Showering test two

No real changes in the most visible dye treatments, apart from Intradene Blue which dropped to 4th place after the second showering test (Table 5). Ten of the eighteen dye treatments (all acid dyes) were not visible following this test.

Table 5: Visibility following heavy rainfall during stump treatment

Dye (commercial name)	*Concentration*	*Visibility score*
Teilmark Green B	0.10%	7.0
Basazol Blue 57L	0.10%	7.0
Basazol Violet 56L	0.10%	6.8
Intradene Blue	0.10%	6.3
Teilmark Green B	0.01%	6.0
Basazol Violet 56L	0.01%	5.2
Basazol Blue 57L	0.01%	4.3
Intradene Blue	0.01%	3.7
Cartasol Red	0.01%	2.5
Forestmark Blue 400	0.10%	1.3

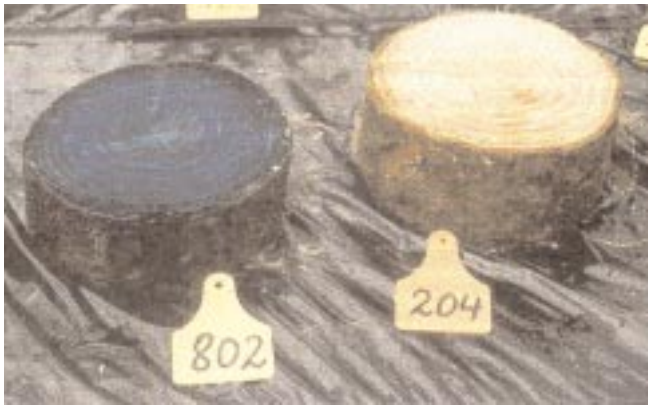
Note: The top ten visible dyes only are presented in this table.

Discussion and recommendations

- Final recommendations for the selection of a dye for use in stump treatment are based on the outcome of the severest test of the dyes i.e. showering with heavy rainfall during painting.

- Four basic dyes are recommended for use in stump treatment. These dyes are recommended for use at 0.01% concentration, with the objective of reducing the use of chemicals in the forest.
  - Teilmark Green B
  - Basazol Blue 57L
  - Basazol Violet 56L
  - Intradene Blue

- All of the above dyes recommended are free from negative health and safety implications at present. However, if basic dyes are not allowed in the future the acid dyes that scored highest in the first test could be retested.



Sample discs from showering trials

Disc 802 = 0.10% Basazol Blue 57L

Disc 204 = 1.0% Teilmark Red

Disc 502 = 0.10% Basazol Violet 56L

Disc 801 = 0.01% Basazol Blue 57L

Dyes capable of bonding with fresh wood for use in stump treatment

Elaine Smyth, Declan Ward and Áine Ní Dhubháin

- **Tree stumps are treated with 20% solution of urea to protect against the butt-rot fungus *Heterobasidium annosum*. A dye is added to this solution so that treated stumps can be easily identified. However, due to heavy rainfall the dye does not always remain visible.**

- **This project sought to find a suitable dye that could be easily seen and would not interfere with the mode of action of urea. After tests described below, the following dyes performed best.**

- **Four basic dyes are recommended for use at 0.01% concentration, with the objective of reducing the use of chemicals in the forest.**

  Teilmark Green B
  Basazol Blue 57L
  Basazol Violet 56L
  Intradene Blue

Introduction

Heterobasidium annosum (Fr.) Bref., the root and butt-rot causing fungus formerly known as *Fomes annosus*, is one of the greatest disease threats to Irish coniferous forests. Protection from infection is practiced by the application of a 20% solution of urea to tree stumps immediately after felling. As this solution is colourless, a dye is added, to allow forest managers to inspect that stumps have been treated. In the past Rhodamine Red was used, however, following a review of health and safety standards, it was removed from use. The dye currently used as an indicator of stump treatment, Forest mark Blue 400, tends not to remain visible on stumps for the minimum time requirement of seven days, especially under wet conditions. Following extensive research that failed to identify a suitable replacement for Rhodamine Red, this project was initiated.

Objectives of study

To identify a dye or dyes that:

- were water-soluble and bright but did not interfere with the mode of action of urea;
- were capable of bonding with fresh wood of Sitka spruce under varying environmental conditions;
- were visible on the stump surface for a minimum of seven days;
- were effective and had no adverse health or safety implications.

Selection of dyes for testing

Dyes were selected for testing following a review of previous tests by Coillte and a discussion of the options with dye specialists. There are two types of dyes - basic and acidic.

Basic dyes *have the following characteristics:*

- Good 'covering power' and remain visible for a long period of time;
- Low solubility in water, possibly improving their ability to withstand wet conditions. Rhodamine red is an example of a basic dye;

Acid dyes *are characterised by:*

- Poor 'covering power' compared to basic dyes;
- Highly soluble in water, therefore, they tend to fade faster especially under wet conditions;
- Used in colour foods, therefore, are considered free of health concerns.

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Ten dyes (five basic and five acidic) were chosen for testing (Table 1).

Table 1: Dyes selected for testing

Dye (commercial name)	**Generic name**	**Group**
Colourform Turquoise	Blue 9	Acid
Teilmark Crimson	Acid Red	Acid
Golden Yellow	Acid Yellow	Acid
Teilmark Blue	Acid Blue	Acid
Forestmark Blue 400	Acids Blue 9	Acid
Teilmark Green B	Basic Green 4	Basic
Basazol Violet 56L	Basic Violet 4	Basic
Intradene Blue	Basic Blue 41	Basic
Basazol Blue 57L	Basic Blue 26	Basic
Cartasol Red	Basic Red 111	Basic

Experiments

The following three tests were conducted.

Test 1: Interference of dyes with the inhibition of H. annosum by urea.

The objective of this test was to determine if any of the dyes interfered with the inhibitory action of urea on the growth of the fungus. Plates were prepared with growth media consisting of 20% fertiliser urea grade, malt agar and dye.

The ten dyes were tested at the following dye concentrations, 0.01%, 0.10%, 0.50% and 1.00%. The effect of dyes on the pH of the malt agar mixture was monitored in an attempt to explain any interference of the dyes with the mode of action of urea.

Test 1 Results

After 14 days, no growth of *H. annosum* was observed on any of the treatment plates. Ten of the treatment plates (five of 0.01% concentration and five of 0.50% concentration) of each dye were monitored for a further 21 days. No growth was observed on any of these plates. The impact of addition of dyes at a range of concentrations on the pH of the urea and malt agar mixture resulted in a tendency for the pH of the agar/urea mixture to decrease as higher concentrations of basic dyes were added. For acid dyes, the pH of the agar mixture tended to increase in accordance with increasing dye concentrations (Table 2).

Test 2: Dye durability in the field

As none of the dyes interfered with the mode of action of urea, each was tested at the same concentration used in test 1. Three replicates of each of the 40 dye treatments were randomly allocated throughout a Sitka spruce clearfell site in Glenealy forest, Co. Wicklow.

Stumps were treated manually with a urea concentration of 20%, and the dye concentration varying according to dye treatment. The visibility of each dye was assessed on days 2,5,7,14 and 20 after application. The visibility was rated subjectively by two readers on a score of 1 to 7.

The scoring system was as follows:

1	Dye invisible on stump surface
2	Dye was visible
3 to 6	Increasing visibility
7	Maximum visibility

Visibility was assessed based on a combination of
a) the strength of the colour on the stump and
b) visibility of that colour in the forest environment.

Table 2: pH of the malt agar/urea/dye mixture over the range of concentrations tested

		Concentration			
Commercial name	**Group**	**0.01%**	**0.10%**	**0.50%**	**1.00%**
Colourform Turquoise	Acid	5.94	5.95	5.98	6.05
Teilmark Crimson	Acid	6.03	6.09	6.22	6.48
Golden Yellow	Acid	5.64	5.75	6.08	6.39
Teilmark Blue	Acid	5.68	6.23	6.51	6.52
Forestmark Blue 400	Acid	5.92	5.97	5.98	6.15
Teilmark Green B	Basic	5.70	4.75	3.07	2.60
Basazol Violet 56L	Basic	5.95	5.16	4.57	3.97
Intradene Blue	Basic	6.05	6.03	5.97	5.91
Basazol Blue 57L	Basic	6.01	5.33	4.17	3.81
Cartasol Red	Basic	5.94	5.19	4.57	3.94

Table 3: Mean visibility of each dye on day 30

		Concentration			
Dye (commercial name)	**Group**	**0.01%**	**0.10%**	**0.50%**	**1.00%**
Cartasol Red	Basic	5.9	7.0	6.8	6.9
Teilmark Green B	Basic	6.3	5.3	4.8	5.0
Basazol Blue 57L	Basic	4.1	5.5	5.0	5.0
Intradene Blue	Basic	3.9	5.3	4.8	4.8
Basazol Violet 56L	Basic	3.8	5.3	4.7	4.7
Forestmark Blue 400	Acid	1.7	1.9	3.9	4.8
Colourform Turquoise	Acid	1.0	1.3	3.3	4.2
Teilmark Blue	Acid	1.0	1.0	2.8	3.7
Teilmark Crimson	Acid	1.0	1.0	1.0	1.0
Golden Yellow	Acid	1.0	1.0	1.0	1.0

Notes: Visibility score scale 1 to 7.
1=Dye invisible on stump surface, 2= Dye was visible, 3 to 6 = increasing visibility, 7= Maximum visibility.

Test 2 Results

A mean visibility score was produced for each treatment. Dye visibility was found to be highly correlated with dye grouping (table 3). Basic dyes were highly visible over the entire period, both at low and high concentrations. Acid dyes tended to fade over time, especially at low concentrations. Thirty days following application, only high concentrations of acid dyes were highly visible on tree stumps. Basic dyes were more visible at low concentrations rather than high concentrations (this was due to the nature of the basic dyes which appeared black when applied to stumps at high concentrations.)

Table 4: Visibility after heavy rainfall following stump treatment

Dye (commercial name)	**Concentration**	**Visibility score**
Teilmark Green B	0.10%	7.0
Intradene Blue	0.10%	7.0
Basazol Blue 57L	0.10%	7.0
Basazol Violet 56L	0.10%	6.8
Teilmark Green B	0.01%	6.3
Basazol Violet 56L	0.01%	5.7
Intredene Blue	0.01%	5.7
Basazol Blue 57L	0.01%	5.2
Colourform Turquoise	0.5%	4.7
Teilmark Blue	0.5%	4.2
Cartasol Red	0.01%	4.0
Teilmark Crimson	1.0%	3.7
Teilmark Crimson	0.5%	3.5
Colourform Turquoise	0.10%	3.3
Teilmark Blue	0.10%	1.8
Forestmark Blue 400	0.10%	1.3
Colourform Turquoise	0.01%	1.0
Forestmark Blue 4000	0.01%	1.0

Test 3: Effect of heavy rainfall on dye durability

The aim of this test was to determine the effect of the intensity of rainfall and timing of rainfall on dye durability. Dyes which were invisible following mild conditions experienced in the field were eliminated from this test. Furthermore, dye combinations scoring under 2, i.e. those which were invisible on stumps, were also excluded. A total of eighteen dye concentration/combinations were selected for testing.

Two showering trials, using a simulated overhead showering system at University College Dublin (UCD), were instigated using tree discs from first thinning of Sitka spruce.

Showering trial one: Tree discs treated with urea and dye solution followed by heavy showering (32 mm per hour) for one hour.

Showering trail two: Tree discs subjected to heavy showering (32 mm per hour) during painting followed by light showering for 40 minutes.

Test 3 Results

Showering test one

All the basic dyes were highly visible following disc showering one hour after application (table 4). Four dyes (all acid dyes) were not visible after showering. Acid dyes performed well only at high concentrations.