HEXAMETHYLDISILAZANE – A CHEMICAL ALTERNATIVE FOR DRYING INSECTS[1]

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ABSTRACT: Two methods of chemically drying softbodied Chalcidoidea (Hymenoptera) are compared: critical-point drying (CPD) and hexamethyldisilazane (HMDS). For three groups of Eulophidae, Encyrtidae and miscellaneous Chalcidoidea, the CPD specimens were of consistently higher quality for all groups, although the overall differences between CPD and HMDS specimens were marginal.

Soft-bodied insect specimens have long been the bane of systematics. Freshly killed and air-dried specimens (Fig. 1) undergo partial to complete collapse of body parts, whereas specimens initially preserved in EtOH fare even worse when subsequently removed from the liquid and air dried (Fig. 2). This is not only a problem of obtaining quality museum specimens but in the past has deterred some systematists from bothering with samples preserved in alcohol, such as those taken in malaise or pan traps. Critical-point drying (CPD) of specimens through a liquid CO_2 intermediate (Gordh & Hall 1979) provides a means of retrieving large numbers of soft-bodied specimens from EtOH and is being widely used for some taxa, especially Chalcidoidea. The primary advantage of using CPD is little or no collapse of soft body parts, including internal muscles and nerves. Secondarily, the structure of muscles, nerve tissue and other internal body parts is maintained, allowing for later survey of these structures from museum specimens (Heraty et al. 1997). The disadvantages with the CPD are that it 1) is relatively expensive to buy the initial equipment ($2,000-8,000), 2) is necessary to obtain specialized CO_2 tanks that must be maintained above 900 psi, 3) is labor intensive, 4) can cause abnormal swelling or occasional bursting of some body parts, and 5) may leave surface residues on specimens.

Several alternatives to air drying or CPD have been proposed, some of which are freeze drying, Peldri II (Brown 1990), acetone vapor (van Noort 1995), xylene (R. Carlson pers. comm.), and hexane (D. Hawks, pers. comm.). A new chemical method involving hexamethyldisilazane (HMDS) has been proposed as a simple and cost-effective means of retrieving high-quality specimens from collections preserved in EtOH (Nation 1983, Brown 1993). Only the CPD and HMDS methods are regularly applied for the retrieval of large collections of Chalcidoidea initially preserved in alcohol, and here we compare the two methods.

METHODS

All specimens were initially killed and preserved in 70-75% EtOH at 4°C.

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Evaluations were of separate collections made from 1990 to 1996 in southeast Asia, the Galapagos Islands and California. Lots that had a high proportion of soft-bodied Chalcidoidea were chosen, and all specimens were scored from each lot. Fourteen separate collections (362 specimens) were evaluated for the CPD method and 5 collections (347 specimens) for HMDS. Overlap in collection time and country for each method occurred only for the southeast Asian collections. Additional specimens of a new species of *Cirrospilus* (Eulophidae) from California were examined as representatives of very soft-bodied Chalcidoidea.

Specimens were scored on a scale of 1 to 5, with 5 being a nearly perfect specimen suitable for scanning electron microscopy (SEM). Scores were based only on the softer body parts. *Cirrospilus* are almost entirely soft-bodied and represent an extreme; in other taxa, for example pteromalids, the head and mesosoma are well-sclerotized and do not collapse under any treatment, but the antennae and gaster will partially or completely collapse. A score of 1 would be typical of air-dried eulophids taken from alcohol: completely shrivelled and collapsed (Fig. 2). A score of 2 was assigned to specimens that had extensive collapse of the softer body structures (head, antennae and gaster) (Fig. 1). A score of 3 was given to specimens with partial collapse of all softer body parts (Figs 4, 5). Freshly killed and air-dried specimens would usually be given a score between 1 and 3, with a score of 3 bordering on acceptable for museum collections or SEM (at least partly shrivelled or collapsed). A score of 4 was given for very minimal collapse of not more than one body part or a slight distortion (wrinkling or bloating) of the gaster (Figs 4, 5). The *Cirrospilus* were not scored for comparative analysis. All material is deposited in the Entomology Research Museum, University of California, Riverside.

CPD method. The liquid vapor interface is the primary destructive force in air-drying specimens, and if not about equal, results in the breakdown of cell walls and collapse of tissue. For CO_2, the identical vapor pressure as a liquid or gas, the critical point, is reached at 31.0°C and 1093 psi (Burstyn and Bartlett 1975). Specimens were dried as outlined by Gordh & Hall (1979) by 1) dehydrating the specimens to 100% EtOH, 2) exchanging fluids through liquid CO_2 under high pressure (900 psi) and low temperature (11-15°C) through a series of soaks and purges until the exhausted dry CO_2 did not leave a liquid residue (complete sublimation), 3) drying under high pressure (1100-1200 psi) until the chamber temperature reached 41-43°C, and then 4) slowly exhausting the gaseous CO_2 to room atmospheric pressure.

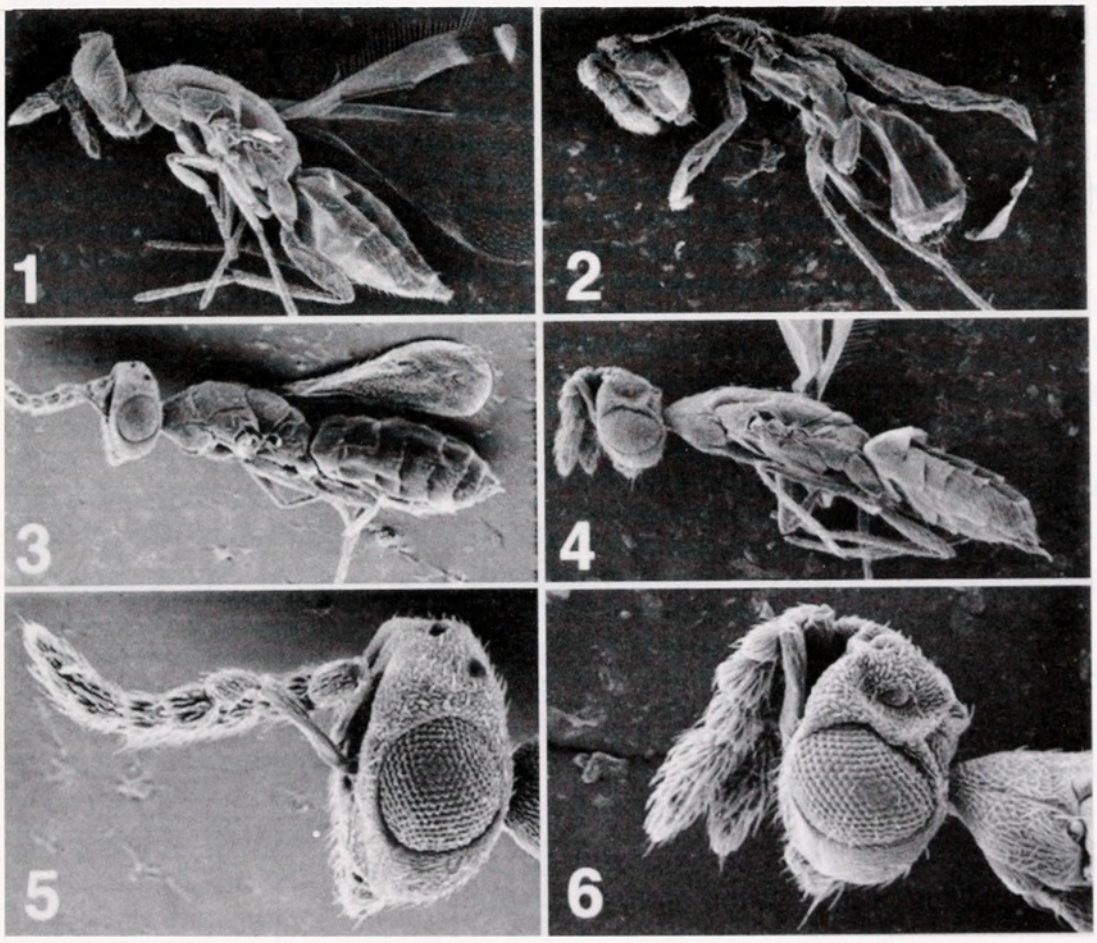
HMDS method. HMDS ($[(CH_3)_3Si]_2NH$) reacts with water to produce hexamethyldisiloxane ($[(CH_3)_3Si]_2O$) and ammonia (NH_3), both of which evaporate from the specimen (Dave Jordon, Polysciences Inc., pers. comm.). Specimens were dried in the manner outlined by Brown (1993) by 1) dehydrating the specimens to 100% EtOH, 2) replacing the alcohol with HMDS for two soaks of 1/2 hour each in a covered glass vial or dish, and 3) after the

second soak, pouring off most of the HMDS and allowing the remaining HMDS to evaporate in a fume hood (or outdoors in a well-ventilated area). Samples can be soaked and dried in glass vials or dishes. Gas buildup in the vials may cause the release of liquid HMDS while being uncapped, but this can be avoided by using smaller volumes of HMDS (less than half of vial) or slowly unscrewing the vial top. We prefer to line the bottom of a glass dish with a fine brass screen and cover each sample with a screen lid during evaporation, thus preventing dried specimens from disappearing into the exhaust. HMDS is a skin irritant, and gloves and eye protection are recommended.

All specimens were card-mounted for examination following Noyes (1982).

RESULTS

Cirrospilus (Eulophidae) was used as an example of a very soft-bodied species that does not fare well under any of the drying methods (Figs 1-6). Air-drying (Fig. 1) resulted in collapse of the antennae, femora, and gaster dor-



Figures 1-6. *Cirrospilus* sp. (Eulophidae): **1,** freezer killed and air dried. **2-6,** killed and preserved in 70% EtOH and then: **2,** air-dried; **3&5,** CPD dried; **4&6,** HMDS dried.

sally and laterally; the mesosoma was relatively undistorted. The specimen illustrated would receive a score of 2, which would be marginally acceptable for use in collections. Air-drying from alcohol (Fig. 2) was disastrous, with general collapse of all body parts (score 1). CPD *Cirrospilus* (Figs 3, 5) showed slight collapse of the scape and scrobes, and distortion but not collapse of the gastral tergites. Such a specimen (Fig. 3) would be scored as a 4 (less than perfect). HMDS *Cirrospilus* (Fig. 4, 6) exhibited a greater degree of collapse of the scape, head and metasoma, with the specimen receiving a score of 3. For extremely soft-bodied specimens, the CPD method was consistently better than the HMDS method, and both were better than air-drying.

Seven families of Chalcidoidea were encountered in the 19 collections evaluated (Table 1). Each family presents a different problem with respect to how they were affected by improper drying. Even when CPD- or HMDS-treated, soft-bodied Eulophidae generally had some collapse or distortion of all body parts (cf. Figs. 3-5). Using either CPD or HMDS, 51% of the

Table 1. Quality of soft-bodied Chalcidoidea dried using critical-point drying (CPD) or hexamethyldisilazane (HMDS). Ranking based on a scale of 1-5, with 5 indicating a near-perfect specimen. Mean values were significantly higher for CPD specimens for all groups (Chi-square, P=0.01). Data were pooled for Aphelinidae, Mymaridae, Pteromalidae, Torymidae and Trichogrammatidae.

		rank						
		5	**4**	**3**	**2**	**1**	**n**	**mean rank**
Eulophidae	CPD	126	114	7	0	0	247	4.48
	HMDS	87	60	22	0	0	169	4.38
Encyrtidae	CPD	46	19	6	0	0	71	4.56
	HMDS	29	32	0	0	0	61	4.48
Aphelinidae	CPD	7	2	0	0	0	44	4.82
	HMDS	56	2	2	2	0	117	4.51
Pteromalidae	CPD	7	4	1	0	0		
	HMDS	13	9	9	1	0		
Mymaridae	CPD	4	4	1	0	0		
	HMDS	6	6	0	0	1		
Trichogrammatidae	CPD	11	1	1	0	0		
	HMDS	8	2	0	0	0		
Torymidae	CPD	1	0	0	0	0		
	HMDS	—	—	—	—	—		

Eulophidae treated had a score of 5, and, although a much higher proportion than the CPD method, only 13.0% of the specimens received a score of 3, and none received a 1 or 2. Many Eulophidae are reasonably well-sclerotized and do not have problems similar to those of *Cirrospilus*. Often the most noticeable artifact was a slight wrinkling of the gastral tergites (score of 4), which was common in both treatments. Pteromalidae generally have a well-sclerotized head and mesosoma, but the gaster of males is particularly susceptible to collapse. Both Trichogrammatidae and Aphelinidae are soft-bodied but responded well to either technique except for some collapse of the antennae, which occurred with use of either method. Other than Eulophidae, all of the chalcidoid groups responded well to either technique, with consistent scores of 4 or 5, both of which are acceptable for museum collections.

For statistical comparisons, Eulophidae and Encyrtidae were common in all samples and were treated separately; results for Aphelinidae, Pteromalidae, Mymaridae, Torymidae and Trichogrammatidae were pooled. In all three comparisons, the CPD specimens were of significantly higher quality (rank) than the HMDS specimens (Chi Square, P=0.01), although the differences in the mean rank scores for each treatment were marginal (Table 1). The CPD method after ethanol fixation also ranked better than HMDS in a study of pre- and post-fixation techniques in four taxa (Swearingen et al. 1997). In contrast to the techniques used by Swearingen et al. (1997), we have not found fixation in osmium tetroxide to be a necessary step in preparation for either museum or SEM specimens.

CPD and HMDS methods left little or no residue on the specimens, as noted by Swearingen et al. (1997). Specimens treated by HMDS appeared to be slightly cleaner, but we could see no way to quantify this characteristic accurately. HMDS also works as a good degreasing agent for some insects such as tiger beetles and robber flies. We also found various labels and ink types (including laser-printed labels) to be unaffected by HMDS, allowing their inclusion during processing. The same is possible for the CPD method, although processing is usually in small capsules making inclusion of larger labels impossible. At $30 U.S. per 400 ml of HMDS and 5 ml per large lot of about 100 chalcidoids, we estimate a cost of about 37.5 cents per run, or 0.4 cents per specimen. We have tried HMDS on a variety of insects, including Collembola, flies, beetles and other Hymenoptera (*Perdita* and *Bombus*), with generally excellent results. Heavily sclerotized individuals processed using HMDS are as good as CPD specimens. Internally, muscles and nerve tissue are preserved in the same manner as using the CPD process. For larger specimens, wings are often crumpled in smaller CPD capsules, but this was not a factor with HMDS. In addition to improved specimen quality, it is also noteworthy that mitochondrial DNA was successfully extracted from dried CPD and HMDS specimens of Ichneumonidae and Encyrtidae (Austin & Dillon 1997).

In summary, the use of HMDS is a viable alternative to use of CPD for retrieving soft-bodied insects from alcohol. CPD specimens are marginally better in quality than those treated with HMDS, but HMDS is cost-effective and less labor intensive than CPD. If the equipment is not available, HMDS may be the preferred technique.

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