PROTOCOL F: DETERMINATION OF TOTAL CYANIDE IN BAMBOO SHOOTS

1. The new leaf matter of the bamboo shoot is removed exposing the edible bamboo shoot (see sketch). The tip section contains more cyanide than the middle section which contains more than the base section[a]. Each section may be analysed by cutting a thin section (about 0.5-1 cm thick across the bamboo shoot and then cutting a small sector from it as shown in the sketch.
2. This sector is ground up in a pestle and mortar. *Note. The enzyme breaks down the taxiphyllin quite rapidly to produce HCN, hence carry through steps 2 to 6 as quickly as possible.*
3. **Immediately** weigh out about 25 mg (accurately) of the ground bamboo shoot in a flat bottomed plastic bottle (supplied in the kit). Weight of sample = z mg.
4. **Immediately** add 0.5 mL of 0.1 M phosphate buffer at pH 6[b].
5. **Immediately** add a yellow picrate paper attached to a plastic strip[c]. The picrate paper must not touch the liquid in the bottle.(STORE PICRATE PAPERS IN THE DEEP FREEZE OF THE REFRIGERATOR).
6. **Immediately** close the bottle with a screw capped lid.
7. Prepare another sample as above but with no bamboo shoot, to serve as a blank.
8. As a control (or standard) to check on the method, place a Whatman filter paper disc loaded with buffer and linamarase (marked with a black spot) in a bottle, add a pink linamarin paper (see below), 0.5 mL water and a yellow picrate paper. Immediately close the bottle with a screw cap lid.
9. Allow the bottles to stand for 16-24 hour at room temperature (20-35^{o} C).
10. Open the bottles and match the colour of the paper against the colour chart supplied.
11. From the colour chart, read off the amount in ppm. The total cyanogen content in ppm is obtained by multiplying the value from the colour chart by 100 / z. Also check that the blank corresponds to zero and the control gives the expected value.

FOR USE IN A LABORATORY EQUIPPED WITH A SPECTROPHOTOMETER

12. Carefully remove the plastic backing sheet (it may be washed and used again) from the picrate paper.
13. Immerse the picrate paper in 5.0 ml of water (measured accurately with a pipette) for about 30 min with occasional gentle shaking.
14. Take the blank picrate paper (see 7 above), remove its plastic sheet and immerse the yellow picrate paper in 5.0 ml of water for about 30 min with occasional gentle shaking.
15. Measure the absorbance at 510 nm of the picrate solution from 13 against the blank from 14.
16. The total cyanogen content in ppm is calculated by the equation[1]

$$\text{total cyanogen content (ppm)} = 396 \times \text{absorbance} \times 100 / z.$$

Weight of ground up bamboo shoot taken = z mg.
17. The cyanogen content obtained for the same sample of bamboo shoot from both measurements 11 and 16, should be in reasonable agreement. Also check that the control value from 8 agrees using both methods.

FOOTNOTES -PREPARATION OF BUFFER AND PICRATE PAPERS

[a] Young bamboo shoots contain large amounts of the cyanogenic glucoside (taxiphyllin) and, in our experience, have sufficient enzyme present to hydrolyse the taxiphyllin which breaks down rapidly to HCN.

[b] A solution of approximately 0.1 M phosphate buffer may be prepared by taking 8 mL

of concentrated phosphoric acid (88% H_3PO_4) and adding about 750 mL of water. A solution of 1 M sodium hydroxide is prepared by dissolving 10 g of sodium hydroxide pellets in water and making up to 250 mL. The sodium hydroxide solution is now added to the phosphoric acid solution with stirring until the pH measured using a pH meter increases up to 6.0. Alternatively the 0.1 M buffer may be prepared as follows: prepare two 0.1 molar solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate by dissolving the calculated amounts of solid NaH_2PO_4 and Na_2HPO_4 to make 0.1 M solutions. Add the acidic sodium dihydrogen phosphate solution to the disodium hydrogen phosphate solution until the pH decreases to 6.0.

c Moist picric acid (1.4 g of BDH Lab Reagent) is weighed out and dissolved in 100 ml of 2.5 % (w/v) sodium carbonate. A square (about10 cm x 10 cm) of Whatman 3MM filter paper is immersed in the yellow picrate solution for about 20 sec and hung up to dry in air. *Note. Wash off with water any yellow picric acid on hands. Wear gloves if available when handling picric acid solution and papers.* Any unevenly coloured sections of the paper are cut off. The paper is cut into 30 mm x 10 mm rectangular pieces. Each piece is glued using one small drop of PVA hobby glue to a plastic strip (10 mm x 50 mm), cut from overhead transparency plastic sheet, so that the upper end of the yellow paper is about 5 mm from one end of the strip. Picrate papers must not be exposed to bright sunlight and should not be left in laboratory light for long periods. STORE THEM IN THE DARK IN IN THE DEEP FREEZE OF THE REFRIGERATOR WHERE THEY ARE STABLE INDEFINITELY[1].At room temperature they gradually darken and should not be used for more than one month with the colour chart, but can still be used with the spectrophotometer

LINAMARIN CONTROL PAPERS TO CHECK METHODOLOGY

Every time that you run a set of experiments, it is important to run a linamarin control sample (step 8). The linamarin is a stable cyanide compound which is broken down at pH 6 by the enzyme linamarase to produce HCN. The enzyme linamarase is immobilised in small filter paper discs which also contain phosphate buffer at pH 6 and are marked with a black spot . In the kit there are pink filter paper discs containing linamarin which gives 50 ppm on the colour chart.

TROUBLE SHOOTING

If the cyanogen content is much less than that expected from using the pink linamarin control sample, then there is clearly some problem with the methodology. Possible causes could be:

(1) Picrate paper is old (has been stored at room temperature for more than 1 month) or has been exposed to bright light or long exposure to laboratory light.[2]

(2) Loss of linamarase activity in the round Whatman 3MM discs included in the kit.

(3) Breakdown of linamarin in the pink control paper[2].

(4) Use of a non-air tight container (e.g. screw cap is cracked), which would allow HCN gas to escape.

COMPONENTS OF KIT F

The kit has the following components:

1. Protocol F, which gives a detailed stepwise method for cyanide analysis in bamboo shoots.
2. Bottles, clear plastic, flat-bottomed, with screw lids (25 mm diam., 50 mm high).
3. Small, graduated 1 ml, plastic pipette.
4. Picric acid papers glued to strips of clear plastic with PVA hobby glue. STORE IN THE DEEP FREEZE OF THE REFRIGERATOR. STABLE FOR ONE MONTH ONLY AT ROOM TEMPERATURE. Picric acid is not supplied because it cannot be sent by air.
5. Colour chart containing 10 entries from yellow to brown.
6. Pink filter papers containing linamarin for controls, equivalent to 50 ppm.
7. Filter paper discs, 21 mm, which contain buffer at pH 6 and linamarase. These papers are identified by a small black spot.

Materials not supplied Small balance for weighing out sample, pestle and mortar, 0.1 M phosphate buffer at pH 6.

References

1 Bradbury, M G., Egan, S.V. and Bradbury, J H (1999) Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. J.Sci. Food Agric., 79, 593-601.

2Egan, S.V., Yeoh, H.H. and Bradbury, J.H. (1998) Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. J. Sci. Food Agric.76, 39-48

Correspondence to Dr J. Howard Bradbury, Division of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia. Tel +61-2-61250775, Fax +61-2-61255573, e-mail <Howard.Bradbury@anu.edu.au> ; 21/6/99, 2/3/00, 29/6/01.