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# AN IMPROVED METHOD OF STAINING WITH FAST GREEN*

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The troubles involved in preparing plant tissues for microscopic study are too well known to need discussion. The following method, developed in connection with researches on the histogenesis of foliar structures, works equally well with anatomical material of diverse types. Through its use the ordinary difficulties such as plasmolysis of tissues, under or over-straining, loss of time due to lengthy schedules, etc. are largely avoided.

1. Material is killed and fixed for 24 hours in formalin-acetic-alcohol (formalin 5%, glacial acetic acid 5%, in 70% ethyl alcohol).
2. Wash for 2 hours in 80% ethyl alcohol, changing at end of first hour.
3. 80% ethyl alcohol 65 parts and tertiary butyl alcohol 35 parts— 1 hour.
4. 90% ethyl alcohol 45 parts and tertiary butyl alcohol 55 parts— 1 hour.
5. Absolute ethyl alcohol 25 parts and tertiary butyl alcohol 75 parts— 1 hour.
6. Tertiary butyl alcohol — 2 or 3 hours, changing at the end of each hour.
7. Infiltrate over night on top of oven.
8. Replace butyl alcohol with paraffin over a period of 6 hours in the oven with at least six changes of paraffin.
9. Imbed, section and mount on slides.
10. Xylol — 6 minutes.
11. Absolute alcohol — 2 minutes.
12. 95% alcohol — rinse 5 or 6 times.
13. 50% alcohol — rinse 5 or 6 times.
14. 1% safranin in 50% alcohol — 6 to 12 hours.
15. Wash in water.
16. 50% alcohol — 2 or 3 minutes. In most cases destaining with acid alcohol is not necessary.
17. 70% alcohol — rinse 5 or 6 times.
18. 95% alcohol — rinse 5 or 6 times.
19. Absolute alcohol — rinse 5 or 6 times.
20. 40% absolute alcohol and 60% xylol — rinse 5 or 6 times.
21. Fast green (prepared by adding a few pipettes of a saturated clove oil solution of fast green FCF to a stender of 40% absolute alcohol and 60% xylol. The solution should be fairly dark green, although the strength may vary considerable. If the solution seems unstable or turbid, let it stand for a day or two and if it does not become clear add a pipette of absolute alcohol.) — *time to be determined by trial.* Try 2 or 3 minutes at first. There is very little danger of over-staining, or of reducing the safranin.

*Contribution from the Botanical Laboratory, University of Oklahoma No. 45

22. 40% absolute alcohol and 60% xylol — rinse 5 or 6 times.
23. Xylol — 5 minutes.
24. Mount in any good medium, preferably neutral balsam.

This schedule has been found to be very satisfactory for practically all types of anatomical material and it gives passable results with semi-cytological items. The use of tertiary butyl alcohol was first suggested by Johansen (1935). The method of making up the fast green solution was suggested by a somewhat similar procedure utilized by E. J. Kraus [described by Conant (1926)] who, however used gentian violet instead of fast green. The advantages of using the green are that it provides a greater contrast with safranin, and it does not fade so rapidly.

LITERATURE CITED

Conant, G. H. 1926. A rapid combination stain for differentiating plant tissues. Turtox News 4: 21-22.
Johansen, D. A. 1935. Dehydration and infiltration. Science 82: 253-254.