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GENERAL METHOD AND THEORY

The cotton strip assay for cellulose decomposition studies in soil: history of the assay and development

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1 *Summary*
The history and development of the cotton strip assay as a field method for ecological use in the International Biological Programme (IBP) and other studies are discussed. The further standardization and progressive refinement of the assay have reduced many of the original uncertainties regarding the data. Use of the cotton strip assay in ecological studies is described.

2 *History of soil burial methods*
The textile industry has long been concerned with the prevention of cloth decay, especially by fungal attack. To test the effectiveness of fungicide treatments, a routine test has been devised in which strips of textile are buried in tanks of soil (Wade 1947; Barr 1988; etc). The loss in tensile strength of the cotton fibres, with time, is used as a quantitative assessment of the rottenness of the fabric and, thus, the effectiveness of the fungicide. The value of the test and its widespread use stimulated the specification of the British Standard 2576 (Anon 1986) for general commercial application of the test.

As the soil burial test became more widely used, the variability inherent in the use of different types of cloth and soil became clear (Schmidt & Ruschmeyer 1958). One way of reducing variation inherent in the test was to standardize the cloth type used. Restriction of cloth type to one general group, for instance unbleached calico, could, however, only be a partial solution, as different fibre mixtures, thread densities and weaving treatments in different mills could produce wide variations in tensile strength (Sagar 1988a). A specialized cloth was, therefore, required and in the 1960s Toegepast Natuurwetenschappelijk Onderzoek (TNO) in the Netherlands produced such a material.

The breakdown of cotton fibres is of interest to a range of textile scientists, and early work is summarized in Thaysen and Bunker (1927) and Siu (1951). Research has been carried out on the soil burial method by the textile industry, to develop an understanding of the test (Barr 1988) and the optimum conditions for microbial attack. Much of this research used cotton, and was carried out at the Shirley Institute, Manchester. Detailed studies were made of the method of attack, both physical (Simpson & Marsh 1960; Kassenbeck 1970) and chemical aspects (Blum & Stahl 1952; Selby 1961; Halliwell 1965; Sternberg *et al.* 1977) and the cellulolytic enzymes involved (Sagar 1988b). A range of information was thus available by the early 1960s about a test which measured the effects of biological attack on a partially processed natural substrate which was generally over 96% pure cellulose.

The decomposition of cotton in soil appears largely to be due to microbial attack (Latter *et al.* 1988), a conclusion to be confirmed for normal soil temperatures by Richard (1945) during storage of cotton at pH 2.

3 *Development of the cotton strip assay for soil studies*
Cellulose comprises the bulk of plant material and is of great significance in the decomposition of soil organic matter by micro-organisms and microfauna, and thus in nutrient cycling, in natural and agricultural ecosystems. Although cellophane film, filter paper and lens tissue have been successfully used in cellulose decomposition experiments, the need for a more robust, flexible, standard substrate for ecological studies in soil was recognized many years ago. As early as 1945, Richard (1945) used cellulose cords of viscose rayon (regenerated cellulose fibre) in alpine areas of Switzerland, whilst in 1948, in Poland, Kuzniar (1948, 1988) used strips of linen (a partially lignified fibre), as do other east European workers still (Strzelczyk *et al.* 1978; Sadanov 1982). Cotton tape was also used in Australian soil studies (Rovira 1953) and for assessing cellulase activity of isolates of the fungi *Pythium* (Taylor & Marsh 1963) and *Chaetomium* (Farrow 1951).

Following on from Kuzniar's experiment and the soil burial method, strips of cotton (unbleached calico) with over 95% cellulose were inserted vertically into soil profiles (Plate 6) for cellulose decomposition studies on Pennine moorland sites (Latter *et al.* 1967). Visual observations showed marked differences in rotting of the cloth between sites, and the assay was quantified by using tensile strength as the measure of cloth decomposition. The purpose of the soil burial test was, in essence, reversed to develop a field ecological test (Table 1 & Plate 9).

The development of the International Biological Programme (IBP) from 1965 onwards included investigations of the decomposer cycle in various ecosystems. In the Tundra Biome, it was decided

Table 1. A comparison of the procedures and purpose of the soil burial test as used in the textile industry and the cotton strip assay used in field ecology

Soil burial textile test	Cotton strip assay as soil test
Procedures:	Procedures:
Standard soil	Variable soils
Standard or selected textiles	Standard textile-cotton
Variable treatment of textile	Standard treatment of textile
Frayed before burial	Frayed after burial
Controlled environment, high incubation temperature and moisture	Varying environment in field, or controlled for experiments
Soil is used to show:	Cotton is used to show:
Rotting of textile with varying treatments, eg fungicides, or textile manufacturing variations	Ability of soil to rot cotton with varying soils or site treatments
Suitability of textile for use under particular environmental conditions	Suitability of soil environment for biological activity

to concentrate on the study of energy flow through various trophic levels (Dahl & Gore 1968). With sites spread over the polar and alpine regions of the world, an assay was needed to provide standardized inter-site comparisons of rates of cellulose decomposition in soil. A paper cellulose board (Rosswall 1974) was used along with cotton textile strips (Heal *et al.* 1974), and the latter became the most widely used method providing, for the first time, comparable data on cellulose decay from the arctic, antarctic and alpine sites (Heal *et al.* 1974). It, thus, became possible to test for the effectiveness of a particular subset (the cellulolytic species) of the cosmopolitan soil flora on a global scale.

To provide some degree of standardization, a single source of cotton, an unbleached commercial calico (Plate 7), continued to be supplied to IBP field scientists from stocks at the Institute of Terrestrial Ecology's Merlewood Research Station. Techniques for field use were later refined and standardized (Latter & Howson 1977). One necessary step was the provision of adequate supplies of a closely specified test cloth, as the TNO fabric was no longer available. The Shirley Institute produced the 'Shirley Soil Burial Test Fabric' (Plate 7) in 1976 (Walton & Allsopp 1977; Sagar 1988a). The cloth was widely advertized and was soon in use for commercial testing and for ecological studies.

The assay has now been used, in particular, for polar studies (eg Davis 1986; Wynn-Williams 1988) and for land management studies (eg Brown 1988; Brown & Howson 1988), and it has become apparent that certain aspects, including some procedures taken over from the soil burial test, need further assessment in the context of the ecological test with its high field variability. Some technical aspects, including autoclaving to sterilize cloth prior to burial, washing to clean retrieved cloth for storage until tensile tested, the best conditions for storage of strips, the effect of width, the need for fraying of substrips for tensile testing, and the inter-comparability of different tensile testing machines, are reported by Latter *et al.* (1988) and Walton (1985). The method for the assay, as we would currently recommend it, is given in Appendix I.

4 *Ecological use of the assay*

The impetus for more widespread use of the cotton strip assay in soil was undoubtedly provided by the successful world-wide experiments in the IBP Tundra Biome. The development of the Shirley Soil Burial Test Fabric and its incorporation into the British Standard 6085 (Anon 1981) commercial test provided, for the first time, a single source of standard textile for all users. A wide variety of uses has ensued.

Commercial users continue to carry out fungicide assessment tests in laboratory soil tanks (Anon 1981). The Shirley Soil Burial Test Fabric has also been widely used ecologically for field comparisons of cellulose decomposition, having been supplied to research workers in UK, USA, New Zealand, Australia, Canada and the Antarctica. During IBP, the earlier type of cloth was also used in Finland, Ireland, Norway, USSR, Sweden and Alaska. Less orthodox usages have included assessments of cellulose decomposition in coal tips, compost heaps, and the rumen of sheep.

Many of the technical problems originally associated with the cotton strip assay have now been adequately documented. We do not suggest that we understand exactly what occurs during biological attack, as much still remains to be learnt about the biochemistry of cellulose decomposition (Sagar 1988b; Howard 1988). Nevertheless, the technique and data analysis now appear sufficiently standardized for users to have confidence in the inter-comparability of data.

The papers in this volume emphasize the practical nature of the assay. It is especially useful for early detection of biological change in soils or in pinpointing aspects of environmental change needing more detailed investigation, eg the marked depression of cellulose decomposition on an oak (*Quercus* spp.) site at 4–12 cm depth during summer (Brown 1988), the enhanced cellulose decomposition in sub-antarctic sites in the vicinity of rush (*Juncus* spp.) roots (Smith & Walton 1988), or the unexpectedly high cellulose decomposition at Rothera Point, Antarctica, possibly due to radiant energy input (Wynn-Williams 1988). Information is currently available for a number of sites world-wide, and one purpose of the Workshop was to bring these data together. The Workshop results are discussed by Ineson *et al.* (1988).

Some evidence is available suggesting that cellulose decomposition relates to soil properties similar to those affecting plant growth (Latter & Harrison 1988).

Cotton strip assay data show similar trends to weight loss of plant litter (French 1988), but a close relationship cannot be expected (Howard 1988) because the decomposition of cellulose in plant litter is influenced in various ways by other chemical constituents to which it is bound or closely allied. Partition of decomposition stages, thus, requires the use of individual standard substrates to simplify the system for examining the various influences of environmental parameters. Decomposition of any single substrate can never be fully representative of any other, and this is particularly true of cellulose. However, Shawky and Hickisch (1984) show virtually the same ratio of weight losses (1.1–1.2) when comparing 2 soils for decomposition of cotton, filter paper or wheat straw by *Trichoderma*.

The use of this type of assay in soil, which integrates decomposing activity over a period of time, partly overcomes a sampling problem when working at widely dispersed sites. Short-term changes in environmental conditions during the time taken to sample remote sites should have no significant effect on an integrated measurement, but could produce large differences in organism activity, if instantaneous measurements, such as respiration or enzyme assays (eg cellulase), are used. It is, however, obvious that any decomposition test involving the addition of a substrate to soil is no absolute measure of activity, but only an index of the potential to attack that added substrate. The same applies when natural litter falls or is incorporated into soil.

As well as using the assay to examine cellulose decomposition unconfounded by other factors, which was the original purpose, the assay is often considered useful as an index of general biological, or decomposer, activity, on the basis that cellulose is a major part of organic matter. Both uses are discussed in various papers in this volume. As different advantages and limitations of using the assay apply in the 2 approaches, the aims of any study and the purpose of using the assay should be examined critically from the start.

Many of the criticisms raised by Howard (1988) are valid, and the suggested need for 'a method for studying the decomposition of cellulose, and other constituents, in plant litter' cannot be disputed, but the current absence of a method suitable for field use means that analogue substances will remain, for the present time, a practical means of assessing certain biological changes. It would certainly be advantageous if similar simple field tests could be developed for other substrates, eg chitin sheet or complexes of lignin and cellulose. We have had no success with silk as a protein substrate as used by Richard (1945), as it appeared to be chemically or photochemically attacked, but chitin sheet and wood veneer (Latter 1984) appeared promising. In the meantime, we consider that useful ecological and management information can be obtained with this cotton strip assay – provided users are well aware, as with any method, of its limitations and that use of a simple method does not imply careless attention to detail.

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