A CRITICAL POINT DRIER USED AS A METHOD OF MOUNTING INSECTS FROM ALCOHOL[1]

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ABSTRACT: The use of a critical point drier for mounting insects from alcohol is described. Advantages over standard methods are given which include: 1) Many specimens can be handled at one time, 2) pigment colors remain life-like, 3) specimens do not collapse, 4) appendages need no teasing and, 5) specimens are not brittle.

A major problem confronting museum workers has been the preparation of material for study which had been collected or stored in alcohol. The "standard" methods of removing insect specimens from alcohol and mounting them on points or minutens has involved the transfer or movement of the material through various liquids, viz. ethyl acetate, xylene and cellosolve (ethylene glycol monoethyl ether) (Sabrosky, 1957, 1966; Vockeroth, 1966). Other chemicals that have been used include chloroform, ether and acetone. The disadvantages of the currently used methods include: 1) Time involved in transferring the specimens or the liquids, 2) necessity of teasing the appendages away from the body, especially the wings, 3) shriveling, 4) resultant hardening or brittleness of the specimens and 5) the toxicity of compounds such as xylene and acetone.

All biological tissue contains water. To maintain three dimensional morphology of tissue or organisms for study, it is necessary to replace the physiological water with another fluid (preservative) or eliminate the water from the specimen without distortion. Drying specimens is achieved by evaporation, freeze drying or critical point drying. The evaporative method of drying is the oldest and most commonly used procedure in museum work, but it is undesirable because surface tension forces developed during drying cause severe distortion of soft tissue and more rigid tissues that contain large amounts of water. Alternatively, freeze drying techniques have been developed to prepare material for study, but the process is time consuming, requires elaborate equipment and is not always successful. Freeze drying can distort or destroy ultrastructure by differential thermal expansion or the formation of ice crystals from unbound water. We believe that the process of critical point drying is ideal for all forms of preparation involving small bodied insects.

The physical principles behind critical point drying are simple. In review, liquefaction of gasses occurs when the cohesive forces binding molecules together are greater than the kinetic energy disrupting them. Two physical parameters are important to the phenomenon – critical temperature and

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pressure. Critical point drying involves passing a specimen through an intermediate fluid (acetone, ETOH, Freon) and into a transitional fluid (CO_2, Freon, nitrous oxide) and subjecting it to the critical temperature and pressure.

We discovered that insect specimens critically point dried emerged in excellent condition for point mounting and subsequent study. The procedures involved are simple and comparatively rapid. Specimens in alcohol, any percentage, are placed in a small mesh screen basket with lid. They are then dehydrated by taking them through a series of increasing concentrations of ethyl alcohol, we use 10% increments, ending in a solution of 100% ETOH. The specimens within the basket are passed through two washes of 100% ETOH. Specimens that have been preserved in 70% ETOH can be taken through the alcohol series without rehydration. We have obtained excellent results by leaving the basket of specimens in concentrations of alcohol for 20 - 30 minutes. In the case of freshly collected specimens in alcohol or small Lepidoptera larvae, a longer period of time in each concentration is required, eg. 1-2 hours. It is safe to leave any specimens for longer periods in concentrations of alcohol above 50%. After removal from the last wash of absolute alcohol, the specimens, with the basket, are placed within the chamber of the critical point drier and are processed according to drier instructions.

There are several critical point driers on the market. Some of the transitional fluids used in critical point driers include Freon, CO_2, and nitrous oxide. The driers we use can be used with either Freon or liquid CO_2. Liquified CO_2, research grade, is the transitional fluid we prefer because it is easiest to use, comparatively inexpensive, is less noxious and provides more uniform results than other fluids. We have used Freon-13 as a transitional fluid and have not noted any differences in specimens treated with this fluid as compared to CO_2. We have not tested Freon-23 or Freon-116, but they may be useful for some biological materials because each has a characteristic critical pressure and temperature. When Freon is used as a transitional fluid, specimens must be run through a series of increasing concentrations of Freon starting with 10% and ending in pure Freon. This adds an additional procedure over the use of CO_2.

There are distinct advantages to critical point dried specimens over the "standard" methods employed. These advantages include: 1) Many specimens can be handled simultaneously, 2) pigment colors remain life-like, 3) specimens do not collapse or shrivel, 4) no manipulation of the appendages is required and 5) the specimens are not brittle. Relative to this latter point the appendages can actually be manipulated and are more supple than in air dried specimens. A further advantage is that the museum preparator is free to conduct other duties while the specimens are being processed.

We have critical point dried Chalcidoidea, Proctotrupoidea and other various

small Hymenoptera with excellent results. The turgid condition of the insect allows us to study not only segmentation of small parts, eg. labial palpi, but also sculptural features of the integument. Diptera belonging to the Nematocera and other small acalyptrate flies have been critical point dried. The setation and pilosity remains as in life and the small bristles are less apt to be broken off. Soft bodied arthropods such as Collembola, Thysanura, small Lepidoptera larvae, spiders and mites have all been critical point dried. In the case of the latter two groups the abdomen remains turgid and the legs do not curl up as in air dried specimens. Cockroach oothecae and various Hymenoptera and Diptera pupae have also been critical point dried with success.

We can foresee, with the use of the critical point drier, at least partial, if not total elimination of alcohol collections in museums. Insects and spiders normally stored in alcohol can be critically point dried then stored, if desired in air-tight vials. This eliminates the need to constantly replenish the alcohol in alcohol stored material. Colors that fade with years of storage in alcohol are preserved with critical point drying. This is especially important for Lepidoptera larvae.

A word of caution: if the specimens are not completely dehydrated or the alcohol is not completely purged from the chamber in the critical point dryer, the specimens will eventually shrivel as in air dried material. If after processing, it is determined the specimens are not completely dried, they can be returned to absolute alcohol for a short time then rerun through the critical point drier.

At present the only disadvantage we have noted is that in general museum use one is limited by the small size of the chamber, 1 inch diameter x 1 inch high. Large larvae must still be stored in some preservative or other until such time that a critical point drier is developed with a large enough chamber to accommodate the larger specimens.

LITERATURE CITED

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