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YELLOW RAIN ANALYSED

Yellow rain — a palynological analysis

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Yellow rain, purported to be an agent of toxin warfare in South-East Asia, contains a high percentage of pollen. Analysis of this pollen in yellow rain samples suggests that they are the faeces of honey bees.

A HIGH pollen content has been reported in samples of yellow rain from South-East Asia[1-7]. We have examined 11 samples of yellow rain allegedly collected at sites of chemical attack in Laos. The samples contain a high proportion of pollen. No two samples, even different spots of yellow rain on the same leaf, have the same composition of pollen types. The plant taxa identified are common in South-East Asia. Seven pollen types in yellow rain samples were also found in *Apis cerana* and honey from Thailand. In general appearance, high pollen content, and differences in pollen composition from one spot to another, the yellow rain samples resemble honey bee faeces.

The first two yellow rain samples we analysed were from small reddish-brown rocks with yellow spots. The third, the ABC News sample, is reported to have been scraped from vegetation by Hmong soldiers in Laos in March 1981 and to contain three trichothecene mycotoxins[8]. The remaining 8 samples were yellow spots from the upper surfaces of narrow lanceolate leaves about 15 cm long. The rocks and leaves were given to Canadian Embassy officials in Thailand in April 1982 by Hmong refugees who said they had collected them at two sites of chemical attack in Laos in March. The records do not specify whether the rock and leaf samples we examined are from one or both sites. All spots were 2–5 mm in diameter, agreeing with earlier descriptions[1,2,7,9]. A 3 mm spot from a leaf weighed 1.5 mg and contained 1.1×10^5 pollen grains. We examined pollen from abdomens of two Asian honey bee species, *A. dorsata and A. cerana*, collected in Thailand by T.D. Seeley, who first suggested that yellow rain samples from South-East Asia might be faeces from cleansing flights of honey bees[7]. We also tested two samples of honey purchased in July 1983 at villages on the Thai-Laotian border, and known faeces of *A. cerana* and *A. dorsata* on leaves collected in July and September, respectively, near Poona, India.

The first rock sample (Table 1, rock 1) was the residue on a piece of paper on which the rocks had been examined. This, and all other samples except as noted were acetolysed before scanning electron microscopy (SEM)[10]. It contained at least six pollen types. None could be confidently identified even to family. The most numerous type, triaperturate and 20–23 μm long, is designated Fagaceae-like. The second sample, rock 2, was from a single spot. Its pollen consisted almost entirely of one of the minor types in rock 1, plus some grass pollen.

The ABC News sample consisted of four yellow fragments of approximately 2 mm^3. SEM of an untreated fragment revealed vague outlines of pollen in an unknown matrix (Fig. 1*a*). Another untreated fragment, mounted in glycerine jelly for light microscopy, appeared to consist mainly of pollen, plus a few bee hairs (setae). The remainder of the sample was acetolysed, revealing about 20 pollen types, some of which are shown in Fig. 1*b, d*. Families identified were Compositae, Dilleniaceae, Elaeocarpaceae, Euphorbiaceae, Icacinaceae, Myrtaceae, Polygalaceae, and Sterculiaceae (Table 1). There was also a small proportion of grains possibly of Melastromataceae/Lythraceae. Grains were found that are indistinguishable from *Dillenia pentagyna* taken from herbarium specimens. Also found (Fig. 1*d*) were grains indistinguishable from those of *D. hookeri*, a species common in Laos[11]. Another type in the ABC News sample (Fig. 1*d*) is indistinguishable from pollen from herbarium specimens of *Macaranga denticulata*, although its rather unspecialized morphology and the existence of some 300 species of *Macaranga* make narrow identification difficult. The most common grain in the ABC News sample is very small, about 10 μm, with three apertures and a smooth/irregularly-pitted surface (Fig. 1*d*). It is classified as Elaeocarpus-like. Also, fungal hyphae and a few setae were seen in acetolysed preparations of the ABC News sample.

Leaf samples 1 and 2 were spots from different leaves, while 3A and 3B were spots a few cm apart on a single leaf, as were the sample pairs 4A/4B and 5A/5B (Table 1). Leaf samples 1 and 2 had three pollen types present in the ABC News sample — *M. denticulata*, Melastomataceae/Lythraceae and a pollen type tentatively placed in *Apodytes* (Icacinaceae). In addition there were hyphae, setae, and stellate, trichome-like structures. An abundant grain in leaf sample 1, probably from Moraceae/Urticaceae (Fig. 1*e*), was not seen in leaf sample 2. Conversely, an abundant grain in leaf sample 2, from *Ilex* (Fig. 1*f*), was not found in leaf sample 1.

Leaf sample 3A contained *M. denticulata, Ilex, Apodytes*, Melastomataceae/Lythraceae and a small proportion possibly of *Nuphar* (Nymphaeaceae). Sample 3B consisted mainly of Fagaceae-like grains, matching those in rock sample 1. The remaining leaf spots 4A, 4B, 5A and 5B, all had *Ilex, M. denticulata, Apodytes* and Melastomataceae/Lythraceae, but in very different propor-

Table 1 Summary of pollen identifications

	YELLOW RAIN												
	Rock 1	Rock 2	ABC News	Leaf 1	Leaf 2	Leaf 3A	Leaf 3B	Leaf 4A	Leaf 4B	Leaf 5A	Leaf 5B	*A. cerana*	Honey 2
AQUIFOLIACEAE *Ilex*					++	+		*	*	*	*		
COMPOSITAE			*										
DILLENIACEAE *Dillenia pentagyna* *Dillenia hookeri*			+ *										* *
EBENACEAE Diospyros-like												++	
ELAEOCARPACEAE Elaeocarpus-like			++										+
EUPHORBIACEAE *Macaranga denticulata*			+	++	++	++		+++	+	++	++		
FAGACEAE-LIKE	++						+++						*
GRAMINEAE		+										*	
ICACINACEAE *Apodytes*			*	++	++	+		*	+++	+	*		
MELASTOMATACEAE / LYTHRACEAE			*	+	+	+		+	+	*	+		*
MORACEAE / URTICACEAE				++									
MYRTACEAE			*										*
POLYGALACEAE			*										
SAPINDACEAE													++
STERCULIACEAE *Sterculia*			*									+	
UNIDENTIFIED, 35-38μ 4 Apertures			+										*
UNIDENTIFIED, 25-35μ Punctate, 3-Colporate	+	+++											
UNIDENTIFIED, 45-50μ Thick Exine										+			

Key: *, minor component (<5%); +, 5–10% of sample; + +, 10–50%; + + +, more than 50% of samples.

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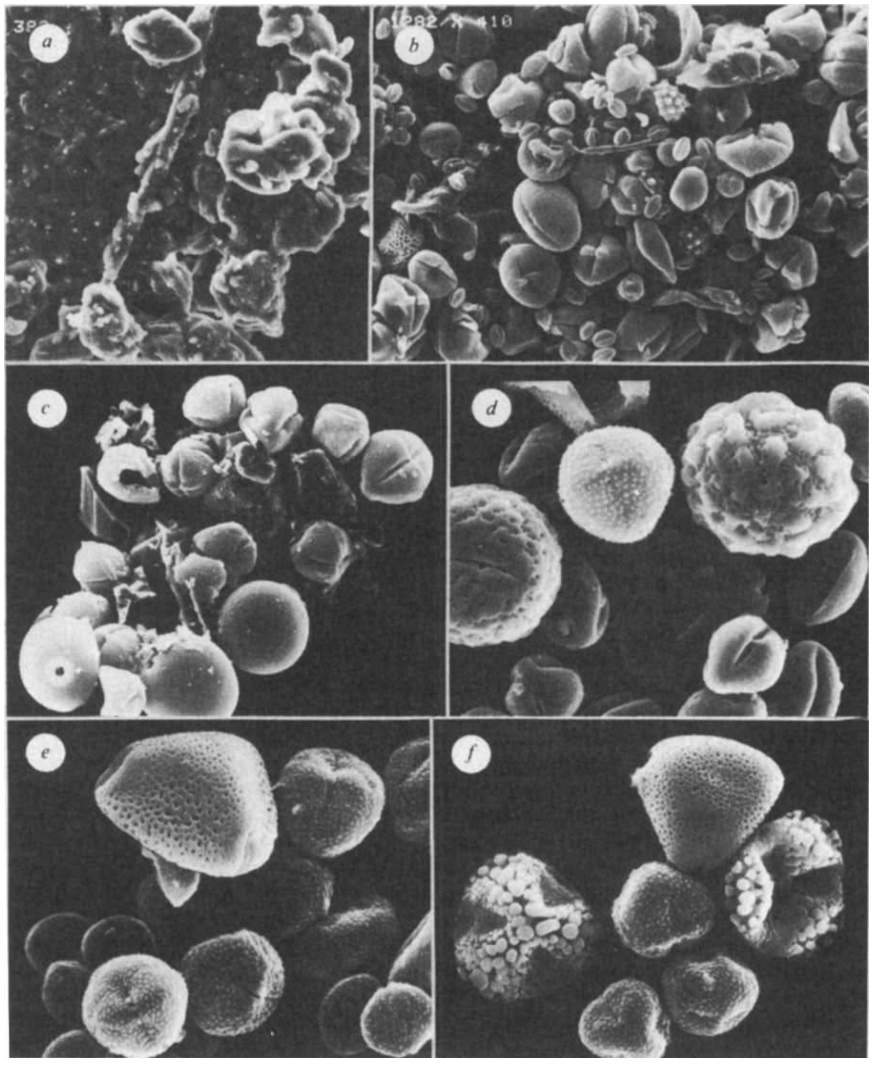

Fig. 1 SEMs of pollen in yellow rain. *a*, ABC news sample, untreated, X250. *b–f*, All acetolysed preparations. *b*, ABC News sample, X270. *c*, Rock sample 2, three spheroidal grains at bottom left are from the grass family, X330. *d*, ABC News sample, larger grain at upper right and partial grain middle left are probably *Dellenia hookeri*; grain between is *Macaranga denticulata*; the triangular grain in background is probably Myrtaceae; numerous smaller gains were classified as Elaeocarpus-like, X1,300. *e*, Leaf sample 1, the largest grain, seen in oblique view is assigned to *Apodytes*; the smallest grains, some showing pores, are Moraceae/Urticaceae; the remaining ones with furrows are *M. denticulata*, X1,170. *f*, Leaf sample 2, the uppermost triangular grain is *Apodytes*, the three small grains are *M. denticulata*, and the remaining two are *Ilex*, the one on the right is partially collapsed, X980.

tions in each case. In addition, 5A had an unidentified type 45–50 μm long, 3–4 aperturate with a thick exine, that was not found in the other samples. The great diversity of the ABC News sample as compared with the leaf and rock samles may be a consequence of collection technique — a number of yellow spots may have been combined.

An abdomen of *Apis cerana* collected in March 1980 contained at least three pollen types: Diospyros-like, a small proportion of grains from the grass family (Gramineae) and a reticulate type 35 μm long, matching in form and range of variation a type in the ABC News sample tentatively assigned to *Sterculia*. *A. dorsata*, collected in March 1980, had pollen probably representing two or three closely related species in *Castanopsis/Lithocarpus* (Fagaceae).

Honey sample 1, from Nakhon Phanom (17° 25′N: 104° 45′E) had at least ten pollen types, but none matched those in the yellow rain samples. Honey sample 2, from Chiang Khan (17° 55′N; 101° 45′E) contained many pollen types including Fagaceae-like, seen in rock sample 1 and leaf sample 3B; the type designated Melastomataceae/Lythraceae, found in the ABC News sample and in leaf samples 1, 2, 3A, 4A, 4B, 5A and 5B; and four more types seen in the ABC sample: *D. pentagyna*, Elaeocarpus-like, and an unidentified four-aperturate grain, 35–38 μm long.

Known faeces of *A. cerana* and *A. dorsata* on leaves collected near Poona, India resembled spots of yellow rain in size, general appearance, high pollen content and the presence of setae and hyphae. Each of three spots of *A. cerana* faeces had at least five pollen types, with only some common to all three. Faeces of *A. dorsata* from a nearby site showed similar diversity from spot to spot.

Our identifications of pollen in yellow rain samples were compared with those in a report from the Australian Defence Department[2]. One of their three samples had mainly Harpullia-like pollen (Sapindaceae); the other two were predominantly Rapanea-like (Myrsinaceae). A Chinese study describes a "yellow rain" phenomenon in September 1976 in Jiangsu[12]. Occurrences generally lasted a few minutes, covering areas of 0.5–20 acres with 2–6mm yellow spots rich in pollen. The authors concluded that the spots were honey bee faeces. Mass cleansing flights of *A. melifera*, causing similar phenomena, have been studied previously[13,14].

All of the plant taxa we identified in samples of yellow rain are common in South-East Asia. The three assignments we made at the species level, *D. hookeri, D. pentagyna* and *M. denticulata*, are trees native only to Southern Asia. Also, a reticulate type in the ABC sample assigned to *Sterculia* was present in *A. cerana* collected in the same season in Thailand. Most significantly, honey sample 2 contained six pollen morphotypes we found in yellow rain, showing that these pollen types are gathered by honey bees in Indochina. No two samples of yellow rain we examined had the same pollen composition; even adjacent spots on the same leaf were different. Pollen diversity from spot to spot was also found for known bee faeces from India. Such differences between spots would not be expected if the spots of yellow rain had been disseminated by an artificial source. Our observations lead us to conclude that the yellow rain samples we examined are probably honey bee faeces.

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