Dyer

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
HARVARD UNIVERSITY

7 Divinity Avenue
Cambridge, Massachusetts 02138

September 9, 1983

Dr. Fred C. Dyer
Central Bee Research Institute
KVIC
Pune INDIA

Dear Dr. Dyer:

Thank you very much for sending me the samples of bee feces. They are the only samples I have of authentic feces of Asian Apis. As you will see from the enclosed draft of a letter to Science, the size of cerana deposits is indistinguishable from that of 'yellow rain' from an alleged attack in Laos. I will soon analyze some of the spots you sent, along with some of the yellow rain spots, for uric acid to see if their main nitrogenous component is the same. Joan Nowicke, at the Smithsonian has started to examine the pollen in the cerana deposits. The first one she looked at contains a high proportion of grass pollen plus several other types. We will be interested to know if spots collected at the same time and in the same place show sizeable differences in the mixture of plant species represented. Am I right in thinking that two bees from the same nest and even in the same cleansing flight may well have eaten quite different pollens stored in the nest?

If you have any more time for helping us we would like to have more samples of cerana and especially dorsata feces, plus some of florea. 30 or 40 representative spots of each on leaves collected and shipped just as you did before would serve very well. If you wish, some naphthalene crystals could be included to kill insects. In addition we would like to examine pollen as it is brought back to the nest by the bees. As you know this can be collected by placing a net of the right mesh at the entrance to the nest, I suppose this will work only for cerana. Emery Sarver of the Army contends that pollen harvested in this way is possibly used as a component in yellow rain. This could be distinguished

from fecal deposits in a number of ways, including chemical and microscopic examination. Also we would be interested in any information you may have or be able to obtain about the defecation behavior of each of the three Asian honey bee species. Is it ever synchronized in time? If so, why? If there are mass cleansing flights, what is the distribution of deposition as a function of distance from the nest? Is there any seasonality in the pattern of defecation? We would be glad to learn anything about the subject of defecation of Asian bees.

Sincerely,

Matt Meselson

Matthew Meselson
Professor of Biochemistry
and Molecular Biology

MM/db

Enclosures

Draft
for Science
September 2, 1983

Natural Origin of Yellow Rain?

Joseph Rosen (letters, 19 August, p.698) has analyzed one of the numerous samples of yellowish spots and powders from sites of alleged chemical warfare in Southeast Asia. He reported the detection by gas chromatography-mass spectrometry of approximately 50 parts per million (ppm) of each of three trichothecene mycotoxins and 265 ppm of zearalenone, compounds produced by certain widely distributed fungi. He also noted a series of peaks shifted by increments of 30 and 44 m/z, suggesting the presence of the CH_2CH_2O repeat of polyethylene glycol (PEG). Rosen argues that this evidence for PEG, a synthetic industrial chemical, 'makes irrelevant' any explanation of yellow rain as a natural phenomenon.

An obvious problem in this is that PEG, commonly known as Carbowax, could have entered the sample as a contaminant. More than 100 million pounds of PEG and its derivatives are made annually for a great many uses including the treatment of wood and paper, the lubrication of molds for forming and rolling rubber, plastic and metal, and as emulsifiers and detergents. Rosen's sample is reported to have been scraped from vegetation by a Hmong soldier in Laos, given to an American in a Thai refugee camp and then to personnel of ABC News. ABC gave it to the Arthur D. Little Corporation of Cambridge, Massachusetts where it was transferred to new vials and sent to Rosen. There is nothing to rule out contamination of the sample before, during or after its collection. Even laboratory contamination is not fully excluded by the blank analysis Rosen performed after analyzing the sample. What is more, the peaks interpreted as evidence of PEG by Rosen have not been reported in mass-spectrometric analyses of yellow rain samples received by the US Government. The origin of yellow rain is too important an issue to be allowed to hinge on an isolated finding on a single possibly contaminated sample.

The one consistent and remarkable finding in all samples of spots and powders from alleged attack sites examined to date is that they consist mainly of pollen. This includes Rosen's sample, all 5 samples examined for pollen by the Army and at least 10 other samples and groups of samples investigated in Australia, Canada, Great Britain, Sweden and Thailand. The pollen identified thus far is consistent with plant families common in Southeast Asia. These families are mainly insect-pollinated, not wind pollinated and their pollen is gathered by bees. US Government investigators have suggested that pollen is being used as a carrier for toxins in weapons. However, according to published descriptions and our own examination of samples, the spots of yellow rain closely resemble, in size, shape, color, texture and high pollen content, the natural excreta of bees of the genus *Apis*. Three species of *Apis*, the true honey bees, occur in Southeast Asia. All three are abundant in the forests of Eastern

Thailand and, undoubtedly, are also common in Laos and Cambodia. Honey bees almost never defecate in their nest, but instead do so in flight. The massive cleansing flights of honey bees in spring, after confinement in winter, are well known to temperate zone bee-keepers. Whether the time pattern of defecation of Asian honey bees includes similar synchronous behavior is unknown, although it might follow prolonged confinement at times of dearth or heavy rainfall or due to the fact that in two species (dorsata and florea) all individuals spend most of their lives in a protective curtain of interlocked bees covering the nest. Honey bee excrement consists largely of the walls or exines of pollen consumed from stores accumulated in the nest. Along with pollen, bees occasionally collect various other materials, including fungal mycelia and spores. The color of the fecal deposits varies but usually is pale to dark yellow or a shade of brown. Their size is also variable, ranging from about 1 to 10 mm. Fecal spots of the one Asian honey bee for which we have samples, Apis cerana do not differ significantly in average diameter from yellow rain spots from an alleged attack in Laos (A. cerana $\bar{x}$=3.3, s.d.=0.9, n=43; yellow rain $\bar{x}$=3.2, s.d.=1.0, n=25). Honey bee fecal deposits are wet and sticky upon deposition, drying to a waxy consistency. Older deposits are of variable texture, from rather hard and brittle to powdery. This matches reported descriptions of yellow rain as well as our examination of aged yellow rain samples. We have also observed occasional bee hairs in yellow rain spots and in authentic bee feces. As we reported to the AAAS Annual Meeting in Detroit on May 31, these and other similarities led us to conclude that the yellow rain may be bee feces. Subsequently, it was reported in Science that Emery Sarver, chief of the Army's analytical team investigating yellow rain, does not rule out the possibility that some or all yellow rain samples might be bee feces (Eliot Marshall, News and Comment, 24 June, p.1356). Seven of the yellow rain samples known to contain pollen have been analyzed for trichothecenes. The toxins were found in three, possibly because the fecal deposits became infected with toxigenic fungi before or after deposition. No toxins were found in the other four.

Quite apart from the close similarity of yellow rain samples and bee feces, difficult to explain for a chemical warfare agent, the evidence for trichothecene warfare in Southeast Asia is far from convincing. Altogether, trichothecenes have been reported in environmental or biomedical samples associated with 17 alleged attacks. The available descriptions of the mode of attack, bearing on the essential question of whether there was a chemical attack at all, are vague and lack consistency. In these 17 attacks delivery of the agent is attributed to low and high-flying aircraft, artillery shells, mines, grenades and simply to passing through an area attacked previously; no means of delivery is specified for 5 of the attacks. No spent or unspent chemical munitions have ever been recovered to confirm these or any of the more than 100 other such accounts even though many accounts refer to such recoverable items as artillery shells and rockets.

Trichothecenes are reported to have been found in five of the environmental samples received by the US from alleged attack sites in

Southeast Asia. All five of these and the sample analyzed by Rosen were collected at the end of the dry season, between mid-February and mid-April, in 1981 and 1982. No trichothecenes were found in the approximately 60 other environmental samples from such sites or in any of 16 control samples from outside of the attack areas. These results give no statistically significant evidence that the toxins are more common at the sites of alleged attack than outside. Moreover, in retrospect, it is seen that the controls are poorly matched. Most or all of them were not collected in the season when the toxin-containing samples were taken and no attempt was made to include spots of bee feces. For Afghanistan, the other part of the world where trichothecene warfare is alleged, the only definite finding of trichothecenes is a few micrograms of T2 on the outer surface of a Soviet gas mask, said to have been purchased in Peshawar, Pakistan.

Although attacks have been alleged in all seasons, the times of collection of the Southeast Asian biomedical samples reported to contain trichothecenes show the same seasonal clustering as the environmental samples, 17 out of 20 having been collected within the same 8-week period at the end of the dry season in 1981, 1982 and 1983. Trichothecenes have been detected in the blood, urine and tissues of 18 victims of alleged attack and have been tentatively identified in two others. The total number of alleged victims testing negative has not been made public. Nine individuals living in or near the camps where the victims were examined were selected as controls and they tested negative. These controls were not matched for diet and they were not reported to have been ill. In contrast, most or all of the alleged victims testing positive for trichothecenes had symptoms of illness at or shortly before the time of sampling. Two were sampled post-mortem. The use of apparently healthy controls risks excluding any naturally occurring trichothecene positives from the control group, since the presence of the toxins in the body is likely to be associated with illness.

Thus, the findings of trichothecenes in environmental and biomedical samples from Southeast Asia do not distinguish between artificial introduction and natural occurrence. A number of observations suggest that the occurrence of these toxins is natural. For example, most of the Southeast Asian blood samples testing positive for intact T2 toxin were drawn between 1 and 10 weeks after the alleged attacks. Available animal studies show a half-life of T2 in the blood of only a few hours, suggesting that the toxin found in refugees originated in moldy food eaten long after the alleged attack. In the only reported autopsy, conducted 5 weeks after the alleged attack, T2 was found at much higher concentrations in the stomach and intestines than in other organs; aflatoxin B1 was also present at high levels in the digestive organs, suggesting the ingestion of moldy food within the previous day or two. That T2 can contaminate food in the Asian tropics is indicated by published reports from India of high levels of the toxin in corn, sorghum and safflower seed.

Although it still believes that trichothecenes are being used as weapons, the US Government should reconsider the possibility that their occurrence is instead a natural phenomenon. One hypothesis that

deserves consideration is that trichothecene-producing fungi occur naturally in the environment and in the diet, perhaps especially at the end of the dry season, and that people sometimes mistake the natural excrement of bees for an agent of chemical warfare. A similar misidentification may have been responsible for the complaints brought before the UN Security Council 19 years ago by the Cambodian government, alleging that US and South Vietnamese planes were spraying lethal yellow powder over Cambodian villages. If the yellow rain is a natural phenomenon, there could still remain a serious and possibly widespread problem of human illness caused by trichothecenes.

Peter S. Ashton

Matthew Meselson

Harvard University
Cambridge, Massachusetts 02138 USA

J.P. Perry Robinson

University of Sussex
Brighton, BN1 9RF England

Thomas D. Seeley

Yale University
New Haven, Connecticut 06520 USA

Peter S. Ashton
Director, Arnold Arboretum
Arnold Professor of Botany
Professor of Dendrology
Harvard University
Cambridge, Massachusetts

Matthew Meselson
Thomas Dudley Cabot Professor
of the Natural Sciences
Harvard University
Cambridge, Massachusetts

J. P. Perry Robinson
Senior Fellow
Science Policy Research Unit
University of Sussex
Brighton, England

Thomas D. Seeley
Assistant Professor of Biology
Department of Biology
Yale University
New Haven, Connecticut

COMPARISON OF YELLOW RAIN AND BEE EXCREMENT

CONTENTS

1. PHOTOGRAPHS AND PHOTOMICROGRAPHS OF SPOTS OF YELLOW RAIN AND BEE EXCREMENT

2. SCANNING ELECTRON MICROGRAPHS OF POLLEN IN YELLOW RAIN AND IN BEE EXCREMENT

3. TABULATION OF YELLOW RAIN SAMPLES FROM SOUTHEAST ASIA

4. TABULATION OF EVIDENCE ON THE ORIGIN OF YELLOW RAIN

Data from forthcoming report by P.S. Ashton (Harvard University), M. Meselson (Harvard University), J.W. Nowicke (Smithsonian Institution), J.P. Robinson (University of Sussex) and T.D. Seeley (Yale University)

May 31, 1983

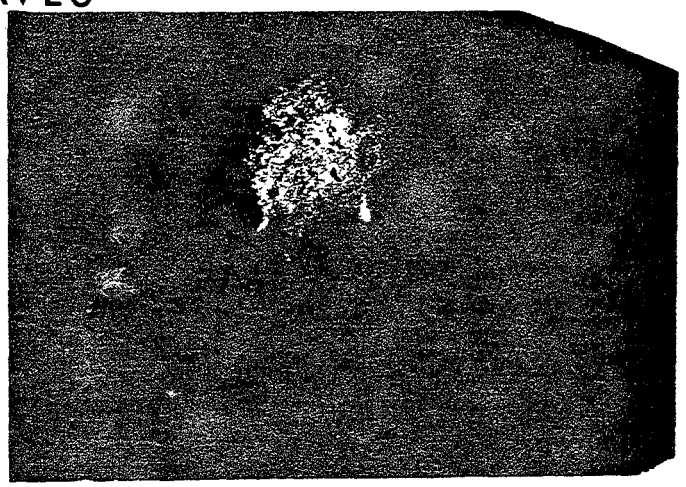
Presented at the AAAS Meeting in Detroit, May 31, 1983

Yellow Rain and Bee Excrement Compared

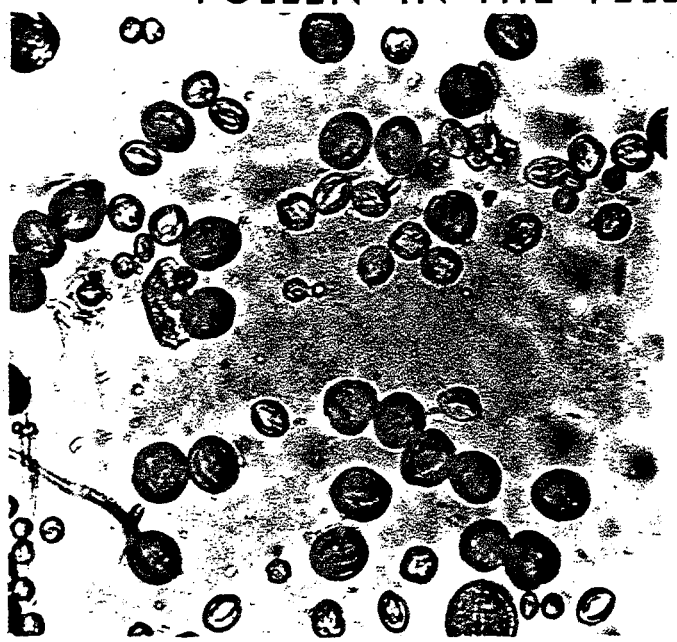
YELLOW RAIN
from alleged chemical attack
Thailand, February 1982

BEE EXCREMENT
from prunus leaf
Massachusetts, 1983

YELLOW SPOTS ON LEAVES



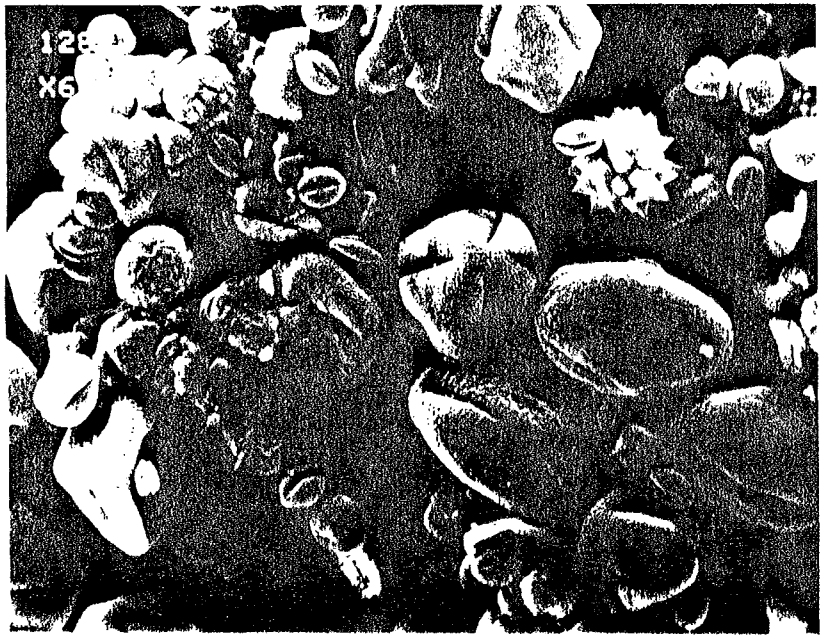
POLLEN IN THE YELLOW SPOTS (photomicrographs)



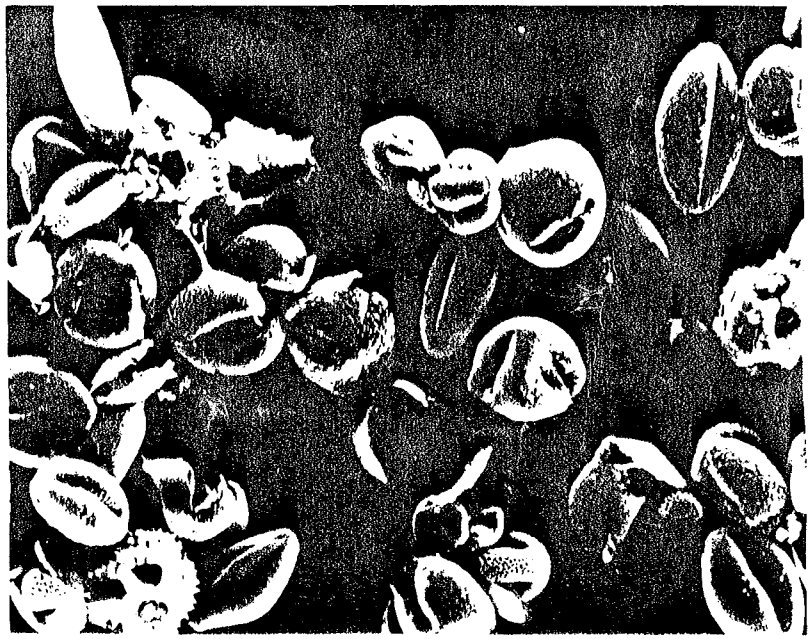
DATA FROM FORTHCOMING REPORT BY P.S. ASHTON (HARVARD UNIV.), M. MESELSON (HARVARD UNIV.), J.W. NOWICKE (SMITHSONIAN INST.), T.D. SEELEY (YALE UNIV.) AND J.P. ROBINSON (UNIV. OF SUSSEX).

Electron Micrographs of Pollen in Yellow Rain and Bee Excrement

YELLOW RAIN
from alleged chemical attack
Laos, March 1981



POLLEN
from spot on automobile
Massachusetts, May 1983



DATA FROM FORTHCOMING REPORT BY P.S. ASHTON (HARVARD UNIV.)
M. MESELSON (HARVARD UNIV.) J.W. NOWICKE (SMITHSONIAN INST.)
T.D. SEELEY (YALE UNIV.) AND J. ROBINSON (UNIV. OF SUSSEX)

EVIDENCE REGARDING THE ORIGIN OF YELLOW RAIN

Evidence Consistent with the Natural Origin of Yellow Rain as Bee Excrement

1. Yellow color, size (a few millimeters in diameter) and general appearance of spots
2. Approximate area in which spots are found in an occurrence (a few adjacent houses in a village)
3. Approximate spacing of spots in affected area, several per square foot
4. Continued appearance of spots over a period of days in an affected area
5. High pollen content of all spots examined
6. Variable diversity of pollen types and sizes in spots from different sites
7. Different pollen types in spots from different sites
8. Pollen in spots from plant families common in Southeast Asian tropics
9. Pollen in spots from plant families visited by bees
10. Bee hairs present in spots

Evidence and Reports Not Explained by Natural Origin of Yellow Rain as Bee Excrement

1. Tricothecene mycotoxins in samples of yellow rain
2. Tricothecene mycotoxins in samples of blood, urine, and tissues of alleged victims
3. Tricothecene mycotoxin on Soviet gas mask from Afganistan and possibly on an additional gas mask and on vegetation
4. Refugee reports of illness and death associated with occurrence of yellow rain
5. Refugee reports of yellow rain following overflights or attacks by aircraft and attacks by artillery and rockets

Conclusion

Whatever the source of mycotoxins in various samples, it is possible that yellow rain is bee excrement.

ENVIRONMENTAL SAMPLES FROM SOUTHEAST ASIA REPORTED TO CONTAIN TRICHOTHECENE MYCOTOXINS

Nature	Origin	Date of Collection	Reported by	Pollen
Leaf and stem	Kampuchea	March 1981	State Dept	not looked for
Water and floating material from same site as above				not looked for
Yellow-green powder scraped from rock	Laos	March 1981	State Dept	yes
Rock scraping	Laos	April 1981	State Dept	not looked for
Yellow powder scraped from vegetation	Laos	March 1981	ABC Television News	yes
Spots on vegetation	Thailand	February 1982	State Dept	yes

ADDITIONAL SAMPLES EXAMINED FOR POLLEN

Leaf samples		received April 1982	Australian Defense Dept	yes
Leaf samples		received April 1982	Australian Defense Dept	yes
Pebbles		received April 1982	Australian Defense Dept	yes
Leaf samples	Thailand	received April 1982	Australian Defense Dept	yes
Yellow powder scraped from rock	Laos	received November 1981	United Nations	yes
Leaf and stem	Laos	October 1981	United Nations	yes

Total Examined for Pollen: 9
Total Containing Pollen: 9

AIR MAIL
PAR AVION

Dyer
CENTER FOR THEORETICAL STUDIES
INDIAN INSTITUTE OF SCIENCE
BANGALORE-560 012, INDIA

Dr. Matthew Meselson
Department of Biochemistry
and Biophysics
Harvard University
Cambridge, Massachusetts
02138
USA

Send Dyer duplicate
copies of our 9 Sept
letter: to Pune and also
to Bangalore.

Include Science draft with
each —

Done -9-30-83