SEED DECOMPOSITION BY SOIL MICROBES

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Introduction

Seeds function as a means of both reproduction and dispersal (Leek et al. 1989); most seeds contain food storage material (Van Etten et al, 1967, Lee et al. 1991) as well as embryo and surrounding protective coats (Fenner 1985). The storage material (starch, protein, fat) is a rich available energy and nutrient source and liable to be attacked by animals and microbes. Nevertheless many seeds survive the attention of these organisms and physical factors such as fire, freezing/thawing, wetting/drying to germinate. There is relatively little information on the quantities of seeds destroyed as a result of microbial activity, or how the microbes (bacteria and fungi) gain access to the seeds, guarded as they are by coats composed of relatively tough, impervious material and/or batteries of chemical defences.

Burrows (1995), like others, e.g. Pitty et al. (1989), has made casual observations about the prolific growth of fungi on seeds of some species during germination studies and noticed that seeds buried in soil germinated less successfully and more slowly than those on moist filter paper. This circumstantial evidence suggests that soil microbes may exploit seed resources although no detailed research in this direction has been reported. Post-harvest losses of stored seeds caused by fungi have been studied for many years (Mohamed-Yosseen et al. 1994) and since dispersed seeds in nature may be cached, or occur in high concentrations in fallen pods, capsules etc, under high humidity, then some microbial activity may be expected.

I report results from simple experiments begin in 1984 in which air dried seeds (0.1-0.5g weight), whole or 'damaged' (nicked to mimic physical damage) were subjected to soil microbial activity under moist (but not wet) conditions at 12°C for up to 70d in the dark. Seed decomposition was assessed in two ways: by weight loss and by the appearance of inorganic nitrogen. These methods are described elsewhere (Greenfield 1993) but a brief account is given below.

Methods

To determine weight loss a known weight of seeds was placed in preweighed glass tubes, moistened and inoculated with a drop of Cass forest soil suspension. Tubes (duplicates) were capped with thin plastic film to allow gas exchange but prevent water loss, and incubated. After incubation

tubes minus caps, were oven dried, cooled and reweighed to determine weight loss due to microbial activity. Some seeds e.g. *Betula*, *Lolium*, showed a tendency to germinate sporadically after 12days or so. Daily inspections allowed for germinating structures to be detached from seeds but all parts were returned to their respective tubes. Tests in which seeds were incubated in the presence of antibiotics to inhibit microbial growth showed that not more than 2% seed weight loss occurred over 30d. These losses may be attributed to seed metabolism and have been subtracted from all weight loss results in Table 1.

Nitrogen (N) release estimated the amount of seed organic N that was transformed into inorganic N by microbes. A known weight of seeds (0.1-0.3g) containing a known amount of organic N was mixed with humus-free coarse sand (5g) and placed in glass flasks. Sufficient water containing a drop of forest soil inoculum (1g Cass beech forest soil + 9 ml water) was added so that the mixtures appeared moist. Flasks were capped and incubated as before. Inorganic N present at the end of incubation was extracted with potassium chloride solution and determined by a distillation procedure (Greenfield 1993). No inorganic N was detected in seeds at the start of the experiment.

Analyses for organic N, ash (mineral matter) and fats were performed by standard methods (Allen et al. 1974).

Results

Results in Table 1 show that the N, ash and fat content of the seeds used fell within the ranges reported elsewhere (Van Etten et al. 1967). All 'damaged' seeds were susceptible to decomposition as judged by weight loss and N release (with the exception of *Betula*, *Cordyline* and *Pinus* for N release). Longer periods of decomposition may cause more extensive weight loss and N release as was beginning to occur for *Betula* after 70d.

In the case of whole, undamaged seeds only *Sophora* showed zero weight and N loss. After 30d these seeds were recovered from tubes and flasks, nicked and replaced whereupon they swelled and attempted to germinate. Whole seeds of *Betula*, *Malus*, *Nothofagus* and *Pinus*, whilst showing little initial decomposition (30d) did undergo more extensive decay with longer incubation periods (70d).

Interestingly, some notorious weed species (whole or 'damaged') e.g. *Carduus*, *Ulex*, were extensively decomposed in my experiments.

Discussion

It is recognised that the conditions of constant moisture and temperature used here are not like those in nature, however these experiments provide hints as to the probable behaviour of seeds in soil seed banks. Soil was not used in the weight loss work due to the difficulty of separating microbial

Table 1 Chemical composition of seeds (% dry weight) and % decomposition (wt organic N basis) of seeds after 30 and 70 days incubation

Species	% organic N	% ash	% fat	30d % wt loss	30d % N release	70d % wt loss	70d % N release
Betula pendula	0.94	2	2	8 (5)	0 (0)	14 (9)	ND
Carduus nutans	2.45	4	26	31 (15)	46 (21)	(26)	(44)
Cirsium arvense	2.26	3	19	33 (16)	46 (17)	(29)	(35)
Cordyline australis	1.47	5	21	30 (12)	0 (0)	ND	ND
Dactylis glomerata	2.58	3	2	40 (22)	38 (1)	(46)	(12)
Digitaria sanguinis	1.99	6	1	52 (30)	22 (17)	(44)	(27)
Lolium perenne	2.30	4	1	62 (34)	23 (2)	(60)	(5)
Malus pumila	5.25	4	22	49 (8)	65 (2)	(32)	(35)
Nothofagus fusca	1.17	3	12	(5)	(0)	ND	ND
N. solandri	1.91	4	21	(9)	(4)	ND	ND
Olearia paniculata	1.89	4	ND	(11)	(18)	ND	ND
Pinus radiata	3.48	6	ND	32 (8)	2 (0)	(12)	(1)
Sophora microphylla	2.71	3	11	51 (0)	9 (0)	ND	ND
Ulex europaeus	5.20	3	8	43 (35)	52 (43)	ND	ND

ND = not determined; ( ) figures in parentheses refer to whole 'undamaged' seeds

metabolism of humus from that of seeds. The use of sand in the N release experiments does approximate a sandy soil but without leaching influences which could remove toxic seed chemicals. However this latter may not have been important for the following reason. It is often argued that many seeds contain chemical compounds (known as allelochemicals) to fend off microbial attack (Mohamed-Yosseen et al. 1994). In the present study aqueous extracts prepared from crushed seeds (1g in 10 mls water) of each species when inoculated with a drop of soil suspension all supported

extensive microbial growth when incubated at 20°C for 5d. This suggests that some soils contain microbes which can utilize or grow in the presence of seed allelochemicals and that other factors are responsible for seed persistence in soil.

Seed persistence may, amongst other things, be a property of the physical structure of the seed coat. *Sophora* seeds, like those of *Discaria* and *Calystegia*, are thick and hard walled. In the present experiments *Sophora* seeds did not imbibe water or become attacked by soil microbes until they were physically damaged. In other tests I have noticed that one or two normal *Sophora* seeds in a batch of 20 collected from the same tree swelled after four months but did not germinate. It was found that squeezing the swollen seed forced out a brown foul-smelling bacterial ooze. This would suggest that in any *Sophora* seed set there are individuals with initially impervious seed coats that are, in time, inexorably subjected to microbial enzymes until the seed wall or micropyle is breached, allowing microbial entry to the rich supply of nutrients.

Gogue and Emino (1979) published scanning electron micrographs of freshly collected *Albizzia julibrissin* seeds which possessed fine cracks wide enough (up to 2 μm) to allow microbial access. They further showed that growth of soil microbes on the seed coat resulted in erosion and further cracking of the seed coat. They found that such scarification increased the rate of seed germination but it can also be adduced from their article that in the longer term a great many germinating seeds were attacked by soil microbes. Lonsdale (1993) is of the view that soil microbes may cause losses of germinating but not dormant mimosa seeds. Mohamed-Yosseen et al. (1994) mention that even seeds with hard seed coats may have natural openings such as the micropyle and cracks which may allow the entry of microbes into the seed with a consequent reduction in germination.

It would appear that many seeds buried in the soil are not as resistant to microbial decomposer activity as has been assumed. My results indicate that seeds scarified by whatever mechanism are most susceptible to microbial invasion and few germinate. Evidently many normal seed populations contain individuals which initially are literally watertight and do not respond to dormancy breaking triggers and/or show great resistance to the battery of enzymes produced by the diverse and ubiquitous soil organisms. However in the long term some of these succumb to these soil organisms. Although this is clearly detrimental for the plant species concerned of course the soil organisms benefit. In the long term this could be beneficial to plants, through recyling of nutrients which they can use.

The fact that some, but not all, seeds of a particular species survive for very much longer periods in the soil may be explained by the possession of perfectly impermeable seed coats, or the absence of the appropriate soil organisms. It is difficult to envisage in the very long term, though, how such

seeds will survive intact and germinate successfully. Perhaps the existence of long-term soil seed banks can be viewed as a function of the biology of the soil into which they enter. Recruitment, loss or survival to become a successful seedling are, from a soil biota viewpoint, continuous processes and those seeds present in soil (assuming they are viable) at any one time represent a balance between these three processes. The fact that we are not knee deep in seeds suggests that seed germination and decomposition are successfully occurring in nature in a balanced fashion. It is perhaps extraordinary that so many seeds successfully germinate in soil and overcome the attention and activities of that most powerful, diverse and dominant group of soil organisms, the microbes - a most vital link between the above and below ground ecosystems.

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