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Devitalisation of imported feed grain by fumigation

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Abstract

The intensive livestock industries in Australia periodically suffer shortfalls in cost effective feedstuffs during drought. The current protocol for the importation of feed grain does not apply beyond capital cities. The feasibility of using the fumigant ethanedinitrile (EDN) for import quarantine treatment of commodities for distribution beyond capital cities was tested. The fumigant was successful in devitalising barley, maize, sorghum, wheat, all surrogate pathogens and the majority of testable weed seeds. The characteristics of the gas on the four commodities and the disparity in tolerance between weeds and the commodities indicated that there would be large differences in the economics of treating the four commodities to devitalise all target organisms. It was concluded that maize would be the most profitable commodity in terms of feed value and fumigant consumption for development of large-scale application of EDN for quarantine import purposes. This would potentially deliver benefits in opening market access up country to imported feed grain during periods of limited supply. This usage would be subject to registration of EDN and successful development and approval of an import protocol based on EDN satisfying Australian quarantine requirements.

Executive summary

The intensive livestock industries in Australia periodically suffer shortfalls in cost effective feedstuffs during drought. The current protocol for the importation of feed grain does not apply beyond capital cities. This work aims to provide proof of concept for a devitalisation protocol based on a fumigant gas ethanedinitrile (EDN) that has the potential to reduce the residual quarantine risks for the importation of feed grain to a level acceptable to Biosecurity Australia.

The experimental work reported here was undertaken over eight months, during which time approximately 250,000 weed and commodity seeds were fumigated and assessed, efficacy of treatment against surrogate pathogens was determined, and detailed studies of the sorption and interaction of EDN with target commodities and materials was undertaken.

The results have demonstrated the "in principle" feasibility of devitalising maize, barley, wheat and sorghum imported from the UK and USA along with many, but not all, exotic seeds associated with these products. In addition, the efficacy of EDN was demonstrated against a selection of surrogate pathogens chosen to represent the pathogens deemed to be quarantine risks associated with the four commodities imported from the UK and USA. The commodities were relatively easy to devitalise, but the pathogens and the weed seeds were more difficult to kill. While assessing the efficacy of EDN, headspace gas loss was measured and found to differ markedly between commodities. This has implications on the final cost of treatment for each commodity; seeds, which are admixed with a commodity with higher headspace gas loss will be exposed to less gas than if they were mixed with a commodity with lower headspace gas losses. On this basis it was concluded that maize would be the most feasible commodity to treat with EDN, followed by wheat and then barley. Given the easy availability of the other commodities, and the variable feed value of sorghum, the management committee did not feel that work on sorghum beyond this project would be justified.

Another major thread of the project was concerned with the compatibility of EDN with materials commonly present in storage structures and other potential fumigation environments. Colour changes and permeability were identified as issues in some tarping material, but CanvaconTM was identified as a suitable material for use with EDN. EDN use was compatible with metals, but there was measurable sorption to concrete and brick, and very high sorption to freshly made concrete. While sorption to fresh concrete is an issue, sorption of EDN to aged concrete structures was significantly less, and this can be factored into fumigation protocols. Where basic compatibility parameters cannot be met, control measures such as barriers will be needed. Residues were ameliorated by aeration for all materials tested.

The potential industry benefit lies in opening market access to feed grain in periods of limited supply. This would have the potential to sustain grazing herd numbers, and provide economic relief to feedlot operations during times of drought. Further work on a larger scale is essential to demonstrate the practical application of an import protocol before industry benefits can be realised. In addition, EDN is not currently registered for use as a fumigant, although, at the time of writing registration is being pursued by a commercial partner of CSIRO Entomology. Nevertheless, the results presented here indicate that EDN fumigation as part of an import protocol has the potential to enable the importation of feed grain to Australian quarantine requirements.

The intensive livestock industry would be the main beneficiary of access to imported feed grain to supplement local shortfalls in supply. Graziers may also benefit from downward price pressure on supplemental feed during times of shortage. Both industries are cyclically dependent on feed grain to support livestock in times of drought conditions. During these times feed prices can increase markedly. Manufacturers and on-sellers of the EDN fumigant, and importing bulk handlers and storers would also stand to benefit from this technology. MLA, as the holders of the intellectual property of this project, may be able to realise benefit from this through the wider use of EDN in other markets that require devitalisation of traded commodities.

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1 Background

Recent years of drought have had a large impact on feed grain prices. Both graziers and feedlot operators have been forced to pay high prices for feed grain in efforts to sustain herds and production, and to maintain export contracts. Climate predictions indicate that such events may occur with greater frequency due to the atmosphere alteration associated with fossil fuel and other man-made emissions.

Previous attempts to import feed grain to alleviate high prices accompanying periods of local grain and feed shortage have been subjected to onerous protocols. One treatment adopted is hammer milling of imported feed grain to effectively devitalise the grain and any potential weeds, followed by steam treatment to remove pathogens. However, grain treated in this way is difficult to handle in feed lot operations. Grain has also been imported under quality specifications and then allowed only limited movement within metropolitan areas for feed manufacturing for chicken production. The only registered fumigant considered a candidate to effectively treat imported grain is methyl bromide, which is being phased out of use because it is a greenhouse gas. Moreover, studies by Cassells *et al.* (1995) showed that only partial devitalisation of maize and sorghum can be achieved with methyl bromide, and they concluded that CT products (concentration × time) in excess of 17,000 mg h L^{-1} would be necessary to effectively treat the grain.

CSIRO has developed a new fumigant, ethanedinitrile (EDN), with the potential to treat imported grain. CSIRO was approached by GrainCo Ltd to provide a proof of concept for an "Emergency Import Permit" during the recent drought. This interest subsequently developed into the current investigation as defined by MLA project FLOT.124.

2 Project objectives

The objectives of FLOT 124 were originally part of a larger proposal formulated to develop an import protocol for feed grain. The objectives reported are designed to provide the initial evaluation of the EDN concept, enabling MLA to assess the merits of full commercialisation of EDN fumigation of grain. The objectives thus describe laboratory scale experiments and seek to address what were perceived to be the three major biosecurity issues associated with imported feed grain:

- to demonstrate the ability of EDN to devitalise the four target grain commodities (maize, wheat, barley and sorghum). This was important as devitalising the grain breaks the life cycle of any obligate pathogens (e.g. many viruses and pathogens).
- to devitalise contaminant weed seeds that might be potentially associated with imported grain; and
- to demonstrate a capability to devitalise pathogens that might be associated with imported grain.

Other aspects, such as compatibility of materials and the behavior of the gas, were also included to discount possible barriers to application, and to formulate a protocol for treatment at a larger scale. The numbering and order of the objectives has been altered from that of the original contract to better reflect the logical sequence of the work.

- Objective 1 – Furnish details as required by AQIS for the development of a (pathogen) incursion risk list for the commodities proposed for importation.
- Objective 2 - Selection of surrogate pathogens.
- Objective 3 – Produce a complete list of weeds of quarantine concern from the UK and USA.
- Objective 4 – Classify weeds into 'testable' (seed available) and 'not testable' (seed not available).

- Objective 5 – Develop treatment schedule for discriminating, one off treatments of all testable weed species, and the four grains, wheat, barley, sorghum and maize, including relative humidity (RH) (water activity), dose exposure time, application technique, filling ratio.
- Objective 6 – Assess compatibility of materials against ethanedinitrile.
- Objective 7 – Refine techniques for study of spore survival of surrogate pathogens.
- Objective 8 – Determine effective dosage for target surrogate pathogens to ethanedinitrile with RH equivalent 10-15% moisture content cereals.
- Objective 9 – Assess one off exposure of testable weeds and the four grains, wheat, barley, sorghum, and maize at constant RH, filling ratio, temperature, dose, exposure time, application followed by germination testing.
- Objective 10 – Develop treatment schedule that completely devitalises commodities according to International Seed Testing Association (ISTA) germination tests.
- Objective 11 – Develop absolute maximum dose schedule that can be applied to commodities, weeds, insects, pathogens and materials.

Proof of concept – Ethanedinitrile has the potential to cause devitalisation of wheat, barley, sorghum and maize, and of associated weed seeds and pathogens, to a level acceptable by quarantine authorities using a dose and method of application likely to be commercially viable and at a cost no greater than $10 per tonne of grain treated. CSIRO will establish the suitability of ethanedinitrile as a potential solution for devitalisation of grain in the proof of concept.

3 Review of incursion risks associated with imported barley, maize, wheat and sorghum from the UK and USA

In order to proceed with testing of weeds and pathogens a comprehensive list of incursion risks was needed. Biosecurity Australia is vested with the task of risk assessment of imported goods for the purposes of quarantine and is in the process of preparing risk assessments for a number of commodities. However, for the purpose of the seed devitalisation project, assessment information was available for only one commodity, maize from USA. In response to CSIRO's interest in this matter Biosecurity Australia requested information on weeds and pathogens associated with wheat and barley from the UK. The following is a compilation of this information and satisfies the requirements of Objectives 1 - 4.

3.1 Exotic Pathogens

An incursion risk assessment (IRA) had been completed for maize prior to commencement of the seed devitalisation work. This IRA documents the pathogen incursion risks for this product. The main pathogens of concern are listed in Table 3.1. However the maize IRA does not comprehensively cover pathogens of other crops. *Tilletia indica* (Karnal bunt of wheat) is mentioned specifically in relation to possible contamination of shared freight facilities. The maize IRA is available on the Australian Forestry Fisheries and Agriculture website at:
http://www.affa.gov.au/corporate_docs/publications/pdf/market_access/biosecurity/plant/final_maize.pdf

In addition to this, CSIRO Entomology has at Biosecurity Australia's request compiled an additional list of pathogens of wheat and barley from the UK. This information was passed onto Biosecurity Australia in March 2003. This body of work is appended as Appendix A. Advice from Biosecurity

Australia as it currently stands is that the pathogens in Table 3.1 are considered of importance in imported feed grain.

Table 3.1 Pathogens considered incursion risks associated with importation of wheat, barley, sorghum and maize from UK and USA.

Pathogen	Common name	Host
Cephalosporium Stripe		
Hymenula cerealis	Cephalosporium stripe	wheat
Downy mildew		
Peronosclerospora sorghi	Sorghum downy mildew	sorghum and maize
Smut fungi		
Tilletia controversa	Dwarf bunt	wheat and barley
Tilletia indica	Karnal bunt	wheat
Ustilago nuda f. sp. *tritici* current name *Ustilago tritici*	Loose smut	wheat
Ustilago zeae	Boil smut	maize
Sporisorium cruentum	Loose kernel smut	sorghum
Sporisorium sorghi	Covered smut	sorghum

3.2 Selection of surrogate pathogens for study within Australia

Biosecurity Australia has advised CSIRO of several fungi that are considered potential risks on wheat, barley, maize and sorghum imported from the UK and USA (Table 3.2). These fungi, identified as part of Objective 1, may be harboured on or within the seed. However, the importation of fungal pathogens for research purposes is subject to quarantine regulation, and it is not practical to study these organisms within Australia. For this reason related organisms were chosen as surrogates for study based on the criteria of taxonomic affinity and morphological similarity. The chosen surrogates are listed in Table 3.2. The rationale behind the selection of surrogates is summarised below for each incursion risk.

3.2.1 Cephalosporium Stripe

Hymenula cerealis Deuteromycotina, (Tuberculariaceae), causes a vascular wilt in wheat and barley. The primary infection source is stubble on which significant inoculum survives for two years. The fungus has very limited saprophytic ability. Sporulation occurs in cool wet periods, and spores germinate to infect roots mainly through injury by insects, freezing or frost heaving (freeze/thaw). The vascular system is compromised, resulting in haying off. Some species escape damage, as they do not suffer from freeze damage or root wounding. Conidia are produced on sporodochia during the saprophytic phase on stubble. It is rarely seed-borne in wheat, but it is common in seed of winter barley (USA). The critical issue regarding testing for efficacy is the dormant mycelium within the seed.

A possible surrogate for cephalosporium stripe is *Fusarium graminearum*. Until recently, two sub-groups were recognized. Group 1 was typically found to be associated with a crown rot of wheat, barley and oats, while Group 2 was associated with ear rot of corn and head scab of wheat, oats and barley. The Group 1 subgroup is now recognized as a separate species; *F. pseudograminearum*. *F. graminearum* is potentially a good substitute for *H. cerealis* as it shares affinity with respect to its ability to cause a seed-borne infection; both cause a vascular wilt, and both are sporodochia producing mitosporic fungi.

3.2.2 Downy mildew

Peronosclerospora sorghi (sorghum downy mildew) is considered a risk to both sorghum and maize production. Two tentative reports of *P. sorghi* into Northern Australia are probably *Peronosclerospora maydis* (Ramsey and Jones, 1988). *P. sorghi* belongs to the Oomycota, now considered taxonomically distinct from the fungal kingdom. Downy mildews produce resilient oospores, which can be present as a seed contaminant. In sorghum downy mildew, oospores form on leaves or glumes, either of which can cause contamination of seed (Bock et al., 1997; Rao et al., 1984). In other species the seed is infected directly (Bains and Jhooty, 1982). Downy mildews are obligate parasites, and the study of oospores is hampered because they cannot be readily generated in the laboratory. This is not a problem with the Pythiales, another group classified within the Oomycota. For this reason *Phytophthora citricola* has been chosen as a surrogate source of oospores. Other potential surrogates for this fungus include *Sclerophthora macrospora,* a downy mildew of wheat, or *Peronospora parasitica* (e.g. from Brassica). However, to date a source of material suitable for study has not been found. In addition, informal advice from Biosecurity Australia has indicated that these would not likely be considered a suitable substitute. *P. citricola* can be cultured and, as it is a homothallic species, readily forms oospores in culture and within colonized seed.

3.2.3 Smut fungi

Several smut fungi are considered incursion risks on imported grain (Table 3.2). The life cycle of a smut fungus in the plant culminates in the formation of a teliospore, typically bunted seed or smutted leaves or flowers or seed. The teliospores represent a significant contamination problem in imported seed. One of the main incursion risks is Karnal bunt (*Tilletia indica*). This disease has a high profile and can be expected to cause losses to production although it would probably cause greater damage in terms of lost market access. *T. tritici* (Common bunt) which is found in Australia has similar teliospore size and structure and has been chosen as a surrogate.

Table 3.2 Fungal species identified by Biosecurity Australia as incursion risks in imported grain from UK and USA alongside their surrogates chosen for this study.

Incursion risk	Surrogate	Comments
Cephalosporium stripe		
Cephalosporium gramineum	*Fusarium graminearum* head blight of wheat	*Fusarium graminearum* is also a mitosporic fungi with similar life cycle and is seed-borne.
Downy mildew		
Peronosclerospora sorghi	*Phytophthora citricola* root rots various species	Readily cultured and produces resilient oospores.
Smut		

Tilletia controversa *Tilletia indica* *Ustilago nuda* f. sp. *tritici* *Ustilago zeae* *Sporisorium cruentum* *Sporisorium sorghi*	*Tilletia tritici* bunt of wheat, barley	Teliospores of *Tilletia tritici* are structurally very similar to those of Karnal Bunt, a high profile incursion risk. Testing a surrogate for all smut fungi listed was considered too ambitious in the allotted time frame.

3.2.4 Collection and maintenance of fungal isolates.

Pathologist groups in WA, Qld, NSW, SA and Vic were contacted in order to source material. Five species of smut fungi were supplied by SARDI and *Phytophthora citricola* is currently represented in CSIRO Entomology's fungal collection. Samples of wheat bunt were supplied by CSIRO Plant Industry. Isolates of *Fusarium graminearum* have been supplied by NSW Agriculture

3.3 Exotic weeds

A list of weeds of quarantine concern in maize from the USA has been compiled by Biosecurity Australia as part of the Maize IRA. This list also essentially covers possible weeds of wheat from the USA. As wheat is grown in rotation with maize, the weeds associated with wheat must be considered possible contaminants of the crop and the supply chain. This list is contained within the Maize IRA at the Australian Forestry Fisheries and Agriculture website at:
http://www.affa.gov.au/content/publications.cfm?Category=Biosecurity%20Australia

However no publicly available list of potential weed species associated with UK wheat (and barley) was available, so a list of potential weeds associated with these commodities was compiled from literature sources. The completed list includes plant species recorded as weeds of wheat crops in the UK, including plant inhabitants of the field margins of wheat crops. The information was obtained from a search of the scientific literature from the early 1970s to date, and a search of the Internet, which was conducted during the week ending 10 January 2003. The assessment was made on the following criteria and methods.

- A weed of quarantine concern has been defined as 'A pest of potential economic importance to Australia and not yet present in Australia, or present but not widely distributed and being officially controlled' (AQIS Import Risk Analysis Process Handbook).
- Weeds listed as 'noxious' by any state or territory legislation are taken to be 'under official control' and may thus be of quarantine concern if present in imported grain.
- The status of listed plant species in Australia was first checked using PlantNET (http://plantnet.rbgsyd.nsw.gov.au/) and then the state floras of South Australia, Tasmania and Victoria.
- At this stage no attempt has been made to assess the difference in herbicide tolerance between strains of weed species that are found in both the UK and Australia.
- For species identified as 'of quarantine concern' no attempt has yet been made to determine whether they have been assessed for weediness and cleared for import by Biosecurity Australia using their 'weed risk assessment' process.
- This list specifically refers to weeds associated with wheat, but it would be expected that the weeds associated with other major cereal crops grown in the UK, in particular barley and oats, would be very similar.

Biosecurity Australia was furnished with the results of the study in the January 2003. This body of work is attached as Appendix B.

3.4 Classification of exotic weeds into 'testable' (seed available) and 'not testable' (seed unavailable)

As research on quarantine weeds is subject to both regulation and availability, not all quarantine weeds could be tested. Those for which both permit and seed have been obtained are listed in Table 3.3. Weed species for which surrogate species have been obtained either locally, or under AQIS permit, are listed in Table 3.4. Table 3.5 lists those species for which no source of seed or suitable surrogate was available. Should a successful protocol be developed for the devitalisation of grain these would be designated as nil tolerance for the purposes of importation. It is possible that some of the species tested via surrogate species in Table 3.4 may also be eventually categorised as nil tolerance following Biosecurity Australia's assessment of the suitability of the surrogates

Table 3.3 Species for which quarantine seeds have been obtained under AQIS permit. Those seed with unacceptably low germination are to be reclassified as non-testable (Table 3.5).

Family	Genus	Species	Surrogate available ex Australian sources	Present in UK/US
Malvaceae	*Abutilon*	*theophrasti*		UK/US
Amaranthanceae	*Amaranthus*	*chlorostachys*	*Amaranthus angeticus*	UK/US
Amaranthanceae	*Amaranthus*	*palmeri* (herbicide resistant)	*Amaranthus tricolour*	UK/US
Amaranthanceae	*Amaranthus*	*retroflexus* (triazine resistant)		UK/US
Asteraceae	*Ambrosia*	*artemisiifolia*		UK/US
Asteraceae	*Ambrosia*	*trifida*		UK/US
Umbelliferae	*Anthriscus*	*sylvestris*	*Anthriscus cerefolium*	UK
Poaceae	*Apera*	*spica-venti*		UK
Asclepiadaceae	*Asclepias*	*syriaca*		UK/US
Brassicaceae	*Brassica*	*juncea* (syn *japonica*)	*Brassica ocephala*	UK/US
Poaceae	*Bromus*	*tectorum*		UK/US
Poaceae	*Bromus*	*commutatus*		UK
Poaceae	*Cenchrus*	*incertus*	*Cenchrus ciliaris*	UK/US
Poaceae	*Cenchrus*	*longispinus*		UK/US
Chenopodiaceae	*Chenopodium*	*album* (atrazine resistant)	*Chenopodium quinoa*	UK/US

Asteraceae	*Chrysanthemum*	*segetum*	*Chrysanthemum parthenium*	UK
Asteraceae	*Cirsium*	*arvense*		UK/US
Asteraceae	*Cirsium*	*vulgare*		UK
Brassicaceae	*Conringia*	*orientalis*		UK/US
Convolvulaceae	*Convolvulus*	*arvensis*		UK/US
Poaceae	*Cynodon*	*dactylon*	*Cynodon dactylon*	UK
Solanaceae	*Datura*	*stramonium*		UK/US
Poaceae	*Echinochloa*	*crus-galli* (herbicide resistant)		UK/US
Lamiaceae	*Galeopsis*	*tetrahit*	*Mentha spicata viridis*	UK
Rubiaceae	*Galium*	*aparine*		UK
Umbelliferae	*Heracleum*	*sphondylium*	*Coriandum sativum*	UK
Convolvulaceae	*Ipomoea*	*lacunosa*	*Ipomoea aquatica*	UK/US
Convolvulaceae	*Ipomoea*	*hederacea*		UK/US
Convolvulaceae	*Ipomoea*	*purpurea*		UK/US
Convolvulaceae	*Jacquemontia*	*tamnifolia*		UK/US
Chenopodiaceae	*Kochia* (*Bassia*)	*scoparia*	*Beta vulgaris crassa*	UK/US
Asteraceae	Matricaria [*Tripleurospermum*]	*perforata*	*Matricaria chamomilla*	UK
Poaceae	*Panicum*	*capillare* (herbicide resistant)		UK/US
Poaceae	*Panicum*	*dichotomiflorum*		UK/US
Poaceae	*Pennisetum* (syn *Setaria*)	*glaucum* (syn *lutescens* (herbicide resistant)	*Pennisetum clandesrinum; P. alopecuroides*	UK
Poaceae	*Phleum*	*pratense* ssp. *bertolonii*		UK
Polygonaceae	*Polygonum*	*aviculare*		UK/US
Polygonaceae	*Polygonum*	*lapathifolium*		UK/US
Polygonaceae	*Polygonum*	*pensylvanicum*		UK/US

Family	Genus	Species	Surrogate available ex Australian sources	Present in UK/US
Brassicaceae	*Raphanus*	*raphanistrum*	*Raphanus sativus*	UK/US
Chenopodiaceae	*Salsola*	*kali (kali* subsp. *ruthenica)*		UK/US
Asteraceae	*Senecio*	*jacobaea*	*Tanacetum vulgare*	UK
Poaceae	*Setaria*	*faberi*		UK/US
Poaceae	*Setaria*	*verticillata*		UK
Poaceae	*Sorghum*	*halepense*		UK/US
Lamiaceae	*Stachys*	*sylvatica*		UK
Brassicaceae	*Thlaspi*	*arvense*	*Iberis amara*	UK/US
Asteraceae	*Xanthium*	*spinosum*		UK/US
Asteraceae	*Xanthium*	*strumarium*		UK/US

Table 3.4 Species for which surrogate species have been obtained either locally or under AQIS permit. The suitability of these surrogates is ultimately to be determined by Biosecurity Australia. Those surrogates failing suitability, or with unacceptably low germination, are to be reclassified as non-testable (Table 3.5).

Family	Genus	Species	Additional substitutes from Australia	Substitute AQIS	Present in UK/US
Asteraceae	*Acanthospermum*	*hispidum*	*Arctium lappa*		UK/US
Fabaceae	*Aeschynomene*	*virginica*	*Dolichos lab lab*		UK/US
Amaranthanceae	*Amaranthus*	*hybridus* (herbicide resistant)	*Amaranthus hypochondriacus*	Table 3.3	UK/US
Amaranthanceae	*Amaranthus*	*rudis* (triazine resistant)		Table 3.3	UK/US
Amaranthanceae	*Amaranthus*	*tamariscinus*		Table 3.3	UK/US
Asteraceae	*Ambrosia*	*grayi*		Table 3.3	UK/US
Asteraceae	*Bidens*	*aurea*	*Lactuca sativa*	*Bidens tripartita*	UK/US
Polygonaceae	*Brunnichia*	*ovata*	*Fagopyrum esculentum*		UK/US
Cyperaceae	*Cyperus*	*esculentus*		*Cyperus iria*	UK/US
Cyperaceae	*Cyperus*	*rotundus*		*Cyperus iria*	UK/US

Family	Genus	Species	Additional substitutes from Australia	Substitute AQIS	Present in UK/US
Solanaceae	*Datura*	*inoxia*		Table 3.3	UK/US
Asteraceae	*Erigeron*	*annuus*	*Echinacea purpurea*	*Erigeron canadensis*	UK/US
Asteraceae	*Eupatorium*	*capillifolium*	*Centaurea cyanus*		UK/US
Euphorbiaceae	*Euphorbia*	*platyphyllos*	*Euphorbia marginata*	*Euphorbia lathyris*	UK
Lamiaceae	*Galeopsis*	*angustifolia*	*Hyssopus officinalis*		UK
Lamiaceae	*Glechoma*	*hederacea*	*Scutellaria baicalensis*		UK
Convolvulaceae	*Ipomoea*	*turbinata*		Table 3.3	UK
Lamiaceae	*Lamium*	*album*		*Lamium purpureum*	UK
Poaceae	*Lolium*	*perenne* sbsp *multiflorum* (herbicide resistant)	*Lolium perenne; Lolium rigidum*	*Lolium perenne*	UK/US
Boraginaceae	*Myosotis*	*avensis*		Table 3.3	UK
Poaceae	*Panicum*	*fasciculatum*		Table 3.3	UK/US
Poaceae	*Panicum*	*ramosum*		Table 3.3	UK/US
Poaceae	*Panicum*	*texanum*		Table 3.3	UK/US
Poaceae	*Paspalum*	*boscianum*	*Paspalum dilatatum*	*Paspalum fasciculatum*	UK/US
Polygonaceae	*Polygonum*	*bungeanum*		Table 3.3	UK/US

Table 3.5 Non-testable weed species (due to unavailability of seed). These seeds are to be categorised as nil tolerance for purposes of importation.

Family	Genus	Species	Present in UK/US
Apocynaceae	*Apocynum*	*cannabinum*	UK/US
Brassicaceae	*Berteroa*	*incana*	UK/US
Euphorbiaceae	*Chamaesyce* (*Euphorbia*)	*maculata* (*supina*)	UK/US
Menispermaceae	*Cocculus*	*carolinus*	UK/US
Asclepiadaceae	*Cyanachum (Ampelamus)*	*laeve*	UK/US
Equisetaceae	*Equisetum*	*arvense*	UK/US
Poaceae	*Eriochloa*	*villosa*	UK/US
Asteraceae	*Helianthus*	*annuus* (herbicide resistant)	UK/US
Poaceae	*Muhlenbergia*	*frondosa*	UK/US
Solanaceae	*Physalis*	*heterophylla*	UK/US
Poaceae	*Setaria*	*lutescens (herbicide resistant)*	US
Cucurbitaceae	*Sicyos*	*angulatus*	UK/US

4 Compatibility of materials with ethanedinitrile

Before a fumigant can be used to disinfest or devitalise certain commodities its interactions with storage structures and plant must be documented. Materials commonly present in storage structures include metals, plastics (PVC pipes, tubing and sheets etc) and concretes (bricks, pavements and silos). The following experiments were devised to investigate;

1. The EDN sorption and desorption pattern on different types of structural materials like metal sheets (copper, aluminum, stainless steel and galvanized steel), plastic (Canvacon™, Canvacon 5000Q™, Land Mark™, PVC and tubing) and concrete (brick, concrete fresh and concrete silo) commonly present in a grain storage facility.
2. The extent of EDN permeation across plastic sheets such as Canvacon™, Canvacon 5000Q™ and Land Mark™.
3. The level of possible retention of EDN residues on the above-mentioned contact materials.
4. Discolouration of test materials following sorption of EDN.

4.1 Methods

4.1.1 Test materials

Metal sheets were supplied by the workshop at CSIRO Entomology, Canberra. The plastic sheets (CanvaconTM, Canvacon5000QTM and Land MarkTM) were obtained from GRAINCO, Qld. The materials available onsite were PVC plastic, teflon tubing, bricks, fresh concrete and silo concrete.

4.1.2 Sorption of EDN on test materials

Metal and plastic sheets were cut into rectangular pieces (3.7cm x 7.5cm). Pieces were transferred to 260 mL cylindrical glass (10 cm x 6 cm dia.) jars and conditioned at 25°C and 70% relative humidity (RH) for 24 h. At the time of EDN application, the jars were closed airtight by screw-capping with plastic lids equipped with a half-hole rubber septum in the center to monitor its sorption with gas chromatography (GC). To eliminate any chance of fumigant leakage from the test jars, lids were further sealed with the application of a layer of masking tape around the lids. The pieces of concrete and brick were added as maximum of 1-2 in number. Test jars were filled up to 30%, 20% and 10% of filling ratio (V/V) for concrete, plastics and metal pieces, respectively. These filling ratios were selected to reflect the rough composition of materials in grain storage facilities.

EDN was generated in the laboratory and checked for its purity using a Gas-Density balance GC. It was applied, in duplicate, to the test material in jars at the rate of 100 mg L-1 using an airtight syringe. An equivalent volume of air was removed with the syringe prior to EDN application. The sorption of EDN was monitored for a minimum of two days by taking at least three readings during the initial four hours from headspace and then two readings per day over the next two days. All the test materials were subjected to EDN sorption in different batches for the sake of ease in subsequent monitoring. Concentration of EDN was determined using an external standard curve. The samples and standards were kept at 25°C when not being monitored.

4.1.3 Discolouration of test materials in response to fumigation of EDN

After monitoring of EDN sorption on test materials, treated and untreated samples were displayed on a laboratory bench in parallel to each other to be visually judged for any significant colour change by five independent staff members/visitors not related to the project and without any knowledge of the treatments. Their response was recorded as ‘yes’ or ‘no’ for significant change of colour.

4.1.4 Desorption of EDN from test material

After the completion of sorption studies the test materials were divided into two parts. The contents of the first part (equal in volume to that used in sorption) was immediately transferred to new glass jars in duplicate and closed airtight similar to sorption study. The headspace concentration of EDN was monitored and quantified similar to the sorption study. However, the second part of material was left open inside the fume hood for 4 d aeration and subsequent monitoring of EDN was carried out similar to the first part. Similarly, all the test materials were investigated for desorption of EDN after 0 and 4 d aeration periods. Data were collected by monitoring headspace concentration of EDN of the glass jars similar to sorption studies.

4.1.5 EDN residues on the test material after fumigation

At the completion of the desorption study, glass jars were opened and the test material was subjected to further investigation for the presence of EDN residues. Ten to twenty grams of test material was weighed accurately and added to new 260 mL glass jars containing 50 mL of Toluene. Glass jars were closed airtight. Residual concentration of EDN on the material was determined by preparing spiked standards in parallel to test samples containing material from the control treatments. Standard jars were spiked with EDN (of known purity) at two rates of 5 and 15 mg L-1. All the jars were prepared in duplicate. Sample and standard jars were gently shaken and incubated at 25°C for 6 h before monitoring of headspace EDN concentration. Residues of EDN were calculated on the basis of sample weight.

4.1.6 Permeation of EDN across plastic sheets

Plastic sheets such as CanvaconTM, Canvacon5000QTM and Land MarkTM were cut into circular pieces with diameter of 19 cm to fit inside the edges of two desiccator lids leaving about 10 mm space from the periphery. High-pressure vacuum grease was applied to the smooth peripheral edges of the lids intended to grasp the outer circular portion of the sheet. Excess grease was applied to the area of lids not holding the sheet but sandwiched between two lids to prevent any leakage of applied EDN. Joined edges of both desiccators were further reinforced by pulling masking tape over them. Both the lids were equipped with QuickfitTM inserts fitted with rubber septa to facilitate sampling of gas exchange across the sheet. The assembled apparatus with sampling ports open was placed in a conditioning room for 24 h prior to use. EDN was applied from one side of the setup at the rate of 100 mg L-1 based on that side of volume but monitored from both source and sink sides over two days. Apparatus was prepared in duplicate for each sheet and kept in the incubator (25°C) when not in use.

4.1.7 Statistical Analysis

All the data were subjected to analysis of variance (ANOVA).

4.2 Results and Discussion

4.2.1 Sorption of EDN on test materials

The test materials were fumigated with EDN at a relative humidity of 70% or higher. Normally, fumigation is carried out at a humidity of less than 70%. However, a worst case scenario was tested, as for many commodities (wheat, barley, paddy and maize) their moisture content of 13.5 to 14.5% (wet basis) is in equilibrium with RH of ~70% at 25°C (Roberts, 1972).

In general, application of EDN to the metals tested (Figure 4.1) had no effect on its sorption when compared to control. However, in contrast to metals, the presence of the plastic and concrete material led to a significant sorption of EDN, with concrete showing more affinity compared to plastics. Maximum sorption of EDN was detected in the case of fresh concrete (Figure 4.2) where EDN absorbed to less than detectable concentration within half an hour followed by silo concrete with comparable time of 18 h. Brick material was less efficient in sorption of EDN.

The sorption of EDN on different plastic materials varied significantly. The maximum rate of sorption was found in Land MarkTM type of material, reducing the headspace concentration to < 5% 18 h after initial application. This was significantly higher than other materials (Figure 6.3). The order of significant sorption among the plastic materials was Land MarkTM > tubing > Canvacon 5000QTM > CanvaconTM > PVC + Control. Land MarkTM resulted in almost complete sorption of applied EDN over 24 h in contrast to the PVC pieces which showed a non-significant level of sorption even after 40 h of application.

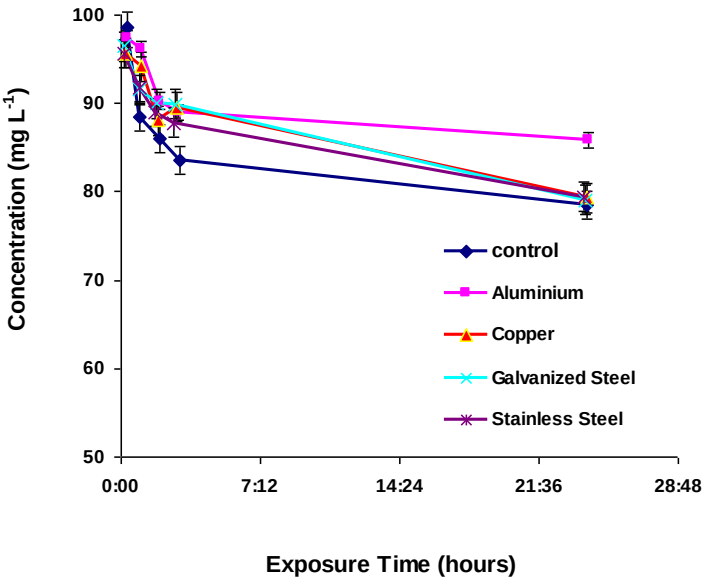

Figure 4.1: Ethanedinitrile (EDN) sorption on metals. Error bars depict standard error of the mean.

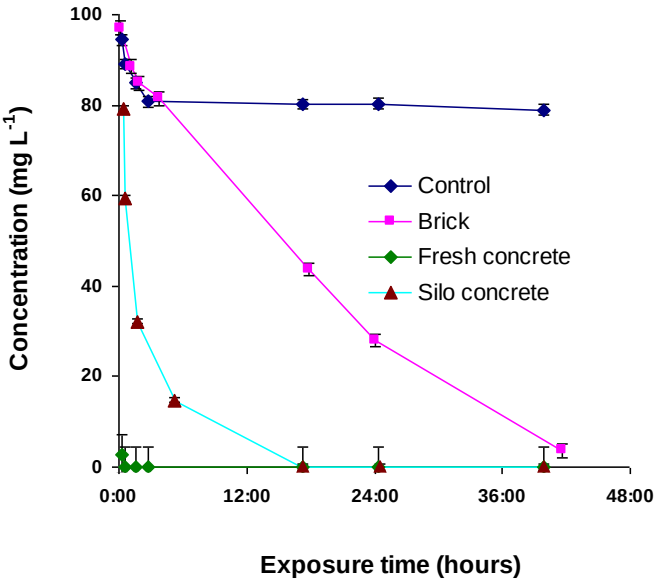

Figure 4.2 Ethanedinitrile (EDN) sorption on concretes. Error bars depict standard error of the mean.

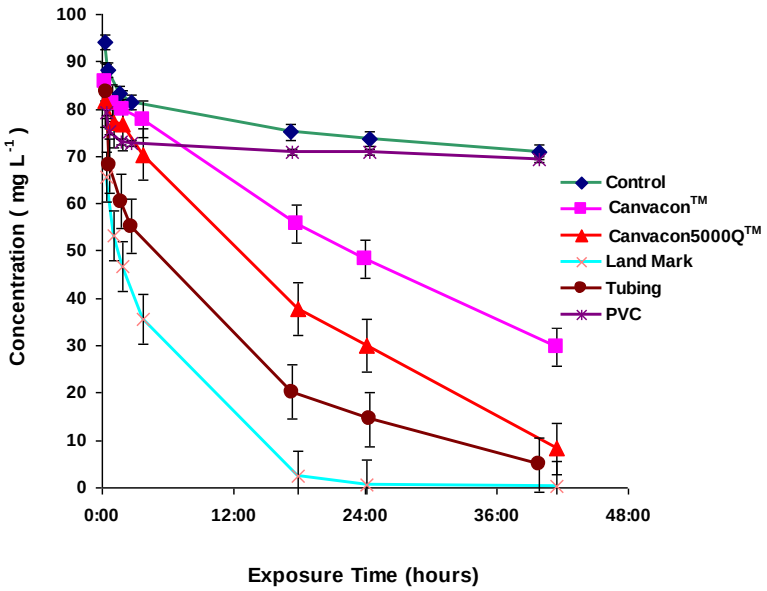

Figure 4.3 Ethanedinitrile (EDN) sorption on plastics. Error bars depict standard error of the mean.

4.2.2 Discolouration of test materials in response to fumigation with EDN

Observation of discolouration of test materials showed no effect of EDN application on the metals and concretes. However, a marked change in colour was observed in some of the plastics tested. For example, the colour of Canvacon 5000QTM changed from sky blue to olivaceous grey and that of tubing from white to brownish green. A subtle change in colour also occurred in Land MarkTM sheet but was judged to be non-significant. It was also noticed that the discolouration, in response to EDN fumigation of plastics, further increased when some of the treated samples (CanvaconTM, Canvacon5000QTM and Land MarkTM) were retreated with an additional dose of 100 mg L-1.

4.2.3 Desorption of EDN from test materials

The extent of EDN desorption from treated materials was monitored immediately and after 4 d aeration following sorption assessment. Results related to desorption are shown in Figures 4.4 to 4.8. It was found that desorption of EDN was significantly higher (>10-fold) from the samples tested without post-sorption aeration compared to those with a 4 d aeration period. Significant variation in desorption of EDN was also found among different types of material within the same group. For instance, EDN desorption from PVC plastic (Figure 4.5) was markedly higher compared to other plastics at 24 h of monitoring. Maximum rate of desorption occurred in plastic material (Figure 4.5) followed by concrete (Figure 4.7) and then metals (Figure 4.4).

Aeration (4 d) of samples in all the test groups led to a significant decrease in desorption. EDN concentration was reduced to less than detectable limit of instrument in case of metals (data not presented) and to < 0.01 mg L-1 in brick (Figure 4.8) of applied concentration (100 mg L-1). EDN was also reduced to lower than detection limit in case of both fresh and silo concretes. Though desorption of EDN in response to 4 d aeration was also reduced significantly, an increased rate of desorption over the period of monitoring (50 h) was observed in PVC, CanvaconTM and Land MarkTM types (Figure 4.6). Interestingly, these types of materials showed less sorption compared to others.

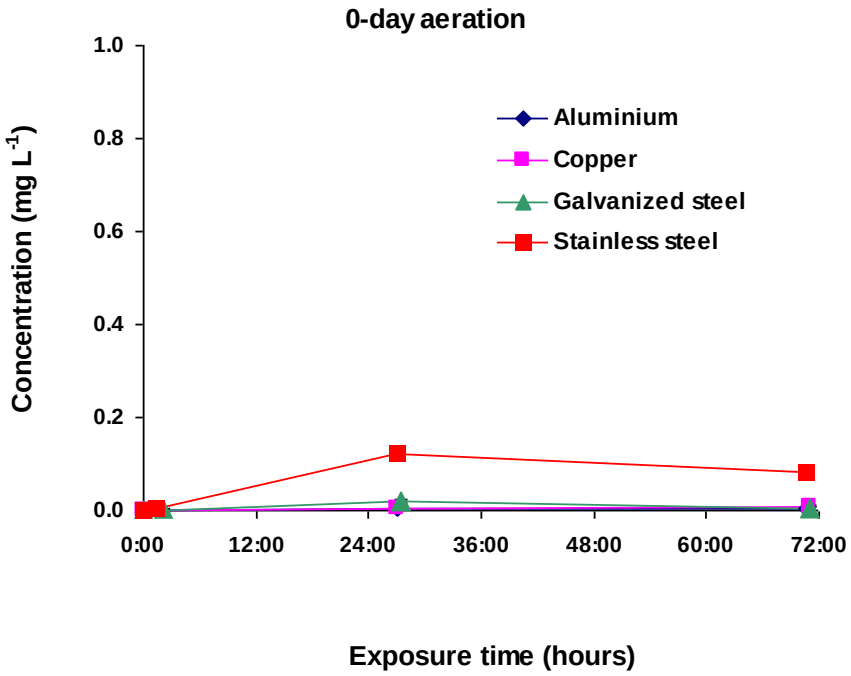

Figure 4.4 Ethanedinitrile (EDN) desorption from metal surfaces. Error bars depict standard error of the mean.

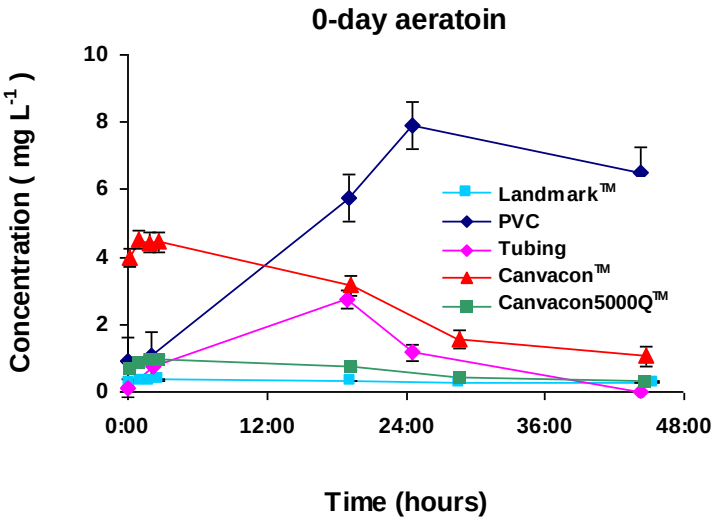

Figure 4.5 Ethanedinitrile (EDN) desorption from plastics. Error bars depict standard error of the mean.

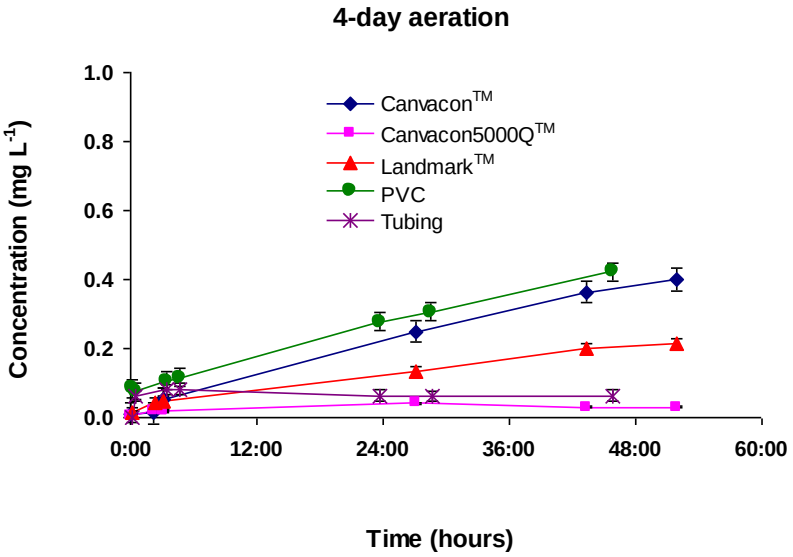

Figure 4.6 Ethanedinitrile (EDN) desorption from plastics. Error bars depict standard error of the mean.

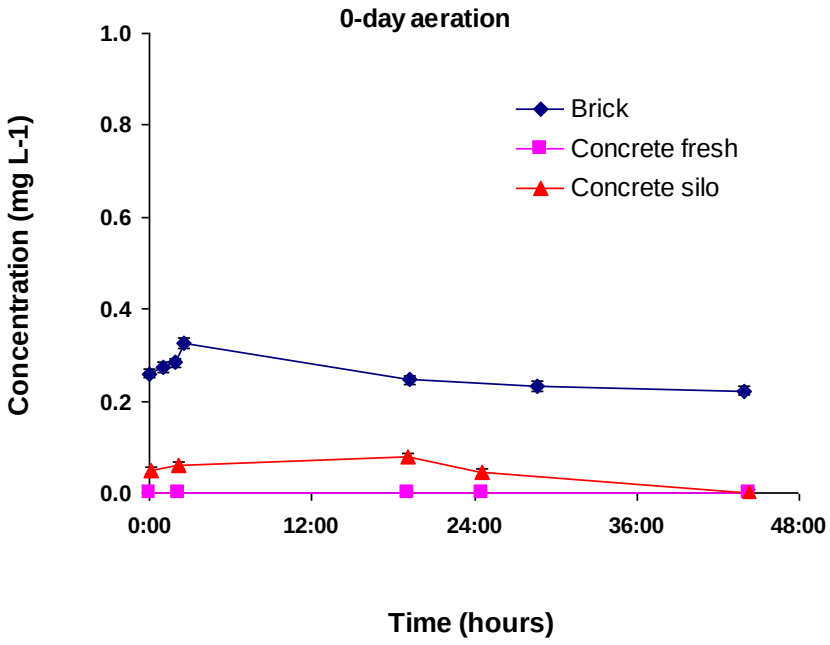

Figure 4.7 Ethanedinitrile (EDN) desorption from concrete and brick. Error bars depict standard error of the mean.

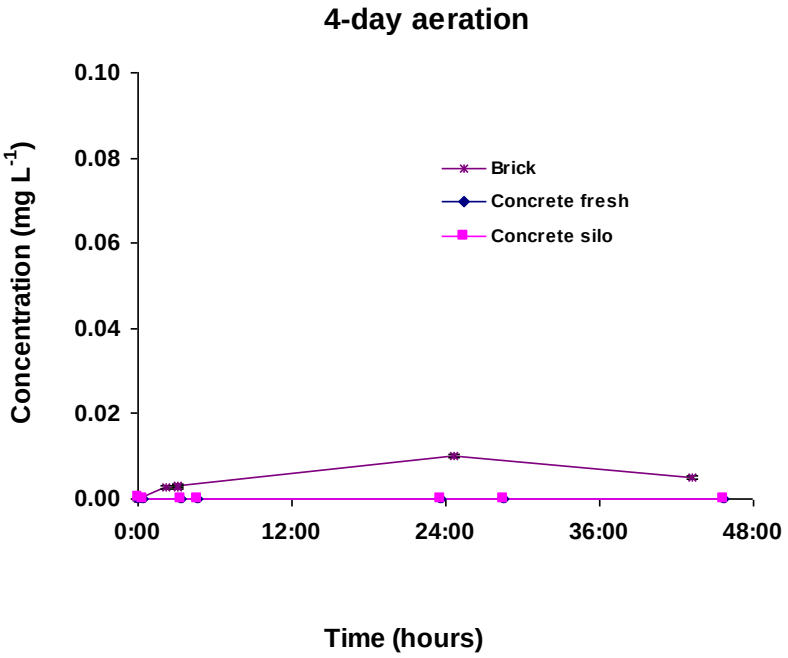

Figure 4.8 Ethanedinitrile (EDN) desorption from concrete and brick. Error bars depict standard error of the mean.

4.2.4 EDN residues on the test material after fumigation

Analysis of the test materials, for the retention of EDN residues after fumigation, indicated a significant difference in their capacity to retain residues as shown in Table 4.1 and Table 4.2. Presence of both forms of concrete (fresh and silo) resulted in significant adsorption of EDN residues compared to brick and all other materials tested from the non-aerated batch. Aeration of samples for 4 d led to significant reduction in residues found on concrete (Table 4.2). Brick showed the least affinity for EDN residues. No residues were found on the metals tested. However, the plastic material tested showed a large range of EDN residues (Table 4.1). A 4-day aeration of plastic samples did not reduce residue concentration greatly when compared with concrete.

Table 4.1 Residues of Ethanedinitrile (EDN) detected on plastic materials after fumigation

Material	Residues after no aeration (μg kg^{-1})	Residues after 4 d aeration (μg kg^{-1})
Canvacon™	252.5	226.7
Canvacon 5000Q™	132.0	130.9
Land mark™	189.4	169.4
PVC	215.2	190.9
Tubing	234.2	183.7
Standard error	20.9	11.2

Table 4.2 Residues of Ethanedinitrile (EDN) detected on concrete materials after fumigation

Material	Residues after no aeration (µg kg^{-1})	Residues after 4-day aeration (µg kg^{-1})
Brick	84.9	72.5
Concrete fresh	399.9	251.9
Concrete silo	361.8	234.2
Standard error	99.2	57.1

4.2.5 Permeation of EDN across plastic sheets

Results summarized in Figures 4.9 to 4.11 show permeability of EDN fumigation across test sheets of typical bunker covers sealed between two desiccator lids. Initial conditions were 100 mg L -1 on one side of the sheet and zero on the other. At equilibrium state, the concentration of EDN should reach approximately 50 mg L-1 in both halves of permeation chamber as it was applied at the rate of 100 mg L-1 based on volume of one side (1/2 volume of total) of chamber.

Canvacon 5000QTM sheet was found to be most permeable (Figure 4.10) where EDN concentration reached near equilibrium state after 24 h of application. CanvaconTM was the least permeable of the test sheets where concentration of EDN in the receiving half of the chamber reached less than 20% of the applied (100 mg L-1) EDN after 24 h of fumigation (Figure 4.9). However, Land MarkTM sheet showed an intermediate permeability to applied EDN (Figure 4.11).

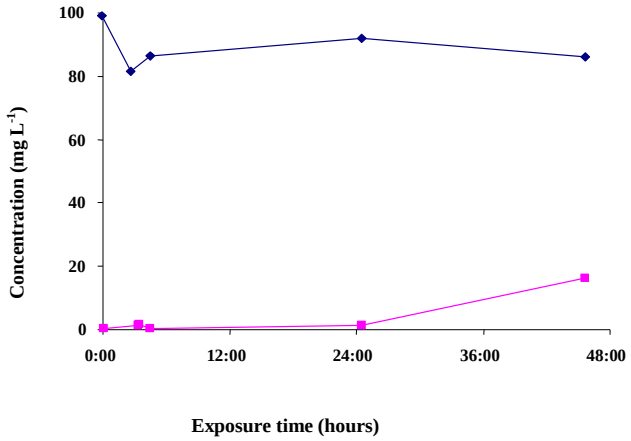

Figure 4.9 Ethanedinitrile (EDN) permeation through Canvacon^{TM} sheet (◆) dosed side, (■) receiving side of the diffusion chamber.

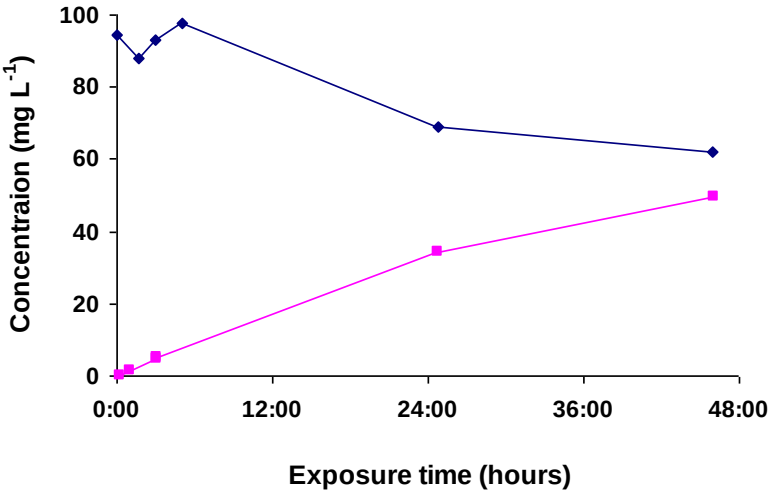

Figure 4.10 Ethanedinitrile (EDN) permeation through Canvacon 5000Q™ sheet (◆) dosed side, (■) receiving side of the diffusion chamber.

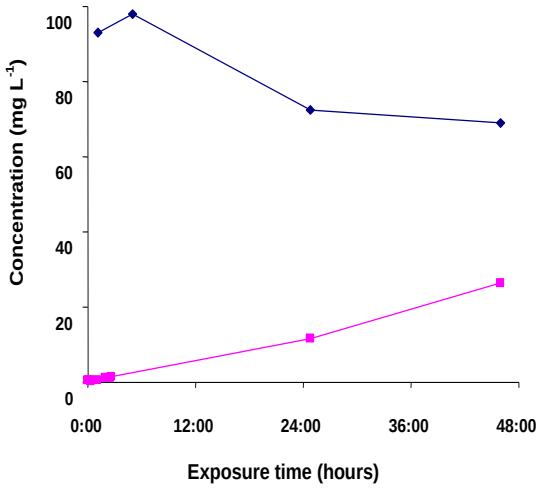

Figure 4.11 Ethanedinitrile (EDN) permeation through Land Mark™ sheet (◆) dosed side, (■) receiving side of the diffusion chamber.

4.3 Conclusions

EDN did not react with metals at prevailing test conditions of temperature (25°C) and relative humidity (70±3 %), and residues were below detection limits. However, very strong sorption of EDN occurred on fresh concrete where added EDN was removed within half an hour of application. The level of desorption was also relatively high for fresh concrete. Sorption on aged silo concrete was not as high with brick showing the least sorption. Plastic materials showed a wide variation in sorption and desorption of EDN as well but were less absorbent than concrete. Once sorbed, the retention of EDN residues was higher on concrete than plastics.

Canvacon 5000Q™ and Land Mark™ sheets of plastic were more permeable to EDN compared to Canvacon™, and sorption of EDN by plastic materials caused a significant colour change in Canvacon 5000QTM and tubing. Aeration of samples for 4 d was very effective in reducing residues in all the test materials.

Use of EDN fumigation for devitalisation of commodities would require compatibility of fumigation facilities to be established to ensure the most efficient and efficacious fumigation. Where basic compatibility parameters cannot be met, control measures such as barriers will need to be put in place prior to fumigation. Colour change in plastics sheets caused by EDN could be avoided by the selection of Canvacon™ tarping, which would ameliorate this problem, as well as optimising fumigation, due to its relatively low permeability.

5 EDN fumigation of the surrogate pathogens

The purpose of this experiment was to prove the ability of EDN to completely devitalise the surrogate fungi *Fusarium graminearum*, *Tilletia tritici* and *Phytophthora citricola* which were identified earlier in this report as surrogates for quarantine incursion risks associated with barley, maize, sorghum and wheat from the UK and USA.

Mycological studies in areas such as epidemiology, ecology, fumigant and fungicide application, routinely assess the viability of fungal spores. This may be achieved by observation of germination frequency; however this may be hampered by spore dormancy. Oospores of *Phytophthora citricola* and teliospores of *Tilletia tritici* spores both possess dormancy, which can potentially hinder assessment of viability by germination methods. In addition germination studies are not as convenient or rapid as vital staining methods. The vital stain MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) has been extensively employed for the study of oospore viability in; *Phytophthora* (Cohen 1984; El-Hamalawi and Erwin 1986; Bowers, Papavizas and Johnston 1990; Jiang and Erwin 1990; Pittis and Shattock 1994; Medina and Platt 1999), *Aphanomyces*, *Pythium* and *Phytophthora* (Sunderland and Cohen 1983), and *Peronospora* (van der Gaag 1994; van der Gaag and Frinking 1997), as well as several other groups of fungi. INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride) was used by Walley and Germida (1995) on vesicular-arbuscular mycorrhizae spores, Weiersbye-Witkowski and Straker (1997) in their study of spore viability of the powdery mildew (*Sphaerotheca fuliginea*) spores, and by Nelson and Olsen (1967) on *Synchytrium endobioticum* spores. Both Walley and Germida (1995) and Nelson and Olsen (1967) concluded that the INT was superior to MTT, and Walley and Germida (1995) found that only the viability estimated with INT was consistent with viability assessed by bioassay. The use of INT also has the advantage that the staining solution itself is colourless, and the variation in staining reactions is much less. While the use of these staining systems on oospores is routine, no studies have evaluated the use of INT or MTT. Therefore the reliability of INT and MTT as vital stains was evaluated on several smut fungi commonly found in Australia, by comparison of the staining reactions of autoclaved and non-autoclaved spores.

Following evaluation of the vital stain on smuts the three surrogate fungi were subjected to EDN fumigation to determine a devitalisation treatment. Assessment of propagules following fumigation

was undertaken by culture of propagules, spore germination assessments by microscopy and vital staining using INT and MTT.

5.1 Methods

5.1.1 Evaluation of vital staining of smut spores

Smut spores of five species were transferred to 1.5 mL Ependorf tubes. The species studied were *Tilletia tritici* (Bunt of wheat and barley), *Urocystis segetum* var. *segetum* (Covered smut of barley and oats), *Urocystis agropyri* (Flag smut of wheat), *Urocystis segetum* var. *avenae* (Loose smut of oats) and *Ustilago tritici* (Loose smut of barley). Half of the tubes of each isolate were autoclaved at 120^{o}C for 20 min, while the other half were retained at room temperature as the control treatment. Spores were then stained with 300 µL of either 0.24 mM MTT (Sigma Chemical Company, St Louis, Missouri, USA) or 0.24 mM INT (Sigma Chemical Company, St Louis, Missouri, USA) in 10mM phosphate buffer, pH 6.0. The staining pH was as recommended by van der Gaag (1994). Viability was then assessed at 1, 2, 3, 5 and 6 days by microscopy based on the combined counts of pink, red and black stained teliospores (viable) and unstained teliospores (non-viable).

5.1.2 Preparation of propagules

The tolerance of the vegetative stage of two of the surrogate pathogens *P. citricola* and *F. graminearum* was assessed using colonized cloth as an inoculum source. Three field collections of *T. tritici* teliospores were used as an inoculum source for testing smut tolerance. Oospores of *P. citricola* (isolate 1320) were generated in clarified V8 juice broth supplemented with β-sitosterol and thiamine (Ribeiro 1978). The oospores were harvested and concentrated by centrifugation (Ribeiro 1978). Extraneous mycelial material was removed enzymatically by the helicase method (Ribeiro 1978). The oospore solution was then smeared onto microscope slides for later vital staining, or smeared onto the internal surface of 1.5 mL Ependorf tubes for later plating onto selective agar medium following the EDN treatment.

5.1.3 Fumigation of surrogates

The various propagules were then placed in open desiccators of measured volume, allowed to equilibrate to 69% relative humidity, sealed, and injected with a test amount of EDN through a gas septum port, having first withdrawn an equivalent volume of air. The EDN was sourced from gas bottles, collected in a Tedlar bag. Percent purity was analysed using a Gas Density Balance, Tracor 220 M (Tracor Inc., Austin, TX, USA), with a 1 m × 5 mm glass column packed with Porapak Q 100/120 mesh (Alltech Associates, Deerfield, IL, USA), run at 105^{o}C with a carrier gas (N_2) flow of 150 mL min^{-1} and using 1,1,1,2 tetrafluoroethane as a reference gas. The quantity of gas needed to achieve target concentrations was calculated after correcting for pressure and temperature.

Desiccators were incubated over periods ranging from 1 to 5 d at 25^{o}C. The headspace concentration of EDN within the desiccators was measured in selected experiments by gas chromatography (GC) using a flame ionisation detector (FID) equipped Shimadzu GC6AM (Shimadzu Seisakusho, Kyoto, Japan). The column used was a 1 m × 3 mm glass column packed with HaysSep Q (Alltech Associates, Deerfield, IL, USA) run at 80^{o}C, with a carrier gas (N_2) flow of 40 mL min^{-1}.

5.1.4 Viability assessments

After treatment, the viability of the mycelium embedded on the colonised mesh cloth was assessed by plating onto potato dextrose agar. Teliospores, and the oospore slide smears were stained with 1% 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) in 0.1 M potassium phosphate buffer (pH 6.5). After a 5 d incubation at 25^{o}C the staining responses of the spores were assessed by microscopy. The combined counts of pink, red and black stained teliospores as a proportion of the total indicated percent viability (unstained teliospores were non-viable). For germination assessments of teliospores, spores were suspended in sterile water, and then a 100 µL

aliquot placed on to the surface of 2% agar supplemented with streptomycin (100 mg L^{-1}) and ampicillin (100 mg L^{-1}). Following equilibration the oospore matrix smeared on the Ependorf tubes formed a paper-like crust. This was removed under aseptic conditions and plated onto a modified *Phytophthora* selective agar medium (Tsao and Guy, 1977; Jeffers and Martin, 1986) to assess viability. Four pieces of the matrix were plated per plate and between five and ten plates were plated per treatment. The medium (P_{10}VPH) consisted of 10 g potato dextrose agar, 12 g agar, 50 mg L^{-1} hymexazole, pentachloronitrbenzene (PCNB) 100 mg L^{-1}, vancomycin 200 mg L^{-1}, pimaricin 30 mg L^{-1}. The agar base was first autoclaved then cooled to 60°C prior to the addition of the antibiotics mixed in 3 mL of acetone.

5.1.5 Statistical analysis

CT products (Concentration by Time) were calculated for the dose based on the headspace concentration measured over the duration of the experiment. These calculations were based on the least squares method. Percent viability was expressed by dividing the value for each treatment by the highest scored germination amongst the relevant control values. This had the effect of rescaling the values to remove differences in the background percent viability of the propagules tested. Proportional data was arcsine square root transformed prior to analysis by general linear model (GLM) after removing outliers and using Tukey's test to determine pairwise differences.

5.2 Results and Discussion

5.2.1 Vital staining of autoclaved and non-autoclaved smut spores

Only *Tilletia tritici* and *Urocystis segetum* var. *segetum* showed viable staining reaction using INT and MTT (Figure 5.1). Not only was the frequency of staining lower in *Urocystis segetum* var. *segetum*, it was more difficult to discern the staining reaction due to the small size of the spores. No germination was noted amongst the species which failed to show staining reactions, and while it is not possible to draw firm conclusions regarding the viability of these spores, it is probable that germinability was too low to be detected. In the case of *Urocystis agropyri* the dark pigmentation and elaborate spore structure would likely have precluded any visualization of staining reactions. Only teliospores of *Tilletia tritici* were considered suitable for routine vital staining with INT or MTT. This species possess teliospores that are similar in size and structure to *Tilletia indica* (Karnal Bunt), which is subject to considerable focus as a quarantine incursion risk in Australia.

Of the two staining systems INT gave the clearest staining reactions with fewer, more difficult to discern rose-coloured staining reactions. The spore viability also differed markedly between the two systems. In the case of MTT stained *Tilletia tritici*, the percentage of viable teliospores steadily increased over time whereas the INT staining system tended to fade towards the end of the experiment and one sample of *Tilletia tritici* declined in viability rather than increased over a three-day period. This was probably associated with the microbiological activity of contaminating organisms depleting the staining capacity of the staining solution. It appeared that the INT solution was more conducive to the growth of these organisms. Of the two systems INT gave the clearest staining results, and the proportion of viable spores assessed by this method was considered sufficient to evaluate the efficacy of fumigant if scored after a three to four day staining period.

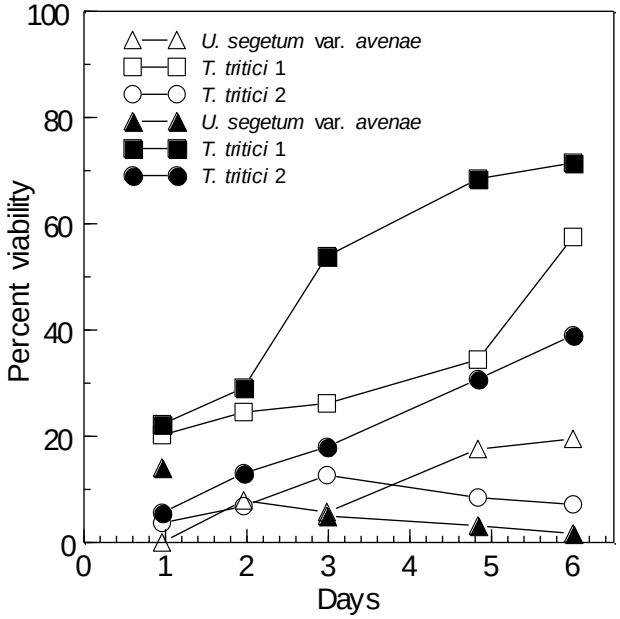

Figure 5.1 Percentage viability non-autoclaved teliospores of *U. segetum* var. *segetum* and two isolates of *Tilletia tritici* as assessed by vital staining for 1-6 d with either INT (open symbols) or MTT (shaded symbols). Viability was adjusted from the staining response of autoclaved control spores.

5.2.2 Surrogate for Cephalosporium stripe

Treatment of the vegetative *F. graminearum* propagules over a 24 h period was highly effective (Table 5.1). Control at even lower CT products was found when the isolate was treated over a 48 h period. No further work was undertaken on this species as it was apparent that its susceptibility was much greater than the other surrogate organisms.

Table 5.1 Proportional viability of *Fusarium graminearum* isolates equilibrated to RH 69% and exposed to EDN over a 24 h period.

	Isolate		
CT product (mg h L^{-1})	**3251**	**3241**	**3402**
0	1.0	1.0	1.0
120	0.3	0.6	0.0
240	0.0	0.0	0.0
480	0.0	0.0	0.0
720	0.0	0.0	0.0
960	0.0	0.0	0.0
1200	0.0	0.0	0.0

Note: Treatment over a 48 h period was even more effective, with no viability detected at 48 mg h L^{-1} and above.

5.2.3 Surrogate for smut fungi

Analysis of transformed smut teliospore germination was highly significant for the factors isolate and dose (GLM, $P < 0.0005$). In pairwise comparisons all doses and the control were significantly different with the exception of the control and a 144 mg h L^{-1} treatment, and a 925 mg h L^{-1} treatment and higher doses between 1210, 1480 and 1825 mg h L^{-1}. The same teliospores assessed by INT staining also showed significant effects (GLM, $P < 0.0005$) for the factors isolate and dose, however the residual viability was much higher and it was only in higher treatments (data not shown) that complete suppression of viability was observed using the INT staining method. The disparity between the viability assessments of the two methods is clearly visible in Figure 5.2.

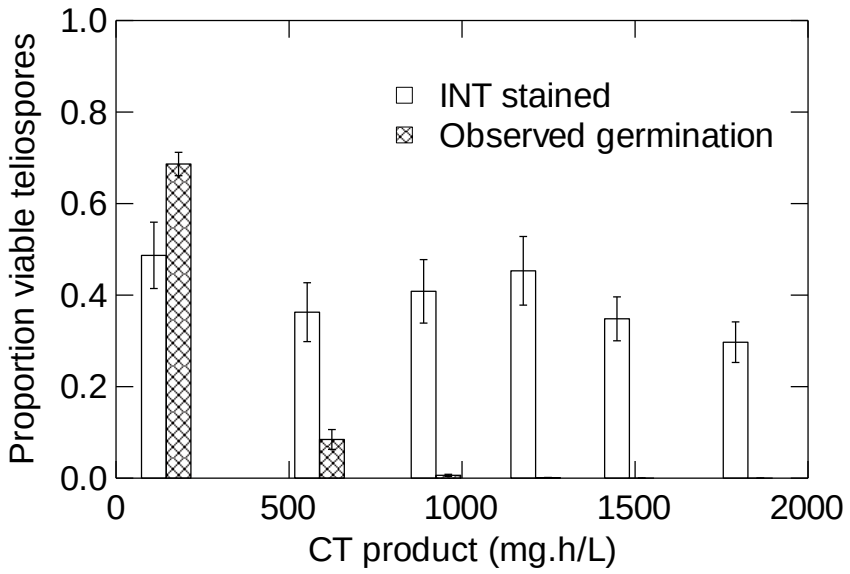

Figure 5.2 Comparison between observed germination and viability assessed by INT staining of *T. tritici* teliospores. Error bars depict standard error of the mean. No viability was detected by observed germination at or above 1500 mg h L^{-1}.

5.2.4 Surrogate for downy mildew

Significant difficulties were encountered in testing the sorghum downy mildew surrogate *P. citricola*. The initial cultures of oospores failed to generate viable oospores. Once this barrier had been overcome a series of increasing test doses were applied to slide smears of oospore matrix. However, even a dose of 36000 mg h L^{-1} failed to fully devitalise the oospores as assessed by INT staining. This was despite the oospore wall structure appearing significantly disrupted at half this value. In an attempt to resolve this, direct plating was adopted. However there was insufficient time to fully explore a range of doses so only limited results are presented in Table 5.2. These show effective control by a dose of 13800 mg h L^{-1}. However the actual value may be lower than this, as the test dose below this is half this value.

Table 5.2 Growth of *P. citricola* oospore matrix following EDN treatment. Error term is standard error of the mean.

CT product (mg h L^{-1})	Viable growth on P_{10}VPH medium
0	0.4±0.2
3300	0.08±0.08
6650	0.06±0.06
13800	0.0
19300	0.0
26500	0.0
40000	0.0

5.3 Conclusions

5.3.1 Vital staining versus direct methods

The disparity between the vital staining and the direct germination methods is interesting as the vital staining is effective in some situations, as evidenced by the autoclaved vs non-autoclaved treatments tested above. However, for EDN fumigation the method does not appear to be reliable. One explanation could be that EDN devitalises the spores without inactivating the pathways responsible for the formation of NADPH and NADH from NADP and NAD. These molecules donate protons to cause the colour change in INT and MTT. For the moment this seems the most likely explanation, and further use of the vital stain for EDN treated spores would be dependant on obtaining a correlation, if at all possible, between actual spore viability and that estimated by INT or MTT.

5.3.2 Devitalising the surrogates

The CT products for the three surrogates varied considerably. The CT products for T. tritici teliospores were considerably higher than that for F. graminearum, although both these figures were in turn much lower than needed to kill the weed seeds (see next section). The effective dose for the devitalisation of P. citricola oospores is likely to lie between 6650 and 13800 mg h L-1. In the event that this project is pursued further, it is likely that a full analysis of the actual pathogen incursion risks would be required. Given that the surrogates are at best a guide, the values obtained here, although not optimal, are sufficient to undertake assessment of the concept, prior to a full evaluation of the actual incursion risks at a later date.

6 EDN tolerance of testable weeds

The task to demonstrate devitalisation of all potential weed seed presented a significant problem, due to the large numbers of weed species to be tested, considerable variation in germination protocols of the different weed species, and the fact that, in many cases, tests require a considerable time to complete. The project plan thus aimed to apply a single ethanedinitrile (EDN) dose, designed to identify the majority of weed species with lower tolerance. This would demonstrate the ability of EDN to devitalise most of the weed species and any future work could proceed more efficiently by only concentrating on the most tolerant weed species.

Unpublished data collected by CSIRO Entomology on commodities and weed seeds equilibrated to RH 60 and 80% and fumigated for 5 d at 5% fill ratios was used to estimate a discriminating dose at fill ratio 5% and RH 70%. RH 70% was selected, as the EDN was deemed too effective at RH 80% to allow a reliable estimate of the discriminating dose, while at RH 60% the effectiveness of the chemical is such that excessive quantities would need to be applied. Using summarised data in Table 6.1 a discriminating dose was determined to be 500 mg/L at RH 60% and 75 mg/L at RH 80% for 5 d. Assuming a linear response between dose and RH, this would indicate a discriminating dose of approximately 300 mg/L at RH 70%.

Table 6.1 Indicative range for 100% kill of weed seeds for various commodities and weeds at 5% fill ratio and 5 days exposure to EDN.

Seed	ED_{100} dose (mg/L) at RH 60 %	ED_{100} dose (mg/L) at RH 80%
Wheat	250 - 350	< 50
Barley	350 - 500	
Maize	300 - 450	< 100
Sorghum	400 - 500	< 50
Cotton	500 - 750	75
Amaranthus	< 500	
Millet	< 50	
Fagopyrum	< 200	
Sesame	300 - 500	
Sunflower	350 - 750	
Foxglove	> 250	
Hyssop	< 100	
Linseed	200 - 500	

6.1 Methods

The test seed was equilibrated at 70% RH and placed in steel micromesh containers so that the seed could not escape confinement. These containers were transferred to a gas tight pressure tested drum, giving a fill ratio of approximately 2.5%. The drum was then sealed and transferred from the quarantine facility to the Entomology laboratories for fumigation, as per the quarantine import conditions, where a revised dose of 261 mg L^{-1} was applied, after a review of previous experimental work. EDN sourced from gas bottles was collected in a Tedlar bag and analysed to determine percent purity. This was undertaken using a Gas Density Balance, Tracor 220 M (Tracor Inc., Austin, TX, USA) with a 1 m × 5 mm glass column packed with Porapak Q 100/120 mesh (Alltech Associates: Deerfield, IL, USA) run at 105°C and a carrier gas (N_2) flow of 150 mL min^{-1} using 1,1,1,2 tetrafluoroethane as a reference gas. The quantity of gas needed to achieve target concentrations was calculated after correcting for pressure and temperature. Based on these calculations a quantity of gas was pumped into the drum. Pressure balance was maintained by the collapse of an internal air bladder ported to the external atmosphere.

The drum was then incubated at 25°C for a period of 5 d. During this time headspace concentration was measured by gas chromatography (GC) using a flame ionisation detector (FID) equipped Shimadzu GC6AM (Shimadzu Seisakusho, Kyoto, Japan). The column used was a 1 m × 3 mm glass column packed with HaysSep Q (Alltech Associates, Deerfield, IL, USA) run at 80°C and with a carrier gas (N_2) flow of 40 mL min^{-1}.

At the completion of the fumigation the drum was first aired, and then re-sealed prior to transfer back to the quarantine facility where germination assessments of the treated seed were compared to those of control seed. Chemical or physical pre-treatments were applied to the seed as dictated by ISTA or published seed germination methods. Germinations were undertaken on wetted filter paper in 15 cm Petri dishes, or on rolled paper in plastic bags, and incubated under appropriate light and temperature conditions. In some cases, alternative seed pre-treatments and surface sterilisation techniques were employed to increase germination. The germinations were assessed according to ISTA rules, and the positive germination results reflect the combined numbers of germinated, germinated low vigour, and germinated abnormal seed. Non-quarantine commodities (wheat, barley, maize and sorghum) were tested separately from the quarantine weeds at a dose of 250 mg L^{-1} using UK wheat as an internal check. A CT (Concentration × Time) product was calculated for the dose based on the headspace concentration measured over the duration of the experiment. The calculation was based on the least squares method. For logistic reasons, locally sourced test seed of the four commodities could not be included in the main dose, so a second dose was applied to these at 250 mg L^{-1}, with UK wheat as an internal check.

6.2 Results and discussion

6.2.1 Sorption

The main dose applied to the weed species was calculated to achieve an initial concentration of 261 mg L-1. Initial sorption measurements were low indicating that mixing of the EDN gas was slow (Figure 6.1). The CT product was calculated using a least squares method, after excluding measurements taken immediately following injection, and before the EDN had mixed. On this basis the dose was equivalent to a CT product of 2358 mg h L-1.

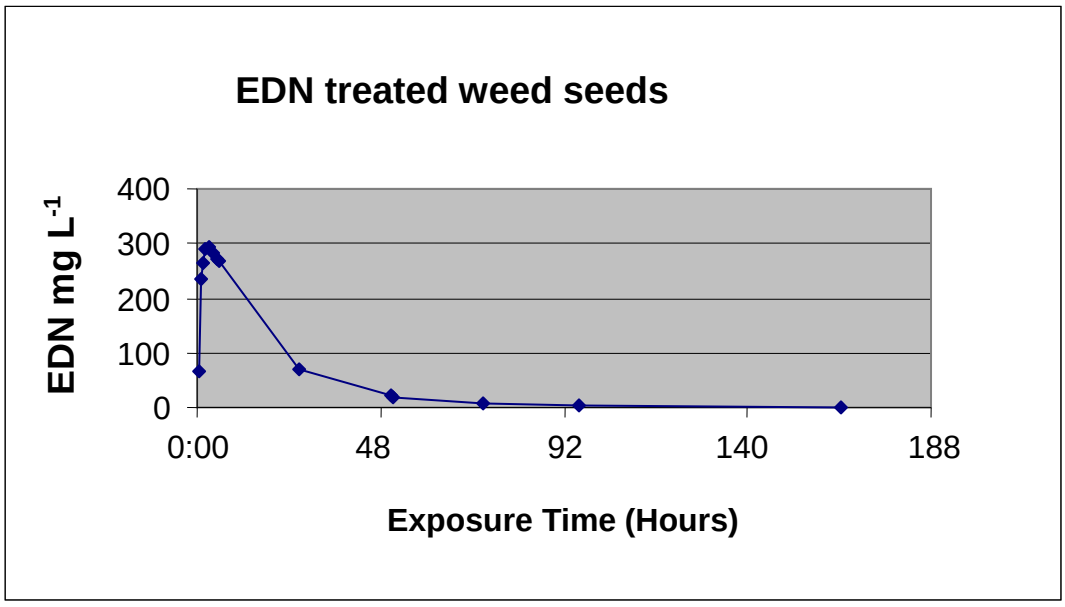

Figure 6.1 Sorption profile for the weed treated initially with EDN to 261 mg L^{-1} then incubated for 5 d (CT product calculated at 2358 mg h L^{-1}).

6.2.2 Tolerance to EDN

A total of 20.6% of the weed species survived the main discriminating dose at 261 mg L^{-1}, and these showed percentage germination ranging from between 0.3 to 100% germination expressed as a percentage of germination of the control treatment (Table 6.2). A further 41.3% of weed species showed no germination after treatment with the EDN (Table 6.2). However 36.5% of the species showed no germination either in the treated or control seed after following recommended germination procedures. These seed were deemed non-testable (Table 6.3). This is a significant increase in the numbers of weed species originally identified as non-testable, due to unavailability of seed (Table 6.4), from 11 to 34. The second discriminating dose (applied to commodities only) of 250 mg L^{-1} for five days was sufficient to kill all of the commodities (Table 6.5). In summary the EDN dose devitalized all of the commodities and approximately 35% of the weed species of quarantine concern. Approximately 20% of the weed species require further testing to determine the lethal dose and a further 45% have been categorised as non-testable.

Many of the non-testable seed were classified as such due to the lack of germination observed in the untreated control seed. This may be due to dormancy, non-viability of the seed or lack of appropriate germination protocols. It was not possible to draw any conclusions from these seed in the EDN discriminating dose test. It was not possible within the timeframe of the project to ensure the germinability of all seeds prior to testing, and this factor is responsible for some of the increase in the number of species that have been re-classified as non-testable.

In many instances the germination tests were affected by fungal contamination. Utilising ISTA paper germination tests can present considerable difficulties when applied to seed with long germination times. Fungal contamination present on the seed from harvest or due to storage conditions can proliferate during the seed germination tests, compromising the viability of the seeds. It is thought that this is the reason many of the untreated seed failed to germinate. Without a germination frequency for the untreated seed it is not possible to separate lack of viability due to EDN from other factors in treated seed. It was also noted in our tests that there was a marked reduction in fungal contamination on many seed that had been treated with EDN, compared to the untreated control seed. On other seed it was apparent that the composition of contaminants had changed, with only a few or single species dominant in the treated seed. This would confirm the contamination was seed borne. Hypochlorite treatment did not overcome germination problems in all fungal affected seed.

Additional doses (Table 6.3) were applied at higher CT products (3000 - 57000 mg h L^{-1}) to the exotic seeds ranked from one to nine in EDN tolerance (Table 6.2). In the discrimination dose these seed had exhibited germination in the 28-100% range. With the exception of *Ipomoea lacunosa* none were completely controlled by any of these treatments, although dosage related responses were observed.

Table 6.2 Results of discriminating dose of 261 mg L^{-1} for 5 days applied to quarantine weeds and UK wheat under quarantine. The SAN number has been allocated by CSIRO for each batch of seed acquired for testing.

Rank	SAN	Germination	Family	Genus/Species	Common name
1	10150	100.0	Convolvulaceae	*Jacquemontia tamnifolia*	small flower morning glory, hairy clustervine
2	10147	99.2	Convolvulaceae	*Ipomoea hederacea*	ivyleaf morning glory
3	10146	97.6	Convolvulaceae	*Convolvulus arvensis*	field bindweed, morning glory, small bindweed

Rank	SAN	Germination	Family	Genus/Species	Common name
4	10158	93.9	Malvaceae	*Abutilon theophrasti*	velvetleaf
5	10139	82.1	Brassicaceae	*Brassica juncea*	Indian mustard, brown mustard, leaf mustard
6	10148	70.5	Convolvulaceae	*Ipomoea lacunosa*	pitted morning glory
7	10122	64.3	Amaranthaceae	*Amaranthus palmeri*	careless weed
8	10141	34.5	Brassicaceae	*Raphanus raphanistrum*	wild radish, jointed charlock, jointed radish, wild raddish
9	10121	28.6	Amaranthaceae	*Amaranthus chlorostachys*	slim amaranth, pigweed
10	10149	17.4	Convolvulaceae	*Ipomoea purpurea*	tall morning-glory, common morning glory
11	10123	16.6	Amaranthaceae	*Amaranthus retroflexus*	redroot amaranth
12	10143	0.9	Chenopodiaceae	*Chenpodium album*	white goosefoot
13	10167	0.4	Poaceae	*Panicum capillare*	witchgrass, old witchgrass, ticklegrass, tumbleweed grass
14	10161	0.3	Poaceae	*Bromus tectorum*	cheatgrass, downy brome,
15	10138	0.3	Asteraceae	*Xanthium strumarium*	Noogoora burr, cocklebur, common cocklebur, rough cocklebur
16	10126	0.0	Apiaceae	*Torilis arvensis*	spreading hedge parsley, hedge parsley
17	10135	0.0	Asteraceae	*Matricaria perforata*	scentless chamomile, mayweed
18	10137	0.0	Asteraceae	*Xanthium spinosum*	spiny cocklebur, Bathurst burr,
19	10140	0.0	Brassicaceae	*Conringia orientalis*	hare's-ear mustard, klinkweed, rabbit ears
20	10144	0.0	Chenopodiaceae	*Kochia scoparia*	kochia, Mexican fireweed, summer cypress, Mexican burning bush
21	10151	0.0	Cyperaceae	*Cyperus iria*	sedge, Rice flatsedge

Rank	SAN	Germination	Family	Genus/Species	Common name
22	10156	0.0	Lamiaceae	*Salvia verbenaca*	salvia, vervain salvia, wild sage, wild clary
23	10164	0.0	Poaceae	*Cynodon dactylon*	Bermuda grass, common couch, couchgrass
24	10165	0.0	Poaceae	*Echinochloa crus-galli* f. *frumentaceae*	barnyard grass, Japanese millet
25	10166	0.0	Poaceae	*Lolium perenne*	perennial ryegrass
26	10172	0.0	Poaceae	*Setaria faberi*	giant foxtail, nodding foxtail, Chinese millet, Chinese foxtail, Japanese bristlegrass
27	10173	0.0	Poaceae	*Setaria verticillata*	bristly foxtail, rough bristle-grass, whorled pigeongrass, lovegrass, foxtail, rough bristlegrass
28	10177	0.0	Polygonaceae	*Polygonum lapathifolium*	pale smartweed, curlytop knotweed
29	10128	0.0	Asteraceae	*Ambrosia artemesifolia*	common ragweed
30	10131	0.0	Asteraceae	*Chrysanthemum segetum*	corn marigold, corn chrysanthemum, corn daisy
31	10142	0.0	Brassicaceae	*Thlaspi arvense*	fanweed, field pennycress, pennycress, stinkweed
32	10145	0.0	Chenopodiaceae	*Salsola kali*	soft roly poly, prickly saltwort, Russian thistle, prickly glasswort
33	10155	0.0	Lamiaceae	*Mentha arvensis*	mint, corn mint, field mint
34	10159	0.0	Poaceae	*Apera spica-venti*	loose silky bent, silky bent grass, windgrass
35	10160	0.0	Poaceae	*Bromus commutatus*	meadow brome
36	10168	0.0	Poaceae	*Panicum dichotomiflorum*	fall panicgrass, western witchgrass
37	10170	0.0	Poaceae	*Pennisetum glaucum*	pearl millet
38	10171	0.0	Poaceae	*Phleum pratense*	Timothy grass, cat's tail grass
39	10174	0.0	Poaceae	*Sorghum halepense*	Johnson grass

Rank	SAN	Germination	Family	Genus/Species	Common name
40	10176	0.0	Polygonaceae	*Polygonum aviculare*	wireweed, prostrate knotweed
41	10178	0.0	Polygonaceae	*Polygonum pensylvanicum*	Pensylvania smartweed, Pink knotweed, pinkweed
42	10182	0.0	Graminae	*Triticum aestivum*	wheat GRADE: UKF

Table 6.3 Weeds deemed non-testable following failure of germination in controls. These seeds are to be categorised as nil-tolerance for purposes of importation in addition to those in Table 6.4, unless further testing overcomes germination inhibition. The SAN number has been allocated by CSIRO for each batch of seed acquired for testing.

N°.	SAN	Family	Genus/Species	Common name
1	10169	Poaceae	*Paspalum fasciculatum*	Mexican crowngrass
2	10124	Apiaceae	*Anthriscus sylvestris*	cow parsley, wild chervil
3	10125	Apiaceae	*Heracleum sphondylium*	Hogweed
4	10127	Asclepiadaceae /Apocynaceae	*Asclepias syriaca*	common milkweed
5	10129	Asteraceae	*Ambrosia trifida*	great ragweed
6	10130	Asteraceae	*Bidens tripartita*	trifid burr-marigold
7	10132	Asteraceae	*Circium arvense*	Canada thistle
8	10133	Asteraceae	*Circium vulgare*	Bull thistle
9	10134	Asteraceae	*Erigeron canadensis*	horseweed, horseweed fleabane
10	10136	Asteraceae	*Senecio jacobea*	tansy ragwort, ragwort
11	10145	Chenopodiaceae	*Salsola kali*	soft roly poly, prickly saltwort, Russian thistle, prickly glasswort
12	10152	Euphorbiaceae	*Euphorbia lathyris*	caper spurge
13	10153	Lamiaceae	*Galeopsis tetrahit*	Hemp-nettle, common hempnettle
14	10154	Lamiaceae	*Lamium purpureum*	purple deadnettle, red deadnettle
15	10157	Lamiaceae	*Stachys sylvatica*	wood woundwort, hedge woundwort
16	10162	Poaceae	*Cenchrus longispinus*	spiny burrgrass, innocent-weed, burrgrass
17	10163	Poaceae	*Cenchrus incertus*	coastal sandbur, mat sandbur, field sandbur, innocent weed

18	10175	Poaceae	*Urochloa panicoides*	liverseed grass, panic liverseed grass, urochloa grass
19	10179	Rubiacea	*Galium aparine*	cleavers, stickywilly
20	10180	Solanaceae	*Datura stramonium*	jimsonweed, Jamestown weed, thornapple, common thornapple
21	10181	Solanaceae	*Solanum nigrum*	common nightshade, black fruited nightshade, blackberry

Table 6.4 Non-testable weed species (due to unavailability of seed). These seeds are to be categorised as nil-tolerance for purposes of importation.

N°.	**Family**	**Genus/Species**
1	Apocynaceae	*Apocynum cannabinum*
2	Brassicaceae	*Berteroa incana*
3	Euphorbiaceae	*Chamaesyce (Euphorbia) maculata (supina)*
4	Menispermaceae	*Cocculus carolinus*
5	Asclepiadaceae	*Cyanachum (Ampelamus) laeve*
6	Equisetaceae	*Equisetum arvense*
7	Poaceae	*Eriochloa villosa*
8	Asteraceae	*Helianthus annuus (herbicide resistant)*
9	Poaceae	*Muhlenbergia frondosa*
10	Solanaceae	*Physalis heterophylla*
11	Cucurbitaceae	*Sicyos angulatus*

6.3 Conclusions

In terms of discriminating between the tolerant and non-tolerant weed species the Objective 9 has been met with success. Nevertheless 23 species of weeds have been deemed as non-testable. Although a good sample of weed tolerance was demonstrated amongst the successful tests, there is a chance that there may be more tolerant weeds amongst the group that was not successfully tested. It is likely that in several cases the barriers to germination will be overcome by testing a second seed source. In addition over half of the weeds deemed to be non-testable (Table 6.3) belong to the families Poaceae, Asteraceae and Lamiaceae, which were all completely devitalised in the testable weeds (Table 6.2). The full significance of these results will ultimately be dependant on a full risk analysis by Biosecurity Australia taking into account factors such as; risk of contamination at source/in transit and from structures and machinery, presence or absence of the quarantine risk at source, seed screening and other methods which can be used to reduce contamination levels, and the level of likely contamination and risk of establishment at the end point. After taking factors such as these into account it may become apparent that only some of the weed species in Tables 6.3 and

6.4 would require a high level of control. Further research could be targeted towards these specific risks.

The purpose of this objective was to provide a basis for additional testing to identify a definitive list of testable and controllable weeds and to define the dose at which control can be achieved. While this additional work falls outside the scope of this project, some progress has nevertheless been achieved. Those seed showing appreciable germination following the discriminating dose (ranked 1 - 9) showed similar responses when tested at much higher doses. The highest level tested was sufficiently high to be considered impractical for large-scale application. On the basis of this it does not seem profitable to conduct further testing on these seed. However four species in the discrimination dose showed germination responses below 1%. It is very likely that further testing of these four species would be controlled by CT product values in the vicinity of 2400 mg h L-1 and further testing would be warranted. In the absence of further research a dose of 2400 mg h L-1 with an additional component for safety could be considered sufficient to devitalise the commodities in Table 6.2 with the exception of those numbered 1-15. In this respect treatment by EDN cannot be considered to be a completely effective against all known exotic weeds which might be associated with imported barley, maize, sorghum and wheat. For these tolerant species and those listed in Tables 6.3 and 6.4 stringent contamination control and tolerance specifications would need to be imposed to the satisfaction of Biosecurity Australia before a protocol for importation could be agreed upon within a risk assessment framework.

7 Devitalisation of commodities

One of the primary objectives of the project is to demonstrate the ability to devitalise the target commodities. This is important, as the commodities are an important link for exotic pathogens. Viruses in particular are not considered to be a viable target for fumigation; however by killing the host the life cycle of a virus may be interrupted. Similarly for obligate fungal pathogens, the absence of the host is an additional level of control over any direct fumigant action on the pathogen.

7.1 Methods

7.1.1 Experimental design

Information on the seed collection used for the study was placed in a database and the seed stored at 4°C prior to study (Table 7.1). Commodities were equilibrated to 14% moisture content prior to experimentation, and one variety of each commodity was also equilibrated to 15 and 16% moisture content. Available water (A_w) estimates were measured using an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Washington, USA) and are tabulated in Table 7.1. Each accession was subjected to a range of doses. Due to the number of commodities, varieties and different moisture contents investigated, treatments were conducted sequentially over a 7-month period. For each batch there was a minimum of three replicates for each accession/dose combination and a control treatment that contained no EDN. In addition, a check flask containing 14% moisture wheat (SAN 10184) was routinely included. A fill ratio of 5% for each seed accession was calculated, and the treatment flasks were filled to this level on a weight basis.

7.1.2 EDN fumigation

Prior to treatment, EDN sourced from gas bottles was collected in a Tedlar bag and analysed to determine the percent purity. This was undertaken using a Gas Density Balance, Tracor 220 M (Tracor Inc., Austin, TX, USA) with a 1 m × 5 mm glass column packed with Porapak Q 100/120 mesh (Alltech Associates, Deerfield, IL, USA) run at 105°C and a carrier gas (N_2) flow of 150 mL min^{-1} using 1,1,1,2 tetrafluoroethane as a reference gas. The quantity of gas needed to achieve target

concentrations was calculated after correcting for pressure and temperature. Based on these calculations air was withdrawn from each flask and replaced with an equivalent volume of EDN.

The flasks were then incubated at 25°C for a period of 5 d. Headspace concentrations were measured by gas chromatography (GC) using a flame ionisation detector (FID) equipped Shimadzu GC6AM (Shimadzu Seisakusho, Kyoto, Japan). The column used was a 1 m × 3 mm glass column packed with HaysSep Q (Alltech Associates, Deerfield, IL, USA) run at 80°C and with a carrier gas (N_2) flow of 40 mL min^{-1}. After treatment, the contents of the flasks were aired, and the seed removed for germination assessment.

Germination assessment was conducted according to International Seed Testing Association methods. Accordingly each of 400 seeds for each commodity dose combination was assessed as either normally germinated, low vigour, abnormal, fresh (normal in appearance but non-germinated), mouldy, hard (not imbibed), or empty. Germination test procedures are described in Appendix C.

7.1.3 Statistical analysis

Prior to analysis of the germination data set, a CT (Concentration by Time) product was calculated for each dose based on the headspace concentration measured over the duration of the experiment. The calculation was based on the least squares method. The CT product was matched to the germinations measurements for each flask. For each accession/dose/replicate combination, percentage viability of the seed was calculated by dividing the sum of the normally, low vigour and abnormal germinations by the total seed tested. For each treatment, the percent viability was rescaled by dividing by the highest control seed percent viability. These values were then arcsine square root transformed. The linearity of CT product and viability for each replicate was checked before performing regression analyses. Coefficients of regression were used to estimate the effective dose at which 50 and 95% of the seed was devitalised (ED_{50} and ED_{95} respectively) for each replicate in every treatment combination tested, using arcsine square root transformed percentage values.

Two analyses were undertaken on the transformed data. The subset of the data containing commodities at all three moistures was analysed based on a general linear model, with moisture content and commodity type as independent variables. This was to test the hypothesis that moisture content affected the efficacy of the fumigation of the four commodities. A second analysis using general linear model was undertaken on the seed tested at 14% moisture content using commodities type and variety as independent variables. Pair wise treatment comparisons were made using Tukey's test for both analyses.

The rate of headspace loss monitored for each flask (dose/commodity combination) was estimated by non-linear regression using the following model:

$$C_t=C_o e^{(-k.t)}$$

where C_t is the gas concentration (mg L^{-1}) at time t, C_0 is the gas concentration at t = 0 (mg L^{-1}), k is the extinction coefficient, and t = time (days).

The extinction coefficients for four of the commodities tested at three moisture contents were analysed by GLM and pairwise comparisons were made using Tukey's test.

Table 7.1 Moisture content (MC) and available water (A_w) measurements for the commodities tested in this study. The SAN N°. refers to the accession number assigned to the seed lot by CSIRO Entomology.

SAN N°.	Crop	Variety	Moisture Content	A_w	Mass @ 5% fill
10183	barley	Schooner	14	0.63	11.8
10189	barley	Gairdner	14	0.56	11.8

SAN Nº.	Crop	Variety	Moisture Content	A_w	Mass @ 5% fill
10190	barley	Sloop	14	0.55	12.9
10077	maize	PAC-345	14	0.62	12.75
10078	maize	Pioneer 317	14	0.66	12.7
10195	maize	QX 8	14	0.62	12.8
10196	maize	QX 6	14	0.55	12.8
10117	sorghum	white sorghum	14	0.64	12.3
10187	sorghum	MR 43	14	0.63	13.1
10118	wheat	Rosella	14	0.62	12.5
10119	wheat	Diamond Bird	14	0.65	13.1
10184	wheat	Diamond Bird	14	0.67	12.8
10185	wheat	Hartog	14	0.69	13.3
10186	wheat	H 45	14	0.68	12.8
10192	wheat	Sunlin	14	0.67	13.2
10193	wheat	Ellison	14	0.67	12.2
10183	barley	Schooner	15	0.67	10.9
10078	maize	Pioneer 317	15	0.68	12.7
10117	sorghum	white sorghum	15	0.69	12.8
10186	wheat	H 45	15	0.73	13.5
10183	barley	Schooner	16	0.75	11.1
10078	maize	Pioneer 317	16	0.75	12.6
10117	sorghum	white sorghum	16	0.73	12.6
10186	wheat	H 45	16	0.74	12.6

7.2 Results and Discussion

7.2.1 Impact of moisture content on EDN efficacy

Regression of transformed seed viability assessments against the calculated CT products for each dose generally gave significant regression coefficients for the 14 and 15% moisture contents ($0.64>R^2<0.97$). At the 16% moisture content the doses tested were very effective, and for maize and

sorghum treatments seed was totally killed at the very lowest dose tested. The ED_{95} estimates are thus based on two point regressions (control and the lowest dose) in these cases. As these values would in any case result in an overestimate of the ED_{95} estimate, it was considered safe to include these results in the analysis.

Commodity type and moisture contents were both highly significant in analysis of the ED_{95} values (GLM, $P < 0.0005$; Table 7.2). There was also a significant interaction between the two factors. In pair wise comparisons there was no significant difference in the ED_{95} values of wheat and either maize or sorghum. Barley was significantly different to the three other commodities and sorghum significantly differed from maize. In responses averaged across moistures the most tolerant commodity was barley followed by maize, wheat and sorghum in that order. ED_{95} values for each moisture commodity combination are presented with standard error of the mean values in Table 7.2. Raw germination data rescaled as a percentage of the highest control germination are presented in Figures 7.1 to 7.4.

Table 7.2 ED_{95} values for EDN treatments of four commodities at three moistures.

N°.	**Crop**	**MC**	**ED50**	**ED95**
10183	barley (Schooner)	14	219±8	456.8±19.5
10078	maize (Pioneer 317)	14	60.6±2.1	120.2±4.5
10117	Sorghum (white)	14	31±0.7	108.6±2.9
10186	wheat (H 45)	14	57±1.8	133.2±1.1
10183	barley (Schooner)	15	94±2.6	167.6±5.1
10078	maize (Pioneer 317)	15	37±1	74±4
10117	Sorghum (white)	15	5±5.3	28.1±0.7
10186	wheat (H 45)	15	29±0.9	70.1±0.9
10183	barley (Schooner)	16	49±0.6	86.4±0.3
10078	maize (Pioneer 317)	16	48±0.6	88.7±0.2
10117	Sorghum (white)	16	4±0.4	10.2±0.1
10186	wheat (H 45)	16	27±0.7	54±0.5

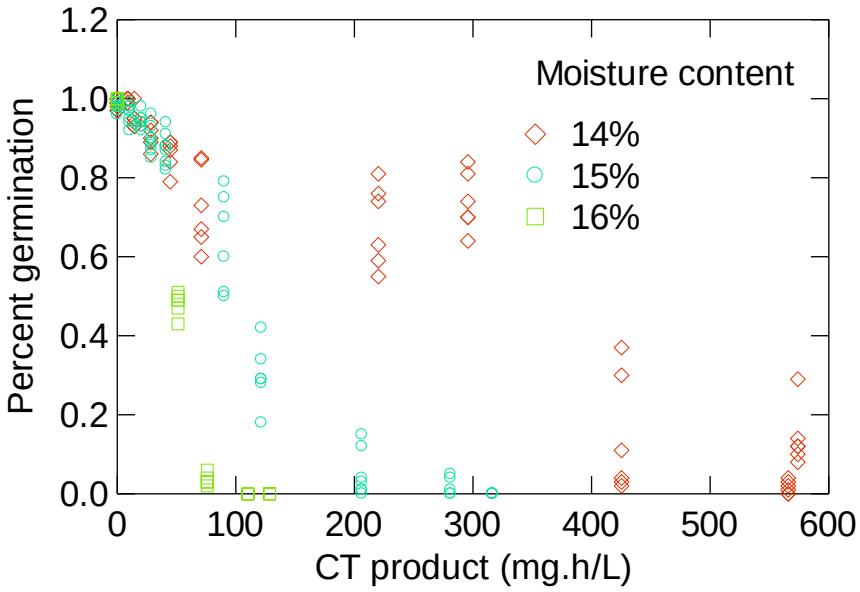

Figure 7.1 Response of barley (cv Schooner) to EDN and moisture. CT products calculated by least squares method from sorption measurements taken over the duration of the trials.

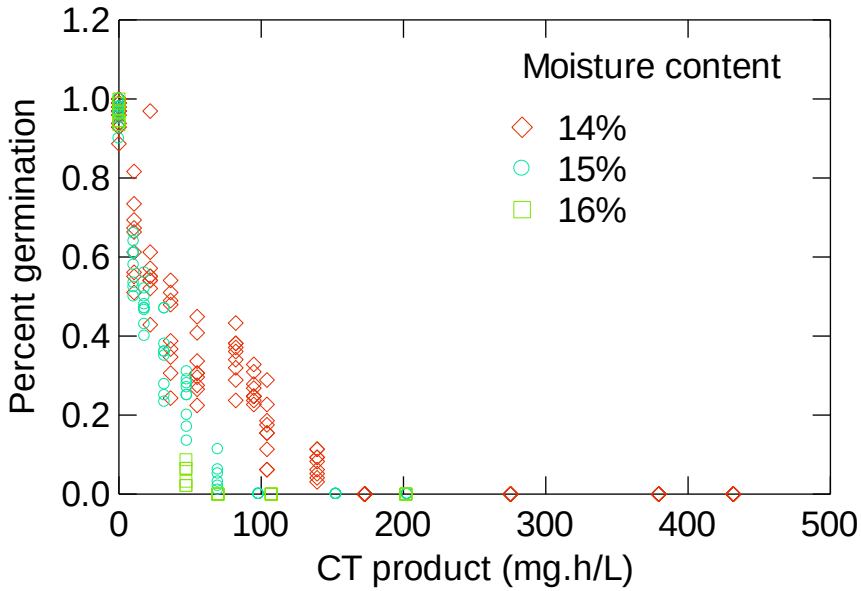

Figure 7.2 Response of wheat (cv H45) to EDN and moisture. CT products calculated by least squares method from sorption measurements taken over the duration of the trials.

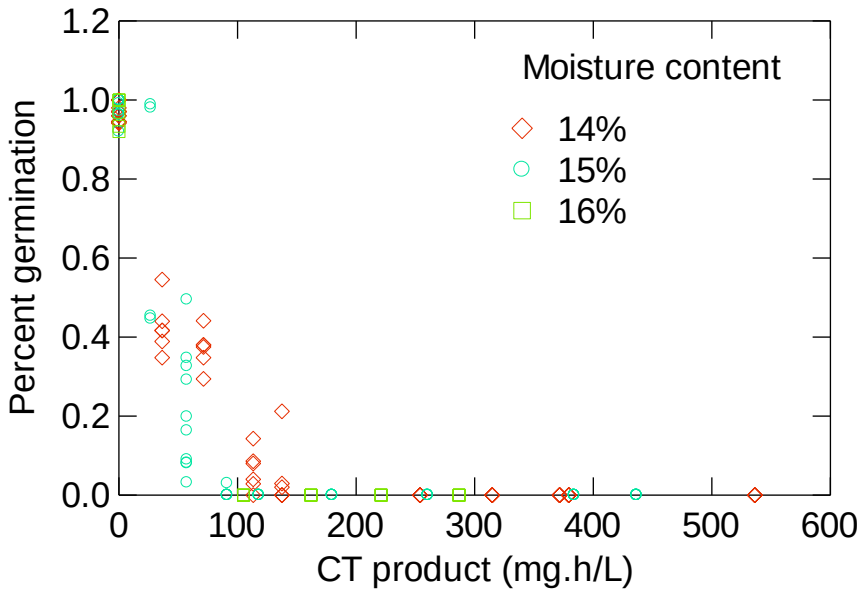

Figure 7.3 Response of maize (cv Pioneer 317) to EDN and moisture. CT products calculated by least squares method from sorption measurements taken over the duration of the trials.

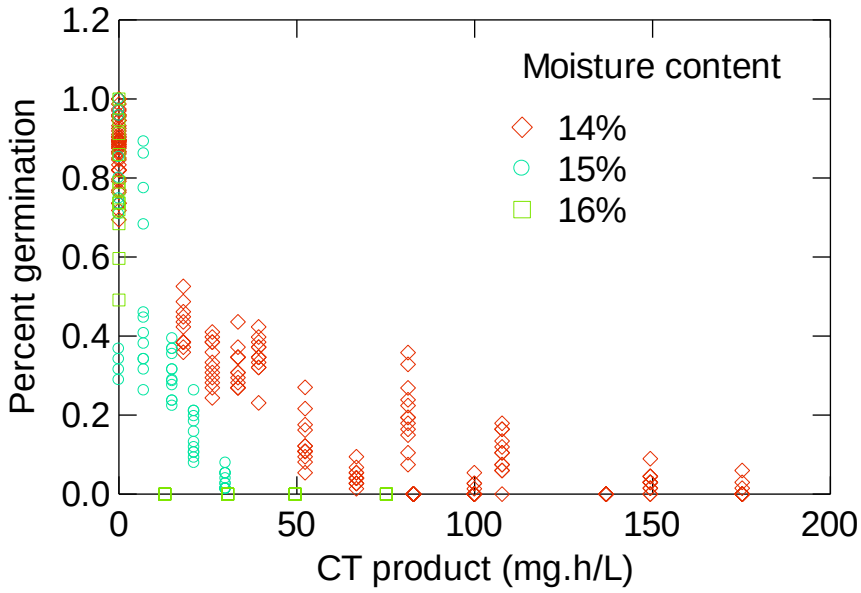

Figure 7.4 Response of white sorghum to EDN and moisture. CT products calculated by least squares method from sorption measurements taken over the duration of the trials.

7.2.2 Efficacy of EDN against commodities – variety responses at 14% moisture

Regression of transformed seed viability assessments against the calculated CT products for each dose generally gave significant regression coefficients for the 14% moisture content commodities ($0.67>R^2<0.99$). For three maize replicates tested the lowest applied dose resulted in complete

devitalisation of the test seed. Thus the ED_{95} estimates for these replicates were based on a poor spread of points. As these values would in any case result in an overestimate of the ED_{95}estimate, it was considered safe to include these results in the analysis.

Commodity type was highly significant in the GLM analysis of ED_{95} values of 14% moisture content commodities (GLM, $P < 0.0005$). Pairwise comparisons (Tukey, $P < 0.0005$) revealed a significant difference between barley and the three other commodities. But there were no significant differences between maize, wheat and sorghum in ED_{95} estimates. Similarly, variety was highly significant (GLM, $P < 0.0005$; Table 7.3). Raw germination data rescaled as a percentage of the highest control germination are presented in Figures 7.5 to 7.8. Variability between the varietal responses is depicted in Figure 7.9.

Table 7.3 Statistics for ED_{95} values calculated for each commodity type at 14% moisture content. SEM is the standard error of the mean.

Commodity	Mean ± SEM (mg h L^{-1})	Range (mg h L^{-1})
Barley	702±103	422-1207
Maize	144±18	53-298
sorghum	162±25	100-224
Wheat	127±11	83-263

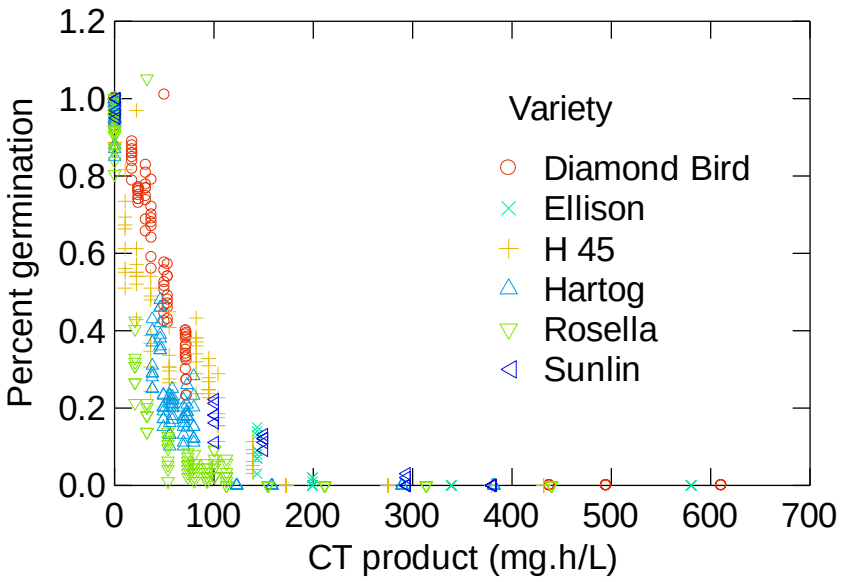

Figure 7.5 Percent germination of wheat varieties treated with EDN. CT products calculated by the least squares method from sorption measurements taken over the duration of the trials.

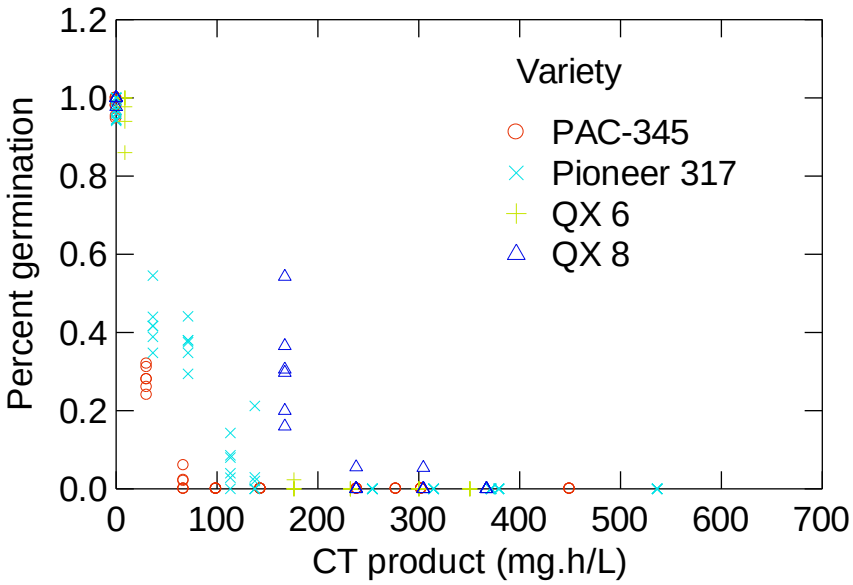

Figure 7.6 Percent germination of maize varieties treated with EDN. CT products calculated by the least squares method from sorption measurements taken over the duration of the trials.

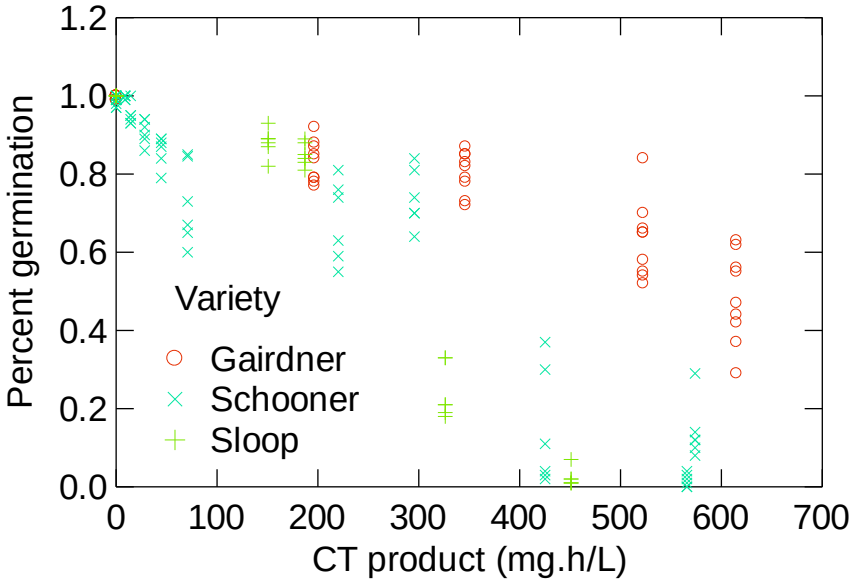

Figure 7.7 Percent germination of barley treated with EDN. CT products calculated by the least squares method from sorption measurements taken over the duration of the trials.

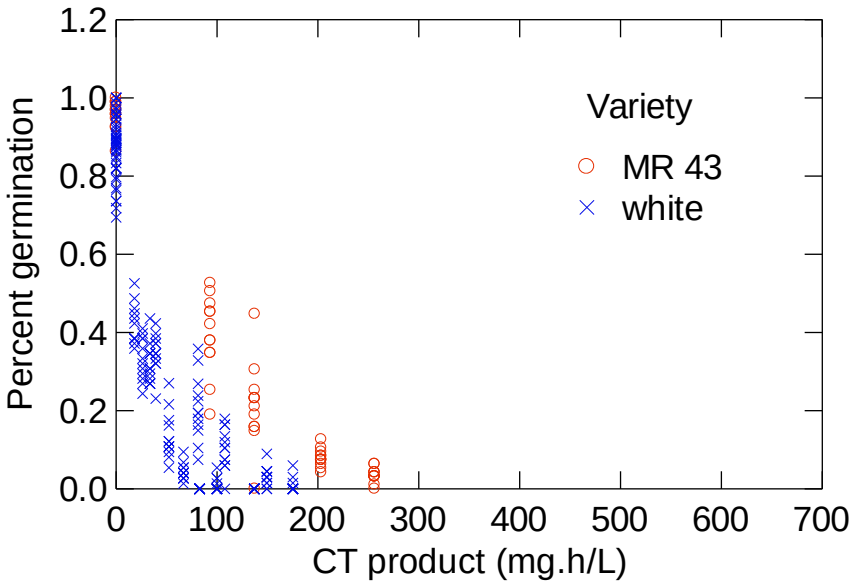

Figure 7.8 Percent germination of sorghum treated with EDN. CT products calculated by the least squares method from sorption measurements taken over the duration of the trials.

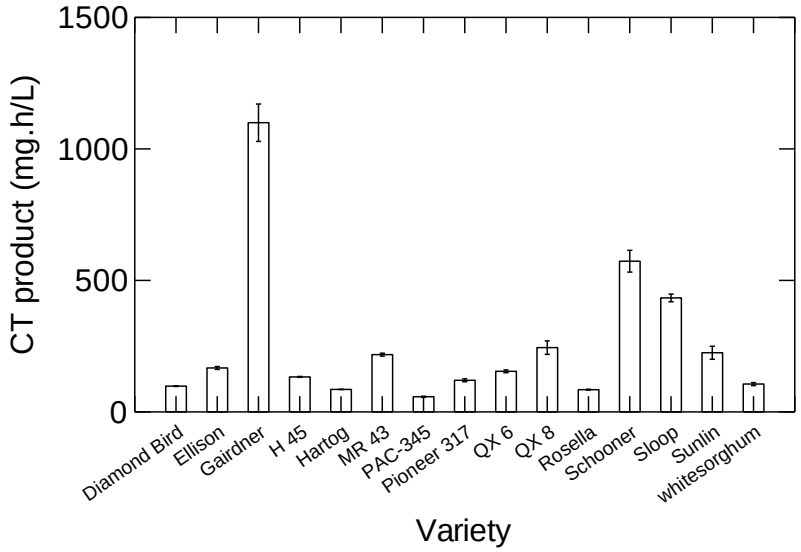

Figure 7.9 ED_{95} values expressed as CT product, mg h L^{-1} for each of the varieties of wheat, barley, maize and sorghum fumigated with ethanedinitrile (EDN) at 14% moisture content. Error bars depict standard error of the mean.

7.2.3 Head space loss (sorption) of EDN on the commodities tested

Analysis by GLM revealed a significant effect of commodity type and moisture content on the extinction coefficient (k) a term that describes the constant rate of headspace loss of EDN ($P = 0.004$, $P \leq 0.0005$ respectively). There was also a significant interaction between moisture content and

commodity type. This is evident in the response of sorghum at 15% which breaks the consistent trend of increase in extinction coefficient with moisture content (Figure 7.10). In pairwise comparisons, all commodities were significantly different (Tukey, $0.005 \geq P \leq 0.032$), with the exception of sorghum and wheat (Tukey, $P = 0.068$). At the critical value of 14% moisture, the commodities ranked maize, barley, wheat and sorghum in order of increasing rate of headspace loss. There was also a relationship between the extinction coefficient (k) and the initial concentration applied (C_o) with the extinction coefficient declining with increasing C_o (Table 7.4).

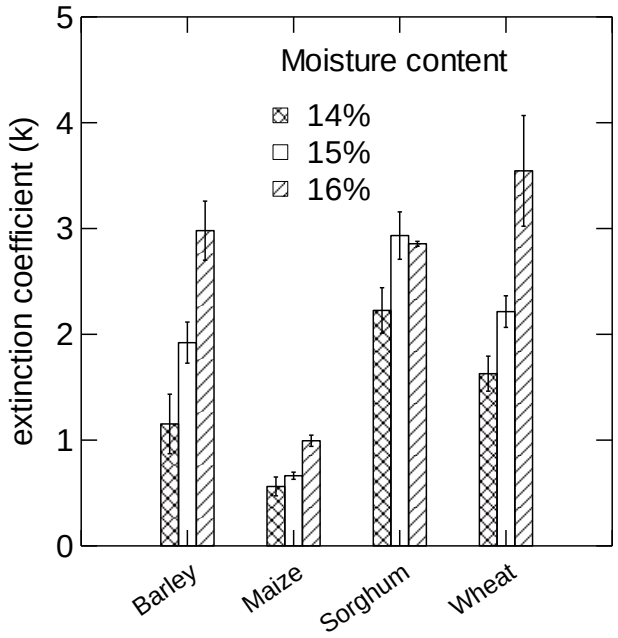

Figure 7.10 Extinction coefficients (k) for barley, maize, sorghum and wheat at three moisture contents. Error bars depict standard error of the mean.

Table 7.4 Extinction coefficients (k) for barley, maize, sorghum and wheat at different moisture contents and initial doses (C_o).

Commodity	Variety	Initial dose (C_o)	Extinction coefficients at Moisture content		
			14%	15%	16%
Wheat	H45	20	2.20	2.52	
Wheat	H45	40	2.12	2.83	
Wheat	H45	60	2.00	2.23	
Wheat	H45	80	1.83	2.01	
Wheat	H45	100	1.99		4.32
Wheat	H45	110	1.89		
Wheat	H45	120	2.01	2.46	
Wheat	H45	140	1.76		4.06

Commodity	Variety	Initial dose (C_o)	Extinction coefficients at Moisture content		
			14%	15%	16%
Wheat	H45	160	1.27	2.23	
Wheat	H45	180			3.44
Wheat	H45	200	1.03	1.80	
Wheat	H45	240	0.73	1.64	2.36
Wheat	H45	260	0.69		
Maize	Pioneer 317	20	0.69	0.80	
Maize	Pioneer 317	40	0.88	0.70	
Maize	Pioneer 317	60	0.67	0.67	
Maize	Pioneer 317	80	0.83	0.70	
Maize	Pioneer 317	100			1.12
Maize	Pioneer 317	120	0.46	0.71	
Maize	Pioneer 317	140	0.30		0.99
Maize	Pioneer 317	160	0.37	0.63	
Maize	Pioneer 317	180			0.92
Maize	Pioneer 317	200	0.30	0.51	
Maize	Pioneer 317	240		0.58	0.94
Barley	Schooner	20	2.45	2.52	
Barley	Schooner	40	2.15	2.35	
Barley	Schooner	60	1.74	2.53	
Barley	Schooner	80	1.37	2.38	
Barley	Schooner	100			3.14
Barley	Schooner	120	0.63	1.90	
Barley	Schooner	140			2.59
Barley	Schooner	160	0.64	1.76	
Barley	Schooner	180			2.62

Commodity	Variety	Initial dose (C_o)	Extinction coefficients at Moisture content		
			14%	15%	16%
Barley	Schooner	200	0.50	1.31	
Barley	Schooner	240	0.40	1.18	3.57
Barley	Schooner	260	0.50	1.38	
Sorghum	white sorghum	20	1.87	3.76	
Sorghum	white sorghum	40	2.46	3.20	
Sorghum	white sorghum	60	2.03	3.62	
Sorghum	white sorghum	80	2.10	3.18	
Sorghum	white sorghum	100			2.89
Sorghum	white sorghum	120	1.75	2.48	
Sorghum	white sorghum	140			2.86
Sorghum	white sorghum	160	1.97	2.66	
Sorghum	white sorghum	180			2.80
Sorghum	white sorghum	200	1.90	2.37	
Sorghum	white sorghum	240	2.21	2.20	2.88
Sorghum	white sorghum	260	3.74		

7.3 Conclusion

Wheat, which comprised the bulk of varieties tested, had the least variability in response to EDN treatment (Figure 7.5). Barley was the most difficult to kill with only the variety Sloop showing complete mortality in the range tested. The variability of response across and within commodities was also a concern but in the light of the higher CTs needed to control exotic weeds, doses in excess of 2400 mg h L^{-1}, it is likely that all commodities would be devitalised. At these levels (see Section 6) barley, the most difficult commodity to devitalise, was completely controlled. The moisture content of the grain had a marked effect on devitalisation by EDN. This could have potential for large scale application where moisture level could be manipulated to increase the susceptibility of the grain. However this would have to be balanced against higher head space loss (sorption onto the grain) at high moisture and how this might affect other target species contaminating the grain bulk. How the moisture affects the efficacy of the fumigant is not understood, and further investigation may provide valuable insight into the optimal application and management of grain fumigation with EDN.

The different headspace losses (sorption) detected between the different commodities is an important result. The differential sorption between the commodities indicates that there will be significant differences in the cost of treating the different commodities. It is clear from the study on

weeds that the dose required to devitalise many of the weeds will be in excess of that required for the commodities. However, if the weed seed is present as a contaminant in a bulk of commodity with high headspace loss (large extinction coefficient), the exposure of the weed to the fumigant would be less than if it had been present in a commodity with lower rate of headspace loss. Therefore, to attain a given treatment (a CT product specification) EDN treatment of maize would likely be more economic than any of the other commodity treatments at 14% moisture content, then barley, wheat and sorghum respectively.

It is also worth noting that the germination estimates used in this experiment are conservative to the extent that they are based on the pooled data from the categories of normal, low vigour and abnormal germination. In practice, this will tend to overestimate the amount of fumigant needed for devitalisation as abnormal seeds are unlikely to develop into a viable plant. A crude recommendation for efficacy would be provided by a doubling of the ED_{95} values presented here for a very high level of confidence.

8 Residues of ethanedinitrile in treated grain

The nature of residues remaining after treatment with EDN need to be considered as this forms an important part of data required for registration and residues may have an impact on the palatability of treated commodities. In this study, unchanged residues were assessed in barley, wheat and maize and changed residues were assessed in these species plus in oats as well.

8.1 Methods

Unchanged residues of EDN remaining after treatment at 70, 140, 210 and 280 mg L^{-1} and aeration over four days were assessed using a micro-wave extraction method (Ren and Desmarchelier 1998). After treatment and airing, 5 g samples of the treated commodity were placed into 250 mL flasks. The flasks were sealed and headspace concentrations of EDN were measured after microwave extraction.

Changed residues were measured in samples of wheat, barley, oats and maize treated with EDN at 90% fill ratio at 200, 400 and 600 mg l. The samples were kept in a sealed container to prevent loss of volatile residues and were measured after four months. The converted residues were assessed by measuring the ammoniacal nitrogen (NH_4-N), nitrate nitrogen (NO_3-N) and nitro nitrogen (NO_2-N) content of treated commodities using a Nitrogen Analyser and comparing these with the levels in untreated controls.

8.2 Results and discussion

Table 8.1 shows that sample levels after treatment with EDN did not have significantly higher levels of nitrate nitrogen (NO_3-N) and nitro nitrogen (NO_2-N) than the controls - a very positive result. The major residue generated by fumigation with EDN was ammoniacal nitrogen (NH_4-N). The levels of ammoniacal nitrogen (NH_4-N) were about two times higher than natural levels in barley, wheat and oats, and 4 times higher in maize. The increase in ammoniacal nitrogen (NH_4-N) residue was only slightly correlated with dose.

Table 8.1. Changed nitrogen residues in barley, wheat, oats and maize treated with ethanedinitrile from 200-600 mg L^{-1} compared to control samples.

Commodity	EDN Dose mg/L	NH_4-N (mg/kg)	NO_3-N+NO_2-N (mg/kg)	NO_2-N (mg/kg)
Barley	200	42.20		0.05

Commodity	EDN Dose mg/L	NH_4-N (mg/kg)	NO_3-N+NO_2-N (mg/kg)	NO_2-N (mg/kg)
	400	55.35	0.12	0.02
	600	59.46	0.19	0.04
	600	60.74	0.02	0.03
	control	24.89	0.00	0.05
	control	25.57	0.04	0.06
Wheat	200	65.35	0.06	0.06
	200	66.95	0.09	0.03
	400	79.74	0.15	0.06
	400	83.74	0.21	0.08
	600	82.91	0.89	0.10
	600	84.30	0.71	0.11
	control	44.24	0.65	0.11
	control	45.56	0.63	0.07
Oats	200	41.03	1.59	0.07
	400	52.49	1.38	0.05
	600	62.79	1.07	0.08
	600	72.91	2.31	0.08
	control	26.98	1.84	0.07
	control	27.15	1.59	0.07
Maize	200	60.41	0.40	0.03
	400	65.73	0.42	0.05
	600	84.02	0.42	0.03
	600	79.69	0.42	0.03

Commodity	EDN Dose mg/L	NH_4-N (mg/kg)	NO_3-N+NO_2-N (mg/kg)	NO_2-N (mg/kg)
	control	18.75	0.40	0.03
	control	19.03	0.41	0.03

Results for unchanged residues shown in Table 8.2 indicate that unchanged residues decline to background levels after 8 days of aeration. For comparison, it should be noted that natural levels of EDN are 0.01 mg/kg in canola and 0.025 mg/kg in canola cake, values that are higher than in the treated commodities after 8 days of aeration.

Table 8.2 Decline over 8 days in unchanged residues microwave-extracted from 5g samples of barley, wheat and maize and estimated from headspace concentration in a sealed 250mL bottle. The commodities were exposed to EDN for 4 days at 5% fill ratio in sealed 305mL flasks.

Grain	EDN Dose (mg/L)	EDN Residue Concentration (mg/kg)			
(Moisture Content))		Day 0	Day 1	Day 4	Day 8
Barley	100	16.8	3.7	0.1	< 0.005
14.46%mc	100	16.9	3.8	0.1	< 0.005
	200	28.9	5.1	0.2	< 0.005
	200	30.9	5.9	0.2	< 0.005
Wheat	100	7.2	0.6	0.06	< 0.005
14.35%mc	100	6.7	0.7	0.05	< 0.005
	200	20.3	4.9	0.1	< 0.005
	200	21.9	4.6	0.1	< 0.005
Maize	100	11.0	1.7	0.1	< 0.005
13.6%mc	100	14.8	2.5	0.1	< 0.005
	200	33.2	6.9	0.3	< 0.005
	200	35.4	6.7	0.3	< 0.005
Sorghum	100	11.7	3.4	0.1	< 0.005

14.4%mc	100	17.4	3.3	0.1	< 0.005
	200	32.9	6.1	0.3	< 0.005
	200	31.4	5.9	0.3	< 0.005

Canola: the natural level of EDN is 0.010mg/kg and of its cake is 0.225mg/kg

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These results are promising from the perspective of both the regulatory and the feed palatability issues. The data indicate that there is little of concern in changed nitrogen residues and given that addition of ammoniacal nitrogen to feedstock in the form of urea is common practice in the feedstock industry, the small elevation of ammoniacal nitrogen should not present a significant palatability problem.

9 Towards a treatment schedule for EDN fumigation of commodities

Objective 11 in the context of the original proposal was to formulate a recommendation that could be used to assess the prospects for further research to develop EDN as a grain fumigant. It was formulated on the basis that a single dose was to be applied to the commodity in a commercial scale application. Under these conditions there are flammability limits that must be considered, and only so much volume of gas can be placed between the grains. However, in the light of the sorption properties of the commodities and the amount of EDN needed to devitalise all target organisms, it has become clear that multiple or continuous dosing would be employed in any large scale EDN application. Under these conditions the 'absolute maximum dose schedule that can be applied' as is stated in Objective 11 is no longer relevant. In fact the amount of chemical that can be applied is limited only by economics and the time available under multiple dose application. For the purpose of planning further work an estimate of an effective devitalising treatment is more relevant.

The commodities were all controlled within the 300-1200 mg h L^{-1} range. This is well below the dose of 2400 mg h L-1 used to screen the weed species. Many weed species were controlled at this dose. However, specifying a dose which can safely devitalise those species is hampered by the fact that the discriminating dose is only the first pass screen. Doubling the dose to 4800 mg h L^{-1} provides a significant margin of safety in the absence of further work. This value would generally be sufficient to control the smut surrogate and the surrogate for cephalosporium stripe. The situation for the downy mildew surrogate is not simple, as the results can at best be considered preliminary and lack precision. The viable growth on agar media was narrowed to a value between 6700 and 13800 mg h L^{-1}. However, further work is needed to fully explore the responses between these values. Finally there is the problem of shielding of the various fungal propagules against the fumigation treatment. This was outside the scope of the FLOT 124 project but should be a priority for future work. Given these uncertainties it is not possible to accurately define a treatment schedule that can achieve complete devitalisation of all target organisms. For the purposes of further study, and in the absence of further research to refine the results collected to date, a CT product of 13800 mg h L^{-1} would be a starting point. However significant cost savings could be made if further research identified a lower effective dose for the downy mildew surrogate. An estimate of the cost of treating maize for bulk prices of EDN ranging from USD$3-20 per kg is based on a CT product of 13,800 mg hL^{-1} and the sorption results of 90% fill ratio tests for maize. The CT was estimated from the measured levels within the flasks over 24 hours using the formula

$$\mathbf{Ct}_{n,n+1} - (T_{n+1} - T_n) \times \square (C_n \,.\, C_{n+1}) \ (g\ h\ m^{-3}) \qquad (1)$$

where

- T_n is the time the first reading was taken in hours
- T_{n+1} is the time the second reading was taken in hours
- C_n is the concentration reading at T_n in gm^{-3}
- C_{n+1} is the concentration reading at T_{n+1} in g m^{-3}
- $\mathbf{Ct}_{n,n+1}$ is the calculated **Ct** product between T_n and $T_{n+}1$ in g h/m^3

Using this information and a stowage factor of 1.7, the average cost of gas was calculated as:

Cost/tonne @ USD$3/kg	Cost/tonne @ USD$10/kg	Cost/tonne @ USD$20/kg
$11.96	$39.86	$79.73

10 Success in achieving objectives

Objectives 1 to 4 were met on time without incident. They are summarised in the first section of the report and provide the foundation for the following experimental work. The incursion risks identified in these objectives and the choice of surrogate organisms for study were devised in consultation with Biosecurity Australia. However, in taking the results beyond the proof of concept there is no guarantee that the risk analysis by Biosecurity Australia would come to the same conclusion as has been reached here, or that new risks may have appeared in the risk assessment environment.Close consultation with Biosecurity Australia has tried to minimise this possibility.

The outcome of material compatibility with ethanedinitrile (EDN) was very favourable. Some issues were highlighted such as the sorption of fresh concrete and the gas permeability of some tarping materials. However these are considered manageable factors in any upscale of fumigation.

The most difficult aspect of the project was the pathogen work (Objectives 7 and 8). Development of a vital stain, previously untested on smuts, proved to be successful for the chosen surrogate based on the responses of autoclaved and non-autoclaved spores. In EDN fumigation tests the surrogates for smuts and cephalosporium stripe were susceptible to fumigation despite the fact that germination studies indicated that the vital staining technique underestimated the devitalisation of the smut spores. However, significant difficulties were encountered in testing the sorghum downy mildew surrogate. Widely used methods to stimulate production of oospore propagules initially failed to generate viable spores. In the short time frame of the project this was a significant setback, as the time to undertake this process is measured in months. When the spores were eventually generated, viability staining indicated that the spores were viable after very high levels of EDN treatment, despite the fact that wall structures appeared significantly disorganised. Limited post treatment growth assessments undertaken late in the project have shown that the oospores can be killed by EDN, but further work is needed. The results are sufficient to assess the merits of taking the project to the next stage and experience has been gained to focus any further work on surrogates, or the incursion risks themselves.

The discriminating dose (comprising Objectives 5 and 9) was very successful in terms of providing a workable ranking of tolerance to identify a subset of the weeds for further study. It also demonstrated complete devitalisation of the four commodities. The outputs for weed species testing have been impressive in view of the volume of testing undertaken and given that there was no time to develop and optimize germination protocols. In terms of the efficacy of the chemical it is apparent

from preliminary testing undertaken following the discriminating dose that a subsection of the weeds tested will not be controlled at feasible levels of EDN treatment. On the positive side the majority of the weed species in the non-testable category belonged to taxonomic families that showed consistent control in the discriminating dose.

Commodity testing was completed on a range of varieties of the four commodities at 14% moisture content and on a subset of the commodities at 15 and 16% moisture. This gave good estimates of variety variability and highlighted the effect of moisture content in increasing efficacy of the EDN fumigation. Intensive headspace sampling undertaken during the fumigations identified differences between the commodities that would translate into significant differences in economy of treatment. This was a very important outcome of the project, as the interaction of the gas with the commodity will be the dominant factor when the commodity treatment is scaled up in size. These issues have been highlighted to the management committee resulting in a shift in attention from wheat to maize as the most promising commodity for future study, and up scaling of EDN fumigation.

The 'proof of concept' and dose schedule (Objective 11) have been achieved within the limits of having only worked on surrogates not actual pathogens, on the basis that not all weed species are controllable by EDN treatment alone. The management committee had waived the $10 per tonne commodity target for EDN treatment during the course of the project.

11 Impact on Meat Industry – Now and In Five Years Time

The impact of the research reported here lies in the future development of EDN fumigation for quarantine treatment of imported grain. If pursued and successful this would fall into a five-year horizon. Immediate benefits are not realisable due to the preliminary nature of this research, lack of an import protocol and the pending registration of the fumigant. If the quarantine treatment of bulk grain can be successfully commercialised then the impacts on the industry are likely to be significant. By opening market access to feed grain in periods of limited supply the feedlot industry could be assured of stabilised grain pricing during periods of drought and graziers may also benefit from downward price pressure on supplemental feed during times of shortage. While there are still significant risks and hurdles to the full development of EDN for quarantine treatment of imported grain the potential benefits to the meat and livestock industry are likely to be significant and far reaching.

12 Conclusions and recommendations

Recent drought conditions have greatly affected the profitability of the meat production industry. If unseasonal weather events become more frequent, the livestock industry will suffer greater exposure to high prices and shortfalls in feed grain. The cost effective fumigation of imported grain could provide relief from these events. At present EDN appears to be the only candidate fumigant that could potentially treat imported grain to a quarantine standard. The ability to devitalise the commodities and pathogens satisfies the main quarantine concerns. While full control of weed species was not achieved at feasible doses, these species might potentially be controlled for quarantine purposes by seed screening and other protocols. The work presented here is only preliminary and whether EDN treatment can be cost effective is yet to be determined. However in light of the potential benefits, it is concluded that the investment risk associated with the further development of EDN treatment for imported grain treatment is justified.

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14 Appendix A

14.1 Pathogens of wheat and barley from the UK

Bacteria associated with wheat from UK					Recorded in								
Family	**Genus**	**Species**	**Disease caused**	**Paper ref source**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed ?**
Rhizobiaceae	*Agrobacterium*	*tumefaciens*	vector for virus	Dale et al. 1988a,b	+	+	+	+	+	+	-	Y	
Enterobacteriaceae	*Erwinia*	*rhapontici*	crown rot (pink grain)	Roberts 1974; Sellwood & Lelliott 1978	+	+	+	+	+	+	+	Y	
Enterobacteriaceae	*Erwinia*	*carotovora*	bacterial root rot	Bergey et al. 1923; Anon 1999	+	+	-	+	+	+	+	Y	
Pseudomonadaceae	*Pseudomonas*	*syringae pv coronafaciens*	halo blight	Young et al. 1978; Anon 1999	+	+	+	+	+	+	+	Y	
Pseudomonadaceae	*Pseudomonas*	*cichorii*	lettuce varnish spot	Stapp 1928; Anon 1999	-	+	-	-	-	+	-	Y	
Pseudomonadaceae	*Pseudomonas*	*fuscovaginae*	sheath rot	Duveiller and Maraite 1990	-	-	-	-	-	-	-	N	
Microbacteriaceae	*Rathayibacter*	*rathayi*	gumming disease	Zgurskaya et al. 1993; Anon 1999	+	+	+	+	+	+	+	Y	
Xanthomonadaceae	*Xanthomonas*	*translucens*	leaf streak	Duveiller 1994b	-	+	-	-	-	-	-	Y	

Nematodes associated with UK wheat						Recorded in								
Family	**Genus**	**Species**	**Disease caused**	**Paper Reference**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk on devitalised seed**
Hoplolaimidae	*Helicotylenchus*	*dihystera*	Common spiral nematode			-	+	-	-	-	+	-	Y	
Pratylenchidae	*Pratylenchus*	*penetrans*	Northern root lesions			+	+	+	+	+	+	+	Y	
Pratylenchidae	*Pratylenchus*	*thornei*	Root cortex damage			+	+	+	+	+	+	+	Y	
Rhabditidae	*Phasmarhabditis*	*hermaphrodita*	none	Wilson et al 1993	control of slugs in wheat	-	-	-	-	-	-	-	N	
Anguinidae	*Anguina*	*tritici*	seed gall	Southey,1972		+	+	+	+	+	+	+	Y	

Viral Pathogens of wheat from UK			Recorded in								
Scientific name	**Disease**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed ?**
Barley mild mosaic bymovirus (BaMMV)	Chlorotic flecks	Boyle et al. 1988	-	-	-	-	-	-	-	N	
Barley stripe mosaic hordei virus (BSMV)	Brown stripes	Wolfe 1987	-	-	-	+	-	+	-	Y	
Barley yellow dwarf luteoviruses (BYDV)	purpling, reddening	Cameron et al. 1988; Starling & Richards 1990; Boyd et al. 1993; Davis et al. 1987	+	+	+	+	+	+	-	Y	
Phleum mottle virus (PhMV)	chlorotic mottle	Jenkyn et al. 1996	-	-	-	-	-	-	-	N	
Soil-borne wheat mosaic virus (SBWMV)	leaf mosaic	Rennie 1987	-	-	-	-	-	-	-	N	
Striate mosaic virus (WSMV)	Striate mosaic	Hide & Read 1991	-	-	-	-	-	-	-	N	
wheat streak mosaic virus (WSMV)	Streak/mottling	McGrath & Bale 1989	-	-	-	-	-	-	-	N	
wheat yellow mosaic virus (WYMV)	yellow mosaic	Peeters et al. 1990	-	-	-	-	-	-	-	N	

Fungi associated with wheat from UK						Recorded in								
Phylum	**Genus**	**Species**	**Disease caused**	**Paper ref**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
Mitosporic fungi	*Alternaria*	*alternata*	blackpoint of grain	Magan N, 1993; Ellis & Gooding 1996		+	+	+	+	+	+	+	Y	
Mitosporic fungi	*Alternaria*	*triticina*	leaf blight	AQIS list		+	+	+	+	+	+	+	Y	N/A
Ascomycota	*Blumeria*	*graminis*	powdery mildew	Anon 1999		+	+	+	+	+	+	+	Y	
Ascomycota	*Botrytis*	*cinerea*		Ushaet al. 1989		-	-	-	-	-	-	-	N	
Basidiomycota	*Ceratobasidium*	*cereale*	Sharp eyespot	Anon 1999		-	-	-	-	-	-	-	N	
Mitosporic fungi	*Cladosporium*	spp.	ear rot	Wainwright et al. 1992; Mantle & Swan 1995; Magan & Lacey 1986; Ajayi & Dewar 1983		-	-	-	-	-	-	-	N	
Ascomycota	*Claviseps*	*purpurea*	ergot	Shaw 1988; Shaw 1986		-	+	+	+	+	+	+	Y	
Ascomycota	*Drechslera*	spp.		Cameron et al. 1988		-	-	-	-	-	-	-	N	
Mitosporic fungi	*Epicoccum*	*nigrum*		Magan & Lacey 1986		-	-	-	-	-	-	-	N	
Ascomycota	*Erysiphe*	*graminis*	mildew	Hu et al. 1988; O'Dell et al. 1989; Kettlewell et al. 1990; Dover & East 1990; Dovgan 1990; Borodanenko &		-	-	-	-	-	-	-	N	

Fungi associated with wheat from UK						Recorded in								
Phylum	**Genus**	**Species**	**Disease caused**	**Paper ref**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
				Spirina 1989; Weltzien 199031										
Ascomycota	*Eurotium*	*amstelodami*	grain mould	Magan 1993		-	-	-	-	-	-	-	N	
Ascomycota	*Fusarium*	*nivale*		Cameron et al. 1988; Usha 1989; Parry 1990; Sharman et al. 1991		-	-	-	-	-	-	-	N	
Mitosporic fungi	*Fusarium*	*culmorum*	stem base rot	Bateman 1993; Pettitt et al. 1993; Parry et al. 1995; Pettitt et al. 1996		+	+	+	+	+	+	+	Y	
Ascomycota	*Fusarium*	*avenaceum*	root rot	WF68		-	-	-	-	-	-	-		
Ascomycota	*Fusarium*	*sporotrichioides*				+	+	+	+	+	+	+	Y	
Ascomycota	*Gaeumannomyces*	*graminis*	Take-all	WF9,16,39,54,55,94		+	+	+	+	+	+	+	Y	
Mitosporic fungi	*Hymenula*	*cerealis*	Cephalosporium stripe	AQIS list		-	-	-	-	-	-	-	N	seed-borne
Ascomycota	*Leptosphaeria*	*nodorum*		WF8		-	-	-	-	-	-	-	N	
Ascomycota	*Microdochium*	*nivale*		WF68,78,131	most common	-	-	-	-	-	-	-		
Ascomycota	*Mycosphaerella*	*graminicola*	leaf spot	WF75,80,220		+	+	+	+	+	+	+	Y	
Basidiomycota	*Neovossia*	*indica*	karnal bunt	WF186		-	-	-	-	-	-	-	N	

Fungi associated with wheat from UK						Recorded in								
Phylum	**Genus**	**Species**	**Disease caused**	**Paper ref**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
Mitosporic fungi	*Penicillium*	*aurantiogriseum*	Grain mould	WF85		-	-	-	-	-	-	-	N	
Plasmodiophoromycota	*Polymixa*	*graminis*	vector of streak mosaic	Anon 1999 , 1999		-		-	-	-	-	-	N	
Ascomycota	*Pseudocercosporella*	*herpotrichoides*	eyeSpot	WF7,10,27,30,35,44,48,146	found on decaying stem and leaf	+	+	+	+	+	+	+	Y	
Basidiomycota	*Puccinia*	*striiformis*	Stripe rust	WF5,41,42,46,53,56,83,358		+	+	+	+	+	+	+	Y	
Basidiomycota	*Puccinia*	*graminis*		WF6,14,36,46,53		+	+	+	+	+	+	+	Y	
Basidiomycota	*Puccinia*	*hordei*	brown rust	WF17		+	+	+	+	+	+	+	Y	
Basidiomycota	*Puccinia*	*recondita*	brown rust	WF17,36,41,46,273,297		+	+	+	+	+	+	+	Y	
Ascomycota	*Pyrenophora*	*teres*	net blotch	WF50		-	-	-	-	-	-	-	N	
Ascomycota	*Pyrenophora*	*tritici-repentis*	tan spot	WF178		+	+	+	+	+	+	+	Y	
Oomycota	*Pythium*	*oligandrum.*	root rot	WF61		+	+	+	+	+	+	+	Y	
Oomycota	*Pythium*	*anguioides*	root rot	WF68		-	-	-	-	-	-	-	N	
Basidiomycota	*Rhizoctonia*	*cerealis*	Sharp eyespot	WF10,279,382	less frequent	-	-	-	-	-	-	-	N	
Mitosporic fungi	*Septoria*	*tritici*	leaf blotch	WF32,45,49		-	-	-	-	-	-	-	N	
Mitosporic fungi	*Septoria*	*nodorum*	glume blotch	WF32,44		-	-	-	-	-	-	-	N	

Fungi associated with wheat from UK						Recorded in								
Phylum	**Genus**	**Species**	**Disease caused**	**Paper ref**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
	Tapesia	*yallundae*	eyespot	WF106,108,132		-	-	-	-	-	-	-	N	
Basidiomycota	*Tilletia*	*tritici*	Bunt of wheat	WF11,36,42	via soil	+	+	+	+	+	+	+	Y	
Basidiomycota	*Tilletia*	*controversa*	Dwarf bunt	AQIS list		+	+	+	+	+	+	+	Y	resting spores
Basidiomycota	*Tilletia*	*caries*	Bunt of wheat	WF69		-	-	-	-	-	-	-	N	
Basidiomycota	*Tilletia*	*indica*	kernal bunt	AQIS list		-	-	-	-	-	-	-	N	Yes
Basidiomycota	*Typhula*	*incarnata*	snow blight	Anon 1999		-	-	-	-	-	-	-	N	
Basidiomycota	*Ustilago*	*nuda var.tritici*	loose smut	WF12,36,47,130	infects seed embryo	-	-	-	-	-	-	-	N	Resting spores
Mitosporic fungi	*Verticillium*	*lecanii*		WF291		-	-	-	-	-	-	-	N	

Bacteria associated with Barley from UK						Recorded in								
Family	**Genus**	**Species**	**Disease caused**	**References**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
Pseudomonadaceae	*Pseudomonas*	*syringae* pv.*syringae*	leaf blight	Georgakopoulos & Sands 1992										Nil ?
Pseudomonadaceae	*Pseudomonas*	*syringae* pv.*striafaciens*	stripe blight	Young & Triggs 1994										
Xanthomonadaceae	*Xanthomonas*	*translucens* pv. *Translucens*	Bacterial blight	Vauterin et al. 1995										

Nematodes associated with Barley from UK						Recorded in								
Family	**Genus**	**Species**	**Disease caused**	**References**	**Notes**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Potential risk for devitalised seed**
Heteroderidae	*Heterodera*	*avenae*	Root stunting	Evans et al. 1993	Cerea Cyst nematode									
Meloidogynidae	*Meloidogyne*	*naasi*	Root gall	Evans et al. 1993	Root Knot									
	Subanguina	*radicicola*	Root gall	Kort 1972										
Pratylenchidae	*Pratylenchus*	*penetrans*	Root lesion										Y	
Pratylenchidae	*Pratylenchus*	*thornei*	Root lesion										Y	

Viral pathogens of barley from UK			Recorded in								
Scientific name	**Disease**	**References**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS**	**Potential risk for devitalised seed ?**
Barley mild mosaic virus (BaMMV)	chlorotic flecks	Adams 1991	-	-	-	-	-	-	-	N	
Barley stripe mosaic hordei virus (BSMV)	brown stripe	Atabekov & Novikov 1989	-	-	-	+	-	+	-	Y	
Barley yellow dwarf viruses (BYDVs)	reddening, purpling, stunting	Starling & Richards 1990	+	+	+	+	+	+	-	Y	
Brome virus (BV)	Yellow/white spots/streaks	Lane 1989.	-	-	-	-	-	-	-	N	
Oat sterile dwarf virus (OSDV)	Dark green stunting	Boccardo & Milne 1980	-	-	-	-	-	-	-	N	
wheat streak mosaic virus (WSMV)	Streaking/mottling	Brakke 1971	-	-	-	-	-	-	-	N	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Ascochyta	*horde*		Ascochyta leaf spot	Anon 1999								NO	NO	
Cephalosporium	***gramineum***	*Hymenula cerealis*	Cephalosporium Stripe	Anon 1999; Anon 1986								NO	YES	
Claviceps	***purpurpea***	*Sphacelia segetum*	Ergot	Anon 1999	x	x		x	x	x		YES	YES	
Cochliobolus	*sativus*		Common Root Rot/ Spot Blotch	Anon 1999	x	x	X	x	x	x		YES	YES	
Colletotrichum	***graminicola***	*Bipolaris sorokiniana, Helminthosporium sativum*	Anthracnose	Anon 1999; Walker, McLeod 1971; Ramsey 1990a,b; Peel 1982	-	x				x		YES	YES	
Drechslera	*wirreganensis*			Wallwork et al. 1992			x					YES	NO	
Erysiphe	***graminis* f. sp. *hordei***	*Blumeria graminis f. sp. Hordei, Oidium monilioides*	Powdery Mildew	Anon 1999; Polley et al. 1993								YES	YES	
Fusarium	*avenaceum*	*Gibberella avenacea*	Scab or Head Blight	Anon 1999; Jenkinson & Parry 1994; Nik & Parbery 1977	x			x				YES	YES	
Fusarium	*poae*		Scab or Head Blight	Anon 1999; Jenkinson & Parry 1994; Ding et al. 1995; Nik & Parbery 1977								YES	YES	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Fusarium	*sporotrichiodes*		Scab or Head Blight	Anon 1999								NF	NF	
Fusarium	*culmorum*		Common Root Rot	Anon 1999; Jenkinson & Parry 1994	x	x		x	x			YES	YES	
Fusarium	*graminearum*	***Gibberella zeae**, Gibberella roseum* f. sp. *cerealis, Fusarium saubinettii*	Crown Rot	Anon 1999; Jenkinson & Parry 1994	x	x	x			x	x	YES	YES	
Fusarium	*nivale*	*Microdochium niviale, Gerlachia nivalis,* ***Monographella nivalis**, micronectriella nivalis, Calonectria nivalis, Griphosphaeria nivalis*	Scab or Head Blight/Snow Mold	Anon 1999, Usha et al. 1989; Jennings & Turner 1996; Baldwin 1990								NO	NO	
Fusarium	*pseudograminearum*		Scab or Head Blight	Anon 1999								YES	YES	
Gaeumannomyces	*graminis var tritici*		Take-all	Anon 1999	x	x	x	x	x	x		YES	YES	
Leptosphaeria	*herpotricoides*		Leptosphaeria Leaf Spot	Anon 1999								NF	NF	
Leptosphaeria	*nodorum*			Anon 1999; Anon 1986	x	x	x	x	x	x		YES	YES	
Pseudocercospere lla	*herpotrichiodes*	*Cercosporella herpotrichiodes,* ***Tapesia yallundae***	Eyespot	Anon 1999; Anon 1986	x	x	x		x			YES	YES	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Puccinia	***Graminis; graminis* f. sp. *tritici***		Stem Rust	Anon 1999; Park & Wellings 1992; McLean 1995	x	x	x	x	x	x		YES		
Puccinia	***hordei***	*Puccinia anomala*	Leaf Rust	Anon 1999; Polley et al 1993			x		x			YES	YES	
Puccinia	***coronata***		crown rust	Anon 1999; Rose & Scattini 1994	x	x	x	x		x		YES	NO	
Puccinia	***striiformis* f. sp. *hordei***	*Puccinia glumarum*	Stripe Rust	Anon 1999; Polley et al. 1993	x	x	x		x			YES	YES	
Puccinia	***recondita***		wheat brown rust	c11,13,29; Johnson et al. 1986	x	x	x	x	x	x		YES	YES	
Pyrenophora	***graminea***	*Drechslera graminea, Helminthosporium gramineum*	Barley Stripe	Anon 1999				x				YES	YES	
Pyrenophora	***teres***	*Drechslera teres, Helminthosporium teres*	Net Blotch	Anon 1999; Khan 1988; Polley et al. 1993; Khan 1989		x	x	x				YES	YES	
Pyrenophora	***semeniperda***	*Drechslera verticillata*	Leaf spot	Kahn 1988; Khan & Young 1989			x					YES	NO	
Pyrenophora	***tritici-repentis***	*Pyrenophora trichostoma, Drechslera tritici-repentis, Helminthosporium tritici-repentis*	Yellow Leaf Spot	Anon 1999; Cook & Yarham 1989	x	x		x		x	x	YES	YES	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Pythium	***iwayamai***		Snow Rot	Anon 1999								NO	NO	
Pythium	***okanoganense***		Snow Rot	Anon 1999								NO	NO	
Pythium	***paddicum***		Snow Rot	Anon 1999								NO	NO	
Pythium	***arrhenomanes***		*Pythium* Root Rot	Anon 1999; Waller 1979		x				x		YES	YES	
Pythium	***graminicola***		*Pythium* Root Rot	Anon 1999; Margarey 1986; Bratolovanu & Wallace 1986						x		YES	YES	
Pythium	***tardicrescens***		*Pythium* Root Rot	Anon 1999								NF	NF	
Rhizoctonia	*cerealis*	***Ceratobasidium gramineum***	Sharp Eyspot	Anon 1999								YES	YES	
Rhizoctonia	*solani* (AG 4 and AG 1)	***Ceratobasidium cereale***	Sharp Eyspot	Anon 1999; Balali et al. 1995; Masuhara et al. 1994								YES	YES	
Rhynchosporium	*secalis*	*Marssonia secalis*	Scald	Anon 1999; Anon 1986; Khan 1988; Polley et al. 1993; Wallwork 1995		x	x	x	x			YES	YES	
Sclerotinia	*rolfsii*	***Athelina rolfsii***, *Corticum rolfsii*										YES	NF	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Sclerotinia	*borealis*	*Myriosclerotinia borealis*	Snow Scald	Anon 1999								No	NF	
Sclerophthora	*rayssiae*	*Sclerospora graminicola, Sclerospora macrospora*	Downy mildew	Anon 1999		x	x	x		x		YES	YES	
Selenophoma	*donacis*	*Septoria donacis, Phyllosticta stomaticola, Selenophoma donacis* var. *stomaticola*	Halo Spot	Anon 1999; Khan 1978, 1979, 1988; Polley et al. 1993				x				YES		
Stagonospora	*avenae f. sp. triticea*	*Septoria avenae f. sp. triticea,* ***Phaeosphaeria avenaria* f.sp. *triticea****, Leptosphaeria avenaria* f. sp. *Triticea*	Septoria Leaf Blotch	Anon 1999								YES	Prob.	
Stagonospora	*nodorum*	*Septoria nodorum,* ***Phaeosphaeria nodorum****, Leptosphaeria nodorum*	Glume Blotch	Anon 1999; Baldwin 1990		x		x				YES	YES	
Septoria	*passerinii*		Septoria Leaf Blotch	Anon 1999; Shivas 1989				x				YES		
Tilletia	***caries***			c57								YES	YES	
Tilletia	***controversa***		Dwarf Bunt	Anon 1999		x	x	x				YES	NO	
Typhula	*idahoensis*		Snow Mold	Anon 1999								NO	NO	
Typhula	*incarnata*	*Typhula itoana, Tyhpula graminum*	Snow Mold	Anon 1999; Baldwin 1990								NO	YES	

Fungal pathogens of barley from UK					Recorded in									
Genus	**Species (Teliomorph in bold)**	**anamorph/synonym or teliomorph not in common usage (Teliomorph in bold)**	**Disease caused**	**Paper ref**	**Vic**	**NSW ACT**	**SA**	**WA**	**Tas**	**Qld**	**NT**	**AUS.**	**Pres in UK**	**Potential risk for devitalised seed**
Typhula	*ishikariensis*		Snow Mold	Anon 1999								NO	NO	
Urocystis	*agropyri*		Flag Smut	Murray & Brown 1987								YES		
Ustilago	***hordei***		Covered Smut	Anon 1999	x	x	x	x	x	x		YES	YES	
Ustilago	***nigra***		False loose Smut	Anon 1999	x		x	x	x	x		YES	NF	
Ustilago	***nuda***		Loose Smut	Anon 1999; Nielsen & Thomas 1982			x					YES	YES	
Ustilago	***nuda* f. sp. *tritici***		True Loose Smut	Anon 1999								?	YES	
Ustilago	***segetum* var. *nuda***			c2,8								YES	YES	
Ustilago	***tritici***		loose smut	c31								YES		
Verticillium	*dahliae*		Verticillium Wilt	Anon 1999								YES	YES	

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15 Appendix B

15.1 Plant species recorded as weeds of wheat crops in the UK

Table 1 Plant species associated with wheat in UK but not considered of potential quarantine concern due to presence in Australia.

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Asteraceae	*Arctium*	*lappa*	greater burdock	B	P3	field margins	X	X				X		YES	NO	NO
Asteraceae	*Artemisia*	*vulgaris*	mugwort	P	P3	field margins	NO	NO	NO					Yes as garden herb	NO	NO
Asteraceae	*Lapsana*	*communis*	nipplewort	A	P3	field margins	X	X	X		X			YES	NO	NO
Asteraceae	*Matricaria [Chamomilla]*	*recutita*	scented mayweed	A	c38,179,203,239,606			X	X					YES	NO	NO
Asteraceae	*Senecio*	*vulgaris*	grounsel	A	c239		X	X	X	X	X	X		YES	NO	NO
Asteraceae	*Sonhcus*	*arvensis*	field sow-thistle	P	P3	field margins	?	NO	X					YES	NO	NO
Asteraceae	*Taraxacum*	*officinalis*	dandelion	P	P3	field margins	X	X	X	X	X	X		YES	NO	NO
Caryophyllaceae	*Agrostemma*	*githago*	corn cockle	A	c172			X	X		X			YES	NO	NO

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Caryophyllaceae	*Cerastium*	*fontanum*	common mouse-ear	P	P2		X	X?		X	X	X		YES	NO	NO
Caryophyllaceae	*Stellaria*	*media*	common chickweed	A	c3,15,20,38,65,87,172,179,188,203,239,606,610,P2		X	X	X	X	X	X		YES	NO	NO
Chenopodiaceae	*Chenopodium*	*album*	fat hen	A	c87,203,P2		X	X	X	X	X	X	X	YES	NO	NO
Cruciferae	*Brassica*	*napus*	rape	A	c239,P2		X	X	X	X	X	X		YES	NO	NO
Cruciferae	*Capsella*	*bursa-pastoris*	shepard's purse	A	c87		X	X	X	X	X	X	X	YES	NO	NO
Cruciferae	*Sinapis*	*arvensis*	charlock, wild mustard	A	c31,38,203,P2		X	X	X	X	X	X	X	YES	NO	NO
Dioscoreaceae	*Tamus*	*communis*	black betony	P	c504		NO	NO	NO					NO	NO	YES
Fumariaceae	*Fumaria*	*officinalis*	common fumitory	A	P2			X	X			X		YES	NO	NO
Geraniaceae	*Geranium*	*dissectum*	cut-leaved crane's-bill	A	P3	field margins	X	X?	X		X			YES	NO	NO
Lamiaceae	*Lamium*	*purpureum*	red dead-nettle	A	c15,52,85,188,239,610,P2		X	X	X		X			YES	NO	NO
Leguminosae	*Medicago*	*lupulina*	black medick	A	P1		X	X	X	X	X			YES	NO	NO

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Leguminosae	*Vicia*	*sativa*	common vetch	A	P1			X	X	X				YES	NO	NO
Papaveraceae	*Papaver*	*hybridum*	rough poppy	A	c38		X	X	X	X			X	YES	NO	NO
Papaveraceae	*Papaver*	*rhoeas*	Field poppy	A	c15,20,38,40,52,85,239,606,P2,P3			X						YES	NO	NO
Poaceae	*Agrostis*	*gigantea*	black bent	P	c533,P3		X	X	X	X	X	X		YES	NO	NO
Poaceae	*Agrostis*	*stolonifera*	creeping bent	P	P3	field margin	X	X		X	X	X		YES	NO	NO
Poaceae	*Alopecurus*	*myosuroides*	blackgrass	P	c3,6,15,44,65,199,203,290,304,326,369,606,610, P1,P3		X	X	X	X				YES	NO	NO
Poaceae	*Arrhenatherum*	*elatius subsp. bulbosum*	onion couch, false oat grass	P	c173		X	X			X			YES	NO	NO
Poaceae	*Arrhenatherum*	*elatius subsp. elatus*	onion couch, false oat grass	P	c173,612,P3		X	X	X		X	X		YES	NO	NO
Poaceae	*Avena*	*fatua*	wild oat	A	c15,65,85,172,220,290,369,610,P1		X	X	X	X	X	X	X	YES	NO	NO
Poaceae	*Avena*	*sterilis / ludoviciana*	winter wild oat	A	c290,369,P1		X	X						YES	NO	NO

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Poaceae	*Bromus*	*diandrus*	great brome	A	c141		X	X	X	X	X	X	X	YES	NO	NO
Poaceae	*Bromus*	*hordeaceus [mollis]*	soft brome	A	c89,P3		X	X	X	X	X	X		YES	NO	NO
Poaceae	*Bromus [Anisantha]*	*sterilis*	barren brome	A	c12,40,44,65,172,199,311,369,708, P3			X		X	X	X		YES	NO	NO
Poaceae	*Dactylis*	*glomeratus*	cock's foot	P	P3	field margins	X	X	X	X	X	X		YES	NO	NO
Poaceae	*Elymus [Agropyron]*	*repens*	common couch		c220,290,312,533,610,612,P3		?	NO	NO		X			YES	NO	NO
Poaceae	*Festuca*	*rubra*	red fescue	P	P3	field margins	X	X			X			YES	NO	NO
Poaceae	*Holcus*	*lanatus*	yorkshire fog	P	P3	field margins	X	X	X		X			YES	NO	NO
Poaceae	*Lolium*	*multiflorum*	Italian ryegrass	A/B	c326		X	X	X	X	X	X		YES	NO	NO
Poaceae	*Lolium*	*perenne*	ryegrass	P	c566, P3		X	X	X	X	X	X	X	YES	NO	NO
Poaceae	*Phalaris*	*paradoxa*			c331	?	X	X	X	X		X		YES	NO	NO
Poaceae	*Phleum*	*pratense*	Timothy	P	c612		X	X	X	X				YES	NO	NO
Poaceae	*Poa*	*annua*	Annual medow grass	A	c20,87,239,326		X	X	X	X	X	X		YES	NO	NO

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Poaceae	*Poa*	*trivialis*	Rough meadow grass	P	c15,239,290,326,369,558,610,p3		X	X	NO					YES	NO	NO
Polygonaceae	*Fallopia*	*convolvulus*	black bindweed	A	c38,610		X	X		X		X		YES	NO	NO
Polygonaceae	*Polygonum*	*aviculare*	Knot-grass	A	c31,38,87,179,203,239,610,P1,P2		X	X		X	X			YES	NO	NO
Polygonaceae	*Polygonum*	*lapathifolium*	Pale persicaria	A	c239,P2		X	X	X		X	X	X	YES	NO	NO
Polygonaceae	*Polygonum*	*pericaria*	Redshank	A	c239,P2		X							YES	NO	NO
Polygonaceae	*Rumex*	*obtusifolius*	broad-leaved dock	P	P3	field margins, UK nox weed	X	X	X		X			YES	NO	NO
Ranunculaceae	*Ranunculus*	*arvensis*	com buttercup, corn crowfoot	A	P1		NO	X			X			YES	NO	NO
Ranunculaceae	*Ranunculus*	*repens*	creeping buttercup	P	P3	field margins	X	X	X	X		X		YES	NO	NO
Rosaceae	*Aphanes*	*arvensis*	parsley-piert	A	c606		X	X			X			YES	NO	NO
Scrophulariaceae	*Veronica*	*hederifolia*	ivy-leaved speedwell	A	c15,38,52,65,85,239,P1		X	X	X		X			YES	NO	NO

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Scrophulariaceae	*Veronica*	*persica*	common field speedwell	A	c3,15,38,52,65,85,172,239,606,690		X	X	X	X	X	X		YES	NO	NO
Umbelliferae	*Scandix*	*pecten-veneris*	shepherd's needle	A	c38		X	X	X		X			YES	NO	NO
Urticaceae	*Urtica*	*dioica*	stinging nettle	P	P3	field margins	NO	X	NO					YES	NO	NO
Violaceae	*Viola*	*arvensis*	field violet	A	c15,52,85,188,203,290,606,610,P2		X	X			X			YES	NO	NO

English name as recorded in UK literature

%% Annual / biannual / perennial

Table 2 Plant species associated with wheat in UK considered a potential quarantine concern due to absence in Australia or under control as a noxious weed

Plant Taxa					UK references		Presence in Australia								Quarantine status in Australia	
Family	**Genus**	**Species**	**English name#**	**A/P /B%%**	**Paper ref**	**Notes**	**Vic**	**NSW/ACT**	**SA**	**WA**	**TAS**	**Qld**	**NT**	**Present in Australia**	**Noxious weed in Australia**	**Potential plant of quarantine concern**
Asteraceae	*Chrysanthemum*	*Segetum*	corn marigold	A	c528,P2			NO	NO		NO			NO	NO	YES
Asteraceae	*Cirsium*	*Arvense*	creeping thistle	P	c210,P3	UK nox weed	X	X	X	X				YES	YES	YES
Asteraceae	*Cirsium*	*Vulgare*	spear thistle	B	P3	field margins, UK nox weed	X	X	X	X	X	X		YES	YES	YES
Asteraceae	*Matricaria [Tripleurospermum]*	*perforata*	scentless mayweed	A	c3,15, P1		NO	NO	NO					NO	NO	YES
Asteraceae	*Senecio*	*jacobaea*	common ragwort	B/P	P3	field margins, UK nox weed	X	X	X	X	X	X		YES	YES	YES
Boraginaceae	*Myosotis*	*avensis*	field forget-me-not	A/B	c15,52,610, P2,P3		?	NO	NO					NO	NO	YES
Convolvulaceae	*Convolvulus*	*arvensis*	bindweed	P	c610,P3		X	X	X	X	X	X		YES	YES	YES
Cruciferae	*Raphanus*	*raphanistrum*	wild radish	A/B	c606		X	X	X	X	X	X	X	YES	YES	YES
Dioscoreaceae	*Tamus*	*communis*	black betony	P	c504		NO	NO	NO					NO	NO	YES
Equisetaceae	*Equisetum*	*arvense*	field horsetail	P	c406,532,P1			X						YES	YES	YES

Euphorbiaceae	*Euphorbia*	*platyphyllos*	broad-leaved spurge	A	c38,369			NO	NO					NO	NO	YES
Lamiaceae	*Galeopsis*	*angustifolia*	red hemp-neetle	A	c38		NO	NO	NO					NO	NO	YES
Lamiaceae	*Galeopsis*	*tetrahit*	common hemp nettle	A	c203,239,P2		NO	NO	NO					NO	NO	YES
Lamiaceae	*Glechoma*	*hederacea*	ground ivy	P	P3	field margins	NO	NO						NO	NO	YES
Lamiaceae	*Lamium*	*album*	white dead-nettle	P	P3	field margins		NO	NO					NO	NO	YES
Lamiaceae	*Stachys*	*sylvatica*	hedge woundwort	P	P3	field margins	NO	NO	NO					NO	NO	YES
Poaceae	*Apera*	*Spica-venti*	silky bent	A	c499			NO	NO					NO	NO	YES
Poaceae	*Bromus*	*commutatus*	meadow brome	A	c12,44,65,199,311			NO	NO					NO	NO	YES
Poaceae	*Phleum*	pratense ssp. bertolonii	smaller catstail	P	P3	field margins		NO						NO	NO	YES
Rosaceae	*Rubus*	*fruiticosus*	bramble	P	P3	field margins	X	X	X	X	X	X		YES	YES	YES
Rubiaceae	*Galium*	*aparine*	clevers	A	c3,4,15,20,40,52,65,85,172,239,290,606,610,G3		X	X	X		X	X	X	YES	YES	YES
Umbelliferae	*Anthriscus*	*sylvestris*	cow parsley	B/P	P3	field margins	NO	NO	NO		NO			NO	NO	YES
Umbelliferae	*Heracleum*	*sphondylium*	hogweed	B/P	P3	field margins	NO	NO			NO			NO	NO	YES

Umbelliferae	*Torilis*	*japonica*	hedge parsley	P	P3	field margins	NO	NO	NO					NO	NO	YES

English name as recorded in UK literature, %% Annual / biannual / perennial

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16 Appendix C

16.1 Germination test procedures used in this study are described here.

Two basic tests were carried out: germination tests and phytotoxicity screens. The germination tests very closely adhered to the International Rules of Seed Testing (1999) by the International Seed Testing Association (ISTA). The phytotoxicity screen is a less restrictive version of the germination tests which allows more flexibility when establishing concentration and exposure times through a series of static phytotoxicity assays.

Germination Tests:

400 seeds were counted out at random from the seed lot. Replicates of 100 seeds, and sub-replicates of 50 or 25 seeds were used as necessary. Work was carried out on well-mixed and thoroughly cleaned pure seed samples, excluding foreign matter and damaged or abnormal seeds. Any high levels of abnormalities were reported. The amount of water added was varied to suit the germination substrate and the size and species of seed to be tested. In many cases, seeds were pre-treated as specified in ISTA and other germination protocols.

Seeds were germinated on top of one or more layers of paper placed in Petrie dishes enclosed in plastic bags placed onto trays and incubated in germination cabinets. Alternatively, seeds were germinated between two layers of paper by placing seeds in rolled towels. The rolled towels were kept sealed in plastic bags, usually 4 replicates (or sub-replicates) per bag. Rolls were placed in an upright position in a germination cabinet. Other methods used included germination on agar medium and in some cases in soil.

Seeds were incubated under the conditions given in germination protocol published in the literature or under protocols developed for this project. Seedlings and seeds were scored as Normal seedlings, Low Vigour seedlings, Abnormal seedlings, Ungerminated seeds, Hard seeds and Fresh seeds according to SGRL Germination testing procedures and to the International Rules of Seed Testing (1999) as follows:

1. *Normal seedlings* are developed seedlings which show no defects to essential structures and are of overall normal appearance.
2. *Low vigour seedlings* (Seedlings with slight defects) are intact seedlings that show limited damage to primary structures
3. *Abnormal seedlings* show at least one severe defect to a primary structure or to the appearance of the seedling as a whole.
4. *Fresh seeds* are seeds which have failed to germinate, which have imbibed water, are firm and free of visible fungal infection
5. *Mouldy seeds* are seeds which have failed to germinate and are visibly mouldy and/or exude liquid when gently squeezed
6. *Hard seeds* are seeds which have failed to germinate and have failed to imbibe at all
7. *Empty seeds* are seeds which have failed to germinate because they do not contain an embryo
8. *Intact seedlings* show well developed root systems, shoot axis, cotyledons and other primary structures appropriate to the species tested.
9. *Developed seedlings* are seedlings which have reached a stage where all essential structures can be accurately assessed.

The following treatments promoting germination were used:

1. Pre-chilling: replicates are set up and incubated at 5˚C for 7 days unless a different pre-chill period was specified in the germination protocol
2. Pre-heating: replicates were stored at 30˚C with free air circulation for 7 days, unless otherwise specified in the protocol, before they are set up for germination.
3. Light: Cool white lamps were used in the germination cabinet throughout the germination period unless otherwise specified in the protocol.
4. Potassium nitrate: 0.2% solution was used to saturate the germination substrate.
5. Gibberellic acid: A 0.05% GA_3 was used to saturate the germination substrate. Alternatively a stronger solution was prepared by dissolving 1.7799 g of Na_2HPO_4 and 1.3799 g of NaH_2PO_4 and 1 g of GA_3 in 1 L of milli-pure water.
6. Sulphuric acid: Seeds were soaked in concentrated H_2SO_4 until they became pitted before test.
7. Nitric acid: Seeds were soaked in 1N HNO_3 before test.
8. Soaking: Seeds were soaked for 24-48 h in milli-pure water
9. Mechanical scarification: Seeds were pierced, chipped, filed or sandpapered immediately above the tips of the cotyledon.
10. Pre-washing: Seeds were washed in running water at 25˚C before test. Dry back at 25˚C.
11. Removal: Outer structures of seed were removed before the test.

Fungal growth was a recurring problem to germination assessment of many seed samples. There are a number of treatments available, but these should only be used if essential to successful assessment. First and foremost attention was payed to adequate spacing of seed, optimum environment, and hygiene. In most other cases the use of sodium hypochloride, 0.5-5%, 2-10 minutes was sufficient to allow assessment. In some rare cases antibiotics were added to the germination medium during the germination period.

Tests were repeated where dormancy was suspected and no appropriate method to break dormancy had been applied, the test was unreliable because of microbial contamination, seedlings could not be evaluated correctly because the protocol used was inappropriate or where there were errors in test conditions or evaluation.

All results were calculated as the average of four 100 seed replicates. Sub-replicates were combined into 100 seed replicates. Germination results were expressed as a percentage by number of normal seedlings calculated to the nearest whole number. Abnormal seedlings, hard, fresh, mouldy and empty seeds were expressed in the same way.

Phytotoxicity Screen

The Phytotoxicity screen used the same procedures as the germination test, except that tests were carried out on less than 400 seeds where sufficient material was not available. The number of replicates was reduced accordingly. The assessment periods were shortened in cases where this was necessary to make a clear assessment. All results were calculated as the average of the number of seeds available for the test.