SHORT COMMUNICATIONS

THE ROLES OF RIBOFLAVIN AND INHIBITORS IN CONIDIAL GERMINATION IN *PERONOSPORA TABACINA* ADAM*

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Shepherd (1962) reported results suggesting that conidia of *Peronospora tabacina* Adam required an exogenous source of riboflavin for germination, but noted that the technique used was open to the objection that material might be leached from the spores during the washing process. Washing was found to be obligatory by Shepherd and Mandryk (1962) in order to obtain good germination and it was suggested that conidia contained a water-soluble auto-inhibitor of germination. Subsequently, Shepherd and Mandryk (1963) showed that washing removed materials inhibitory to spore germination from the surfaces of tobacco leaves. It was of interest, therefore, to determine whether the postulated auto-inhibitory materials were, in fact, picked up from the leaf surface during spore removal or were endogenous to conidia and also to examine further the riboflavin requirement of conidia removed from the leaf under more natural conditions than were used previously.

Three methods of preparing conidial suspensions were used:

(1) As described by Shepherd and Mandryk (1962), conidia were removed from the leaf by immersion in distilled water and subsequently washed three times by centrifugation. Suspension was diluted to contain between 5×10^4 and 10×10^4 conidia/ml. A portion (0·05 ml) of suspension was placed on the surface of a 2% agar block. Suspensions prepared in this manner are referred to below as "washed suspensions".

(2) A sporulating leaf was placed, ventral side downwards, on a 0·5 in. stainless steel mesh support and the conidia dislodged by gentle tapping, so that they deposited directly on to an agar surface. Blocks were cut from the agar and a drop of water added to cover the deposited conidia. Suspensions prepared in this manner are referred to below as "tapped suspensions". By trial and error, suspension densities were prepared corresponding to those used in method (1).

(3) As in (2) above conidia were dislodged by tapping, but in this case were deposited into water. The resulting suspension was concentrated, washed three times by centrifugation, and adjusted to contain 5×10^4–10×10^4 conidia/ml. Such suspensions are referred to below as "tapped and washed suspensions".

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Suspensions were placed on blocks of either 2% Difco Bacto agar, or on 2% "washed" agar, prepared according to Ryan, Beadle, and Tatum (1943). Incubation was for 6 hr at 15°C in all cases. The degree of germination was assessed according to Shepherd (1962).

The germination of conidial suspensions prepared by the above three methods was tested on Difco and washed agars, the results being shown in Table 1. The values given in the table are the means of 17 separate experiments.

Conidia removed by tapping give a high level of germination on both agars used; thus these conidia do not contain an auto-inhibitor and the results previously reported by Shepherd and Mandryk (1962) can be interpreted in the light of the subsequent paper by the same authors (1963) as being due to contamination of the conidial suspensions with the inhibitors present on the leaf surface. Furthermore,

TABLE 1

EFFECT OF METHOD OF PREPARATION ON GERMINATION OF CONIDIAL SUSPENSIONS

Method of Preparation	Germination (%) after 6 hr at 15°C on:	
	Difco Agar	Washed Agar
Washed	85·8±9·43	65·2±10·26
Tapped	85·2±8·50	82·8± 9·01
Tapped and washed	73·2±11·92	54·8±14·30
Tapped and washed+ 25 μg/ml riboflavin	—	70·2± 9·54

the similarity in degrees of germination of tapped spores on Difco and washed agars indicates that the endogenous level of riboflavin is not limiting. In the case of both washed conidial preparations, the level of germination on washed agar was considerbly lower than that on Difco agar, suggesting that washing had led to removal of riboflavin which was supplied exogenously in the Difco agar, but not the washed agar. The suggestion is confirmed by the result obtained on the addition of riboflavin to washed spores on washed agar (Table 1).

Washing conidia leads to a decrease in germination and this effect is reversed by the addition of riboflavin.

Positive evidence for the presence of riboflavin in the washings from conidia was obtained by spectrofluorometric examination. By using both fluorescence emission and excitation the presence of riboflavin was established, the amount present being very low (less than 3 μg riboflavin being obtained from 50 mg dry weight of conidia). *Peronospora tabacina* strains APT1 and APT2 (Hill 1963) and a new strain APT3 (Mandryk 1966) all behaved similarly. In various experiments, differences were observed in the degree of germination of washed conidia on washed agar (mean = 65·2±10·26 with extreme values of 41·0 and 90·3). These differences may be interpreted as due to variation of the endogenous riboflavin level in differing lots of conidia. Thus, with a high endogenous riboflavin level, the degree of washing given

does not reduce germination significantly, but with a low endogenous level, the washing given leads to removal of sufficient riboflavin to significantly suppress germination.

There is little doubt that the presence of auto-inhibitors and the riboflavin requirement reported previously were due to the particular technique used. However, in view of the results reported by Shepherd and Mandryk (1963) and the fact that riboflavin was very readily removed by washing in the above experiments, inhibition on the leaf surface and suboptimal riboflavin levels cannot be eliminated as factors possibly affecting the germination of conidia on leaves in the field.

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