A study of germination inhibition in fruits

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The procedure described here for the extraction and bioassay of natural germination inhibitors, requires only inexpensive equipment and minimal experimental skill. The method has been successfully used to demonstrate germination inhibition in a qualitative manner and to provide a framework for quantitative investigations

An illustration of the mechanism preventing the germination of seeds inside fruits despite apparently suitable conditions of water, temperature, and oxygen would be most useful to many school and college students (15–19 year olds). Some may have seen the simple demonstration given by unwashed tomato seeds which fail to germinate, while washed controls proceed uninterrupted (Wareing and Phillips, 1970): few, however, appear to have taken the experiment further. The method described here has been developed over a number of years and may be used to demonstrate qualitative and quantitative differences in germination inhibitor levels in a variety of different fruits or in different tissues within a single fruit. The results presented were obtained by a sixth form (17-year-old) student and are quite typical.

Methods

1. Extraction of germination inhibitor

A method modified from Kenworthy, Aston, and Bucknall (1972) was used. Fresh weight of the tissue containing the inhibitor (e.g. endocarp of orange) equivalent to 1 g dry weight (established by oven-drying of a similar sample at 105 °C to constant weight) was homogenized with adequate methanol to provide 25 cm^3 total solvent volume. The resulting homogenate was filtered and 5 cm^3 aliquots were pipetted on to filter paper discs inside Petri dishes. The solvent was allowed to evaporate in a ventilated cupboard and the dishes stored in a refrigerator until required.

2. Bioassay technique

Twenty cress seeds (cv. curled) were evenly spaced out on the filter paper discs prepared as described above. These were moistened with 2 cm^3 of a 0.1 per cent *w/v* solution of 'Fungizone'[1] (Amphotericin–B, an anti-fungal substance produced by *Streptomyces nodosus*). Control plates were prepared as in 1 above using 5 cm^3 meths instead of the extract. Plates were incubated at 25 °C and re-moistened as necessary to maintain the quantity of water at 2 cm^3. Regular observation using a binocular microscope revealed germination which was recorded when radicle emergence caused cracking of the testa. In addition to the cress seeds the original seeds from the fruits may be thoroughly washed and sowed in dishes in a similar manner.

[1]'Fungizone' may be obtained from Sigma Chemical Company, Fancy Road, Poole, Dorset BH17 7NH. Although the use of this preparation is not essential to the procedure, it does result in a great reduction in losses due to fungal spoilage of the plates.

Results

1. Germination of cress seeds in orange extracts (figures 1 and 3)

Germination inhibitor concentration in the endocarp (pith) of oranges was found to be substantially greater than in any other tissue. The germination of cress seeds in the placenta and mesocarp extracts was very similar to the controls. The cress seeds in the endocarp extract showed only 15 per cent germination 80 hours after sowing, while the control, placenta, and mesocarp

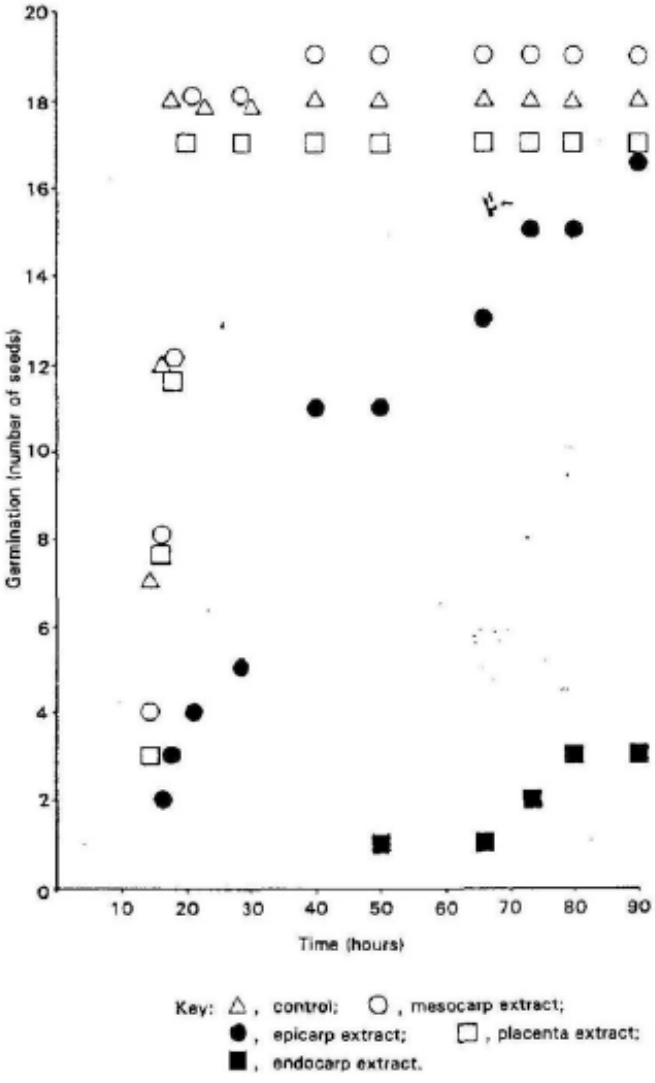

Figure 1 Germination of cress seeds in orange extracts.

extract batches had exceeded this level within 16 hours. In the presence of epicarp extract, 50 per cent germination was reached after 40 hours.

2. Germination of cress seeds in tomato extracts (figures 2 and 3)

The rate of germination of the cress seeds in all three extracts was substantially slower than that of controls. The epicarp extract showed the greatest germination inhibition with only 50 per cent of the seeds germinating within 160 hours. The placenta extract showed the next highest inhibitor level with seeds taking 80 hours to reach 50 per cent germination, and the endocarp extract showed the lowest inhibitor level with 50 per cent seed germination achieved after 43 hours.

Discussion

The experimental procedure described enables investigations of germination control by fruits to be made. The inhibitor substances causing the effect are not identified in this procedure, but the standard comparison of both different fruits and different tissues in a single fruit which may be made outweigh this drawback.

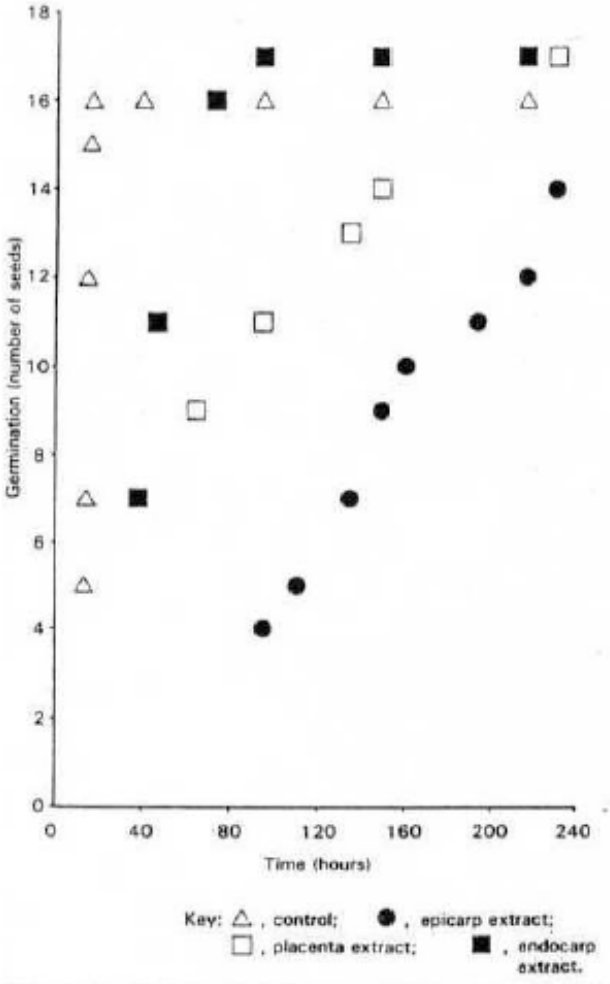

Figure 2 Germination of cress seeds in tomato extracts.

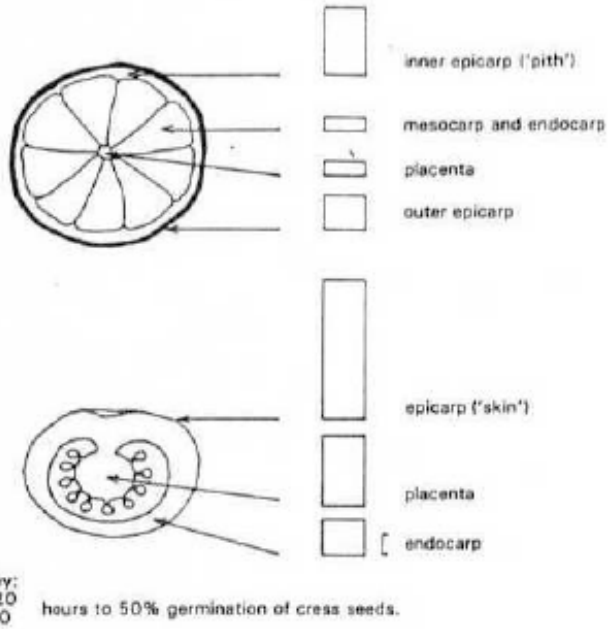

Figure 3 Germination inhibitor levels in orange and tomato extract.

The significance of the results has promoted considerable discussion and links between the experimental results and the life cycle of the plant have been suggested. It is interesting to note that the highest level of germination inhibitor in tomatoes is found in the epicarp which is the most persistent tissue of the fruit. By this means that part which rots last exercises the greatest control over the germination of the enclosed seeds.

The investigation described may well have wider applications in the teaching of control mechanisms in plants or in ecological contexts.

References

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