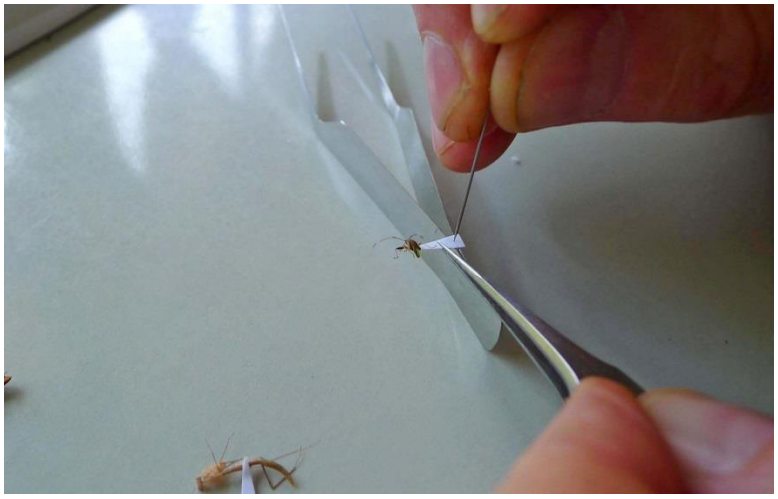




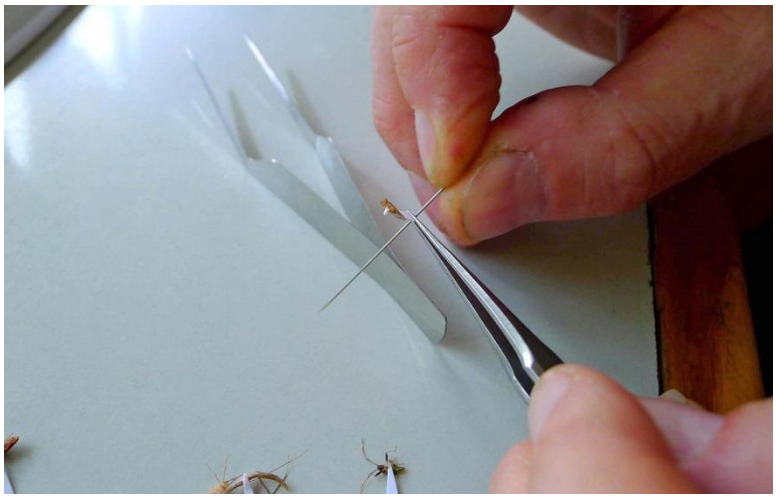
Alternate point mounting method for heavier insects

Many insects although small, are too heavy to sit on the point card before it dries and slide off. Therefore an alternate method is to lay the insect upside down, glue the specimen to the point card, wait for the glue to dry, and then place the pin through the card using forceps to assist as shown in image series below. Two pairs of forceps required for this technique:

- lay specimens on back or left side
- glue bent tip of point card to right side of thorax between mid and hind legs
- wait for glue to dry



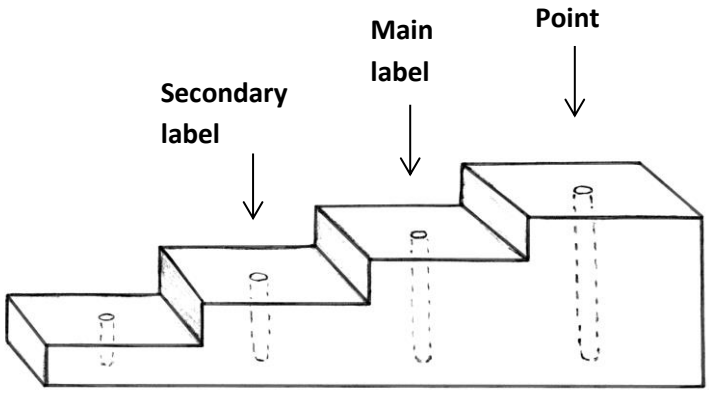
- pick up pointed insect with forceps from the middle of the point
- position end of point over base of forceps
- place pin through end of point card and down between gap in forceps
- **note:** using forceps as a guide to place the pin prevents the point card bending when the pin goes through.



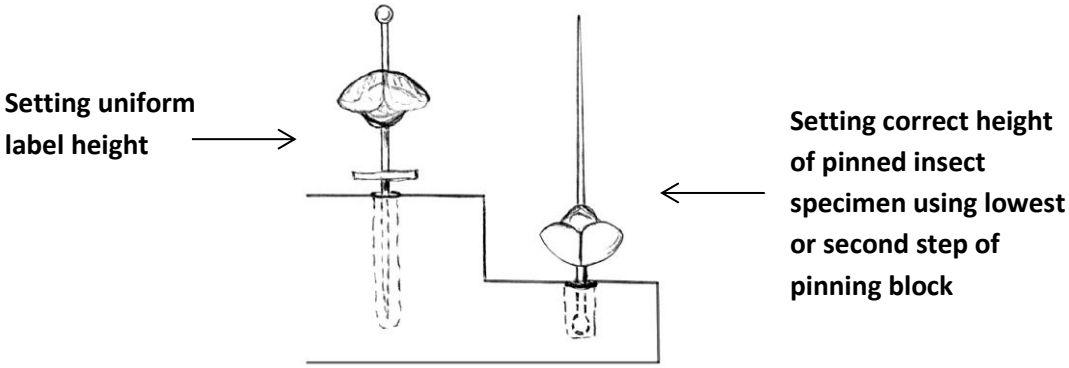
- move the point up the pin to the correct height, ¼ from top of pin

The pinning block

Using a pinning block ensures uniform height of points and labels on pins across a collection. These are generally made of wood and purchased from entomological suppliers but can be easily made with soft timber and a fine saw and drill tip. Drill depths for a four step block for use with standard insect pins (38 mm in height) could be 5, 12, 19, and 27 mm. The last will bring the point to ¾ of the way up the pin. Refer to suggested references at the end of this section which also contain dimensions and specifications for such pinning gauges.



The pinning block can also be used to gauge the correct height for pinned insects from the top of the pin by turning them upside down and placing the pin head in the lowest hole and lowering the specimen carefully along the pin (it can be a good idea to use forceps especially for smaller specimens) until it meets the surface of the pinning block:



Temporary labelling of pinned specimens

Always keep temporary field labels with pinned specimens until permanent labels are written or printed, as shown below. Here, the temporary label from the collecting tube (see page 13) with the collection code, is placed in the pinning tray in front of all specimens from that collection event, until they are permanently labelled. Transfer of the original label will reduce transcription error.

Place the field label at the start of a series of specimens in the unit tray until permanent labels are prepared, as shown below (note: the pinned specimens would be placed facing head up in the unit tray once permanent labels are attached). Before removing, double check that all the information on the permanent labels printed corresponds with the field labels and leave temporary labels with the specimens if possible until the collections are sorted.

Drying pinned and mounted specimens

Especially for larger specimens this can be essential in tropical climates. A drying cupboard can be used and can be easily constructed from plywood with open lattice shelves and fittings for incandescent 40 watt light bulbs installed at the base.