

be attributed to the addition of methyl-paraben.<sup>4</sup>

The acceptability of this diet to the castor semi-looper *Achaea janata* L. was also tested. It is interesting to note that the larvæ feed well on this diet.

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#### LIGHT AND SPORULATION IN *CERCOSPORA PERSONATA* (BERK. ET CURT.) ELL. ET EVERH.\*

*Cercospora personata* sporulates poorly in culture and loses sporulating character on repeated subculturing.<sup>1</sup> Selective subculturing from spores as well as special media have been recommended to secure sporulation in *Cercospora*. Leach<sup>2</sup> reported that light (visible or near ultraviolet) can induce sporulation in a variety of fungi. Recently, this was found to be effective in the case of *C. beticola*.<sup>3</sup> Two poorly sporulating isolates of *C. personata* made in 1959 and 1967 respectively by one of us (R. N. S.) and maintained on potato-sucrose agar were also induced to sporulate by light. No sporulation occurred if the cultures were incubated in total darkness but profuse sporulation was noticed when plates seeded with mycelial fragments from dark-grown cultures were kept under fluorescent lamps. Sporulation commenced even by the end of 48 hours if the mycelial bits used as inoculum were sufficiently large. If they were smaller, 3-4 days were required for sporulation to start. The medium used apparently had no influence on this since it occurred even on water agar.

It was also of interest to see whether the fungus needed light to sporulate on infected groundnut leaves. Leaves of groundnut variety TMV 2 showing lesions, about 4 mm. in diameter (25 days after inoculation), were washed in distilled water so as to remove any spores already present, blotted dry, and incubated in

moist chambers in light and in darkness. After 48 hours the lesions were punched out carefully, suspended in a small volume of water and spores were brought into suspension by shaking with a few glass beads. Spore numbers were then determined with a haemocytometer. The results are shown in Table I.

TABLE I  
Effect of light on spore production by  
*C. personata* on groundnut leaves

| Expt. | No. of spores/lesion |                |
|-------|----------------------|----------------|
|       | Light                | Darkness       |
| 1     | 136,800 ± 7,100      | 68,200 ± 8,500 |
| 2     | 140,900 ± 4,000      | 63,600 ± 4,100 |

It is seen that light is not essential for sporulation on the host. However, it increases spore production. In *Helminthosporium gramineum* Houston and Oswald<sup>4</sup> have observed that light is not required for spore production on the host tissues though needed in pure culture. In the present experiment with infected leaves the light treatment was given only after formation of sporophores unlike in experiments on artificial media. This might be the reason why sporulation occurs even in darkness on leaves. This and other aspects of sporulation in *C. personata* are under investigation.

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#### IDENTIFICATION OF SUBSTITUTED BENZOIC AND CINNAMIC ACIDS IN POLLEN GRAINS

THE accumulation of phenolic acids in various forms, free or as glycosides and esters, is known to occur in almost all plant tissues. These organic acids, which are generally synthesized<sup>1,2</sup> from aromatic amino-acid phenylalanine, have been regarded as metabolically inert substances because of their irreversible biosynthetic pathways. Hence, these acids behave as characteristic stable end products.



Although phenolic acids are common constituents of pollen grains, not much work has so far been done in this field. The presence of a number of flavonoids such as quercetin in *Zea mays*,<sup>3</sup> rutin in *Forsythia* sp.,<sup>4</sup> dactylin in *timothy*<sup>5</sup> pollen and a mixture of quercetin, rutin, myricetin, prunin and narigenin in *Acacia dealbata*<sup>6</sup> has been reported. The phenolic acid make-up of some pollen grains, with special reference to phylogeny, has recently been published by Ibrahim.<sup>7</sup>

The results of the investigations on identification of phenolic acids in pollen of *Zea mays*, *Triticum aestivum* (var. Sonora 64), *Brassica nigra*, *Sorghum vulgare* and *Pennisetum typhoides* are reported in this paper.

Pollen was collected immediately after dehiscence, crushed and refluxed with 80% ethanol for 3 hrs. The alcoholic extract was evaporated to dryness and residue dissolved in small quantity of distilled water and filtered.

About half of this filtrate was adjusted to pH 4 by 6 N HCl, extracted with ether, washed with 25% aqueous sodium carbonate solution, the aqueous layer acidified and re-extracted with ether to isolate free phenolic acids.

The remnant filtrate was hydrolysed by 2 N NaOH at room temperature for 5 hours, acidified with 6 N HCl to pH 2, refluxed for 1 hour, extracted with ether and ether extract washed with 25% aqueous sodium carbonate solution. The final extract contained phenolic acids from esters or glycosides.

The ether extracts containing phenolic acids were evaporated to dryness on water-bath, moistened with few drops of 50% ethyl alcohol and spotted on Whatman No. 1, filter-paper for identification by descending paper chromatography. The solvent systems used were, upper phase of a mixture of benzene-acetic acid-water, 6:7:1 v/v in one direction and a mixture of sodium formate-formic acid-water, 10:1:200 v/v in the other direction. After drying the chromatograms, identification was done either by viewing under UV light for characteristic fluorescence or by colour reactions with various phenolic sprays.<sup>8</sup>

The results presented in Table I indicate the occurrence of *p*-hydroxy benzoic acid, ferulic (3-methoxy 4-hydroxy cinnamic) acid, caffeic (3,4-dihydroxy cinnamic) acid, sinapic (3,5-dimethoxy, 4-hydroxy cinnamic) acid in all the pollen studied. *Zea mays* pollen contain seven phenolic acids while *P. typhoides*, have only four acids. A fifth unidentified spot near the starting point in the first solvent system has

also been observed. It was noted that the ferulic acid gave two spots which may be due to the occurrence of its *cis* and *trans* isomers in solution as an equilibrium mixture. In case of caffeic acid, the duplicate spots may be due to conversion of this compound to related coumarin, esculetin in presence of Fe<sup>++</sup> ions and UV light. The formation of loose complexes by phenolics with each other in crude plant extracts may also result in multiple spots.

TABLE I

Identification of phenolic acids in pollen grains

| Sl. No. | Phenolic acid                           | <i>Z. mays</i> | <i>T. aestivum</i> | <i>B. nigra</i> | <i>S. vulgare</i> | <i>P. typhoides</i> |
|---------|---|----------------|--------------------|-----------------|-------------------|---------------------|
| 1.      | <i>p</i> -Hydroxy benzoic acid ..       | +              | +                  | +               | +                 | +                   |
| 2.      | 3-Methoxy-4-hydroxy-benzoic acid        | +              | -                  | -               | -                 | -                   |
| 3.      | 3, 5-Dimethoxy-4-hydroxy benzoic acid   | -              | +                  | -               | -                 | -                   |
| 4.      | 3-Methoxy-4-hydroxy cinnamic acid       | +              | +                  | +               | +                 | +                   |
| 5.      | 2, 5-Dihydroxy benzoic acid ..          | -              | -                  | +               | +                 | -                   |
| 6.      | 3, 4-Dihydroxy benzoic acid ..          | +              | +                  | -               | -                 | -                   |
| 7.      | <i>o</i> -Hydroxy cinnamic acid ..      | +              | -                  | +               | -                 | -                   |
| 8.      | 3, 5-Dimethoxy, 4-hydroxy cinnamic acid | +              | +                  | +               | +                 | +                   |
| 9.      | 3, 4-Dihydroxy cinnamic acid            | +              | +                  | +               | +                 | +                   |

+ Present, - Absent.

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