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CORRELATION OF SEED UNSATURATED FATTY ACIDS WITH SEED PERSISTENCE IN THE SOIL

M. Mumtaz Khan, *K. Thompson and Muhammad Usman
Department of Horticulture, University of Agriculture, Faisalabad, Pakistan
*Climate Change Impact Lab. Buxton S10 2TN, University of Sheffield U.K.
*Correspondence: mumtaz59pk@hotmail.com

The analyses described here were done to determine chemical composition of seed unsaturated fatty acids in a wide range of species and persistence in the soil. Results showed no relationship between seed lipids and persistence in the soil. Therefore, there was no evidence to support the hypothesis that the oily seeds may be short lived and the relationship between oil content and seed longevity hypothesis is complex.

Keywords: seed chemistry, seed persistence

INTRODUCTION

The longevity of seeds in the soil is of considerable importance to both agronomists and ecologists. Many buried weed seeds can remain viable for years, posing serious problems of control for the farmer. To the ecologist, on the other hand, the persistence of seed banks in the soil is a major component of plant succession and plays a substantial role in the development of plant communities (Grime, 1979; Cook, 1980; Roberts, 1981).

Chemically, seed storage lipids are mainly triacylglycerols, most of which are oils, i.e., they are liquid above about 20°C. Some seeds may also contain appreciable quantities of phospholipids, glycolipids, and sterols generally associated with membranes. The predominant fatty acids in some seeds are unsaturated ones, and of these oleic (18:1) and linoleic (18:2) account for more than 60% by weight of all oils in some oil seed crops. These seed oils are of nutritional value because of their higher unsaturated fatty acid content. However, the seeds with oils rich in unsaturated fatty acids might pay the price of declining seed viability (Kaloyereas, 1958). In the presence of oxygen, fatty acid hydrocarbon chains may spontaneously oxidise producing highly reactive free radical intermediates, a class of compound called hydroperoxides, and a wide variety of secondary products from hydroperoxide decomposition. The rate of this reaction is greatly accelerated by lipoxygenases which are found in many seeds. Once a free radical reaction is produced, usually by oxygen attack, a chain reaction is initiated which creates additional reaction cycles and free radicals. However the lipids in seed tissue may be protected by natural antioxidants which, by scavenging free radicals, can break the chain reaction cycle (Tappel, 1980).

Oxidative damage to membranes caused by free radicals has been suggested as one causative factor of poor seed longevity (Parish & Leopold, 1978; Pearce & Abdel Samad, 1980; Bewly, 1986). In the presence of oxygen, ageing of seeds has been associated with peroxidation of polyunsaturated fatty acids (Stewart & Bewly, 1980; Wilson & McDonald, 1986; Hendry, 1993). As free radical induced damage is believed to be greater in oily seeds, there would be a strong selection pressure for reduction of lipid content in persistent seeds (Ponquett *et el.,* 1992). However, the higher energetic costs of lipid production compared to carbohydrate synthesis could produce a selection pressure against energy storage in seeds in the form of fats.

The mechanisms that determine long-term seed persistence in the soil are largely unknown. Some physical attributes, such as seed size, shape and chemical composition (e.g. ortho-dihydroxyphenol content) have already been discussed in relation to persistence (Thompson, 1987; Thompson *et al., 1993;* Hendry *et al.,* 1994). The relationship between seed persistence and seed lipids (unsaturated fatty acids) was analysed in the present study. Specifically, we tested the hypothesis that the greater danger of oxidation damage in oily seeds leads to a negative relationship between seed persistence and fatty acid content.

MATERIALS AND METHODS

Plant Material

ISP and other species seeds

More recent material of 43 *ISP* (Integrated Screening Program) species and 9 other species were from the seed stocks of the Unit of Comparative Plant Ecology. Most of these seeds were collected from semi-natural sites within a 25 km radius around Sheffield, UK. The list of species is shown in Table 1.

Biochemical analyses

Fatty acid analysis

Unsaturated fatty acids were determined ?S described by Hendry and Thorpe (1993) where 50 mg of ground tissue was extracted with 1.5 ml of borate buffer pH

9.0, 3 ml of KOH was added to 1 ml of extract and incubated in sealed tubes for 6 hours at 80°C. Following centrifugation at 3000 x g for 3 minutes, the saponified extract was incubated with lipoxidase enzyme (60,000 U/ml; Sigma Chemicals) for 20 minutes at 25°C. Absorbance was recorded at 234 nm for both active and boiled enzyme and the treatment responses estimated against a lineolic acid (Sigma Chemicals) standard. All replications consist of 5 samples.

Data source

Seed *chemistry and persistence*

In order to determine whether the fatty acid composition of seed lipids could be correlated with seed survival, the unsaturated fatty acid content of a wide range of species was determined and compared with information on persistence of these species.

Data on seed persistence were taken from Thompson, Bakker and Bekker (1996). The mean seed persistence class (where 1 is transient <1 year), 2 is short-term persistent (>1 and <5 years) and 3 is long-term persistent (> 4 years) over all measurements was calculated and used as a measure of seed persistence.

RESULTS

In the series of experiments, marked differences were observed in the concentrations of unsaturated fatty acids (UFA) in seeds of 52 species. The maximum concentration recorded was 35.0% of dry seed weight in *Urtica dioica* and the minimum was 0.80 % of dry seed weight in *Galium aparine.* The percentage contents of UFA and seed persistence are summarised in table 1. No correlation was found between seed UFA and persistence in the soil (Fig. 1). There were marked differences in unsaturated fatty acid content in monocot and dicot species seeds; most monocots have less unsaturated fatty acids than dicots (Fig. 2).

DISCUSSION

This study provides no evidence to support the hypothesis that long-lived seeds would be less likely to store energy in the form of unsaturated fats. However, some interesting differences in unsaturated fatty acids contents between monocots and dicots emerge from Fig. 2. Results showed that dicot species seeds have generally more lipids (UFA) than monocots. This is only the most obvious example of the role of phylogeny in determining seed chemical composition. The

Table. List of 52 species tested for unsaturated fatty acid contents. Nomenclature follows Stace (1991).

Agrostis capillaris	*Dactylis glomerata*	*Lotus comiculatus*
Anisantha sterilis	*Deschampsia flexuosa*	*Lycopus europaeus*
Anthoxanthum odoratum	*Catapodium rigidum*	*Mentha arvensis*
Anthriscus sylvestris	*Digitalis purpurea*	*Origanum vulgare*
Arabidopsis thaliana	*Dryas octopetala*	*Poa annua*
Arrhenatherum elatius	*Epilobium hirsutum*	*Poa trivialis*
Bidens tripartita	*Eriophorum vaginatum*	*Plantago lanceolata*
Brachypodium pinnatum	*Eupatorium cannabinum*	*Pilosella officinarum*
Briza media	*Festuca ovina*	*Persicaria maculosa*
Bromopsis erecta	*Festuca rubra*	*Pulicaria dysenterica*
Campanula rotundifolia	*Galium aparine*	*Rorippa islandica*
Carex flacca	*Helianthus annus*	*Rumex acetosella*
Centaurea scabiosa	*Helictotrichon pratensis*	*Scirpus sylvestris*
Cerastium fontanum	*Helianthemum nummularium*	*Thymus polytrichus*
Chamerion angustifolium	*Holcus lanatus*	*Urtica dioica*
Chenopodium album	*Koeleria macrantha*	*Zea mays*
Chenopodium rubrum	*Leontodon hispidus*	
Conyza canadensis	*Lolium perenne*	

Compositae are generally high in fats, while *Gramineae* are generally low. The simple correlation in Fig. 1 does not take account of these phylogenetic constraints. However, an analysis conducted by Hodkinson (1996), using a large number of species and modern phylogenetically independent contrast (PIC) methods (Purvis & Rambaut 1995a) also failed to reveal any relationship between seed persistence and fatty acid content.

Given the higher metabolic costs associated with energy storage as lipids one might expect storage as starch (which may be more common in monocots than in dicots) to be selected, independently of any selection on the basis of longevity. Lipids, however, are a more efficient carbon storage form, with an energy yield approximately double that of carbohydrate and lipid storage may be favored in small seeds. The relationship between seed size and lipid content has not been investigated.

In study of 7 species, Ponquett *et al.* (1992) found no correlation between longevity in dry storage and total oil content, but there was a significant relationship between the ratio of linoleic acid to tocopherols (an antioxidant) and longevity. Extrapolation from this study is limited, firstly because of the small number of species considered and secondly because it included 6 species of legume and is therefore phylogenetically unbalanced. Furthermore, lonqevity in dry storage may be unrelated to persistence in the field.

It has been shown that dormant imbibed (buried) seeds can respire, and can carry out protein synthesis in the formation of various cellular organelles and membranes. There is little doubt, therefore, that such seeds can also undertake at least some of the cellular repair and maintenance activities which are part of the normal metabolism of all tissues. The fluid environment provided by water allows for the diffusion of substrates to active sites of enzymes and also serves as a protectant of macromolecular structure (Vertucci & Farrant 1995). Villiers (1974) demonstrated that fully-imbibed seeds can maintain tissues cytoplasmic and nuclear components in good order by repair mechanisms and turn-over. It is probable that repair and turn-over would be unlikely in dry stored seeds, but may be possible in fully hydrated, but non-germinating, seeds.

The relationship between fats and persistence have mostly been concerned with dry storage. The records for dry storage and prolonged longevity are held mostly by legumes with starchy seeds (Harrington, 1972). Seventeen different crop seeds were stored over a period of twelve years and in general, leguminous seeds were found to maintain longest viability (Sonavne, 1934). Oily seeds usually exhibit poor storage life, e.g. sesame, mustard and linseed stored up to 12 months showed marked decrease in germinability (Nandi *et al.,* 1982). Cotton and lettuce Calmer cultivar seeds showed significant decline in viability, when stored for 3 to 6 years in a warehouse (Towers & Harrison, 1949, Harrington, 1973). Reduction of seed lipid content is not the only solution to the problem of reducing oxidative damage - it is also possible to increase the levels of antioxidants. The above observations by Ponquett *et al. (1992)* suggested that reducing the ratio of lipids to antioxidants may be a key to increased seed tonqevity of lipid storing seeds. In various types of plant tissues, "protective solutes" e. g. sugars and polysaccharides have been shown to decrease membrane damage in conditions of environmental stress. In a study of *Inga* species, Pritchard *et al.* (1995) showed that sensitivity to desiccation stress of *Inga* embryos was associated with low internal levels of soluble sugars. Depending upon the evolutionary and ecological constraints upon a species, seed persistence may be facilitated by either changing the energy storage chemical or by increasing antioxidant production. The latter possibility will confound any relationship between seed longevity and seed lipid content.

Of course seed persistence in the soil is dependent on many factors. Seed size, shape, colour, dormancy mechanism, soil environment and resistance to pathogens also contribute to persistence (Thompson *et al.,* 1993; Khan *et al.,* 1996; Kremer, 1986; Hendry *et al.,* 1994). It would be of interest to investigate the proportions of polysaccharides and total lipids in seed reserves of the species examined here for a better understanding of seed persistence mechanisms in the soil.

Fig. [illegible] Seed [illegible] content vs seed persistence score

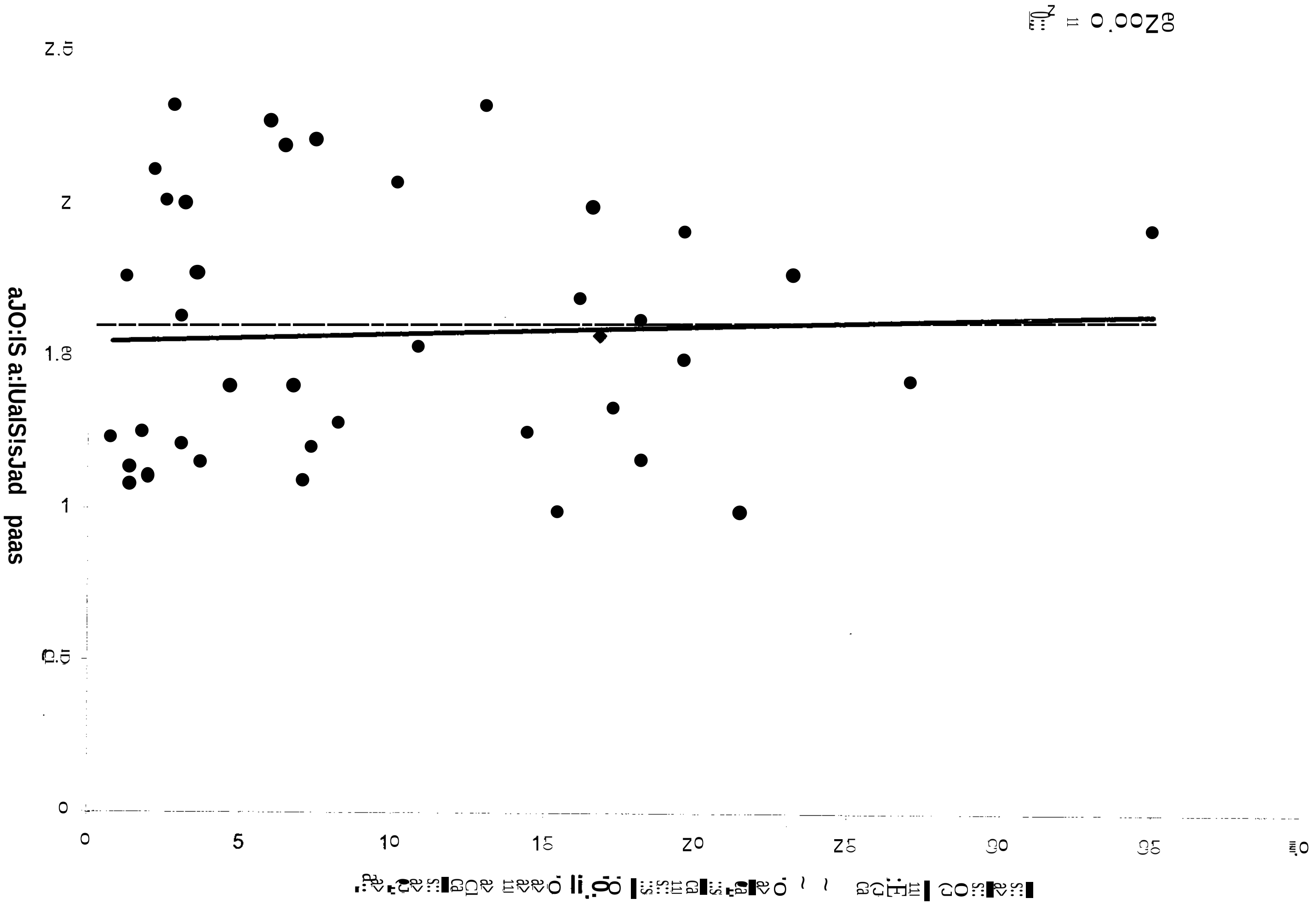

Fig. 2. Percentage Unsaturated Fatty Acids Content of Monocot and Dicot Species Seeds

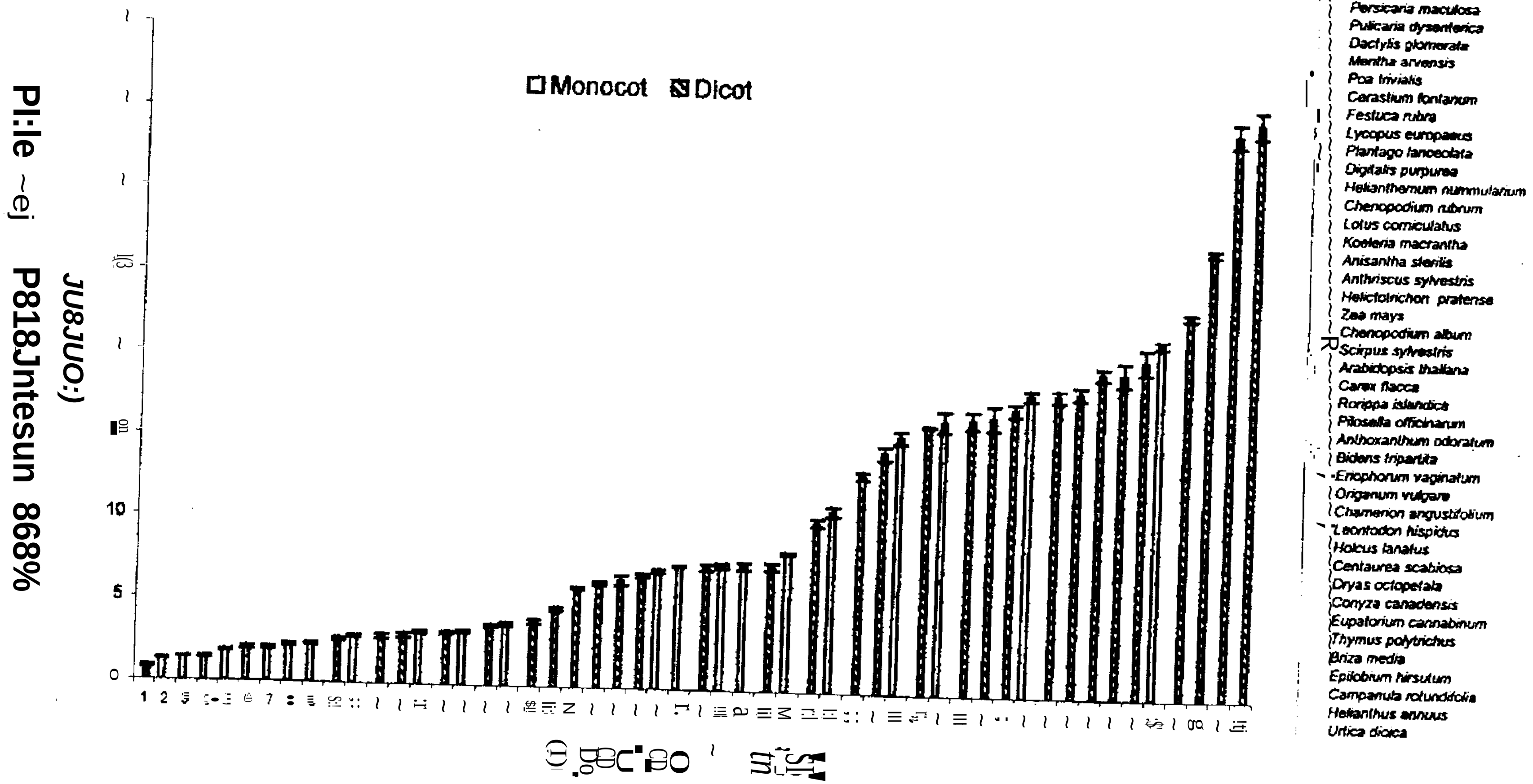

Table 1. Unaturated fatty acids) and seed persistence score of 52 species. Persistence scores are given only for species with at least 10 records.

Species	% oil (UFA) content	Persistence score
Agrostis capillaries	1.3 ± 0.02	1.77
Anisantha sterilis	7.4 ± 0.06	1.21
Anthoxanthum odoratum	15.5 ± 0.34	1.00
Anthriscus sylvestris	7.4±0.18	1.21
Arabidopsis thaliana	10.2 ± 0.23	2.08
Arrhenatherum elatius	1.4 ± 0.02	1.14
Bidens tripartite	16.2 ± 0.05	1.70
Brachypodium pinnatum	1.4 ± 0.06	105
Briza media	21.5 ± 0.22	1.00
Bromopsis erecta	2.3 ± 0.05	
Campanula rotundifolia	27.1 ±0.18	1.43
Carex flacca	10.9 ± 0.30	1.54
Catapodium rigidum	2.8 ± 0.07	
Centaurea scabiosa	18.2 ± 0.35	1.17
Cerastium fontanum	3.6 ± 0.09	1.78
Chamerion angustifolium	16.8 ± 0.74	1.57
Chenopodium album	7.5 ± 0.22	2.22
Chenopodium rubrum	6.5 ± 0.30	2.20
Conyza Canadensis	19.6 ± 0.36	1.92
Dactylis glomerata	3.1 ± 0.09	1.22
Deschampsia flexuosa	2.0 ± 0.08	1.11
Digitalis purpurea	6.0 ± 0.06	2.28
Dryas octopetala	18.4 ± 0.37	
Epilobium hirsutum	23.2±0.17	1.78
Eriophorum vaginatum	16.5 ± 0.64	
Eupatorium cannabinum	19.6 ± 0.72	1.50
Festuca ovina	2.0 ± 0.07	1.12
Festuca rubra	3.7 ± 0.09	1.16
Galium aparine	0.8 ± 0.04	1.24
Helianthus annuus	34.3 ± 0.71	
Helictotrichon pretense	7.5±0.16	
Helianthemum nummularium	6.3 ± 0.10	
Holcus lanatus	18.2 ± 0.30	1.63
Koeleria macrantha	7.1 ± 0.12	1.10
Leontodon hispidus	17.3±0.38	1.34
Lolium perenne	1.8 ± 0.05	1.26
Lotus comiculatus	6.8 ± 0.13	1.41
Lycopus europaeus	3.9±0.14	
Mentha arvensis	3.1 ± 0.09	1.64
Origanum vulgare	16.6 ± 0.53	2.00
Persicaria maculosa	2.8 ± 0.13	2.33
Pilosella officinarum	14.5+0.41	1.26
Plantago lanceolata	4.7±0.11	1.41
Poa annua	2.2 ± 0.06	2.12
Poa trivialis	3.2 ± 0.08	2.01
Pulicaria dysenterica	2.9 ± 0.15	
Rorippa islandica	13.1 ± 0.22	2.33
Rumex acetosella	2.6 ± 0.07	2.02
Scirpus sylvestris	8.3 ± 0.08	1.29
Thymus polytrichus	20.4 ± 0.77	
Urtica dioica	35.0 ± 0.79	1.93
lea mays	7.5 ± 0.23	

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