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PROGRESS REPORT

to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

on the progress of NASA Contract NAS-9-12622

to investigate

# "The Interaction of Lunar Material with the Sterol Metabolism in Tobacco Tissue Cultures"

by

John D. Weete, Staff Scientist
Lunar Science Institute
Houston, Texas

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INTRODUCTION

It should be pointed out that delays in the progress of this investigation have been caused by two principal factors in the administration of NASA contract NAS-9-12622:

1) a five month delay in the funding (from the date funds were available for this investigation only four months remained for research prior to the deadline for this progress report)
2) although a sample security plan has been submitted and a sample number assigned, no lunar sample has been received by the principal investigator for this study.

RESULTS OBTAINED FROM THE DATE OF FUNDING (JUNE, 1972) TO OCTOBER 1, 1972

Before these studies involving lunar sample are initiated, it is imperative that sufficient preliminary investigations with earth materials be first carried out. During the course of this investigation, callus pine (Pinus elloittii) tissue cultures grown in contact with Apollo 15 lunar material, a specially prepared earth basalt material, and Iowa soil became available from the bio-characterization studies (quarantine). This provided tissues from a higher plant species whose response to lunar material had not previously been studied and perhaps more importantly, provided an opportunity to do further studies with tissues

grown in contact with earth materials. Additional control tissues which had no particulates added to the culture were also provided.

A. An investigation of the lipid components of pine tissues (*Pinus elloittii*).

Since the studies with lunar materials involve callus tissue cultures rather than intact plants, it was necessary to establish that the tissue cultures were "normal" with respect to the fatty acid and sterol composition by comparison to the intact plants (leaves, shoots, roots). Thus, before studies on the interaction of lunar material with the formation of certain lipid constituents of pine tissue cultures could be made, it was necessary to do a gas chromatographic-mass spectrometric investigation of the individual fatty acid and sterol constituents of tissue cultures, seedlings, and seeds.

1) Fatty acids of slash pine tissues: A comparative study was made of the total fatty acid constituents of slash pine tissue cultures, seedling, and seed tissues. The results of this investigation have been accepted by PHYTOCHEMISTRY for publication (see enclosed preprint).

2) Sterols of slash pine tissues: A gas chromatographic-mass spectrometric investigation was also carried out on the sterol constituents of slash pine tissue

cultures, seedlings, and seeds. The sterols which have been identified and their relative concentrations as they occur in these tissues are given in table 1. The GLC separation obtained during these analyses are illustrated in figure 1. A manuscript for the publication of these results is presently being prepared.

In each of the above studies (parts 1 and 2), no indications were found which suggests that callus pine tissue cultures grown under germ-free conditions were abnormal with respect to the formation or composition of fatty acid and sterol components.

B. A study on the response by slash pine tissue cultures to growth in contact with Apollo 15 lunar soil, earth basalts, and Iowa soil.

When compared to the untreated control, each of the particulate materials added on top of the tissues caused a reduction of total accumulated lipids. Total lipid levels of 5.0, 5.4, and 5.3 percent (by dry weight) were found in the tissues grown in contact with lunar soil, earth basalts, and Iowa soil, respectively. An average of 39.2 percent reduction in the total lipid concentrations was found in the tissues grown in the presence of particulates.

When the total fatty acid and sterol components of these same tissues were examined, each of the particulate materials added to the tissues again resulted in a significant reduction in the

TABLE 1. STEROLS OF SLASH PINE TISSUE CULTURES, SEEDLINGS, AND SEEDS.

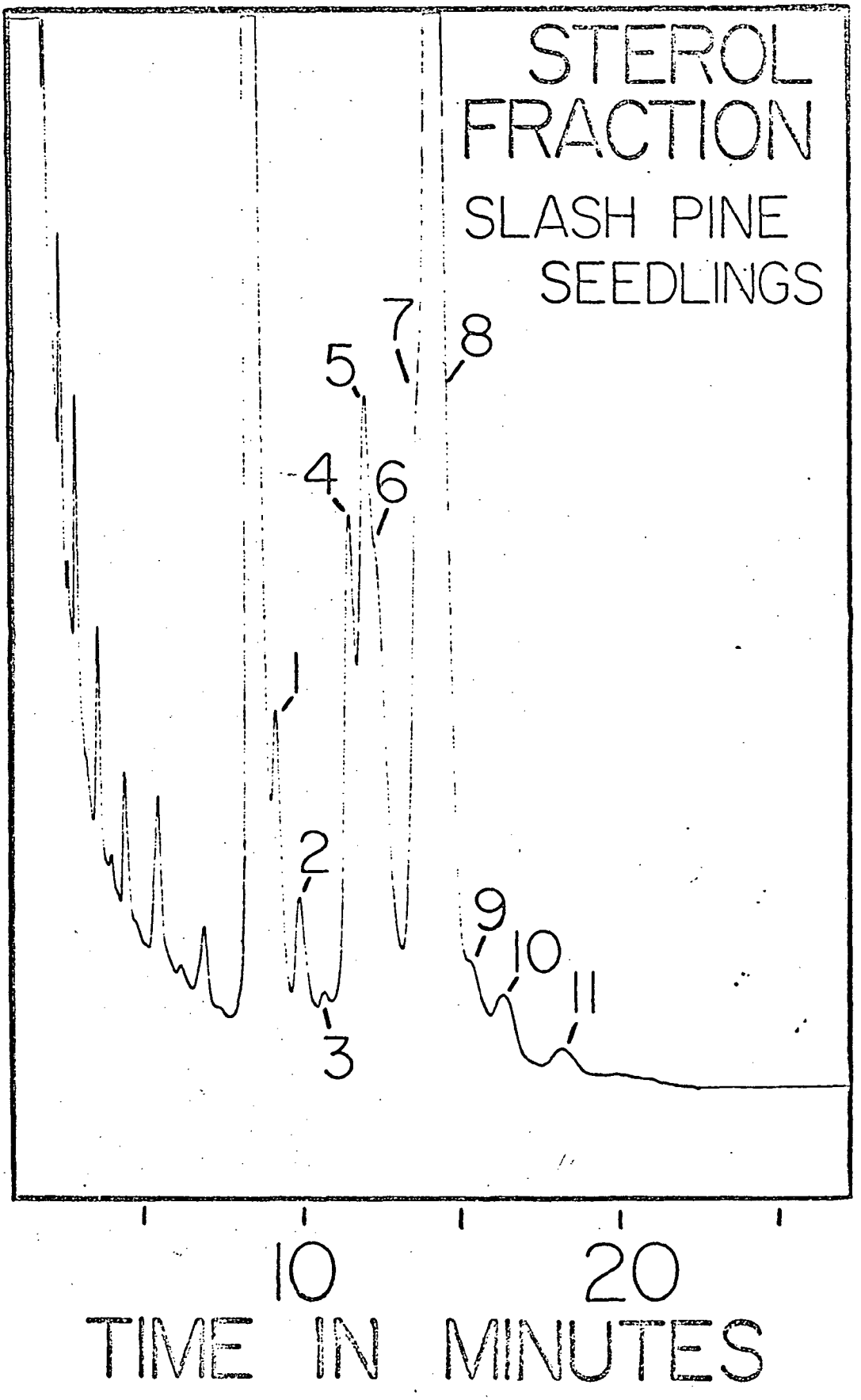
| COMPOUND NO. | STEROL | RELATIVE PERCENT* | | |
|---|---|---|---|---|
| | | Tissue Culture | Seeds | Seedlings |
| 1 | CHOLESTEROL | 0.34 | 5.25 | 2.90 |
| 2 | DESMOSTEROL | 0.15 | 0.66 | 1.74 |
| 3 | LOPHENOL | - | 2.64 | 0.67 |
| 4 | CAMPESTEROL | 5.56 | 7.90 | 6.95 |
| 5 | STIGMASTEROL | 6.69 | 1.31 | 8.70 |
| 6 | UNKNOWN | - | 0.52 | 6.90 |
| 7 | 24-METHYLENE LOPHENOL | tr | 31.85 | 16.13 |
| 8 | β-SITOSTEROL** | 80.78 | 38.07 | 47.83 |
| 9 | CYCLOEUCALENOL | 2.01 | 6.58 | 4.63 |
| 10 | CYCLOARTENOL | 4.47 | 3.96 | 2.83 |
| 11 | 24-METHYLENE CYCLOARTANOL | tr | 1.26 | 0.72 |
| 12 | 24-ETHYLENE LOPHENOL | - | - | tr |

*Expressed as relative percent (%) of the sterols from gas chromatographic data.

tr = less than 0.1%.

**Mass spectrometric data suggest that 28-isofucosterol may be present in small quantities.

FIGURE 1. Gas chromatographic separation of the sterols (TMS derivatives) isolated from slash pine (Pinus elliottii) seedlings.


STEROL
FRACTION
SLASH PINE
SEEDLINGS
1
2
3
4
5
6
7
8
9
10
11
10
20
TIME IN MINUTES

concentrations of these constituents when compared to the untreated controls (Table 2).

When the total fatty acid and sterol concentration values of tissues grown in contact with lunar soil were compared to the particulate controls, a 66.1 and 27.1 percent greater reduction in these constituents were found, respectively. This is in contrast to the results of studies carried out using tobacco tissue cultures.

The individual sterol components of the pine tissue cultures used in this study were identified by gas chromatographic and mass spectrometric techniques as: cholesterol, desmosterol, campesterol, stigmasterol, β-sitosterol, 24-isofucosterol, and 24-methylene cycloartenol. (Cycloartenol and 24-methylene lophenol were identified later). When the individual sterol components from the untreated control, lunar soil, earth basalt, and Iowa soil treated pine tissues were compared, very little variation in the relative concentrations was noted. It should be pointed out that desmosterol, a precursor to cholesterol and possibly the other sterol constituents, was found in only trace (< 0.01%) quantities in the tissues grown in contact with lunar soil (Table 3).

The results of these studies support the previous observations and studies that particulate materials significantly affect the lipid composition of higher plant tissue cultures. It is also significant to note that the lunar material produced the greatest response by these tissues by causing a reduction in the lipid levels by almost a factor of two over the earth particulates. It

Table 2. Total extractable lipids, fatty acids and sterols from slash pine tissues grown in contact with Apollo 15 lunar material, earth basalts, and Iowa soil. Tissues with no particulates added serve as the baseline controls.

| Culture Conditions | Dry Weight Extracted (gms.) | Total Extractable (% of Dry Weight) Lipids | Fatty Acids | Sterols |
|---|---|---|---|---|
| Control | 1.08 | 8.6 | 0.75 | 0.42 |
| Lunar | 1.85 | 5.0 | 0.40 (46.7% Reduction) | 0.20 (52.2% Reduction) |
| Earth Basalts | 1.55 | 5.4 | 0.61 (20.0% Reduction) | 0.26 (38.1% Reduction) |
| Iowa soil | 1.48 | 5.3 | 0.59 (21.5% Reduction) | 0.26 (38.1% Reduction) |

Table 3. Relative percent distribution of sterols from pine tissue cultures grown in contact with Apollo 15 lunar material, earth basalts, and Iowa soil. The results of tissues grown in the absence of particulates are also included.

| GC Peak Number | Compound | Culture Conditions | | | |
|---|---|---|---|---|---|
| | | Control | Lunar | Earth basalt | Iowa soil |
| 1 | Cholesterol | 0.34 | 0.51 | 0.49 | 0.40 |
| 2 | Desmosterol | 0.12 | Tr | 2.99 | 0.38 |
| 3 | Campesterol | 5.56 | 6.05 | 5.84 | 5.75 |
| 4 | Stigmasterol | 6.70 | 6.39 | 6.17 | 6.17 |
| 5 | β-Sitosterol + 24-isofucosterol(Tr) | 82.79 | 81.71 | 81.04 | 83.17 |
| 6 | 24-Methylene-cycloartenol | 4.47 | 3.74 | 3.43 | 4.10 |

(Tr) = less than .01%

should not be overlooked, however, that the response by the pine tissues was the opposite of that by the tobacco tissue cultures. This suggests that different higher plant species respond differently to the lunar material and/or plant tissues respond differently according to the variations in composition and structure of the lunar material from the various lunar landing sites.

The results of these studies support the continuation of studies to define the components of lunar materials which are responsible for the metabolic alterations in higher plant tissues.

PUBLICATIONS RESULTING FROM PREVIOUS WORK ON LUNAR MATERIAL BY THE PRINCIPAL INVESTIGATOR (reprints included)

1) Weete, J. D. and C. H. Walkinshaw. Apollo 12 lunar material: effects on plant pigments. CAN. J. BOTANY 50(1), 101 (1972).

2) Weete, J. D., C. H. Walkinshaw, and J. L. Laseter. Apollo 12 lunar material: effects on lipid levels of tobacco tissue cultures. SCIENCE 175, 623 (1972).

3) Weete, J. D., C. H. Walkinshaw, J. L. Laseter. Response of tobacco tissue cultures growing in contact with lunar fines. SPACE LIFE SCIENCE 3(4) (1972).

PUBLICATIONS RESULTING FROM STUDIES DURING THE PERIOD JUNE, 1972 TO OCTOBER 1, 1972 (4 month period covered by this progress report)

4) Laseter, J. L., G. C. Lawler, C. H. Walkinshaw, J. D. Weete. Fatty acids of slash pine tissues. PHYTOCHEMISTRY (in press). copy enclosed.

5) Laseter, J. L., G. C. Lawler, C. H. Walkinshaw, J. D. Weete. Gas chromatographic-mass spectrometric study of slash pine sterols. PHYTOCHEMISTRY (in preparation).

6) Weete, J. D., G. C. Lawler, C. H. Walkinshaw, J. L. Laseter. Growth of pine tissue cultures in contact with lunar soil and other particulates (in preparation).