The microbial decomposition of seeds

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Abstract

Loss of seeds in soil seed banks has largely been examined from plant ecological perspectives with little attention being devoted to the possibility that soil microbes may cause large losses. The decomposition of a variety of seed species, (three native (*Corynocarpus laevigatus*, *Nothofagus fusca* and *Sophora microphylla*) and six exotic (*Arachis hypogea*, *Carduus nutans*, *Dactylis glomerata*, *Lolium perenne*, *Trifolium pratense* and *Ulex europea*) whole or ground, was studied under fluctuating temperatures but constant moisture conditions in the presence of soil microorganisms for seventy days. Seeds lost weight and released mineral nitrogen under these conditions. Seeds appearing to be intact after incubation failed to germinate, unlike their control counterparts. All aqueous seed extracts supported prolific growth of soil microbes. It is suggested that losses of seeds due to soil microbial action may be substantial and comparable to those caused by animal predation and environmental extremes. Additionally, with some plants producing large numbers of seed per annum the release of mineral nutrients during seed decomposition may be of considerable importance in the transfer and recycling of nutrients between below- and above- ground ecosystem components.

Additional key words: *nitrogen release, seed decomposition, soil microbes*

Introduction

Much work exists concerning the functional ecology of seeds (Leck *et al.*, 1989) and although the factors affecting seed survival in soil remain obscure, it would appear that most attention has been in the direction of seed persistence rather than seed loss. Most seeds are rich in nutrient and energy sources (Van Etten *et al.*, 1967; Lee *et al.*, 1991) and much of the work on seed loss has considered losses caused by germination, age, predators and decomposers (Fenner,1992; Lonsdale, 1993). On close inspection of the literature, many scientists working on seeds do not make a clear distinction between the terms loss and decomposition. This has probably occurred because most work has involved animal predation and the assumed enzymatic digestion, to varying degrees, of the ingested seed material. Here, loss and decomposition are synonymous but loss used too loosely in the sense of disappearance does not mean decomposition.

Decomposition or decay is commonly used by seed scientists and plant ecologists who, after accounting for the losses of seeds caused by animal digestion and physical factors, attempt to invoke the likely role of soil microbes in causing the remaining seed losses in soil seed banks. Indeed, many colleagues use the specialised term pathogen in communicating the possibility that these microbes cause the depletion of seeds in soil seed banks.

Even if one could ascertain the viability of dormant seeds in soil, the use of the term soil microbes in a general sense is preferred because microbial pathogens are somewhat specialised in their host ranges and are not very successful, spatially at least, in the intensively competitive soil environment which, apart from its indigenous soil animals, contains a vast array of microbial species (Baker, 1972). Current belief from RNA analyses is that less than 5 % of total soil microbes have been successfully isolated into pure culture and identified (Borneman and Triplett, 1997).

There is an extensive literature on seed pathology (Baker, 1972) including the transmission of economically important seed borne microbial diseases. Surprisingly, little detailed work has been reported in the plant or microbiology literature on the microbial decomposition of seeds in soil. Pitty *et al.* (1987), using an *in vitro* petri dish method, showed that soil fungi could prevent foxtail seed germination and this was linked to the reduction in the number of weed seeds in Iowa topsoils. Lonsdale (1993) suggested that soil microbes caused minimal losses of *Mimosa* seeds in Australian soils.

Apart from the paucity of work on soil seed loss caused by soil microbes there is no work on a related aspect of this topic, i.e., the mineralization or release of

nutrients from seeds that are being decomposed. It is only recently that plant and soil scientists have begun to appreciate that reproductive "litter", e.g., pollen, petals, stamens, seeds etc., which invariably enter soil systems at fairly regular intervals constitutes a considerable proportion of the weight and nutrient content of the total annual litter return (Pregitzer and Burton, 1991). Regardless of so called mast years, the amounts of seed and seed nitrogen (N) returned to soil can be considerable, e.g., Kawada and Maruyama (1986) found 861 kg *Fagus crenata* seeds containing 52 kg N /ha/annum.

It is not known to what extent soil microbes can access these nutrients. If a large proportion of the seeds reaching the soil do not germinate then it is important from a soil ecology viewpoint to learn the fate of the nutrients in these seeds. In the case of N, the element most frequently limiting growth, the large amounts of this element tied up (unavailable) in ungerminated seeds has profound implications on the processes in soils and plants involved in supplying such large amounts of this element for plant use. Even the most fertile soils could not supply in such a short time the large amounts of N needed by deciduous trees for leaf, flower and seed production. This being so, it may suggest that apart from internal translocation (Millard and Proe, 1992) the action of soil microbes in addition to animal predation (Crawley, 1992) may be a mechanism not only for decomposing seeds and releasing nutrients but also an important factor causing seed loss in soil seed banks in addition to those caused by physical factors.

The experiments reported here attempted to ascertain the microbial decomposition of seeds from a variety of plant species using weight loss and N release as indicators of decomposition.

Materials and Methods

Mature seeds were used either whole or after being ground to a powder. Samples of air dried seed equivalent to 0.1-0.5g oven dry weight were placed in pre-weighed 50 mL glass centrifuge tubes, moistened but not saturated with distilled water and inoculated with a drop of forest or agricultural soil suspension (1g soil:10 mL water). Preliminary work indicated no difference in weight losses from seeds using inocula from either of these soils. Tubes (triplicates) were capped with thin plastic film to allow gas exchange but prevent water loss and incubated for up to 70 d under a daily regime of 12h, 12oC; 6h, 4oC in the light and 6h, 4oC in the dark. During incubation in the light, the temperature was allowed to reach 20oC, on ten occasions, for no longer than 2h each time; during the dark incubation the temperature was allowed to reach minus 2oC for no longer than 2h on ten occasions. The incubation regime attempted to mimic the early spring conditions in New Zealand.

During daily inspections any germinating structures were detached, crushed and returned to their respective tubes since the purpose of the experiment was to examine whether soil microbes could attack seeds and prevent seed germination and removal of these germinating structures would have inflated weight losses. Following incubation, tubes were oven dried, cooled and reweighed to determine weight loss due to microbial activity In other experiments, tubes were not dried but whole seeds removed, 'nicked' to open the seed coat, and placed on moist filter paper in petri dishes for up to 10d at 15oC to assess germinability.

Controls consisted of seeds incubated in the presence of streptomycin, penicillin and actidione (each at 1 % sample weight) antibiotics to prevent microbial growth. Over 30 d not more than a 2 % loss in seed fresh weight occurred and these were attributed to seed metabolism and have been subtracted from the weight losses reported in Table 1.

Mobilization of N was estimated from the amount of seed organic N that was transformed to inorganic N by microbial activity. Using the methods described in Greenfield (1993), a known weight of seeds containing a known amount of organic N, usually 5-10 mg was mixed with 5g coarse sand or agricultural soil in a 100 ml flask. Water contents of these mixtures were adjusted to 20 % by weight so that they looked moist but not wet. Flasks (triplicates) were capped and incubated as described in weight loss experiments. Inorganic N present at the end of incubation was extracted with 2M KCl and determined by a distillation procedure (Greenfield, 1993). Controls consisted of determining inorganic N in sand/soil mixtures minus seeds incubated for similar times and subtracting these values from those from experimental flasks. No mineral N was found in seeds at the start of the experiment.

Water soluble substances were prepared by extracting 1g of ground seed or seed coat material with 20 mL water for 48h at 12oC. After filtering, through a GFA filter, filtrates were divided into two equal portions, to each of which was added one drop of forest soil suspension. One mL of chloroform was also added to one portion to prevent microbial growth. Flasks were capped, incubated on an orbital shaker for 5d at 15oC and then assessed for microbial growth (turbidity, microscopic examination of flocs).

Results

The N content of seeds (Table 1) fell within the ranges reported by Van Etten *et al.* (1967). All seeds, intact or ground, were susceptible to decomposition as judged by weight loss and N release. Although whole seeds of *Nothofagus* and *Sophora* showed little initial decomposition after 30d, there was extensive decomposition after 70d. In general, little visible fungal growth was observed but the foul odours noticed when caps were occasionally removed suggested a bacterially dominated decomposition. Most of the whole seeds when removed at the end of an incubation period and squeezed, emitted a brown ooze which, on microscopic examination, was found to be dominated by bacteria. Scanning electron microscope examination of these seeds showed extensive microbial colonization of the seed coats (unpublished observations). With the exception of a few *Sophora* seeds, no seeds from any other species taken from experimental flasks after 30 or 70 days incubation germinated when the seed coat was 'nicked' and the seeds placed on moist filter paper at 15°C for ten days. At the start of the experiment, 'nicked' seeds showed >85 % germination. It is noteworthy that some weed species, e.g., *Carduus, Ulex* were extensively decomposed in this study. All water soluble extracts supported microbial growth after five days (results not shown), resulting in an increase in pH by at least 2 pH units, except in the case of *Dactylis* and *Nothofagus* where the pH decreased by 1 unit.

Table 1. Mean percentage weight loss and nitrogen release from seeds after 30 and 70 days incubation.

		30 days				70 days			
	% seed N	% wt. loss		% N release		% wt. loss		% N release	
Carduus nutans	2.45	31±5	(15)±2	46±3	(21)±2	ND	(26)±2	ND	(44)±4
Corynocarpus laevigatus	2.13	42±2	ND	30±3	ND	ND	ND	ND	ND
Dactylis glomerata	2.58	40±3	(22)±1	38±2	(1)	ND	(46)±3	ND	(12)±1
Lolium perenne	2.30	62±6	(34)±2	23±2	(2)±1	ND	(60)±3	ND	(5)±1
Nothofagus fusca	1.17	ND	(5)±1	ND	(0)	ND	(35)±1	ND	(8)±2
Sophora microphylla	2.71	51±7	(0)	3±1	(0)	63±4	(28)±2	36±3	(24)±3
Trifolium pratense	5.50	52±4	(50)±4	65±5	(57)±4	ND	ND	ND	ND
Ulex europea	5.20	43±4	(35)±3	52±4	(43)±3	ND	ND	ND	ND

*Standard error (n=3). Values in parentheses refer to whole seeds. ND = not determined.

Discussion

Although there is much information on soil seed banks (Leck *et al.*, 1989), little deals directly with seed decomposition by soil microbes and none has been reported concerning likely nutritional effects arising from decaying seed on the soil-plant ecosystem. It has been suggested (van Leeuwen, 1981) that soil microbes, largely fungal, stimulate *Cirsium vulgare* seeds to germinate where many of these seedlings may be subsequently killed by physical conditions and soil microbes.

Gogue and Emino (1979) reported that heat and several soil fungi, separately, scarified *Albizzia* seed coats causing high germination rates. Perhaps a proportion of these germinated seeds would succumb to the conditions described by van Leewen (1981). Lonsdale (1993) suggested that soil microbes might be important in causing losses of germinating mimosa seed but provided only circumstantial evidence to support this idea. Pitty *et al.* (1987) observed that soil fungi could prevent or reduce germination of *Setaria* seeds but did not indicate whether the seeds were killed. It is known that many plant derived chemicals may inhibit or stimulate germination (Karssen and Hilhorst, 1992). Kremer (1986) found that *Abutilon theophrasti* seeds contained a water soluble substance that inhibited the growth of 117 species of soil bacteria and 39 species of soil fungi. Warr *et al.* (1992) observed that only one out of four soil fungi tested were inhibited by water soluble seed coat extracts. Such inhibition of single microbial species by single plant extracts is common in laboratory studies but these observations rarely indicate whether the soil microbe is killed and do not take into account the fact that pure cultures of soil microbes do not exist in nature and that soil teems with a very diverse and large microbial population.

In my study, quite concentrated aqueous seed extracts (10 mg/ml) supported microbial growth. This suggests

that there are soil microbial community members with the necessary enzymatic ability to utilize these extracts. Paradoxically, microbial studies should not necessarily invoke the usual pure culture approach (see above) and in this study the use of the entire soil microbial population was regarded as a more natural approach. Initially, seeds in soil would be subjected to rain water leaching and would be expected to release all types of substances, inhibitory to some, but not all soil microbes.

Seeds have been shown to be decomposed by soil microbes resulting in complete loss of germinability together with the release of large amounts of mineral N (Table 1). Such N would be expected to be available to soil biota and plant roots. Recently Zackrisson *et al.* (1999) added killed germinated Norway spruce seeds to soils in N. Sweden and suggested that Scots pine seedlings and *Pleurozium schreberi* moss acquired most of the N released from the dead seeds. These workers used germinated seeds that would be expected to be vulnerable to soil microbes *sensu* (van Leeuwen, 1981; Lonsdale, 1993). This study shows that soil microbes can actually decompose intact seeds either by attacking the seed coat or by gaining entry via a natural opening, e.g., micropyle. This is followed by the utilization of the seed contents which often manifests itself as brown bacterial ooze.

The results in this paper may seem to be at variance with the question: "Why do soil seed banks occur?". I have used microcosms that were continuously moist; such constancy is unusual in nature as was the lack of leaching and wet/dry cyles in the microcosms. In nature, soils covered with humus can remain moist for quite long periods of time and it seems reasonable to suggest that the present work shows the likelihood that a proportion of seed reaching the soil may be lost by direct microbial decomposition. Further work is required along the lines suggested by Simpson *et al.* (1989) and Thompson (1992) and before the proportion of seeds in soil banks which are lost by microbial decomposition can be ascertained. When this is known there may be important implications regarding the seasonal acquisition and allocation of nutrient resources within elements of an ecosystem, e.g., plants at varying growth phases and the origin and transfer of nutrients within the soil ecosystem. The fact that we are not knee deep in seeds suggests that there is a natural balance between seed germination and decomposition and given the amazing diversity of soil microbes it is surprising that so many seeds germinate and escape the attention of these organisms!

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