# HEARTWOOD DIFFERENTIATION IN *PINUS* SPECIES — A MODIFIED AZO-DYE TEST

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ABSTRACT

A modified azo-dye reaction test for heartwood in **Pinus** species using a non-carcinogenic dye precursor para-amino-NN-dimethylaniline (PANDA) in place of benzidine or ortho-tolidine is described. Sensitivity and ease of differentiation of heartwood and sapwood are essentially similar to those of the ortho-tolidine reaction. The presence of boron and copper-chrome-arsenic preservatives does not adversely affect the test.

## INTRODUCTION

For many years heartwood and sapwood in *Pinus* species have been differentiated by the azo-dye coupling reaction of pinosylvin and methyl pinosylvin, phenolic constituents of *Pinus* heartwoods with a diazotised solution of benzidine or ortho-tolidine hydrochloride to give an intense red to red-brown colouration. The exact colour varies with the species and the age of the test specimen.

Research at the Chester Beatty Research Institute, Royal Cancer Hospital, London (Anon., 1966) showed positively that both benzidine and ortho-tolidine can cause a cancerous condition of the epithelial lining of the human kidney, ureter and urinary bladder.

The United Kingdom National Insurance Industrial Injuries Act Prescribed Disease No. 39 Regulations laid down that workers in the United Kingdom who handle these substances and develop the condition described above shall be eligible for benefit under the Prescribed Diseases Regulations of the National Insurance Industrial Injuries Act. Subsequent legislation has prohibited the industrial manufacture and use of several chemically similar substances, notably benzidine and alpha and beta-naphthylamines.

For these reasons, and, in addition, because both benzidine and ortho-tolidine are powerful irritants and allergens, it became essential to find an alternative less hazardous reagent.

## EXPERIMENTAL

In principle, any aromatic amine capable of forming a diazonium salt should be capable of coupling with the heartwood phenols to give a coloured substance. In practice most of the coloured substances so formed, particularly with the simpler aromatic compounds such as aniline and sulphanilic acid do not sufficiently contrast with the

natural wood colour to differentiate rapidly and positively between heartwood and sapwood for routine use by other than skilled chemical laboratory staff.

Use of para-amino-NN-dimethylaniline (PANDA) was first considered when the substitution of its diethyl homologue was made for ortho-tolidine in testing water for free chlorine. An initial trial was disappointing; very little colour developed although there was some slight differentiation of heartwood and sapwood zones.

Testing for diazonium compounds by the dye coupling reaction usually involves the use of an alkaline solution of a phenol, typically 2-naphthol in sodium hydroxide, and accordingly tests were made using wood samples whose surfaces had been pre-treated with sodium hydroxide solution. Following application of diazotised PANDA solution, a purplish colour developed slowly in the heartwood areas, but the colour was comparatively pale, although it provided a better contrast than when no alkali was used.

When an ortho-tolidine heart-sap test is made on wood samples previously tested by the ammonia and rubeanic acid method for preservatives containing copper (Cummins, 1966) considerable darkening of the ortho-tolidine treated material occurs. Samples of wood were pre-treated by spraying the surface with a 5% ammonia solution which was allowed to soak in. This was followed by an application of diazotised PANDA solution. A strong red-purple colour developed after a few minutes in the heartwood zone; no colour at all developed in the sapwood.

Specimens of other *Pinus* species were obtained and subjected to this test and to the ortho-tolidine test. (Initial work was done on *Pinus radiata* D. Don because this species is the most important in New Zealand exotic forestry.) Colours obtained varied with species, and are listed, together with the ortho-tolidine colours, in Appendix 1.

Heart-sap differentiation tests are used a great deal at this Institute in connection with wood preservation work; so a large number of specimens of *P. radiata* and a smaller number of *P. nigra* Arnold "laricio" which had all been treated commercially with copper-chrome-arsenic preservatives (Timber Preservation Authority, 1969; Formulations F2.1 and F2.8) and a further set treated by commercial boron diffusion (ibid., Formulation F3,3) processes were tested; neither preservative interfered with the heartwood differentiation.

## DISCUSSION

The azo-dye coupling reaction with benzidine and later with ortho-tolidine has been established for many years as a highly satisfactory test for differentiating heartwood and sapwood in most *Pinus* species. Tabulated results in Appendix 1 confirm the utility of the method put forward in this paper as a replacement test of the same type. The mixed diazonium salt of PANDA hydrochloride is unusual in this class of compound in that it retains its activity for at least 14 days at the normal laboratory temperature of 21°C, and for considerably longer if kept in a refrigerator. In this laboratory the stock is normally used up before it has deteriorated; recommended practice is to store stock solutions (b) and (c) (*see* Appendix 2) and the mixture in a refrigerator at about 4°C. Mixed reagent is used up approximately weekly and more made as required. The mixture should be stored in a partly filled bottle, as some gas pressure develops during storage.

The heartwood colour produced, as with benzidine and ortho-tolidine, is stable for some months provided the specimens are kept dry and out of direct sunlight.

A note of caution must be sounded on the use of PANDA; although not a carcinogen, as shown by its use over many years in the fur trade for dyeing and the use of closely related compounds in hair dyes for human cosmetic use, it is poisonous, producing symptoms similar to those of aniline poisoning, methaemoglobinaemia and depression of central nervous system activity, often accompanied by a fall in blood pressure and disturbance of heart rhythm. It is unlikely that these symptoms would develop from the use of the low concentrations employed in this test; however, it is possible that sensitive subjects may show allergic reactions, and appropriate handling precautions are essential.

## CONCLUSION

The azo-dye coupling test using diazotised PANDA solution on wood specimens previously sensitised with ammonia solution enables heartwood and sapwood of most *Pinus* species to be readily differentiated.

The test offers the advantage of an established method with the removal of the carcinogenicity hazard associated with the benzidine and ortho-tolidine reagents previously used.

## REFERENCES

ANON. 1966: "Precautions for Laboratory Workers who Handle Carcinogenic Aromatic Amines." The Chester Beatty Research Institute, Royal Cancer Hospital, London.

CUMMINS, N. H. O. 1966: Spot tests for wood preservatives: testing for copper with rubeanic acid. **New Zealand Forest Service, Forest Research Institute Research Leaflet 14.**

TIMBER PRESERVATION AUTHORITY, 1969: "Timber Preservation in New Zealand: Specifications" Timber Preservation Authority, Wellington.

## APPENDIX 1

### RESULTS OF ORTHO-TOLIDINE AND PANDA TESTS

| **Pinus** Species Name | Common Name of Pine | O-tolidine Colour on Heartwood | PANDA Colour on Heartwood | No. of Test Specimens |
|---|---|---|---|---|
| **P. contorta** | Lodgepole | bright red | purple-brown | 2 |
| **P. elliottii** | Slash | pale red-brown | faint purple | 1 |
| **P. excelsa** | White | dark red-brown | purple | 1 |
| **P. halipensis** | Aleppo | pale red-brown | pale brownish-grey | 1 |
| **P. nigra** | Corsican | dark red-brown | dark red-brown | 10 |
| **P. patula** | Patula | orange-brown | purple | 2 |
| **P. ponderosa** | Ponderosa | orange-brown | purple | 1 |
| **P. radiata** | Radiata | deep red | red-purple | 30 |
| **P. rigida** | Pitch | deep red | purple | 1 |
| **P. strobus** | Strobus | reddish-brown | brown | 1 |
| **P. taeda** | Loblolly | deep red-brown | purple-brown | 1 |
| **P. torreyana** | Torrey | pale red-brown | pale brown | 1 |

All the above information refers to New Zealand-grown material which in general is faster grown than in the country of origin of the species.

Ortho-tolidine and benzidine have been used on many thousands of specimens of **P. radiata** and **P. nigra** during work on wood preservation in the Institute, and upon lesser numbers of the other species listed for work on wood quality and strength testing where it has been necessary to differentiate heartwood and sapwood, and have been found thoroughly satisfactory.

## APPENDIX 2

### REAGENTS

(a) Ammonia solution, 5% $NH_3$ equivalent
Dilute 1 volume of 0.91 s.g. ammonia with 4 parts distilled or de-ionised water (0.880 ammonia, 1 part to 6).

(b) Para-amino-NN-dimethylaniline hydrochloride reagent.
Dissolve 5 g of the solid in 15 ml of A.R. grade concentrated hydrochloric acid; make up to 1 litre with distilled or de-ionised water.

(c) Sodium nitrite solution
Dissolve 10% by weight of the solid salt in distilled or de-ionised water.

### METHOD

Wet the specimen with solution (a) by spraying or dipping; allow to soak in. Prepare the diazonium reagent by adding an equal volume of solution (c) to solution (b), apply the mixed reagent to the specimen. The contrasting colour of the heartwood begins to develop almost at once and reaches its full intensity in about 5 min.

The mixed diazonium reagent is active for at least 21 days at room temperature, and for very much longer if stored in a refrigerator. The dark precipitate which forms on initial mixing is of no consequence and may be left in the mixed reagent. Solution (b) is of limited shelf life at room temperature and recommended practice is that both (b) and (c) be stored in a refrigerator, where their shelf life is indefinite.

Best results are obtained from dry fresh-cut material: green fresh-cut material contaminated with bark particles can give colours similar to the heartwood colour. The presence of common wood preservatives such as boron compounds or copper-chrome-arsenate does not affect the test.