THE CONTROL OF SEED-BORNE FUNGI BY FUMIGATION

A thesis presented by

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A. ABSTRACT

The literature relating to the use of fumigants for the control of seed-borne microorganisms is reviewed in detail.

The experimental work was an endeavour to control the deep-seated infections of Ustilago nuda and Ustilago tritici, by the fumigation, at 20°C., of infected grain with defined moisture contents, in 20 l. bins, or in 4.3 l. desiccators with the less volatile compounds. The efficacy of the treatments was assessed in glasshouse and field trials.

In the barley experiments, the fumigation of grain of 14 per cent. moisture content with methanol for 2.5 - 6.0 hr., satisfactorily reduced the proportion of infected plants to less than 0.5 per cent., with reductions in plant establishment of 8.6 - 15.0 per cent. Acetic acid, allyl alcohol, allyl isothiocyanate and an 80 hr. fumigation with methyl bromide (C.T. product: 24,000 mg.h./l.), were also judged satisfactory. In one trial, acrylonitrile and diethyl ether were as effective as allyl alcohol. Allyl bromide, allyl chloride, n-butanethiol and chloropicrin were more phytotoxic, and were regarded as unsuitable.

Ustilago tritici was not appreciably controlled by the fumigation of infected wheat grain with methanol, methyl bromide, hydrogen cyanide or the slightly volatile (2:4:5-trichlorophenoxythio) trichloromethane.

Experimental evidence indicated that the control of barley loose smut by fumigation with allyl alcohol, was due to the reduction in viability of the infected grains, but a different mode of action was proposed for methanol.

The ease of control of the pathogen was slightly, but significantly, influenced by the grain moisture content. The storage of fumigated grain for 10 - 12 months only slightly improved the degree of control.

The sorption of methanol in excess of 1 g./100 g. grain was associated with a satisfactory control of the pathogen. In the large scale treatment of grain, such high proportions of the compound would be more conveniently applied as a liquid, than by fumigation.

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CONTENTS

B. INTRODUCTION

One of the most interesting and urgent tasks of seed pathology is the development of disinfection techniques as alternatives to the existing water-soak treatments. Walker, in particular, (1948, 1957), has drawn attention to this relatively neglected aspect of applied microbiology.

During the last 50 years, several groups of compounds have been used for treating seeds. The most important of these to be used for controlling seed-borne pathogens, is the group of organomercury derivatives. These materials have been applied to the seed by means of a water soak, a small quantity of concentrated solution, a slurry, and of a powder. At the present time the most widely used method involves the application of a concentrated solution of the compound, often combined with an insecticide, at a low volume rate of about 2 ml. per kilogram. The seed is usually treated in a rotating drum, to ensure an even distribution. A limited amount of redistribution of the fungicide occurs in bulks of treated seed, in the few hours immediately after treatment, by virtue of the slight volatility of the alkyl and aryl mercury derivatives currently in use.

Several other groups of compounds which are used to varying extents for treating seeds, are virtually ineffective against seed-borne pathogens, but nevertheless protect seedlings from infection by soil-borne pathogens. In this category of protectant fungicides, may be considered the derivatives of the hypothetical dithiocarbamic acid, such as tetramethylthiuram disulfide (thiram), the halogenated benzoquinones and naphthoquinones, and also compounds with the (trichloromethylthio) group,

such as captan. Thiram is also effective in controlling some seed-borne pathogens such as Diplodia sp. causing seedling blight on maize.

It is evident that the organomercurials, having a broad spectrum of toxicity, are the most effective group of fungicides for controlling a wide variety of seed-borne pathogens. Compounds such as the chlorinated benzenes and nitrobenzenes and tetrachloronitroanisole (Purdy, 1963), have a much more limited use. The organomercurials, however, do not diffuse into the seed a distance of more than about 100 microns (Lindström, 1958, 1959 a, b). In consequence, they are toxic only to spores on the surface of the seed and to mycelium in the superficial tissues of the seed. Such materials are described as having a disinfesting action. In considering cereal diseases, examples of seedling-infecting pathogens that are controlled by the more volatile organomercury compounds include, Tilletia caries (bunt of wheat); Ustilago hordei (covered smut of barley); Ustilago nigra (false or black loose smut of barley); Helminthosporium gramineum (leaf stripe of barley); and Cochliobolus sativus (seedling blight of wheat and barley). The common names for the diseases caused by these fungi, have been obtained from the list of seed-borne diseases compiled by Noble, de Tempe and Neergaard (1958).

Organomercurials do not diffuse into the seed sufficiently to control such internal pathogens as those causing the loose smut diseases of wheat and barley, Ustilago tritici and U. nuda respectively, nor do they control two important pathogens of the garden pea (Pisum sativum), namely Ascochyta pinodella and Ascochyta pisi. Furthermore, under circumstances

when disinfestant and protectant fungicides do penetrate deeply into the seed, such as when the seed is mechanically damaged, or when penetration is assisted by the use of solvents, then these compounds usually prove to be so highly phytotoxic as to preclude their use as disinfectants.

To control deep-seated infections, resort has to be made to either the hot water treatment (Jensen, 1888) or the anaerobic water soak treatments (Zalessky, 1935; Tyner, 1953; Hebert, 1955). There are, however, several disadvantages to these methods, such as the often lengthy presoaking period, the critical temperature control necessary in the hot water treatment to avoid killing the seed, and finally, the necessity to dry the seed after treatment to prevent rapid deterioration. Because of these cumbersome and costly limitations to these methods, especially when very large quantities of seed are involved, increasing attention is being paid to the breeding of disease-resistant varieties, and to field inspection schemes, whereby the amount of infected seed material entering commercial channels may be substantially reduced.

Fumigation methods, in which the toxicant is applied in the gaseous phase, have been suggested as a rapid and convenient way of controlling seed-borne pathogens. In particular, a dry, non-phytotoxic treatment suitable for controlling internal pathogens would be a very welcome alternative to the water soak methods. With a suitable fumigant large quantities of seed might be treated <u>in situ</u> such as in silos, or in stacks of bags, in the manner that has now become routine, in some countries, for the control of insect pests of stored products. In international

trade, there is an increasing use of fumigants for the treatment of fruit, vegetables and timber, as a quarantine procedure to reduce the chance introduction of insect pests. If similar methods were available for controlling phytopathogenic microorganisms, a greater international exchange of plant products such as seeds, tubers, growing plants and timber, would be possible.

The chemical reactivity, biological specificity and also the physical properties of individual fumigants vary widely. A certain amount of arbitrariness in the initial choice of fumigants is therefore inevitable, although an examination of the extensive literature on the use of fumigants to control insects, nematodes and soil microorganisms, has enabled a fairly logical choice to be made. Four of the more important physical factors influencing the rate of diffusion of fumigants into bulk quantities of seeds, are the partial pressure of the fumigant, its water solubility, the total gaseous pressure (Page and Blackith, 1954) and the temperature. The sorption of fumigants by intact seeds, by processes of adsorption and absorption, differs appreciably from the sorption on inorganic sorbents such as charcoal or silicates, by being reduced in amount, and taking longer to reach equilibrium (Lubatti, 1945; Lubatti and Harrison, 1944). The moisture and oil contents of seeds influence the amount of fumigant sorbed, and to some extent, also the amount that becomes chemically altered (Lubatti and Smith, 1948). These two factors determine to a very large extent, the susceptibility of the seed to fumigant toxicity, for example, seeds of a high moisture content are more susceptible than those of a low

moisture content (Lubatti and Blackith, 1957; Whitney et al., 1958; Strong and Lindgren, 1959 a, b; Blackith and Lubatti, 1960).

This study has been directed towards an improved understanding of the effect of fungitoxic fumigants upon the control of internal seed-borne fungi.

The grain of several varieties of barley naturally infected with the loose smut pathogen, Ustilago nuda (Jens.) Rostr. and one variety of wheat artificially inoculated with Ustilago tritici (Pers.) Rostr., have been adjusted to several moisture contents, and exposed to a number of fumigants. Observations of the types of seedling abnormality resulting from fumigation treatments have been made under controlled conditions. The greater part of this study is concerned with the degree of loose smut control, and the extent of any associated phytotoxicity as observed in field trials. The influence of grain size and the effect of post-treatment storage are also considered. In conclusion, the results of this investigation are contrasted with the results obtained with the currently practised methods of controlling internal seed-borne pathogens.

C. LITERATURE SURVEY

C.1. A Survey of the Uses of Volatile Toxicants.

Before considering, in detail, the literature relating to the control of seed-borne pathogens, by fumigation, it will be of value to review, briefly, the use of fumigants in the control of insects and nematodes associated with seeds, and of soil-borne microorganisms.

C.1.1. Insecticidal fumigants.

This very extensive subject has recently been reviewed by Frear (1955), Cotton (1956, 1958), Monro (1961), Lindgren and Vincent (1962) and by Page and Lubatti (1963). In addition a valuable compendium of toxicological data has been edited by Negherbon (1959).

Two of the earliest fumigants to be used for insecticidal purposes were carbon disulphide, and hydrogen cyanide. Carbon disulphide entered into general use in the late 1870's chiefly as a soil fumigant for the control of the insect pest Phylloxera sp. on vine in France and Germany, but was also used as a grain fumigant in 1879. Hydrogen cyanide was first used in 1886 and has been extensively employed both in the disinfestation of stored products and in the fumigation of citrus trees under tents, to control scale insects.

The number of fumigants suitable for controlling insect pests is about 30, of which only about ten are in common use. Methyl bromide, the insecticidal properties of which were demonstrated in 1932, is

now used very extensively and has largely replaced hydrogen cyanide, ethylene oxide and chloropicrin, by virtue of its greater volatility and more rapid penetration of materials. Methyl bromide is less sorbed than most of the other fumigants, and undesirable residues are also less of a problem.

Ethylene dichloride and carbon disulphide are still used on an extensive scale for grain fumigations in small farm bins and in some larger storage structures. Both of these fumigants are flammable, but when mixed in regulated proportions with carbon tetrachloride, this risk can be minimized. Ethylene dibromide is currently used on a large scale in quarantine practices especially in America, for the fumigation of fresh fruit and vegetables. Several other fumigants are used to a limited extent, for example, in the spot-fumigations of isolated corners of seed stores or mills, and include chloropicrin, acrylonitrile and allyl bromide.

Sulfuryl fluoride and phosphine are two insecticidal fumigants which are being evaluated. The former has a very low boiling point and is normally transported in metal cylinders as a highly compressed gas. In contrast, phosphine may be generated, in the presence of water vapour, from mixtures of aluminium phosphide and ammonium carbamate compressed into tablet form, and distributed in the bulk of the seed, in the manner used by the firm of Degesh in Germany.

C.1.2. Nematicidal fumigants.

Much of the recent interest (Moje, 1960) in both volatile and

non-volatile nematicides dates from the successful use of chloropicrin in the early 1930's, to control root knot nematode and several soil fungi pathogenic on the pineapple in Hawaii. Chloropicrin has been superseded by a mixture of approximately equal proportions of 1,3-dichloropropene and 1,2-dichloropropane ("D-D"), and by 1,3-dichloropropene in a more concentrated form, ethylene dibromide, and more recently by 1,2-dibromo-3-chloropropane.

An increasing use is being made of derivatives of dithiocarbamic acid, thought to be effective through the release of isothiocyanates, and of some organophosphorus compounds. Methyl bromide is used on a small scale to control soil-borne nematodes, but because of its high volatility, diffusion barriers such as water seals or gas-proof sheets (Hague et al., 1964), must be used to achieve a satisfactory kill near the soil surface.

There are a few instances of plant parasitic nematodes occurring in association with seeds. The stem eelworm Ditylenchus dipsaci (Kühn) Filipjev, has been found in a preadult stage in a desiccated condition on the surface of onion seed (Goodey, T., 1945) and red clover seed (Goodey, J.B., 1949) and its presence on lucerne seed, or associated debris, was suspected by Brown (1957). In seeds of teazel (Dipsacus fullonum L.), Ditylenchus dipsaci occupies the internal region of the seed (Goodey, J.B., 1949), in contrast to Anguina agrostis (Steinbuch, 1799) Filipjev, which occurs in seed galls associated with the normal viable seed of Agrostis tenuis.

Small scale fumigations with methyl bromide have controlled these

nematodes without seriously impairing seed germination (Goodey, T., 1945; Goodey, J.B., 1949; Hague and Clark, 1959; Hague, 1963). Page et al. (1959) have described large scale fumigations of lucerne seed, with a moisture content of 12.5%, with concentration-time products of 1250 and 2500 mg.h./l., which reduced the amount of eelworm infestation in field plots sown with the fumigated seed (Brown, 1957). Subsequent observations (Page, private communication) suggested that some of the eelworm infestations in the plots were not of seed origin, and that the fumigations were therefore more successful than would appear from the published results.

C.1.3. Fumigants toxic to soil microorganisms.

The toxicity of fumigants to soil microorganisms has been very thoroughly reviewed by several authors including Newhall (1955), Kendrick and Zentmyer (1957), Kreutzer (1960, 1963) and Vaartaja (1964). Hemwall (1962) has briefly considered the influence of physical factors upon the mobility of fumigants in soil.

The fumigants currently used to control soil microorganisms are also highly phytotoxic to actively growing plants. Soil fumigations are therefore conducted in unoccupied soil, several days or weeks before the crop is planted. This high degree of phytotoxicity has been put to advantage as, for example, with chloropicrin and allyl alcohol, which, in addition to their high fungicidal activity, are used to kill weed seeds present in the soil.

Although there are differences of detail, the various groups of

microorganisms show a surprisingly similar order of susceptibility to different fumigants. Thus ethylene dibromide, a mixture of dichloropropene and dichloropropane, and carbon disulphide have a rather mild action on the soil microflora, and kill or inhibit only fungi such as Phytophthora spp. and Pythium spp., and the nitrifying and cellulose decomposing bacteria (Wensley, 1953). Methyl bromide, formaldehyde and allyl alcohol are toxic to a broader spectrum of organisms including Sclerotinia sclerotiorum, Thielaviopsis basicola and Rhizoctonia solani. Among the most resistant pathogenic fungi are the Verticillium spp., and the various physiological forms of Fusarium oxysporum, which may however be controlled by chloropicrin and allyl bromide (Christie, 1947; Schmitt, 1949; Zentmyer and Kendrick, 1949), and chlorobromopropene (Kreutzer and Montagne, 1950).

Some non-pathogenic fungi do survive the more severe treatments, such as species in the Penicillium luteum series, and Trichoderma viride (Overman and Burgis, 1956; Garrett, 1957; Saksena, 1960), and appear to influence not only a further control of microorganisms, but also the rate of re-establishment of microorganisms in the soil, by their production of antibiotic substances.

With the exception of the nitrifying and cellulose decomposing bacteria (Wensley, 1953), bacteria are fairly resistant to fumigation treatments, and their growth may even be stimulated (Klemmer, 1957). Soil-borne actinomycetes appear to be even more tolerant of fumigants (Wensley, 1953).

Allyl alcohol and 1-chloro-3-bromopropene-1, are two of the

recently introduced soil fumigants. Other developments have concerned derivatives of dithiocarbamic acid, such as 3-(p-chlorophenyl)-5-methyl rhodanine, and 2-thio-3,5-dimethyl tetrahydro-1,3,5-thiadiazine ("Mylone"). There is evidence that these compounds undergo degradation in the soil to form isothiocyanates which are highly fungicidal. The fungitoxicity of disodium ethylene bisdithiocarbamate (nabam) was also attributed to similar reaction products, but recent work has indicated that carbonyl sulphide is also involved (Moje et al., 1964).

C.1.4. Additional uses of fumigants.

Smith (1962) has recently reviewed the use of fumigants and non-volatile chemicals to reduce fruit rots during storage and in transit. The fumigation of vinifera grapes with sulphur dioxide to inhibit sporulation and spore germination of Botrytis cinerea, is now standard practice in the U.S.A. The fruit is treated shortly after harvest with 1 per cent. SO_2 for 20 - 25 minutes, and the process is repeated every 7 - 10 days of storage, with 0.25 per cent. SO_2 for the same exposure period.

Sulphur dioxide is too phytotoxic to be used for controlling storage rots on other fruits and vegetables or indeed on other American varieties of grape. Couey and Uota (1961) have investigated, in detail, the influence of SO_2 concentration, period of exposure, temperature and humidity upon the viability of B. cinerea spores.

Nitrogen trichloride has been shown to be very toxic to a number of fungi (Klotz, 1936), and has been used in the control of Diplodia natalensis, Penicillium italicum and P. digitatum on lemons (Ryall and

Godfrey, 1948), and of other rotting organisms on oranges and tomatoes. Ryall and Godfrey (1948) used concentrations of only 0.003 - 0.04 p.p.m. for periods of 4 hours, every 24 - 48 hours, while the lemons were being "coloured" by treatment with ethylene, in fumigation rooms.

Attention has been directed to the possible use of ammonia and ammonia-generating powders (Eckert et al., 1963), for the control of rots on stored fruit, but without much success. Another approach has been to use slightly volatile organic compounds, applied either to storage boxes, or to the papers with which the fruits are wrapped or the boxes lined. Biphenyl has been used for its fungistatic properties but efforts have been made to find superior compounds which are more fungicidal, less phytotoxic, and less liable to taint the fruit, such as paraformaldehyde, acetaldehyde, glyoxal, tetrachlorethylene, methyl borate etc. (Klotz and Roistacher, 1954) and dibromotetrachloroethane (Eckert et al., 1962). Slightly volatile substituted (aryloxythio) trichloromethane compounds have been shown to have some commercial value in controlling rots caused by Gloeosporium spp. on applies, (Fawcett, et al., 1958; Spencer and Wain, 1961).

Methyl bromide, chloropicrin and propylene oxide were shown by Partridge (1961) to control Ceratocystis fagacearum, the causal fungus of the important oak wilt disease, when infected logs were fumigated. Fumigants which were either insufficiently fungitoxic or failed to penetrate the infected timber, included acetone, carbon monoxide, ethylene dibromide, chlorine, methanol and xylol. Carbon tetrachloride and formaldehyde were of intermediate effectiveness. Fumigation of large logs with methyl

bromide (Jones, 1963) completely controlled the fungus and indicates a possible way of overcoming the present export restrictions on oak from areas in the U.S.A. where the fungus is endemic.

MacLachlan et al. (1953), investigated the possibility of fumigating used jute bags to control the ring rot bacterium of potato, Corynebacterium sepedonicum. Ammonia, chlorine, hydrogen sulphide, sulphur dioxide, and even chloropicrin and methyl bromide failed to kill the pathogen at dosages of 50, 100 and 150 mg./l. after 16 hours. However, ethylene oxide was toxic at 100 mg./l. after 16 hours, and at 150 mg./l. after 8 hours. Bacteria located in the centre of pressed bales of 500 bags were killed at a concentration of 80 mg./l. within 24 hr. and within 5 hr. under partial vacuum. Richardson and Monro (1962) have claimed an improved control of C. sepedonicum in the centre of bales by the use of a mixture of 5 per cent. ethylene oxide and 10 per cent. methyl bromide.

Ethylene oxide has also been used in biological and medical practice, for the sterilization of instruments, utensils and media (Hansen and Snyder, 1947; Phillips, 1957). Phillips and Kaye have investigated the influence of temperature, relative humidity and concentration of ethylene oxide and several other compounds with 3-membered heterocyclic ring structures, including the very toxic ethylene imine, upon the spores of Bacillus globigii (Phillips and Kaye, 1949; Phillips, 1949; Kaye, 1949; Kaye and Phillips, 1949). Of particular interest is the reduction of the concentration-time product (for 90 per cent. toxicity using an ethylene oxide concentration of 44.2 mg./l.) from 1,380 mg.h./l. at 5^{0}C.

to 146 mg.h./l. at 25°C. and finally to 39.8 mg.h./l. at 37°C. (Phillips, 1949). B. globigii spores were more susceptible at low humidities, and at 28 per cent. relative humidity they were killed about 4 times more rapidly than at 65 per cent., and about 10 times more rapidly than at 97 per cent. R.H. (Kaye and Phillips, 1949). This feature contrasts not only with the toxicity of formaldehyde, for example, to bacteria under similar conditions, but also with the toxicity of ethylene oxide to spores of Alternaria solani (Sampson and Ludwig, 1956) which was greater at the higher relative humidities.

In concluding this section, two other uses of fumigants might be mentioned, namely, the fumigation of food and contaminated storage rooms.

Fumigants have been used for sterilizing foods and food additives, such as ethylene oxide for the disinfestation of spices contaminated with coliform bacteria. This compound has also been used to reduce microbiological spoilage on stored fruit (Whelton et al., 1946a, b). Although undesirable residues seldom if ever result from ethylene oxide fumigations, there may be losses of thiamine, riboflavin, pyridoxine, niacin and folic acid in some treated tissues (quoted by Phillips, 1957). Storage moulds of grains have been controlled by treatments with methyl bromide, chloropicrin, ethylene oxide and to a lesser extent by ethylene dibromide (Majumder, et al., 1955; Srinivasan and Majumder, 1961; Yanai, et al., 1964).

Because of its fungicidal properties, chloropicrin has been used in large scale fumigations of empty rooms, used for storing sweet potatoes,

to reduce the quantity of spoilage organisms (Nusbaum, 1944). The atmosphere in the room was saturated with water vapour to improve the toxicity of the fumigant. This control measure does not appear to have been used to any great extent, which may reflect the unpopularity of chloropicrin, due to its lachrymatory and nauseating properties.

The uses of fumigants for the control of seed-borne pathogens will now be reviewed in detail.

C.2. The control of seed-borne fungi by fumigation.

In order to facilitate the comparison of treatments, a quantity known as the concentration-time product has been calculated wherever possible, and included in the text. An introduction to the concept and units of the concentration-time product is given in Section D. 4.

The first volatile compound to be used extensively for treating seeds was formaldehyde, which was introduced by Guether in 1895 (quoted by Walker, 1948). Its successful use in controlling Ustilago avenae and Ustilago kolleri, respectively the loose and covered smuts of oats, was later reported by Bolley in 1897. The formaldehyde was applied as a dilute aqueous solution, so that the treatment itself was not a fumigation, although it is probable that the distribution of the gas in the bulk of moistened grain was improved by its volatility. Dillon Weston (1940) gave the details of one of the preferred treatments, which involved applying a 1 in 320 dilution of formalin (40 per cent. aqueous solution of formaldehyde), at the rate of 3 - 6 per cent. by weight of the grain. The moistened grain was covered and left undisturbed for 4 hours, and then rapidly dried and sown within 2 days to avoid the adverse effects of formaldehyde upon seed germination.

Davydoff (1931) designed an apparatus in which a 1 per cent. formalin solution was boiled, and the vapours of water and formaldehyde were used to control Ustilago avenae on oats and Tilletia caries on wheat. The grain was exposed to this moist treatment for 1 - 10 minutes, and then

covered with tarpaulins to restrict desorption, for a period of 1 - 2 hours, after which it was sown immediately.

In general, formaldehyde is useful for controlling the superficial fungi, including those such as Ustilago avenae located between the floral parts (lemma and palea) and the pericarp. It is not effective to any great extent in controlling the internal pathogens. There are a few anomalous accounts such as that by Tisdale et al. (1923), in which formalin was reported to be as effective as the hot water treatment in controlling loose smut of barley. The most likely explanation in such cases, is that the loose smut fungus involved, was not the deep-seated Ustilago nuda, but the superficially situated Ustilago nigra. The recognition of these two, distinct barley loose smut fungi was not made until 1932 (Tapke, 1932, 1943).

Hydrogen sulphide and various other sulphides were investigated in considerable detail by Russian workers in the 1930's, for their possible value in crop protection, including seed treatment (Kvashnina, 1935).

Kiashko (1935) showed that a 72 hour fumigation with an initial dose of hydrogen sulphide of 300 - 600 g./cu.m. (maximum C.T.P. 637 - 1274 m M.h./l.) completely controlled Ustilago hordei on barley grain and Tilletia caries on wheat grain, but that Ustilago avenae on oats and Ustilago panici-miliacei (Sphacelotheca destruens) on millet grain, were less easily controlled. These treatments reduced the germination of some seeds, such as beet, maize and cotton. Thus, although Xanthomonas malvacearum, an economically important seed-borne bacterial pathogen of cotton, is killed

by a 72 hr. exposure to 50 per cent. hydrogen sulphide (maximum C.T.P. about 1600 mM.h./l.) (Vzoroff, 1935), it appears unlikely that this fumigant could be used for treating infected cotton seed because of the likelihood of reducing the viability of the seed.

Hydrogen sulphide fumigations with doses of 400 g./cu.m. for 72 hr. (max. C.T.P. 842 mM.h./l.) were used by Kvashnina and Etmisheva (1936), who demonstrated a reduction of the incidence of Ustilago hordei, from 2.5 per cent. in the check, to 0.6 per cent. and with no adverse effect upon the yield (22.8 and 24.8 centners/hectare, respectively). Tilletia caries, on wheat, was reduced in frequency by the same treatment, but in all cases the control was less satisfactory than that resulting from a treatment with a 0.3 per cent. formalin solution. None of these treatments seriously reduced yield, and may even have increased it.

Lobik (1937) found that fumigation with hydrogen sulphide controlled neither Ustilago nuda nor U. tritici. However, Kvashnina and Etmisheva (1936) obtained a fair degree of control of loose smut in the barley variety Pallidum 96-23, by treating grain for 4 days with hydrogen sulphide at an initial concentration of 400 g./cu.m. (max. C.T.P. 1129 mM.h./l.). The frequency of loose smut was reduced from 7.7 to 0.8 per cent. in one sample, and from 8.8 to 3.3 per cent. in a second, and with no appreciable reduction in grain yield.

Petrova (1937) described a series of experiments in which wheat grain of 13.7 per cent. moisture content, was fumigated at 20°C. with hydrogen sulphide, and subsequently observed for frequency of germination

and loose smut infection (Table 1). The concentration-time products have been calculated and corrected for temperature. Loose smut was controlled to some extent, but the treatments were also appreciably phytotoxic. A 3-day fumigation with an initial concentration of 40 per cent. hydrogen sulphide, reduced the incidence of loose smut from 3.7 to 0.5 per cent., but there was an associated reduction in grain germination from 85.0 to 68.5 per cent.

Table 1. The control of loose smut of wheat, by fumigation of infected grain with hydrogen sulphide (abridged from Petrova, 1937).

	Per cent. Concentration H_2S	Duration of Fumigation (hours)	C.T.P. mM.h./l.	Per cent. Germination	Per cent. Loose Smut Infection
1.	0	0	0	85.0	3.7
1.	40	72	1197	68.5	0.5
2.	0	0	0	85.5	0.4
2.	57	72	1706	74.0	0.1
3.	0	0	0	85.5	3.7
3.	70	72	2096	81.0	0.9

Hydrogen sulphide fumigations are effective in controlling some superficial pathogens, but are rather less effective against the deep-seated ones. The high doses of fumigant and the lengthy fumigations, which in most cases were of 3 - 4 days duration, as well as the risk of severe

phytotoxicity, makes this a very unattractive control method.

More recent Russian work, has shown nitrogen peroxide to be a very useful fumigant for disinfesting seeds. A patent in the names of Trzhetsetskaya, Polyakov, Arbuzov and Chepurov (1960), describes a method of sterilizing maize and cotton seed, in a chamber, at concentrations of 0.1 - 0.5 g./l., with short durations of only 3 - 5 minutes at 15 - 20°C. The maximum concentration-time product would be only 0.9 mM.h./l. Nitrogen peroxide is therefore about a thousand times more toxic than hydrogen sulphide, to the superficial seed-borne pathogens. Chepurov et al. (1960) have described the methods, and Polyakov et al. (1960) have briefly discussed the results of laboratory and field investigations.

Cotton seed inoculated with the causal bacterium of gummosis (Xanthomonas malvacearum) was fumigated with nitrogen peroxide at 100 g./cu.m. for 5 minutes (max. C.T.P. 0.2 mM.h./l.) and upon culturing in a suitable medium, was shown to be free of bacteria. Similarly, maize grain inoculated with spores of the blister smut fungus (Ustilago maydis), was fumigated with nitrogen peroxide at the highest rates, and sown in field plots, where only one infected plant was observed among the several thousand which developed from fumigated seed, in contrast to an incidence of 4 - 6 per cent. infection in the untreated lots. For both maize and cotton, the development of plants from fumigated seed was comparable with that from unfumigated seed.

Chlorine gas was used as a fumigant by Leukel and Nelson (1939, 1940) to control superficial fungal pathogens on the grain of barley, wheat,

oats and sorghum. Spores of Tilletia sp. and Sphacelotheca sorghi were dusted on to the grain of wheat and sorghum respectively, and were fairly satisfactorily controlled by subsequent fumigation treatments. The spores of Ustilago hordei and Ustilago levis, were applied as spore suspensions, by a vacuum method, to the grain of barley and oats, and by contrast, were not appreciably controlled by fumigation. Fumigations of 5 minutes duration with 50 and 100 per cent. chlorine failed to kill all the surface-borne spores and were also highly phytotoxic, reducing grain germination from 90 per cent. in the checks to about 40 per cent. Assuming that the temperature at which the fumigation was conducted was 20°C., and that the chlorine concentration remained at 100 per cent. for the 5 minute period, then the concentration-time product was 3.5 mM. h./l.

Fumigations of longer duration with lower concentrations of chlorine, were subsequently found to give a satisfactory kill of the fungal spores, with only a slight associated phytotoxicity. Inoculated wheat grain was exposed for 2 hours, to chlorine of an initial concentration of 9 per cent. (max. C.T.P. 7.5 mM.h./l.). Spore germination was reduced from 50 to less than 1 per cent., and the frequency of plants infected with Tilletia sp. showed a corresponding reduction from 72 to 2, while the field germination of the treated grain was 77 per cent. as against 78 per cent. in the untreated grain. By comparison, treatment with ethyl mercury phosphate (N.I. Ceresan) gave a superior control of the pathogen and slightly improved germination. The same fumigation treatment (max. C.T.P. 7.5 mM. h./l.) failed to reduce the incidence of U. hordei in barley, and resulted

in only a marginal reduction in the frequency of oat plants infected with U. levis, from 92 to 73. Treatment with ethyl mercury phosphate resulted in complete control of U. hordei, and the frequency of plants infected with U. levis, was reduced to 3. In addition, grain germination was markedly superior to that of the fumigated lots and to the untreated lots.

Leukel and Nelson showed that the percentage volume of pure chlorine, relative to the volume of the grain being fumigated, was important and found, under the conditions of their experiments, that values between 20 and 40 per cent. resulted in optimum fungitoxicity and minimum phytotoxicity. This indicates that chlorine is readily sorbed by the grain to an extent that results in phytotoxicity at the higher percentages of relative volume.

Chlorine was not a very effective fumigant for controlling superficial pathogens, at treatments of about 7.5 mM.h./l. Its effect upon internal pathogens, such as Ustilago nuda, was not investigated, contrary to an account in one of the abstracting journals (Rev. appl. Mycol., 19: 75, 1940).

Ethylene oxide has been used extensively for sterilizing surgical equipment (Phillips, 1957) culture media and food, but there are only a few reports of its use for controlling microorganisms associated with seeds. One such report is that by Steinkraus et al. (1959) who fumigated seeds of a wide selection of agricultural crops, that were naturally contaminated with fungi and bacteria.

Infested seed that had been air-dried to a moisture content

within the range 5 - 10 per cent., was exposed to pure (100 per cent.) ethylene oxide vapour at 27°C. for periods up to one hour. A sample of barley grain with 96 per cent. germination and with 45 per cent. of the grains infested with Alternaria tenuis, was reduced in germination to 73 per cent. and freed of A. tenuis by a 5 minute fumigation (max. C.T.P. 3.4 mM.h./l.). However an exposure of 30 minutes (max. C.T.P. 20.4 mM.h./l.), which reduced the germination to 61 per cent., was necessary to free the grain of all the associated mesophilic bacteria. A 30 minute fumigation also completely controlled bacteria on the seeds of samples of mung bean, garden pea, dandelion and aster, but in these cases, germination was reduced by not more than 8 per cent. However different seed samples of the same crop species showed marked differences in their susceptibility to fumigation damage and in the ease of control of the associated bacteria.

The possible use of ethylene oxide in plant quarantine practices for the sterilization of wheat grain, to prevent the introduction into the U.S.A. of Urocystis tritici, causal fungus of flag smut, has been considered by Schoen (1962). It is not apparent from this account whether the dose of 300 mg./l. refers to pure ethylene oxide or to a mixture containing 12 per cent. ethylene oxide. In either case, the concentration-time product that was used (max. C.T.P. 436 mM.h./l. or 52.3 mM.h./l.) was considerably in excess of what Steinkraus et al. (1959) had found was sufficient to control Alternaria tenuis on barley (3.4 mM.h./l.). Indeed, the C.T. products used by Schoen were so high as to render the wheat inviable as well as sterile. In a subsequent evaluation, the baking

characteristics of wheat were shown to have been seriously worsened by an 18 hour fumigation at 26 - 28°C., with an initial application of fumigant equivalent to approximately 560 mg./l. (max. C.T.P. 229 mM.h./l. or 27.5 mM.h./l.). On the basis of this demonstration, Schoen regarded ethylene oxide as unsuitable for sterilizing wheat intended for flour and baking.

Kennedy (1959) investigated the efficacy of ethylene oxide for controlling Ascochyta spp. in seeds of the garden pea (Pisum sativum), a high proportion of which contained internal infections. Seed of 10.3, 13.7 and 17.7 per cent. moisture content was fumigated at 15°C. for 20 hours, with doses of ethylene oxide that yielded C.T. products of 500, 1000 and 2000 mg.h./l. (equivalent to 11.4, 22.7 and 45.4 mM.h./l.). The results (Table 2) indicate an increased susceptibility to fumigation injury at the higher moisture contents. The frequency of Ascochyta spp., shown in parenthesis, was reduced with increasing C.T. products but these values were closely paralleled by a severely reduced seed germination.

Although the germination of another variety of pea was less reduced at these C.T. products, it was concluded that ethylene oxide was too phytotoxic to be used for controlling these particular deep-seated pathogens of the garden pea.

Chloropicrin has been used extensively for soil fumigation because of its high fungitoxicity (Christie, 1947; Schmitt, 1949; Wilhelm and Ferguson, 1953), and has been investigated recently for its suitability in controlling seed-borne bacteria and fungi.

Nugent and Cook (1938, 1939) inoculated kale seed with a suspension

Table 2. The effect of ethylene oxide fumigations at 15°C. upon percentage pea seed germination, and the percentage of seeds infected with Ascochyta spp., in parenthesis, at three seed moisture contents (abridged from Kennedy, 1959).

C.T.P. mM.h./l.	Per cent. Germination after 2 Weeks (Var. Zelka)		
	Per cent. Moisture Content		
	10.3	13.7	17.7
0	53 (51)	59 (59)	62 (34)
11.4	47 (59)	33 (35)	0 (17)
22.7	38 (57)	22 (26)	1 (0)
45.4	37 (44)	14 (17)	0 (0)

of Xanthomonas campestris and subsequently exposed the dried seed, with a moisture content of 6.5 per cent., to the vapour of chloropicrin. A 24 hour fumigation in an atmosphere probably saturated with chloropicrin (max. C.T.P. at 20°C. = 26.2 mM.h./l.) reduced the number of seeds infested with bacteria from 71.1 and 66.8 per cent. to 23.1 and 16.4 per cent., and increased the number of sterile seeds by a larger amount through the control of seed-borne fungi. Seed receiving a similar fumigation at 20 - 23°C. for 24 hours, was sown in soil, where it germinated 67 per cent., against 75 per cent. in the check. X. campestris was completely controlled by the treatment, although 14.4 per cent. of the seeds, cultured on media, remained associated with other species of bacteria. The mercuric chloride

treatment, which was the standard control method proved to be marginally superior. Nugent and Cook found that an increase in moisture content of the seed and an increase of temperature, resulted in a reduction of seed viability, but that the frequency of sterile seeds was not increased above 86.9 per cent.

Chloropicrin was also investigated by Stark (1948), for its efficacy in controlling a heavy infestation of spores of Alternaria sp. on celery seed. A 24 hour fumigation in an atmosphere supposedly saturated with chloropicrin, and with a relative humidity of 20 per cent., reduced the incidence of Alternaria sp. from 53 per cent. to only 45 per cent. and produced a reduction in germination from 53 to 51 per cent. Fumigation at 90 per cent. relative humidity was completely toxic to both fungus and seed.

A more extensive investigation of the fungicidal properties of chloropicrin was undertaken by Kennedy (1959, 1961), and subsequently by Mukkath (1961). In both instances endeavours were made to control deep-seated pathogens. Kennedy showed that fumigations of long duration, of about 72 hours at saturation concentrations of chloropicrin at 20°C. (max. C.T.P. 78.5 mM.h./l.), were necessary to control deep-seated infections of Ascochyta spp., which was accounted for by the slow penetration of the chloropicrin to the inner regions of the seed. Severe phytotoxicity was again encountered with treatments which appreciably reduced the incidence of Ascochyta spp. However, an investigation of the effect of seed moisture content revealed that at about 16 per cent. moisture (and 20°C.), a satisfactory fungicidal effect was obtained by reducing the percentage of

seeds associated with viable Ascochyta spp., from 26 to 6 per cent., with an associated reduction in germination from 75 to 62 per cent. Subsequent experiments with the same variety (Zelka), but fumigated at 13.9 per cent. moisture content, showed it to be more susceptible to fumigant injury than a variety of 'Blue' peas, and further that Ascochyta spp. were less easily controlled in Zelka, probably because the seed was much more heavily infected, than in the seed of the 'Blue' variety.

Mukkath (1961) fumigated celery "seed" with chloropicrin and observed its effect upon seed germination and upon the viability of Septoria apii spores within pycnidia, partially embedded in the wall (pericarp) of the seed. Spore viability was determined by moistening fumigated seed and collecting the spore masses that exuded from the pycnidia, and using these for spore germination tests. Infected seed of 8.7 per cent. moisture content was fumigated for 3 days in an atmosphere saturated with chloropicrin at 20°C. (C.T.P. 78.5 mM.h./l.), resulting in a reduction of spore germination from 12 to 0 per cent., with an associated reduction of seed germination from 46 to 28 per cent. The germination of a less infected sample of celery seed, was less adversely affected by a fumigation of the same duration.

Two interesting points arise from this work. Firstly, there was an appreciable, and reproduceable, increase in the germination of spores recovered from seed fumigated for 2 hours, and secondly a phenomenon which may be described as a delayed loss of spore viability, as a result of the fumigation treatment. These results are shown in Table 3.

Table 3. The influence of duration of fumigation with chloropicrin at 20°C. upon the germination of spores of Septoria apii, and celery seed of 8.7 per cent. moisture content (abridged from Mukkath, 1961).

Duration of Fumigation (Hours)	Per cent. Spore Germination 3 Days after Fumigation	Per cent. Spore Germination 3 Weeks after Fumigation	Per cent. Seed Germination 3 Days after Fumigation
0	12	17	46
2	28	1	42
4	15	1	38
8	19	0	24
24	18	0	34
48	8	0	36
72	0	0	28

Spores recovered from seed fumigated for up to 24 hours showed no reduction in viability when tested immediately (2 - 3 days) after the fumigation, but after 3 weeks storage, the germination was reduced to 1 per cent. or less, while that of the unfumigated spores remained high.

The explanation of this rapid loss of spore viability can only be conjectural at this stage. However, celery seeds have appreciable oil reserves in the region of the fruit occupied by the pycnidia, and chloropicrin is known to be far more soluble in organic solvents and resins, than in water (Jackson, 1934). It is possible that chloropicrin, dissolved in the oil, continued to have a slow fumigating effect within the seed, long

after the seed had been removed from the fumigation chamber, and despite an airing period of 24 hours.

Isaac and Heale (1961) investigated the ease of control of Verticillium albo-atrum associated with lucerne seed. On the basis of a preliminary assay, allyl alcohol, allyl bromide, chloropicrin and formaldehyde (as formalin), were selected for their rapidity of action. Seed inoculated with conidia of V. albo-atrum, and other samples consisting of seed associated with small pieces of moribund lucerne, infected with resting mycelium, were fumigated at saturation concentrations of the four fumigants. The conidia were killed by allyl bromide in 10 minutes (0.9 mM. h./l.), by chloropicrin in 20 minutes (0.4 mM.h./l.), by allyl alcohol in 1 hour (1.0 mM.h./l.) and by formalin in 2.5 hours. The resting mycelium, in the plant debris was less easily controlled, which necessitated using longer exposures:- allyl alcohol, 1.5 hr. (1.5 mM.h./l.), allyl bromide 2.0 hr. (10.9 mM.h./l.), formalin 4 hr. and chloropicrin 8 hr. (8.7 mM.h./l.). Fumigation of seed of 16.4 per cent. moisture with the fumigant and time combinations, just mentioned, resulted in some loss of seed viability after 8 months of post-fumigation storage at 15°C. However, there were no major differences of phytotoxicity between fumigants, since percentage germination was within the range 75 - 68 per cent., with only 77 per cent. for the unfumigated seed stored at 16.4 per cent. moisture, in contrast to 90 per cent. germination of seed stored at 8.9 per cent. moisture.

Fumigations which controlled the resting mycelium of Verticillium albo-atrum were sufficiently non-phytotoxic to justify a further considerat-

ion of the control method and also of the fumigants involved. Allyl alcohol was more toxic to the resting mycelium, than either allyl bromide or chloropicrin, both in terms of the concentration-time product expressed in moles (1.5 as against 10.9 and 8.7 mM.h./l. respectively), and also as regards the duration of the treatment (1.5 hr. as against 2.0 hr. and 8.0 hr. respectively).

Dusts which release fumigants when applied to grain have been tried on a limited scale, but are only effective against superficial pathogens. Satisfactory control of Ustilago avenae and U. levis was obtained using a 25 per cent. formalin dust and a 5 per cent. iodine dust, applied at 3 oz./bushel (Sayre and Thomas, 1927). Sevryukova (1957) has more recently demonstrated the effectiveness of furfural in 5 - 10 per cent. mixtures with soil or superphosphate, as a non-phytotoxic seed application to control Tilletia caries. The slightly volatile organomercury compounds are now used in most instances to control such superficial pathogens.

One of the most important developments in the last decade, in the control of internal seed-borne pathogens, has been made by Wagner, working in Germany, using solutions of methanol applied as a spray to barley grain, to control Ustilago nuda. Since the preparations were very volatile, it is appropriate to include this work in a consideration of fumigation methods of seed disinfection.

Methanol solutions of various compositions have been used. In the early investigations, satisfactory control was obtained using methanol

of more than 96 per cent. purity, or solutions of other, in themselves ineffective organic compounds such as acetone, containing more than 60 per cent. methanol (Wagner, 1958, 1959). The preparation was applied to the grain at the rate of 2 - 3 litres/100 kg. (approximately 2 - 3 per cent. weight/weight) and the treated grain was stored under airtight conditions, such as in polythene sacks, for 24 hours at temperatures of +5 -10°C. (1960a, 1961b). In general, the results were very encouraging. The degree of control of U. nuda was comparable with that obtained with the hot water treatment of 11 minutes at 51 - 52°C. (Wagner, 1963c), and the subsequent grain yield from field experiments was usually superior to the hot water treated grain (Wagner, 1960a). The various treatments reduced the laboratory germination from 87 - 100 per cent. for the untreated grain, to as low as 57 per cent., but the grain yields were generally not less than 90 per cent. of the checks, and were sometimes superior (Wagner, 1961b). Treated grain also produced plants of a darker colour (Wagner, 1961a).

This early work indicated that there were marked differences in the degree of phytotoxicity which resulted from treating grain of different moisture contents and physiological quality, and preliminary treatments of subsamples were recommended as a precaution (Wagner, 1960a). However, these difficulties have been overcome, to some extent, by adjusting the duration of the treatment to suit the temperature (Wagner, 1963a, b), and by the use of inorganic salts in aqueous solutions of methanol (Wagner, 1960b). Sodium, potassium and ammonium nitrates have been found satisfactory, and are used in larger proportions if the water content of the preparation is

high. One particular combination was suggested in Wagner's most recent patent (Wagner, 1960b), namely, a solution containing 800 ml. methanol, 200 ml. water, and 90 g. ammonium nitrate applied at the rate of 5 litres to 100 kg. of barley grain of 14.5 per cent. moisture content. The treatment should last 8 hours at temperatures within the range 5 - 10°C. Formulations with this general composition have been made available, commercially, as "Ustilgon", by the firm of E. Merck, AG., Darmstadt, Germany.

In the earlier treatments the weight of preparation was only 2 - 3 per cent. of the grain, and after 24 hours treatment, this was soon lost by volatilization when grain was transferred by means of a grain blower (Wagner, 1961b). With these more recently developed preparations, the quantity used to treat the same quantity of grain has been doubled, and would appear to be appreciably less volatile. This may raise drying problems, that were also inherent disadvantages with the formalin drench and hot water treatment methods.

Wagner (1963a) has noted that this disinfection technique is only suitable for 2-rowed spring barley varieties, and in its present form, is too phytotoxic for use on grain of some winter barley varieties, or on wheat. Bartos and Zemanek (1963) have confirmed the efficacy of methanol for controlling Ustilago nuda in barley grains, but found that a number of Czechoslovak varieties treated with this compound, showed reductions of germination of as much as 50 per cent.

SUMMARY

Several fumigants have been used for controlling seed-borne fungi, amongst which nitrogen peroxide, ethylene oxide, allyl alcohol, allyl bromide, chloropicrin, formaldehyde and the organomercury compounds, have satisfactorily controlled superficial pathogens in treatments which were not seriously phytotoxic. Less attention has been directed to the control of internal infections. Hydrogen sulphide, ethylene oxide and to a lesser extent, chloropicrin, are all severely phytotoxic at fungicidal levels, but a very effective control of Ustilago nuda has been obtained with formulations of methanol.

Methyl bromide, despite its extensive use in the control of pest infestations of grain, in the control of pathogenic fungi in soil and even of storage moulds of grain (Srinivasan and Majumder, 1961; Yanai et al., 1964) does not appear to have been investigated for its efficacy in controlling deep-seated infections of viable grain.

D. MATERIALS AND METHODS

D.1. Details of cereal varieties

Grain of four varieties of barley (Hordeum vulgare L.) and one variety of wheat (Triticum aestivum L.) infected with Ustilago nuda and U. tritici respectively, were used in these studies. An additional uninfected stock of Rika barley, and grain of three other varieties of wheat were used for preliminary and comparative investigations.

The details of the infected seed stocks are given in Table 4. Where sufficient data were available the means and the 95 per cent. confidence limits (in parenthesis), have been calculated, as for example with the percentage of normal seedling development in the 20°C. sand test, the percentage embryo infection, and the percentage of loose smut infection of plants in the field. These values were calculated using an angular transformation of the percentages (Snedecor, 1956, Table 11.12.1).

In the description of barley varieties, the word "hulled" refers to grains in which the lemma and palea are normally adherent to the pericarp. The term "sprouted" has been used to denote those embryos which showed elongation of the seminal roots or of the coleoptile, indicating that the grain had either ripened or been stored under such wet conditions that it had already commenced germination before having been dried to a lower moisture content. Early stages of germination are not easily recognized by examination of intact grains, but are obvious in the excised embryos, which were examined, more particularly, for an estimate of the frequency of embryo infection by the loose smut fungus.

Table 4. Details of cereal varieties.

(Values in parenthesis in columns 5, 6, 7 and 8 are 95 per cent. confidence limits, as described in the text).

Variety \ Column	1. Type	2. Origin	3. Year of Harvest	4. 1000 Grain Weight (g.) (ca.13 Per Cent. Moisture)
	Barley			
1. Arès	6-rowed, hulled, winter variety.	France, semi-commercial stock	1961	43.0
2. Rika	2-rowed, hulled, spring variety.	Sweden, commercial stock	1960 or 1961	40.1
3. Edda II	6-rowed, hulled, spring variety.	Sweden, commercial stock	1960 or 1961	34.0
4. Donaria	2-rowed, hulled, spring variety.	Germany, semi-commercial stock	1961	36.8
	Wheat			
5. Fylgia II	Spring variety.	England, experimental, inoculated, recleaned.	1961	26.4

Table 4 continued.

	5. 20°C. Sand Test. Per Cent.Normal Seedlings	6. Per Cent.Embryo Infection U. nuda or U.tritici	7. Per Cent.Probability Sprouted Grain (P = 0.025)	8. Per Cent. Loose Smut Infection Field Test	9. Per Cent. Probability U. nigra (P = 0.05)
1.	97.5 (96.7-98.1)	9.15 (7.68-10.74)	None found: less than 0.31	6.50 (5.89-7.14)	None found: less than 0.16
2.	approx.66 (emergence approx. 87)	7.36 (5.63-9.31)	None found: less than 0.38	5.76 (3.83-8.06)	None found: less than 1.19
3.	80.1 (68.0-89.9)	8.94 (6.51-11.72)	10.62 (5.08-17.88) (95 per cent.limits)	5.54 (2.83-9.09)	None found: less than 0.93
4.	96.6 (95.2-97.7)	1.16 (0.73-1.68)	None found: less than 0.16	1.05 (0.89-1.22)	None found: less than 0.09
5.	95.8 (91.3-98.7)	9.54 (8.32-10.83)	None found: less than 0.31	9.23 (5.41-13.95)	Non-pathogenic

A measure of the possible degree of sprouting in those varieties in which it was not observed, possibly because insufficient grains were examined, was calculated by reference to the table of Poisson Limits given by Fisher and Yates (1957, Table VIII.1). The Poisson distribution was considered appropriate in this case, where the probability of finding sprouted grains had been shown to be small.

The probability, p, of the occurrence of sprouted grain was estimated at a fiducial probability level ($P = 0.025$), such that despite their theoretical existence in the grain lot, sprouted grains would be absent in only 2.5 per cent. of the samples examined. The probability, p, was expressed relative to 100, and as such was referred to as a "percentage probability".

D.2. The nomenclature of the loose smut fungi of barley and wheat.

Fischer and Shaw (1953) proposed several consolidations of the North American smut fungi, and suggested binominals that would be valid according to the International Rules of Botanical Nomenclature. Under this broadened concept, "fungi of similar morphology and symptomatology, parasitizing the same or different species and genera of the same host family, would be regarded as belonging to one and the same species" (Fischer & Holton, 1957).

While recognizing the taxonomic value of these changes, the established names have been used in this study, to avoid the use of identical binominals, or possibly trinominals, in reference to the loose smut fungi of barley and wheat.

Binominal used in Thesis	Proposed Binominal (Fischer & Shaw, 1953)
Loose smut of barley *Ustilago nuda* (Jens.) Rostr.	*Ustilago nuda* (Jens.) Rostr.
Loose smut of wheat *Ustilago tritici* (Pers.) Rostr.	*Ustilago nuda* (Jens.) Rostr.
False or black loose smut of barley *Ustilago nigra* Tapke	*Ustilago avenae* (Pers.) Rostr.

D.3. The identification and significance of *Ustilago nigra* in field trials of barley.

Ears of barley covered with the mature sori of *Ustilago nigra* have an almost identical appearance to those infected by *Ustilago nuda*. However, *U. nigra* is only associated with the superficial tissues of the grain and is relatively easily controlled, by applications of volatile organo-mercury compounds, in contrast to the embryo-infecting *U. nuda*. For this reason the fumigation treatments used in these studies, were also expected to control *U. nigra* more easily than *U. nuda*. Therefore, a measure of the proportion of the control of loose smut, that may have been due to the elimination of *U. nigra*, was obtained by calculating the possible frequency of *U. nigra* in the crop (Table 4, column 9) and assuming that it would have been more easily killed by fumigation than *U. nuda*.

The possible occurrence of *U. nigra* in the field trials, was of further interest, because this species has not been recognized in England (Moore, W.C. and Moore, F.J., 1961), and it was feared that it might have been introduced with the four barley varieties received from Sweden, France

and Germany. Although it is rare in Western Europe, Ustilago nigra has recently been recorded in Denmark (Pedersen, 1957), Italy (Grasso et al., 1957), France (Darpoux and Ponchet, 1957) and in Germany by Niemann (1961) although Frauenstein (1962) failed to find it in Eastern Germany.

The differentiation between Ustilago nuda and U. nigra.

Infected ears were collected from the field and stored separately in paper envelopes. From each ear a small quantity of spores ~~were~~ was removed, suspended in sterile, distilled water, and streaked on 2 per cent. potato dextrose agar contained in a 9 cm. petri dish. The cultures were incubated at 20°C. for 36 hours in darkness, and examined microscopically for the type of spore germination. The numbers of viable cultures examined were: Arês - 168; Rika - 25; Edda II - 36 and Donaria - 52. All of the spores examined were echinulate and germinated by the production of hyphae - features diagnostic of U. nuda. Both Ustilago nigra and U. hordei germinate to produce masses of sporidia, as illustrated by Tapke (1943), but no such examples were found in the present investigations.

Calculation of the upper limit of probability for Ustilago nigra in barley.

Ustilago nigra was recognized in neither the standing barley crop nor in the spore germination tests, but it may still have been present with a low frequency. The occurrence of such infected plants in samples taken from the population (developed from untreated grain) was expected to be similar to the Poisson distribution. Reference has been made, therefore, to the table of Poisson Limits given by Fisher and Yates (1957, Table VIII.1)

and the calculation of the upper limit of probability follows the principle discussed in Section D.1, in connection with sprouted grains.

A number of spore collections (c) were made at random, from a population, and then the least number (N) of plants (infected and uninfected), from which these spores had been effectively sampled, was calculated from the 95 per cent. upper confidence limit (Y) for the percentage frequency of loose smut-infected plants in the same population. For example, from a population with an estimated upper frequency (P = 0.05) of loose smut-infected plants of Y per 100, 'c' spore collections were made of which 'a' were identified as U. nigra. The effective number of plants from which these collections were made was N, where $N = \frac{c}{Y} \times 100$ plants

since U. nigra was not identified, 'a' = 0.

In the absence of a column of fiducial probability for P = 0.05, the P = 0.025 column has been used. Therefore if 'a' is zero and N is large, the value corresponding to P = 0.025 (in the part of the table for estimating the upper limit of probability) approaches 3.69, which will be written here as (ca.3.69).

The probability of the occurrence of U. nigra:-

$$= p = \frac{(ca.\ 3.69)}{N}, \text{ where } N = \frac{100c}{Y}$$

$$= \frac{(ca.\ 3.69)}{c} \times \frac{Y}{100}$$

If the probability is expressed relative to 100, the resulting quantity may be termed the "percentage probability".

i.e. Percentage Probability ($P = 0.05$) for $a = 0$, $= \frac{(ca.\ 3.69)}{c}$. Y per cent.

Since the value Y was calculated with a fiducial probability of $P = 0.05$, this level of certainty is also implied in the "percentage probability". This upper limit of probability of U. nigra, expressed as a percentage, was calculated for each of the four varieties of barley (Table 4, column 9).

D.4. The concept of the concentration-time product and its units.

The toxicity of a fumigant to an organism is influenced by many factors, but more particularly by the concentration (C) of the fumigant and the duration of the exposure (T). Haber (1924), quoted by Martin (1959, p.64), was able to show that the proportionalities of these factors were such that their product was a constant ($C \times T = K$), which represented the degree of fumigant toxicity in the gaseous phase. Hemwall (1962) has recently discussed this concept in relation to soil fumigation.

The concentration-time product (C.T.P.), is a useful measure over fairly broad ranges of time and concentration, but often cannot be applied in circumstances where extreme values of either factor are used.

In some fumigation operations, as for example in gas-tight chambers involving fumigants which are only slightly sorbed, the decline in concentration with time is often slight. The C.T. product is then approximately equal to the product of the average fumigant concentration, and the duration of the fumigation.

However, in large scale fumigations of grain or soil, for example, the decline in fumigant concentration at the sampling point is often rapid, due to gas leakage and sorption. In these circumstances, the C.T. product may be calculated by measuring the area under the concentration-time curve, in the appropriate units.

The concentration-time product may be expressed in many different units, but more usually in those of either the metric or the avoir dupois (general) systems. On the metric system, the preferred units of concentration are the milligram/litre or the gram/cubic metre. These quantities are numerically identical, and by a fortunate coincidence, agree to within 1 - 2 per cent. with the quantity expressed in ounces/1000 cubic feet, on the avoir dupois system. The unit of time most usually favoured is one hour, so that an example of the metric units of the concentration-time product, might be:

milligram/litre x hours

or milligram x hours/litre, abbreviated as mg.h./l.

These units have immediate practical utility, when the mass of the fumigant involved is considered. However, in studies of the physical properties, or of the chemical reactivities and toxicities of fumigants, it is often more appropriate to make comparisons in terms of the numbers of molecules, rather than of the mass of the fumigant (Monro et al., 1952).

To facilitate such a comparison the concentration in units of mass (grams), per unit volume, is divided by the gram molecular weight of the gas (or vapour), to give a concentration in moles per unit volume.

The number of molecules per mole is known as the Avogadro number (N), with an accepted value of 6.023×10^{23}.

The concentration-time product may then be expressed as:

mole. hours/litre, M.h./l.

or millimole. hours/litre, mM.h./l.

where $\text{mM.h./l.} = \frac{\text{mg.h./l.}}{\text{M}}$

where M is the gram molecular weight of the gas.

If the fumigant is one of two gases, then the second gas will have a concentration of n_2 moles/unit volume, whereas the concentration of the fumigant (gas 1), will be n_1 moles/unit volume. By an extension of Dalton's law of partial pressures (where the total pressure, P, of a mixture of gases is equal to the sum of the partial pressures of the constituent gases), the total concentration is $(n_1 + n_2)$ moles and the mole fraction of gas 1 is:

$$\frac{n_1}{n_1 + n_2} = \text{mole fraction of gas 1.}$$

This is a useful quantity since the composition of the gas mixture by volume, can be expressed as parts per hundred (per cent.) by multiplying it by 10^2, or as parts per million by multiplying it by 10^6. These measures are used extensively in mammalian toxicology.

When only the mole concentration of gas 1 is known and its value is corrected to correspond to 22.4 litres at S.T.P. (273°K, 760 mm Hg.), then the sum of the mole concentrations of the constituent gases equals

unity, and n_1 is the mole fraction of gas 1,

that is: $\frac{n_1}{(n_1 + n_2)} = \frac{n_1}{1}$, where $(n_1 + n_2) = 1$, at S.T.P.

Therefore, when the fumigant concentration in mg./l. has been corrected to S.T.P., the mole fraction may be calculated in the following manner:

$$\text{mole fraction} = \frac{\text{(mg./l.) S.T.P. x 22.4}}{\text{Molecular weight x 1000}}$$

Conversion of percentage volume to concentration/unit volume.

If x is the percentage volume of a gas at t°C. and 760 mm. then the percentage volume corrected to 0°C. $= x \times \frac{273}{273+t}$.

the corresponding mole fraction $= \frac{x}{100} \times \frac{273}{273+t}$

and the concentration in moles/l. $= \frac{x}{100} \times \frac{273}{273+t} \times \frac{1}{22.4}$ M./l.

or expressed in millimoles/l. $= \frac{x}{100} \times \frac{273}{273+t} \times \frac{1000}{22.4}$, mM./l.

The concentration in milligrams per litre is obtained by substituting the gram molecular weight of the gas for M in the equation:

$$\text{concentration} = \frac{x}{100} \times \frac{273}{273+t} \times \frac{1000}{22.4} \times M, \text{ mg./l.}$$

where x is the percentage volume of the gas at t°C.

Conversion of partial pressure to concentration/unit volume.

Assuming that Dalton's law of partial pressures holds, then the partial pressure of gas 1 in a mixture of two gases is given by the product of the mole fraction of gas 1, and the total pressure P,

where $p_1 = \frac{n_1}{n_1 + n_2} . P$

at S.T.P. $p_1 \times \frac{273}{273+t} = \frac{n_1}{n_1 + n_2} \times 760$ mm.Hg.

but at S.T.P. $(n_1 + n_2)$ is unity and n_1 is the mole fraction of gas 1, thus:

$$n_1 = \frac{p_1}{760} \times \frac{273}{273+t}$$

the concentration in millimoles/litre at t°C.

$$= \frac{p_1}{760} \times \frac{273}{273+t} \times \frac{1000}{22.4}, \quad \text{mM./l.}$$

and the concentration in milligrams/litre at t°C.

$$= \frac{p_1}{760} \times \frac{273}{273+t} \times \frac{1000}{22.4} \times M, \quad \text{mg./l.}$$

where M is the gram molecular weight of the gas.

These equations are based on the assumption that the fumigant behaves as an ideal gas. Since the actual values are likely to deviate appreciably from those of an ideal gas, as the partial pressure approaches the saturation pressure of the vapour, the calculated values have been regarded as only estimates.

D.5. Fumigations conducted in 20 litre bins.

Fumigations of barley and wheat grain with methyl bromide, and of wheat grain with hydrogen cyanide, were conducted in 20 litre mild steel bins, as illustrated in Plate 1. The inner surfaces were painted, and the fitting of the lid was made airtight by screwing it down tight, against

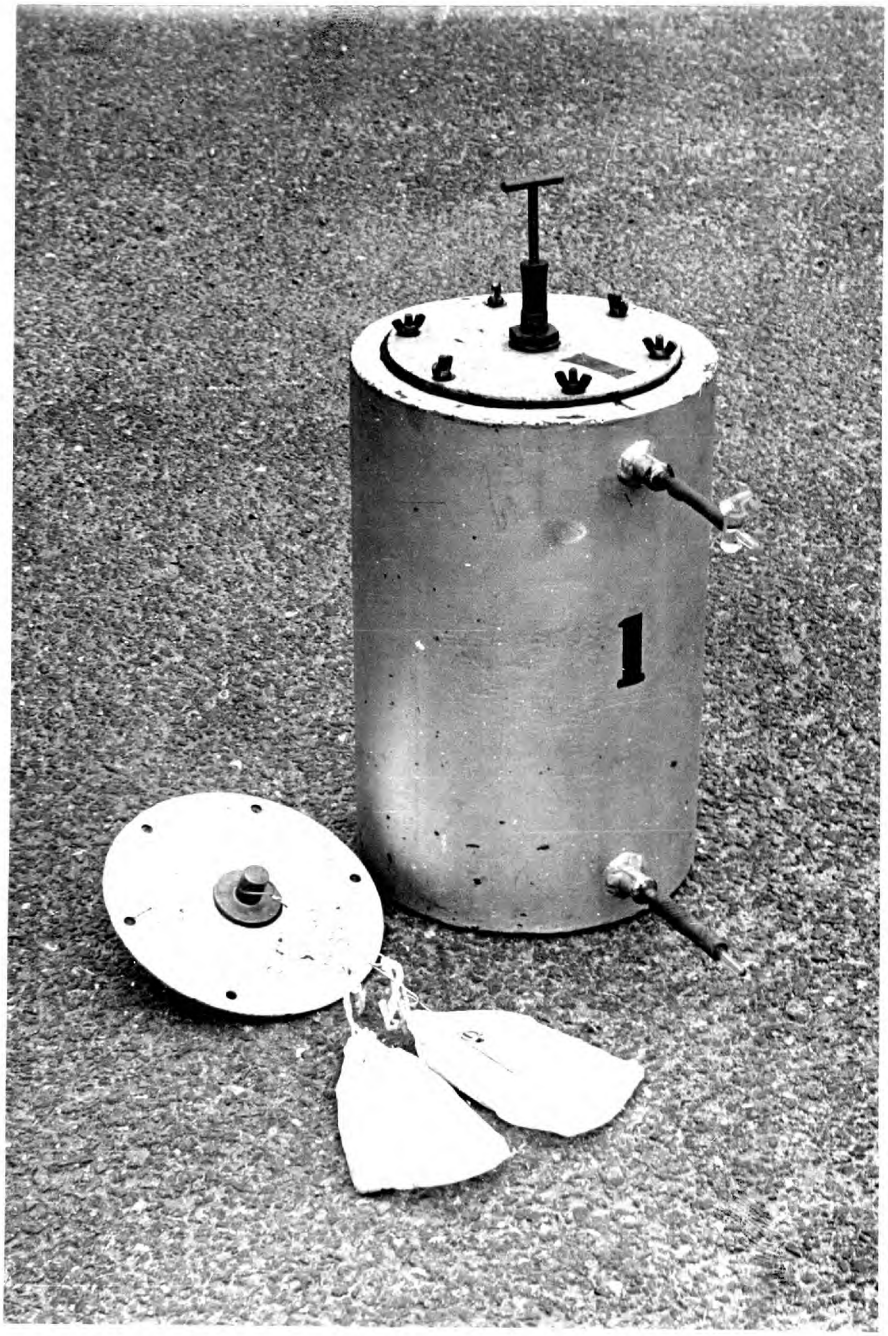


Plate 1. A 20 l. bin as used in the fumigations with methyl bromide and hydrogen cyanide.

a thin foam-rubber gasket, by means of six wing-nuts. The bin was provided with two side tubes, through which fumigant was introduced in some of the methyl bromide fumigations, and through which gas samples were also taken for concentration determinations.

The bin lid was fitted with a device for crushing glass ampoules so that the released fumigant escaped into the bin. It consisted of an 8 cm. length of brass tubing of 1.5 cm. internal diameter, and carried a plunger which moved through a tightly fitting rubber bung. That part of the tube which projected into the bin was sealed at the end, and the side perforated to allow the fumigant to escape when the ampoule was fractured. This device was large enough to accommodate two ampoules, each of 1.5 ml. capacity.

A length of looped wire was supported from the bin lid, and to this were attached numbered muslin bags, containing 30 - 50 g. of grain.

Grain samples were placed in a bin which was then made airtight, 15 - 20 hours prior to the fumigation, so as to allow the moisture content of the grain to equilibrate with the air humidity in the bin. Preliminary experiments had shown that the change of moisture content of a 100 g. sample of grain of 14.0 per cent. moisture, under these conditions was less than 0.1 per cent. All fumigations were conducted at 20°C. and approximately 760 mm.Hg. pressure. The grain was also stored at 20°C. for at least 7 days prior to fumigation.

Small, accurately known weights of fumigant were conveniently introduced into a bin by crushing an ampoule, previously filled with

approximately the appropriate amount. Volumes of liquid fumigant more than 3 ml. were used in some of the barley - methyl bromide fumigations. These were measured in a stout 10 ml. burette, attached to a cylinder of liquid methyl bromide. Upon releasing a valve the liquid fumigant entered the burette under its own pressure, and by closing this valve and opening another, a measured volume was delivered, through a heating coil which assisted the vaporization of the liquid, to a fumigation bin. Prior to the introduction of fumigant, a bin was evacuated by an amount sufficient to leave a slight pressure deficit, after the fumigant had been added. For the largest quantities of methyl bromide (17.3 ml. per 20 l. bin), the pressure in the bin was initially reduced by about 35 cm. of mercury.

Following the introduction of the fumigant, the bin was inverted several times with a rotary motion, in order to agitate the central piece of wire carrying the muslin bags, which functioned as a stirring device, so as to improve the distribution of the fumigant.

Upon completion of the fumigation, the grain samples were removed from the bin, aired for 2 days at 20°C. and 50 per cent. relative humidity, and subsequently stored in perforated paper envelopes at 20°C.

D.6. The determination of concentrations of methyl bromide and hydrogen cyanide.

Methyl bromide.

Concentrations of methyl bromide were determined by means of a thermal conductivity meter that had been calibrated against chemically

determined concentrations of the fumigant. Heseltine (1961) has described the construction and operation of such an instrument.

The thermal conductivity meter, with a cell of 5 ml. capacity and fitted with soda-lime filters, was calibrated to cover the range 0 - 120 mg./l. with a coarse adjustment, and 0 - 60 mg./l. with a fine adjustment. Higher concentrations were determined with the same instrument, after the gas sample had been diluted with air, by a known amount, in an intermediate mixing chamber of 1 l. capacity.

The chemical determinations of methyl bromide had been made by the catalytic combustion method described by Lubatti and Blackith (1956a). The sodium bromide was titrated with 0.05 N silver nitrate, and the equivalence point determined electrometrically by a mains-operated voltmeter of the type described by Scroggie (1952).

Hydrogen cyanide.

In these fumigations 200 g. of grain of 14.0 per cent. moisture content were fumigated, in 20 l. bins of the type used for the methyl bromide studies, just described. Ampoules containing the appropriate quantities of liquid hydrogen cyanide were prepared and subsequently fractured in the ampoule-breaking device, but the resulting concentrations were not determined. Instead an estimate was made from a knowledge of the weight of fumigant introduced. The concentration-time products were assumed to have been 80 per cent. of the theoretical maximum value, and are shown below, corresponding to the approximate initial concentrations and

fumigation durations that were used:

22.7 hr. fumigation at initial concentration 27.5 mg./l. = approx. 500 mg.h./l.

22.7 hr. " " " " 55.0 mg./l. = approx. 1000 mg.h./l.

45.4 hr. " " " " 55.0 mg./l. = approx. 2000 mg.h./l.

However, it is now thought probable that the C.T. products were appreciably less than these calculated values, due to sorption of hydrogen cyanide on the grain.

D.7. Fumigations conducted in desiccators.

Ten of the fumigants that have been used, had boiling points between + 34.6°C. (diethyl ether) and + 150.7°C. (allyl isothiocyanate), and were characterized by correspondingly low concentrations in the vapour phase at 20°C., in comparison with such volatile compounds as methyl bromide, with a boiling point of 3.6°C. In order to achieve a given effect in the shortest possible time, without raising the temperature, fumigations were conducted at the highest possible stable vapour concentration, that is, the saturation vapour concentration (or the saturation vapour pressure, expressed in units of pressure).

In these fumigations an excess of the fumigant was used, which therefore remained as a liquid, but which evaporated to maintain a saturated vapour phase, following losses through leakage or sorption, or when the

Plate 2. Modified 4.3 l. desiccators used in the fumigation of grain at the saturation vapour concentration of several fumigants.

fumigation chamber was opened. Since the concentration in the vapour phase remained constant, time-consuming determinations of the changes of concentration during the course of a fumigation, were avoided.

Glass vacuum desiccators (Plate 2) of 4.3 l. capacity were found to function very satisfactorily as fumigation units, and were suitable for treating up to about 100 g. of grain. A large area of absorbent paper was supported inside the desiccator on a framework of 16 s.w.g. copper wire. This was saturated with liquid fumigant before the grain sample was introduced. A 10.0 × 3.5 cm. strip of thick polythene was supported from the lid, and could be moved sideways by moving the desiccator fairly abruptly. The movement of this strip adequately stirred the vapour phase, which was demonstrated by observing the distribution of a small quantity of tobacco smoke, introduced through the tap in the desiccator lid.

Grain samples were supported in layers approximately one grain deep, on each of the three tiers of the carrier shown in the centre of Plate 2, which consisted of a copper wire frame with nylon mesh trays.

Operation.

The internal surfaces of the desiccator were fully saturated with fumigant at about 18°C., and a visible excess of the liquid was added before the fumigation was commenced. Grain which had been stored for more than a week at 20°C., was rapidly transferred to the grain carrier, which was equally rapidly placed in the desiccator, and the lid secured with two or three small clamps. The ground glass surfaces were lubricated with low melting point, high vacuum silicon grease. The desiccator was transferred

to a 20°C. constant temperature, ventilated room, and for several periods during the initial 30 minutes, the polythene stirrer was vigorously agitated to improve the distribution of the fumigant vapour. The pressure was maintained at about 760 mm. of mercury, by periodically opening the tap to the atmosphere.

Upon completion of the exposure, the desiccator was removed to a well-ventilated space, opened, and the grain carrier removed. The grain was aired for 0.5 hours, and then placed in perforated paper envelopes, in which airing was continued for a further two days at 15 - 22°C. and 40 - 70 per cent. relative humidity. The grain samples were finally stored in perforated envelopes at 20°C. and about 50 per cent. relative humidity.

Disadvantages.

The most serious disadvantage of this method of fumigation, is the likelihood of fumigant condensation on the surface of the grain, which would then raise its effective concentration at the grain surface by as much as 1000 times. This possibility was reduced by conducting fumigations in a controlled temperature room of 20 $\pm$ 0.3°C., and in general, condensation was not a serious problem except with methanol. With this particular water soluble compound, condensation was much more rapid (by a factor of at least 30) on grain of 18 per cent. than of 10 per cent. moisture content.

The second disadvantage results from the moisture content of the grain, not being in equilibrium with the humidity of the air in the desiccator at the commencement of the fumigation.

D.8. Physical properties of selected fumigants.

Thirteen fumigants were evaluated in these studies, of which ten were used at saturation concentrations in the fumigations conducted in modified desiccators (Section D.7). The names and some of the physical constants of these compounds are given in Table 5, of which columns 2,3,4, 5,6,7 and 10 have been compiled from the Handbook of Chemistry and Physics, (Hodgman, 1962).

The saturation vapour pressures at 20°C. (column 8) were obtained from a publication by Jordan (1954), and the corresponding concentrations in millimoles/litre were calculated using the formula given in Section D.4. Additional sources of information have been indicated by numbers:

(2) - Monro, H.A.U. (1961)

(3) - Jackson, K.E. (1934)

(4) - Negherbon, W.O. (1959)

(5) - Fawcett, C.H. (1958)

(6) - Jordan, T.E. (1954)

(7) - Patterson, A.M. (1933)

Details of the vapour pressure of two compounds were not found, but were expected to be comparable to compounds with similar boiling points.

(x) vapour pressure of allyl bromide was taken to be similar to that of acrylonitrile.

(y) vapour pressure of n-butanethiol was taken to be similar to that of allyl alcohol.

Compounds 1 - 5, 8 - 9 and 11 were obtained from Hopkin and Williams

Ltd.; 6 and 7 from The British Drug Houses Ltd.; 10 from Imperial Chemical Industries; 12 from May and Baker, Ltd., and compound 13 was kindly supplied by Professor R.L. Wain.

The purity of compound 12 (methyl bromide) was better than 98 per cent., and the melting point of compound 13, agreed with the published value. For compounds 1 - 8 and 11, their identity and high degree of purity were confirmed by density and boiling point determinations.

The densities of liquids were determined at 20°C. using a 1 ml. pyknometer, made from a 1 ml. pipette, the volume of which was determined accurately by filling with distilled water. Within the experimental error of these determinations, the density of water at 20°C. did not differ from that at 4°C., so that the specific gravity measurements of liquids, were used as estimates of their densities.

Boiling point determinations were made using approximately 0.2 ml. of liquid, according to the method of Smith and Menzies (1910, quoted by Reilly and Rae, 1954a). A small bulb was made at the end of a capillary tube 3 - 4 cm. long and of 1 - 2 mm. internal diameter. The liquid was introduced, and the capillary bent close to the bulb. The capillary was supported in a heating bath in an inverted 'U' position, and the boiling point was determined as the temperature at which a steady stream of bubbles issued from the open end.

Table 5. Nomenclature and physical properties of selected fumigants.

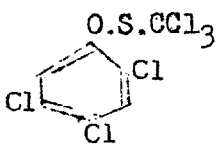
No.	1. Name of Compound	2. Name approved by International Union of Chemistry. 1930 (ref. 7)	3. Structural Formula	4. Molecular Weight	5. Density g./ml.
1.	Acetic acid	ethanoic acid	CH_3COOH	60.05	$\frac{20}{4}$ 1.049
2.	Acrylonitrile	propenenitrile	$CH_2.CHCN$	53.06	$\frac{20}{4}$ 0.797
3.	Allyl alcohol	2-propen-1-ol.	$CH_2{:}CH.CH_2OH$	58.08	$\frac{20}{4}$ 0.855
4.	Allyl bromide	3-bromopropene	$CH_2{:}CHCH_2Br.$	120.99	$\frac{20}{4}$ 1.398
5.	Allyl chloride	3-chloropropene	$CH_2{:}CHCH_2Cl.$	76.53	$\frac{20}{4}$ 0.938
6.	Allyl isothiocyanate	2-propenyl isothiocyanate	$CH_2{:}CHCH_2NCS$	99.15	$\frac{15}{4}$ 1.016
7.	Butyl mercaptan	1-butanethiol	$CH_3(CH_2)_2CH_2SH$	90.18	$\frac{0}{4}$ 0.858
8.	Chloropicrin	trichloronitromethane	$C.Cl_3NO_2$	164.39	$\frac{20}{4}$ 1.651
9.	Ethyl ether	ethoxyethane	$C_2H_5OC_2H_5$	74.12	$\frac{20}{4}$ 0.714
10.	Hydrogen cyanide	-	HCN	27.03	$\frac{20}{}$ 0.699
11.	Methyl alcohol	methanol	CH_3OH	32.04	$\frac{15}{}$ 0.796
12.	Methyl bromide	bromomethane	$CH_3Br.$	94.95	$\frac{0}{0}$ 1.732
13.	(2:4:5-trichlorophenoxythio) trichloromethane. (5)		$C_6H_2Cl_3.O.S.CCl_3.$ 	346.87	-

Table 5 continued.

No.	6. Melting Point, °C.	7. Boiling Point, °C. 760mm.Hg.	8. Vapour Pressure at 20°C.(mm.Hg.) (ref. 6)	9(based on 8) Vapour Concentration, 20°C.(mM./l.)	10. Water Solubility.g./100 ml. 20°C.
1.	+16.6	118.1	11.7	0.64	Inf. sol.
2.	-82	+78-79	100	5.47	7.5 at 25°C. (2)
3.	-129	+96-97	18.3	1.00	Inf. sol.
4.	-119.4	+71.3	100 (x)	5.47 (x)	Insol.
5.	-136.4	+44.6	300	16.41	Insol.
6.	-100	+150.7	3.6	0.197	0.2
7.	-115.9	+98	18.3 (y)	1.00 (y)	Slightly sol.
8.	-69	+112	20.0	1.09	0.16 at 25°C. (3)
9.	α-116.3 β-123.3	+34.6	442.2	24.19	7.5
10.	-14	+26	620	33.91	Inf. sol.
11.	-97.8	+64.6	95.0	5.20	Inf. sol.
12.	-93.66	+3.56	1824 at 25°C. (4)	41.57≡760mm. Hg. at 20°C.	0.09
13.	+46-47 (5)	-	-	-	-

D.9. The determination and adjustment of the moisture content of grain.

The moisture content of grain was determined, by grinding, and subsequent drying of the ground material, by the Air-oven 130°C. method, as recommended in the International Rules for Seed Testing (International Seed Testing Association, 1959).

Two Stage Method.

A sample of grain of approximately 30 g. was taken at random from a larger bulk, and transferred to a tin 10 cm. in diameter and 2.0 cm. in depth. The weight (A) of the grain at the initial moisture content (a) was determined, and the sample was then exposed in the laboratory for 24 - 48 hours to allow the moisture content to equilibrate with the air humidity. At the end of this period, the weight (B) of equilibrated grain was determined.

The grain was ground in a hand mill ("Spong") to a consistency that satisfied the Rules, and as it issued from the mill, approximately 4.5 g. subsamples were collected in small aluminium ointment tins of 5 cm. diameter, 1.9 cm. depth, and with tightly fitting lids. Four subsamples were generally collected and used for the second stage of the determination.

The weight (C) of ground material in each of the ointment tins was accurately determined to the nearest milligram. The lid was removed and the subsample was placed in a ventilated oven, previously heated to 130 $\pm$ 2°C. The tin remained there for precisely 1 hour, after the temperature had again reached 130°, after which time the lid was replaced and the tin

was transferred to a desiccator containing dry silica gel coloured with cobalt chloride. After allowing at least an hour for the tins to cool to room temperature, the weight (D) of the dried, ground material was ascertained.

In the present determinations, it was found that if the subsamples were returned to the oven and heated at 130°C. for a second period of 1 hour, the resulting increase in the value of the moisture content was not greater than 0.1 per cent.

The initial moisture content (a) was calculated as a percentage of the wet (or fresh) weight of the sample using the formula:

$$a = \frac{AB - CD}{AB} \times 100\%$$, where A = wt. of unequilibrated sample.
B = wt. of equilibrated sample.
C = wt. of undried subsample.
D = wt. of dried subsample.

When the initial moisture content of the sample was approximately in equilibrium with the laboratory humidity, the first stage of the determination was omitted.

Adjustment of moisture content of grain.

The moisture content of a grain sample was reduced by drying at temperatures not exceeding 27°C. in a ventilated oven.

The moisture content was increased by the addition of liquid water, in aliquots equivalent to an increase of not more than 1 per cent. moisture of the grain. The increase in moisture content during a 24 hour

period was also restricted to 1 per cent. Following the addition of water, the grain was mixed by a tumbling action, by slowly inverting the container several times.

Grain was stored in bags made of polyethylene "lay-flat" tubing, of 5 thou. inch, single-sheet thickness. The reduction in moisture content of grain of 18 per cent. moisture, stored in a polythene bag at 20°C., and 50 per cent. relative humidity for 2 weeks, was less than 0.1 per cent.

The weight of water required to increase the moisture content of a quantity of grain was calculated from the formula:

$$Q = \frac{A.\ (b-a)}{100-b}, \quad \text{where } Q = \text{weight of water required.}$$

A = initial weight of sample.

a = initial moisture content of sample.

b = desired final moisture content of sample

In a similar manner, the expected weight of a grain sample after drying to a desired moisture content, b, was calculated as:

$$B = \frac{A\ (100-a)}{100-b}, \quad \text{where A, a and b, are as above,}$$

B = final weight of the sample after drying.

D.10. Extraction and examination of embryos and small inflorescences.

1. Embryo examination.

The method for extracting embryos was based on accounts by Simmonds (1946) and Popp (1958). Barley and wheat grains were soaked overnight in five times their volume of 10 per cent. sodium hydroxide

solution. The following morning, any remaining solution was decanted, and the swollen grain suspended in twice its volume of water, and allowed to stand at 20°C. for 2 hr. The next stage involved gently warming the suspension for 10 - 15 minutes, which loosened the embryos, and the latter were then recovered either by rinsing through a nest of sieves with hot - boiling water, and collecting them on the penultimate screen, or alternatively by picking the embryos out individually with a dropping pipette with a rubber bulb. Where a complete recovery of all embryos was required, the latter method, though tedious, was preferred.

The extracted embryos were rinsed several times in distilled water, and suspended in 1 : 1 : 1 : 1, lactophenol with a composition of equal volumes of phenol, lactic acid, glycerol and water, and a refractive index (n_D) of 1.434 at 20°C. (n_D for water = 1.333). The lactophenol was slowly steamed until reduced to half its original volume, taking 20 - 30 minutes. The composition of the resulting solution was not known, but its refractive index at 20°C. was approximately 1.468. When embryos were mounted in this medium and examined microscopically, the host tissues appeared almost transparent, and the brown, highly refractive hyphae of the infecting loose smut fungi, were relatively easily seen.

The identification of the fungus was easiest in the tissue of the scutellum, and as an aid towards orientating the embryos with the scutellum nearest the microscope objective lens, the embryos were lightly stained in 0.05 per cent. methyl blue (or a similar stain of this triphenylmethane group). The stain was prepared as a 0.025 per cent. solution in 1 : 1 : 1 : 1

lactophenol, and reduced by evaporation to half its original volume. This ensured that its refractive index was the same as the clearing fluid.

The transfer of embryos from the clearing fluid to the stain, and sequently back to the clearing fluid, which was also used as the temporary mountant, was facilitated by using small, soldered, copper mesh baskets. The embryos were manipulated by means of a pipette and blunt needles.

2. Examination of small seedlings.

Seedlings of only a few days post-germination development, and with the grain still attached, were trimmed of unnecessary root and shoot parts, dried at 40°C., and stored in paper envelopes until required. Dried seedlings were submerged overnight in 5 per cent. sodium hydroxide solution, which was subsequently replaced by water. After standing for 2 hours at 20°C., the seedlings were carefully dissected from the grain, so that the scutellum remained attached to the rest of the shoot. The seedlings were washed and cleared in 1 : 1 : 1 : 1 lactophenol and examined unstained.

Greater care was necessary when embryos were extracted from inviable grains which had been exposed in a germination test for several days. In these cases the cytolytic activity of saprophytic microorganisms had weakened the structure of the embryo. In general, the grains were soaked in a 2 per cent. sodium hydroxide solution for only a few hours, but the details varied with the state of integrity of the embryonic tissues.

3. Examination of small inflorescences.

Barley inflorescences larger than about 8 mm., could be identified as infected by their greyish-brown colour or by their shortened and deformed awns. Inflorescences smaller than this were excised from the freshly collected plants, with a scapel, and preserved in 1 : 1 : 1 : 1 lactophenol. Subsequently the inflorescences were supported in a copper mesh basket, and cleared in lactophenol. They were stained in 0.05 per cent. methyl blue in lactophenol (n_D = 1.468), and subsequently mounted in the original clearing fluid.

In this instance the mycelium was readily stained and easily recognized, in contrast to the difficulty of staining the mycelium in the extracted embryos.

The refractive index of lactophenol was determined with a refractometer, similar in design to the Spencer refractometer, figured by Reilly and Rae (1954b, p. 349).

D.11. Laboratory germination tests.

1. 20°C. Tension Plate Test.

A piece of sheet-glass, 25 x 29 cm. with triangular pieces cut away from the four corners, was covered with four sheets of water-absorbent tissue paper, which draped over the sides. This was supported inside a stout polythene bowl, 27 x 31 cm. by 12 cm. deep, upon a 4 cm. high 'H' shaped piece of perspex. The bowl was covered with 2.5 thou. inch thick,

polythene sheeting attached with adhesive tape.

The paper was moistened with aerated tap water, and the plate sown with 200 - 300 grains. Excess water was introduced to the bottom of the bowl, with which the paper made contact and acted as a wick. The vertical distance between the grains and the water, on the floor of the bowl, was 3 - 4 cm.

The chief advantage of the tension plate technique is the small amount of attention necessary, as compared with petri dishes, since sufficient water may be added initially for the whole germination period (2 weeks). The initial stages of germination and types of early seedling abnormality may be viewed through the polythene sheeting, but these would be below the surface, in the sand test, and therefore unobserved. However, grains take about one day longer to germinate on tension plates than in moist sand, due to differences of the area of moist medium in contact with the grain. The seedlings were grown in darkness, except for the occasional examination.

2. 20°C. Sand Test.

Grade 60 (0.8 - 0.05 mm. diameter particles) river sand, suitably cleaned, washed and dried, was weighed into lots of 5 kg., and sterilized at 160°C. for at least 3 hours. When cool, each lot of sand was transferred to a 29 x 31 x 12 cm. polythene bowl, and mixed with aerated tap water, (575 ml.) to a nominal moisture content of 11.5 per cent., (dry weight basis). When lightly compacted, the sand covered the base of the bowl to a depth of about 4 cm. Holes, 2 cm. deep were made in a systematic

manner, usually 200 holes per bowl, and the grains were planted in them, with the embryo region at the base of the grain, i.e. with the coleoptile directed upwards. The sand moisture content at the level of the embryos was 10 per cent. (dry weight basis). After planting, the surface of the sand was smoothed over, and the bowl covered with 2.5 thou. inch polythene sheeting, secured with adhesive tape, and transferred to a 20°C. ventilated, controlled temperature room. The seedlings were grown in darkness, except for occasional examination, in order to observe the extent of maximum development of the etiolated structures.

D.12. Glasshouse trials.

Wooden trays, 4 ft. x 4 ft. and 6 in. deep were arranged on trestles in an artificially heated glasshouse. Sandy loam field soil, was freshly collected, sieved to remove large objects, and was lightly compacted to a depth of 3.5 in., in the trays. Grain was sown at the rate of 300 grains per 3 sq. ft. in rows 3 in. apart, and then covered with 2 in. of lightly compacted soil, and maintained moist. The air temperature in the glasshouse during the trial, fluctuated between 13° and 23°C.

The trial was made in the spring, and to hasten flowering, long daylength conditions were created by supporting a number of 100 watt tungsten filament bulbs, wired to an automatic time switch, above the crop. This supplementary lighting was of 20 - 25 ft. candles intensity, and extended the daylength to 18 hours.

As a prevention against Erysiphe graminis, the crop was sprayed every 5 days with a suspension of a wettable form of colloidal sulphur. The plants were also sprayed with pyrethrum to control aphids.

At anthesis, the plants were dug up and counted.

D.13. Field Trials.

All of the field trials considered in this thesis were conducted at the Imperial College Field Station, Sunninghill, in Berkshire, in the sandy loam soil, with pebble flints and clay lenses, on the Bracklesham beds. This is a very light soil which dries rapidly, and, depending upon previous management, is usually low in nitrogen and phosphorus compounds.

Plots were usually of 500 or 600 grains, with the exception of the trial of variety Donaria, where plots of 2,000 grains were sown. The rate of sowing was always 100 grains per 6 ft. row, irrespective of the anticipated viability and resulting plant density. Grain was planted 2 in. deep, in drills 8 - 9 in. apart.

In trials made in late spring or summer, it was usually necessary to irrigate before and after sowing, and also at regular intervals during the growth of the plants. For this purpose a portable, rotary sprinkler was used, which delivered about 1 in. of water over the irrigated area, in 30 minutes. Granular fertilizer was applied as a top dressing at twice the normal agricultural rate, to seedlings in the 2 - 3 leaf stage.

Severe damage to seedlings by pheasants and to some extent by crows was threatened, the plots were therefore always immediately covered

with netting for 4 weeks after sowing. Additional and very severe bird damage was encountered on the ripening crop, due chiefly to sparrows and greenfinches, however, a nylon net of mesh $\frac{3}{4}$ in. x $\frac{3}{4}$ in. square, was an effective barrier.

Erysiphe graminis was a serious pathogen on all the barley varieties, but was not controlled appreciably, by heavy applications of colloidal sulphur at rates of 1.5 oz./gallon/6000 plants or 500 sq. feet, most probably because it has very poor tenacity under damp conditions.

Two large field trials were sown in mid-late summer, 1962 and 1963, but, because of the rapidly shortening solar daylength, it was feared that the plants would receive insufficient photo-induction to lead to flowering before the advent of frost. Artificial illumination was therefore provided, and consisted of 100 watt tungsten filament bulbs, covered with wide-angle white shades, attached to lengths of stout electric cable (Plate 3).

Lengths of cable 45 - 60 ft. long and carrying bulbs at 8 - 10 ft. intervals were stretched across the plot and supported at the two ends and in the centre by 5 ft. 5 in. high wooden stakes. The cable was moved on alternate days between two adjacent sets of stakes, 6 ft. apart, and connected by cross-wires, so as to even out the light intensity over the plot. At any one time, the furthest horizontal distance between a plant and the nearest bulb was 9 ft., in which position the light intensity was 3 - 4 ft. candles. The highest light intensity of 12 - 13 ft. candles

Plate 3. The installation of electric lighting to increase culm extension in a late-summer field trial.

was received by plants 4 ft. 6 in. vertically below a bulb. The lights were switched on automatically by a time switch for a 4 hr. period in the middle of the dark period (22 - 02 hr. G.M.T.), when the plants were most sensitive to a light break.

Plants of the spring varieties of barley and wheat initiated and developed inflorescences, even when the grain was sown as late as mid-August, and the plants had received no artificial photo-induction. However the degree of elongation of the upper internodes and the emergence of the ear from the flag leaf, varied with the variety, being almost normal in Edda II, but appreciably increased by artificial illumination of 3 - 13 ft. candles, in Rika, Donaria and especially in Svenno wheat. The emergence of the ear from the flag leaf, very greatly minimized the labour involved in deciding whether or not the plants were infected by a loose smut fungus.

At anthesis, the plants were dug up, counted and classed as smutted or non-smutted. In cases where it was difficult to differentiate between a plant having several shoots, and several plants growing very closely together, with interlocking roots, it was usually possible to decide between these possibilities, by examining the root systems to find the primary axes, to which the expended grains were often still attached.

Small, incompletely developed inflorescences were cleared, stained, and examined microscopically for evidence of smut infection (Section D.10.3).

D.14. Definitions: germination; emergence; normality; survival and frequency of smut infection.

1. Germination.

This term has been used to describe any morphological development of the initially dormant grain.

2. Emergence.

In the laboratory tests, this term was applied to seedlings with shoots more than 2 cm. long. In the sand test, this means any seedling development visible above the sand surface.

Emergence in the field refers to seedling development visible above the soil surface, either normal or abnormal, and which therefore represents a shoot development of 2 in. or 5 cm. in length, below the soil surface.

3. Normality.

This term was used to describe seedlings with a shoot, of more than 2 cm. length, of which neither the coleoptile nor the vegetative leaves were atypically split, shortened, discoloured or deformed in any other way. Less attention was generally paid to root development, which usually appeared to be less affected by fumigation treatments, than the shoot.

4. Survival.

The term "survival" or "survived", was applied to plants in which

an inflorescence had been initiated, and in most cases it was used to describe plants with fully developed inflorescences at anthesis or later stages.

5. Frequency of smut infection.

The proportion of smut infection in the crop may be expressed as numbers of plants or as numbers of ears. It has been shown that the proportion of smutted ears in an infected crop is lower than the proportion of smutted plants (Batts and Jeater, 1958; Doling, 1964) and furthermore that smutted plants are more adversely affected by plant competition, and produce fewer tillers than uninfected plants (Doling, 1964).

In the present experiments, several of the trials were grown out of season, and the numbers, and stages of development, of shoots were appreciably different from those expected under normal agricultural practice. The incompletely developed plants, often resulting from infestation by stem-boring insect larvae, were also examined for the presence of the loose smut fungus, as a precaution against the possibility that such insects might be more damaging to infected than to uninfected plants.

It was appreciated that in agricultural practice, the important proportion is the number of infected ears, sufficiently exposed to release chlamydospores, relative to the number of ears with ripe grain. However, in the present studies, the proportion of smut infection has been based on the number of plants involved, and although the labour involved was very great, it was considered that this type of presentation, eased the comparison between the various trials, and also emphasized the important effect of fumigation upon plant survival.

E. THE FUMIGATION OF BARLEY GRAIN TO CONTROL USTILAGO NUDA.

E.1. Fumigation of grain of variety Arès with chloropicrin.

1.1. Introduction.

Chloropicrin has been used widely as a general biocide, and of particular relevance is its use as a highly fungicidal soil fumigant (Newhall, 1955; Section C.1.3). It has been found effective in controlling Xanthomonas campestris on kale seed (Nugent and Cook, 1938, 1939) and also Septoria apii spores in pycnidia, associated with the seeds of celery (Mukkath, 1961). Kennedy (1959, 1961) endeavoured to control Ascochyta spp., present in the seeds of the garden pea, but found that treatments which were satisfactorily fungicidal were also highly phytotoxic, and severely reduced seed germination. However, there was evidence that at seed moisture contents of about 16 per cent., the amount of phytotoxicity associated with an effective elimination of the pathogens, was less than at other moisture contents.

In view of this promising control of Ascochyta spp. in pea seeds with chloropicrin, this experiment was designed to investigate the possibility of controlling Ustilago nuda, present in the embryos of infected barley grains, with the same fumigant. Attention was directed towards the influence of grain moisture content upon the levels of phytotoxicity and the ease of control of the pathogen.

1.2. Materials and methods.

Arès grain of 14.2 per cent. moisture content was adjusted to 10.0, 13.9, 17.9 and 21.5 per cent. (Section D.9) and fumigated, in 4.3 l. desiccators (Section D.7), in an atmosphere saturated with chloropicrin at 20°C., for 1.5, 6.0 and 24.0 hours, and compared with unfumigated lots. Each fumigation treatment was separately replicated three times, making a total of 16 treatments and 48 lots of grain. From each lot, 200 grains were used in a 20°C. sand test, to determine seedling emergence and normality (seedlings with no gross morphological damage, such as split coleoptiles or shortened leaves (Section D.14)). Another 600 grains were sown in the field in early April 1962, and covered with netting as a precaution against bird damage. Plots in the field trial were completely randomized, but were not arranged in blocks. Five weeks after sowing, the emerged seedlings were counted, and in August - September, the trial was dug up and the plants counted.

Direct observations of the yield of grain per plot were not made, but the effect of fumigation of grain of 21.5 per cent. moisture upon shoot production was measured. In addition, from the several hundreds of plants in one of the plots sown with unfumigated grain at this moisture content, 36 plants with one shoot, 18 with two shoots and 12 with three shoots, were sampled at random, and the ripe, fully developed grain was carefully removed from each ear and weighed.

1.3. Results.

The results of both the laboratory sand test and the field trial are presented in Appendix Table A.1, and partially summarized in Tables 6 and 7. The field data of plant survival and that of plant infection were calculated as percentages, transformed to angles and examined by two separate analyses of variance, for any significant effects of the various treatment combinations. The two analysis of variance tables are included in the appendix (Tables A.2 and A.3) together with the appropriate least significant differences.

Variations of both the moisture content of the grain and the duration of the fumigation resulted in large and highly significant ($P = 0.001$) reductions in both percentage plant survival and percentage loose smut infection. However it is evident in Tables 6 and 7, that the greatest effects were obtained with combinations of high moisture content and long durations, and in both analyses, this interaction was found to be highly significant ($P = 0.01$). In comparison with this interaction effect, moisture content differences resulted in a significant ($P = 0.05$) response in both percentage survival and percentage smut, but the variance due to differences of fumigation duration did not differ significantly at the 5 per cent. level of probability.

The only significant ($P = 0.05$) reduction in loose smut frequency was obtained with 1.5, 6.0 and 24.0 hr. fumigations of grain at 21.5 per cent. moisture content, but very severe reductions of plant survival of 16.3, 47.4 and 81.0 per cent. also resulted from these treatments. Twenty-

Table 6. The influence of the moisture content of grain of Arès barley, and the duration of fumigation with chloropicrin at 20°C. upon seedling development, and upon plant survival and infection by Ustilago nuda.

Per Cent. Moisture Content	Fumg'n. Dur'n. (Hours)	20°C. Sand Test	Field Test*		
		Per Cent. Normality (600 Grains)	Per Cent. Emerg. (1800 Grains)	Per Cent. Survival (1800 Grains)	Per Cent. Smut Infect'n. (Survived Plants)
10.0	0	97.0	86.6	86.3ab.....	6.58jk...
	1.5	95.7	85.7	83.7abcd...	6.91j....
	6.0	92.7	80.2	80.9.bcde..	6.43jk...
	24.0	84.3	80.2	79.3..cdef.	6.26jk...
13.9	0	97.0	86.9	87.6a.....	6.41jk...
	1.5	96.2	84.2	81.6.bcde..	7.38j....
	6.0	86.5	82.3	82.5abcde..	5.38jkl..
	24.0	79.5	78.2	77.3....ef.	6.13jk...
17.9	0	97.5	90.1	86.6abc....	6.16jk...
	1.5	89.2	83.1	78.2...def.	7.07j....
	6.0	85.3	80.7	78.1...def.	5.46jkl..
	24.0	61.7	76.8	72.5.....fg	4.05.kl..
21.5	0	98.0	90.1	82.7abcde..	6.92j....
	1.5	70.0	68.7	66.4.....g	3.17..l..
	6.0	26.0	39.6	35.3.....h	1.24...m.
	24.0	0.0	2.7	1.7.....i	0.35....n
				For comparison-	0.00....n

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

Table 7. The control of Ustilago nuda in variety Arès, by the fumigation of infected grain, at four moisture contents, with chloropicrin.

Fumigation Duration (Hours)	Percentage of Plants infected with Ustilago nuda			
	Per Cent. Moisture Content			
	10.0	13.9	17.9	21.5
0	6.6	6.4	6.2	6.9
1.5	6.9	7.4	7.1	3.2*
6.0	6.4	5.4	5.5	1.2*
24.0	6.3	6.1	4.1	0.4*

* Significantly different (P = 0.05) from the unfumigated check.

four hour fumigations of grain of 10.0, 13.9 and 17.9 per cent. moisture contents, resulted in significant reductions of percentage survival of 7.0, 10.3 and 14.1 per cent., respectively, but with no significant (P = 0.05) reduction in percentage loose smut infection when compared with the appropriate unfumigated checks.

From these data it is not possible to gauge at which moisture content the pathogen was controlled with the minimum of phytotoxicity, because the fumigations at the three lowest moisture contents were insufficiently phytotoxic to reduce survival to levels comparable with the grain of 21.5 per cent. moisture. However, plants that developed from grain of 17.9 per cent. moisture fumigated for 24 hours, and grain

of 21.5 per cent. fumigated for 1.5 hours, showed no significant difference (P = 0.05) of percentage survival or of percentage smut infection, indicating that at both of these moisture contents, the control of U. nuda was associated with similar levels of phytotoxicity.

The percentage of normal seedlings in the sand test agreed reasonably well with the field emergence (Table 6), and could be used to predict the latter, except that in the sand test the highest values, of 95 - 98 per cent. normality, corresponded to only 84 - 90 per cent. emergence in the field. The close similarity of percentage survival with percentage emergence (Table 6), shows that most of the plants that emerged, continued to develop satisfactorily, with no evidence of any delayed toxic effect of the fumigation treatments.

In considering the effect of fumigation upon plant productivity, it is evident, in Table 8, that as the number of plants per plot (or plant survival) was reduced, as a result of the fumigation treatment, the number of shoots per plant increased (column 4). However, this increase was not large enough to maintain the number of shoots per plot (or per grain), at a constant value. Furthermore, for plants developed from unfumigated grain, the yield of grain per shoot decreased as the number of shoots per plant increased (Table 9).

These two facts of a reduction of the number of shoots, and a reduction of the grain yield per shoot with increased tillering, tends to confirm the visual expectation that a reduction of plant survival was most probably accompanied by an appreciable reduction in the yield of grain.

Table 8. The influence of the duration of fumigation of grain of 21.5 per cent. moisture content, with chloropicrin, upon the numbers of plants and shoots that survived, and were infected by Ustilago nuda.

1. Fumigation Duration (Hours)	2. Per cent. Plant Survival	3. Shoots per Grain (1800 grains)	4. Shoots per Plant	5. Per cent. Plants Smutted	6. Per cent. Shoots Smutted
0	82.7	1.34	1.62	6.92	8.89
1.5	66.4	1.13	1.70	3.17	3.00
6.0	35.3	0.73	2.07	1.24	1.67

Table 9. The relationship between the number of shoots and grain yield, of plants developed from unfumigated grain.

1. No. Plants	2. No.Shoots per Plant	3. Wt.Grain from 36 Shoots(g.)	4. Yield per Plant(g.)	5. Yield per Shoot(g.)	6. Relative Yield per Shoot
36	1	38.601	1.072	1.072	100
18	2	28.846	1.603	0.801	74.7
12	3	27.745	2.312	0.771	71.9

This association will be closest when grain is sown lightly, and when plants tiller freely, and would not tiller any more profusely despite a reduction in the plant density.

In this experiment the percentage of smutted ears agreed fairly closely with the percentage of smutted plants (Table 8, columns 5 and 6).

1.4. Summary of results.

The higher the moisture content of grain, the greater was its susceptibility to toxicity by a fumigation, of a fixed duration, with chloropicrin.

Loose smut was significantly ($P = 0.05$) controlled, only at the highest moisture content of 21.5 per cent., and this control was associated with a reduction of plant survival of at least 16.3 per cent. A reduction of the smut incidence from 6.9 to 1.2 per cent., by a 6 hr. fumigation, was accompanied by a reduction of plant survival from 82.7 to 35.3 per cent. There was an indication that the control of loose smut in grain of 17.9 and 21.5 per cent. moisture content, might be associated with comparable reductions in plant survival.

A 24 hr. fumigation of grain of 13.9 per cent. moisture content reduced plant survival by 10.3 per cent., but with no significant control of the pathogen.

From evidence of the tillering rate of plants developed from fumigated grain, it appears probable that a reduction in the grain yield would follow, fairly closely, any reduction of plant survival due to phytotoxicity.

E.2. The fumigation of infected grain of Rika barley, with several fumigants, to control Ustilago nuda.

2.1. Introduction.

In the previous experiment chloropicrin was shown to be unsuitable for controlling U. nuda in grain of Arès barley, because of the high degree of phytotoxicity associated with any substantial elimination of the pathogen. At a temperature of 20°C., the duration of fumigation to control U. nuda in grain of 10 - 18 per cent. moisture content, would need to exceed 24 hours. This experiment was designed to find a more fungicidal, less phytotoxic, and a quicker acting fumigant than chloropicrin.

Eight fumigants were chosen, in addition to chloropicrin, which was included for comparative purposes. Allyl bromide and allyl alcohol were selected because of their efficacy as fungicidal soil fumigants (Christie, 1947; Schmitt, 1949; Ferguson and Wilhelm, 1951; Overman and Burgis, 1956; Yatazawa, et al., 1960) and also because of their superior performance to chloropicrin in controlling the resting mycelium of Verticillium albo-atrum associated with lucerne seed (Isaac and Heale, 1961). Allyl chloride, though reputedly less fungicidal than allyl bromide (Schmitt, 1949; Isaac and Heale, 1961) was included for a comparison with the latter compound, in order to ascertain the influence of the halogen upon the toxicity of these compounds.

Allyl isothiocyanate was rejected by Isaac and Heale (1961) in their preliminary studies, because it was slower acting than several of

the other fumigants. However, Walker et al., (1937) and Pryor et al. (1940) have testified to its high degree of fungitoxicity in laboratory tests involving agar cultures of several fungi including Colletotrichum circinans and Aspergillus niger, and for this reason it was chosen for evaluation in this experiment.

Another two compounds, n-butanethiol and acetic acid, were selected for use, in an attempt to simulate the chemical changes within the grain, that are thought to result from the long-water-soak, and also from the hot-water treatments, which are used to control internal seed-borne pathogens.

Gassner (1933) concluded that the efficacy of the hot-water treatment depended upon an oxygen deficit within the grain, and the formation, under the resulting anaerobic conditions, of incompletely oxidised compounds such as alcohols. The effectiveness of this treatment was shown to be improved by the use of dilute aqueous solutions of methanol, ethanol, iso-propanol and other organic compounds in place of water. This approach has resulted in two important lines of development, namely, the anaerobic method (Hebert, 1955) and the use of concentrated solutions of methanol (Wagner, 1958 - 1963).

In an endeavour to reduce the degree of oxidation of unmoistened grain, a highly reduced compound, n-butanethiol, was chosen in the expectation that it might have a superior fungitoxicity to hydrogen sulphide, which was shown to be relatively ineffective in controlling Ustilago nuda (Kvashnina and Etmisheva, 1936) and U. tritici (Petrova, 1937) (discussed

in Section C.2). Some justification for this expectancy was gained from the work of Walker *et al.*, (1937), in which it was shown that the vapours of methanethiol and ethanethiol were about as fungitoxic as allyl isothiocyanate to spores of *Colletotrichum circinans*.

A very different mechanism for the water-soak treatment has been suggested, and involves organic acids which have been shown to accumulate in the treatment water (Leben *et al.*, 1956). Formic and/or acetic, butyric and succinic acids have been identified, and are thought to arise from the metabolism of microorganisms, probably saprophytic, on the seed surface. However, the control of *Helminthosporium sativum* on barley grain, and *H. victoriae* on oat grain with solutions of known acidic composition was poor compared with that obtained with an application of an organomercury compound. Nevertheless, where phytotoxicity is not a limiting criterion, organic acids have been shown to be very fungicidal. Low molecular weight normal aliphatic acids are very much more toxic than the corresponding alcohols, although this difference is reduced as the number of carbon atoms increases, until at C_9 the toxicities of acid and alcohol to *Fomes annosus* are almost identical (Baechler, 1939).

Because of the fungitoxicity of acetic acid and of its possible involvement in the control of internal seed-borne pathogens by the water-soak treatment, it was selected as a fumigant for use in this experiment.

Acrylonitrile was chosen because it has been used as an insecticidal grain fumigant, and also because it is an unsaturated compound with a cyanide group, and it was of interest to observe its degree of toxicity

to host and pathogen, possibly as a respiratory inhibitor.

Finally diethyl ether was used in the hope of causing a more serious disruption of the membranes of the fungal mycelium, than of the host tissue.

In the previous experiment the degree of control of U. nuda was closely associated with the degree of phytotoxicity, and no significant control of the pathogen occurred without a reduction of at least 16.3 per cent. in the percentage plant survival. Further comparisons of the fungitoxicities of fumigants will be made therefore, at comparable levels of phytotoxicity.

2.2. Materials and methods.

Infected grain of Rika barley, received from Sweden, (specifications given in Table 4, Section D.1) was adjusted to 15.4 per cent. moisture content (Section D.9), and fumigated in an atmosphere saturated with fumigant vapour, in a 4.3 l. desiccator in the manner described in Section D.7. Grain lots were fumigated for 2, 3 or 4 different durations, which were chosen to introduce a graded series of phytotoxicity and plant survival. Because the quantity of infected grain was small, preliminary fumigations were conducted using an uninfected stock of English-grown Rika barley, to determine suitable durations. Both of the grain lots gave low percentage emergence and normality of seedlings, and showed similar degrees of phytotoxicity in response to similar fumigation treatments.

The durations of the fumigations are given in Table 10, and the

chemical structure and physical properties of the fumigants are shown in Table 5, Section D.8. Each of the 28 fumigation treatments was separately repeated once, totalling 56 lots of grain, which were compared with 4 lots of unfumigated grain. Laboratory germination tests were not conducted on this occasion, but from each lot, 500 grains were selected, randomly, and field-sown in plots, consisting of 5 rows each 6 ft. long, arranged in one large randomized design. The grain was sown on 2nd. August 1962, and seedling emergence was recorded 5 weeks later. The trial was ultimately dug up and the plants classed and counted during October - November. Artificial illumination was used to promote the emergence of the inflorescence from the flag leaf (Section D.13). A small proportion of plants possessed inflorescences less than 8 mm. in length, which were examined microscopically for evidence of loose smut infection (Section D.10.3).

2.3. Results.

The numbers of seedlings that emerged in the field, and also the number that developed abnormally or wilted, are presented as percentages in Appendix Table A.4, and the emergence figures are summarized as percentages in Table 10. The number of smutted plants, and the total number of plants that survived in each plot, are given in Appendix Table A.6 (1962 results), and are also summarized as percentages in Table 10. Two separate analyses of variances were performed upon angular transformations of the percentage survival and percentage loose smut infection, and these are summarized in Appendix Tables A.7 and A.8, to each of which are

appended the two least significant differences. One is for differences between means of 2 items each (i.e. any two fumigation treatments) and the other for differences between means of 2 and 4 items (i.e. a fumigation treatment compared with the unfumigated check).

There were six treatments in which loose smut-infected plants were found in neither of the replicates, and one treatment in which no plants emerged in either replicate as a result of severe phytotoxicity. In these instances the values for the replicates were identical and added nothing to the error sums of squares. Therefore the corresponding degrees of freedom were subtracted from the total, treatments and error terms, so as to keep the error mean square (variance) as large as possible, thereby maintaining the reliability of the test for significant treatment differences.

The error variance for percentage smut infection was large, because the number of smutted plants in any replicate was small (and very variable), due to the initial low level of infection in the grain, and also because relatively few plants survived from each lot of 500 grains. Consequently, only gross differences of percentage infection could be shown to be significantly different, so that although the mean infection of plants from unfumigated grain was 5.8 per cent., only levels of infection of less than 2.2 per cent. represented a significant ($P = 0.05$) control of the disease.

Of the 28 treatment effects in Table 10, only 13 showed a significant ($P = 0.05$) control of loose smut, and these have been arranged

Table 10. The effect of treatment of infected grain of Rika barley, with nine fumigants, upon the emergence, survival and loose smut infection of the resulting plants.

1. Fumigant	2. Fumigation Duration (Hours)	3. Per Cent. Emergence (1000 Grains)	4. * Per Cent. Survival (1000 Grains)	5. * Per Cent. Loose Smut (Survived Plants)
Unfumigated Check (2000 grains)				
	0	87.3	69.4 a.....	5.75 l.....
Acetic acid				
	0.5	76.1	58.9 ..c.....	2.90 lmno..
	1.5	45.2	32.9 .....fgh.	0.00 .....q
	4.5	0.0	0.0 .....k	0.00 .....q
Acrylonitrile				
	1.5	80.1	65.8 abc.....	5.42 lm....
	6.0	35.5	28.4 .....ghi	0.19 ....pq
	24.0	0.9	0.7 .....k	0.00 .....q
Allyl alcohol				
	2.0	47.8	38.2 ....efg..	0.53 ...opq
	6.0	29.9	23.8 .....hi	0.00 .....q
	12.0	7.4	4.9 .....j	0.97 ...opq
Allyl bromide				
	3.0	44.7	33.1 .....fgh.	1.49 ..nop.
	12.0	43.1	33.3 .....fgh.	0.15 ....pq
	48.0	25.4	20.8 i	0.00 .....q
Allyl chloride				
	3.0	70.1	55.6 ..cd.....	4.46 lmn...
	6.0	52.1	43.0 ....ef...	5.08 lmn...
	12.0	32.8	24.8 .....hi	2.73 lmno..
Allyl isothiocyanate				
	0.5	79.8	70.2 ab.....	5.99 l.....
	2.0	68.5	60.1 abc.....	6.12 l.....
	8.0	50.8	46.6 ...de....	5.93 l.....
	36.0	45.9	39.6 ....ef...	1.66 .mnop.
n-Butanethiol				
	0.5	67.1	64.4 abc.....	5.32 lm....
	2.0	81.0	70.3 ab.....	5.13 lmn...
	8.0	79.3	65.5 abc.....	6.85 l.....
	36.0	65.1	59.3 .bc.....	6.42 l.....
Chloropicrin				
	3.0	46.3	42.9 ....ef...	5.35 lm....
	12.0	35.1	33.3 .....fgh.	3.08 lmno..
	48.0	31.1	27.4 .....ghi	0.74 ...opq
Diethyl ether				
	24.0	50.8	46.7 ...de....	5.92 l.....
	96.0	36.1	32.8 .....fgh.	0.00 .....q

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

in Table 11, in two groups. Group 1 contains treatments in which the level of loose smut is significantly different from zero, while Group 2 is restricted to treatments showing a slightly superior control, in which the level of smut is not significantly different (P = 0.05) from zero. In addition, loose smut frequencies in this Group are not significantly different from one another.

The eleven treatments in Group 2 have been arranged in descending order of decreasing plant survival, (i.e. increasing phytotoxicity). It is evident that the upper six entries differ insignificantly (P = 0.05) from one another (indicated by the letter 'c') and represent six treatments, which, in this experiment, equally effected the greatest control of loose smut, with the minimum of associated phytotoxicity. It is possible to make comparisons between these six treatments in terms of their effectiveness, gauged either by the duration of the fumigation, or by the magnitude of the concentration-time product (Table 11, column 3) (see Section D.4). The latter was calculated as the product of the duration of the fumigation in hours, and the saturation vapour concentration, at 20°C., in millimoles/litre (Table 5, column 9). Taking the C.T. product as the criterion, a 1.5 hr. fumigation with acetic acid (1.0 mM.h./l.) and a 2 hr. fumigation with allyl alcohol (2.0 mM.h./l.), were much more effective than longer fumigations with acrylonitrile (32.8 mM.h./l.), chloropicrin (52.3 mM.h./l.) and allyl bromide (65.6 mM.h./l.). The very large C.T. product (2322 mM.h./l.) resulting from a 96 hr. fumigation with diethyl ether indicates that this compound is either not very toxic, or perhaps does not penetrate

Table 11. The association between percentage plant survival and the control of loose smut in Rika barley (derived from Table 10).

1. Fumigant	2. Fumigation Duration (Hours)	3. C.T.P. (mM.h./l.)	4. * Per Cent. Survival (1000 Grains)	5. * Per Cent. Loose Smut (Survived Plants)
Unfumigated Check:	0	0	69.4 a.....	5.8 h..
Group 1.				
Allyl isothiocyanate	36.0	7.1	39.6 .b.....	1.7 .i.
Allyl bromide	3.0	16.4	33.1 .bcd...	1.5 .i.
Group 2.				
2.Allyl alcohol	2.0	2.0	38.2 .bc....	0.5 .ij
5.Allyl bromide	12.0	65.6	33.3 .bcd...	0.2 .ij
1.Acetic acid	1.5	1.0	32.9 .bcd...	0.0 ..j
6.Diethyl ether	96.0	2322.0	32.8 .bcd...	0.0 ..j
3.Acrylonitrile	6.0	32.8	28.4 ..cde..	0.2 .ij
4.Chloropicrin	48.0	52.3	27.4 ..cde..	0.7 .ij
Allyl alcohol	6.0	6.0	23.8 ...de..	0.0 ..j
Allyl bromide	48.0	262.6	20.8 ....e..	0.0 ..j
Allyl alcohol	12.0	12.0	4.9 .....f.	1.0 .ij
Acrylonitrile	24.0	131.3	0.7 .....g	0.0 ..j
Acetic acid	4.5	2.9	0.0 .....g	0.0 ..j

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

into seed tissue very readily.

The fact that both acetic acid and allyl alcohol are infinitely soluble in water (Table 5, column 10), whereas the other four compounds have water solubilities at 20°C. of less than 7.5 per cent., suggests that this is an important factor which possibly influences the mobility of the

fumigant within the grain, and also its toxicity.

The numbers of abnormally developed or wilted seedlings seldom exceeded 5 per cent. of the emerged seedlings. These were low frequencies, the significance of which will be discussed in Section I. Despite this satisfactory start to the trial, the numbers of plants that survived to produce inflorescences was surprisingly low, for example, there was a loss of 17.9 per cent. of plants in the unfumigated check plots (Table 10). In view of the late sowing (August) of this trial, it is probable that many of these plants died from a combination of heavy infestations of both stem boring insect larvae, and Erysiphe graminis. The mildew was not appreciably controlled by heavy applications of colloidal sulphur (Section D.13).

2.4. Summary of results.

None of the eight fumigants was significantly more fungicidal and, at the same time, less phytotoxic than chloropicrin, although acetic acid, allyl alcohol, acrylonitrile, allyl bromide, and diethyl ether, were all as effective as chloropicrin. Acetic acid and allyl alcohol were quicker acting and had considerably lower concentration-time products, than the other four fumigants. It is suggested that this greater effectiveness was associated with the greater water solubilities of these two compounds.

Significant control of loose smut was associated with a very severe reduction in plant survival, in excess of 30 per cent., compared

with a plant survival of only 69.4 per cent. in the unfumigated check.

Allyl chloride was significantly less fungicidal than allyl bromide, at comparable levels of phytotoxicity.

A 36 hr. fumigation with n-butanethiol (36.0 mM.h./l.) failed to control loose smut, so that the compound was appreciably less toxic than allyl isothiocyanate, which effected a significant control of loose smut (associated with a reduction in plant survival of 30.6 per cent.) as a result of a fumigation of 36 hr. duration (7.1 mM.h./l.).

Large discrepancies between percentage emergence and percentage survival were attributed to severe infestations of stem-boring insect larvae and Erysiphe graminis, which probably killed many of the small plants.

E.3. The fumigation of infected grain of Arès barley with methyl bromide to control Ustilago nuda.

3.1. Introduction.

Methyl bromide has been widely employed as a toxicant for the control of insect and nematode pests (Sections C.1.1 and C.1.2), and also of soil-borne pathogenic fungi and bacteria (Wensley, 1953; McKeen, 1954; Munnecke and Ferguson, 1960; Baines et al., 1962). Munnecke et al. (1959) determined its toxicity to spores of Alternaria solani, and showed that with high concentrations, methyl bromide was more toxic at high than at low relative humidities. In one example, spores showed complete mortality

after a fumigation at 50 per cent. humidity, with 4.5 per cent. methyl bromide, which lasted 4 hours (C.T.P. 712 mg.h./l.). At 20 per cent. humidity, the mortality was only 44 per cent. Yanai et al. (1964) found that similar C.T. products were necessary to control the mycelium of Penicillium islandicum, within the grain of rice (45 mg./l. x 18 hr. at 25°C., giving C.T.P. 810 mg.h./l.).

Pea seed infected by Ascochyta spp. was fumigated with methyl bromide, in a preliminary experiment, by Kennedy (1959). A C.T. product of 600 mg.h./l. (20hr., 15°C.) applied to seed of 12.8 per cent. moisture content, was not toxic to either the pathogens or the seeds. However, a C.T. product of 2000 mg.h./l. was slightly fungicidal, but was also very phytotoxic to seed of 16.2 per cent. moisture.

Methyl bromide has also been used as a fungicide to control storage moulds of grain (Majumder et al., 1955; Srinivasan and Majumder, 1961).

In a study of the toxicity of methyl bromide to cereal grains, Lubatti and Blackith (1957) found that grain of barley varieties Procter and Herta, at moisture contents between 8.0 and 17.5 per cent., exhibited only a slight reduction in germination after 24 hr. fumigations with C.T. products of 600 and 1200 mg.h./l. In contrast, grain of wheat, oats, rye and maize, at the highest moisture content and fumigated at the highest rate, was rendered completely inviable.

Whitney et al. (1958) confirmed that barley had a high degree of tolerance, and demonstrated that grain of 14 per cent. moisture content

fumigated for 24 hours at a concentration of 127 oz./1000 cu. ft. (C.T.P. 3050 mg.h./l.), was reduced in viability from 98.3 to only 45.6 per cent. Similarly, Strong and Lindgren (1959b) found that the fumigation of barley grain of several varieties at 14 per cent. moisture content for 72 hours at 70°F. (21°C.), with a dose of 3.0 lb./1000 cu. ft. (C.T.P. 3460 mg.h./l.) reduced the germination by only 13 - 18 per cent. of the unfumigated check.

In view of the fungitoxicity of methyl bromide and of the high tolerance of barley grain to this fumigant, the present experiment was designed to establish the suitability of methyl bromide for controlling Ustilago nuda within barley grains. As in the fumigation of Arès with chloropicrin, grain of several moisture contents was used to ascertain the influence of this factor upon the ease of control of the pathogen. Comparisons of effective fungitoxicity were made at comparable levels of phytotoxicity, as in the previous experiment with infected Rika grain. However, because of the very marked increase of the susceptibility of Arès grain to methyl bromide at the high moisture contents, the treatments were adjusted, upon the basis of preliminary tests, to give similar graded series of phytotoxicity at each moisture content. A stock of uninfected Rika grain was used for a comparison with Arès.

3.2. Materials and methods.

Samples of infected Arès grain, of 2.5 kg. each, were adjusted to 14.1, 17.9 and 22.0 per cent. moisture, and similar quantities of uninfected Rika grain were adjusted to 14.0, 17.8 and 22.0 per cent.

moisture content (Section D.9). Subsamples of 80 g. were transferred to muslin bags and suspended in airtight 20 l. mild steel bins and subsequently fumigated for 18 - 20 hours, with the exception of two treatments which involved fumigations of 40 and 80 hours. The details of the construction of the bin and method of introduction of the fumigant are given in Section D.5. Fumigant concentrations were determined with a thermal conductivity meter (Section D.6). The concentration-time products of the 17 treatments are given in Table 12. Each of these treatments was separately repeated twice, making a total of 51 lots of grain for both varieties. The fumigations were conducted during May and June, 1963.

From each of the Arès lots, 600 grains were randomly selected, and sown in a field trial of randomized design in June 1963, in the belief that Arès was a spring variety with a long vegetative period. The plants, however, did not flower within the succeeding four months, and only the seedling emergence was recorded.

In 1964, 500 grains were selected from the remainder of each lot, and field-sown in plots, 5 rows each 6 ft. long, arranged in a randomized block design. The trial was sown in early April, and seedling emergence was scored five weeks later. Selected plots were dug up, and the plants classed and counted in August.

From each of the 51 lots of both varieties, 100 grains were randomly selected and tested for viability and abnormality in the 20°C. sand test, which ran for three weeks in this experiment (Section D.11.2).

3.3. Results.

The results of seedling emergence and normality in the sand test, and all of the field results, are given in Appendix Table A.9. Summaries of these data, expressed as percentages, are presented in Tables 12 and 13.

Rika grain was not of such a high quality as Arès, and showed a lower percentage of normal seedlings. However, at 14 per cent. moisture content, it showed about the same proportional reduction in seedling normality as Arès, in response to the most severe treatments.

There was a rapid and unexpected loss of viability of grain of 22.0 per cent. moisture, stored at 20°C. for 3 - 4 weeks prior to fumigation. This was particularly evident in Rika, which also showed appreciable deterioration at 17.8 per cent. moisture content.

The increased susceptibility of grain to loss of viability through fumigation, with an increase of moisture content was particularly striking. For example, the C.T. product necessary to reduce plant survival to about 60 per cent., in the 1964 trial with Arès grain, was 24,000 mg.h./l. (over 20 hours) at 14.1 per cent. moisture, 2,300 mg.h./l. at 17.9 per cent. and 275 mg.h./l., at 22.0 per cent. (Table 13). For both of these intervals of 4 per cent. moisture content, the C.T. product altered by a factor of about 10 times.

The storage of fumigated grain for 10 months, resulted in a slight reduction of field emergence of about 4 per cent., with appreciably greater reductions for grain fumigated at the highest moisture content. This period of storage was not expected to have altered the frequency of

loose smut in the resulting crop to any great extent. The evidence for this view is considered in Section H.

Percentage survival agreed fairly well with percentage field emergence (Table 13), and did not differ by more than 8.2 per cent. This supports the contention, outlined previously, that fumigation does not have any appreciable, delayed toxic effects, and that most of the seedlings that emerge, continue to develop satisfactorily to the flowering stage. The percentage survival was also fairly reliably indicated by the percentage of normal seedlings in the 20°C. sand test. Due to loss of viability during storage, a close agreement between the 1963 field emergence results, and seedling normality in the 1964 sand test was neither expected nor found.

The 1964 field data have been statistically analysed in the manner used in the two previous sections, namely by two separate analyses of variance of angular transformations of percentages. These analyses have been summarized in Appendix Tables A.10 and A.11, to which are appended the least significant differences. The variance due to the block arrangement was shown to be small and not significant ($P = 0.05$), so that only a very slight increase in the precision of these tests resulted from this particular design.

The treatment means with indications of their significant differences, are shown in Table 13. A significant ($P = 0.05$) control of loose smut was obtained in grain of 14.1, 17.9 and 22.0 per cent. moisture content with C.T. products of 12,000, 2,300 and 325 mg.h./l., respectively.

Table 12. The influence of methyl bromide fumigation of grain of varieties Rika and Arès, upon seedling development in the laboratory and field.

Per Cent. Moisture Content	Treatment C.T.P. (mg.h./l.)	Rika Sand Test (1964) Per Cent. Seedlings (300 Grains)		Arès Sand Test (1964) Per Cent. Seedlings (300 Grains)		Arès Field Results Per Cent. Emergence	
		Emerged	Normal	Emerged	Normal	1963 (1800 Grains)	1964 (1500 Grains)
Arès 14.1, Rika 14.0							
	0	86.0	74.7	98.7	97.3	90.1	89.3
	2,300	75.0	65.7	90.3	88.3	76.1	80.3
	12,000	69.7	59.7	82.7	82.0	78.5	77.3
	18,000	77.3	66.3	83.7	81.7	74.9	76.1
	24,000 (20 hr.)	72.3	59.3	83.7	82.3	76.2	72.3
	24,000 (40 hr.)	71.0	57.0	83.3	80.0	79.2	75.3
	24,000 (80 hr.)	69.7	57.3	79.7	78.3	78.8	75.2
Arès 17.9, Rika 17.8							
	0	72.7	53.7	97.7	97.3	90.6	87.0
	2,300	55.0	35.7	77.3	64.3	68.1	63.0
	6,500	24.0	8.3	34.7	20.3	32.1	27.6
	8,900	21.7	11.3	37.7	22.3	28.3	24.0
	12,000	19.3	8.3	27.0	16.0	19.4	14.5
Arès 22.0, Rika 22.0							
	0	55.7	36.3	91.3	90.3	83.2	79.9
	175	49.7	8.7	87.0	85.7	78.4	79.3
	275	28.0	0.3	80.7	48.0	75.9	63.3
	325	8.7	0.0	65.0	15.7	57.8	39.2
	375	1.7	0.0	28.7	1.7	22.4	8.1

Table 13. The influence of methyl bromide fumigation of grain of Arès barley, at three moisture contents, upon percentage emergence, survival and loose smut infection of plants in the field.

Per Cent. Moisture Content	Treatment C.T.P. (mg.h./l.)	Per Cent. Emergence (1500 Grains)	Per Cent.* Survival (1500 Grains)	Per Cent.* Loose Smut (Survived Plants)
14.1	0	89.3	85.1 a.....	5.17 g.....
	2,300	80.3	73.0 .bc...	5.62 g.....
	12,000	77.3	70.8 .bc...	2.12 .hi....
	24,000(20 hr.)	72.3	66.0 ..cde.	1.31 .hi....
	24,000(40 hr.)	75.3	71.1 .bc...	0.89 ..ijk..
	24,000(80 hr.)	75.2	68.9 .bcd..	0.06 .....lm
17.9	0	87.0	78.8 ab....	5.64 g.....
	2,300	63.0	56.8 ...de.	0.17 ....klm
	6,500	27.6	25.6 .....f	0.00 ......m
22.0	0	79.9	73.0 .bc...	2.47 .h.....
	175	79.3	75.2 abc...	1.22 .hij...
	275	63.3	56.7 ....e.	1.20 .hij...
	325	39.2	33.6 .....f	0.36 ...jkl.

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

However, as in the previous trials, reductions in plant survival of more than 14 per cent. were associated with this degree of control.

With grain of 14.1 per cent. moisture content treated at a concentration-time product of 24,000 mg.h./l., a slight, significant (P = 0.05) improvement in the control of loose smut was achieved with no further reduction

in plant survival, by increasing the duration of the fumigation from 20 to 80 hours, and by reducing the concentration of methyl bromide by a corresponding proportion.

The elimination of loose smut was shown to be marginally superior at a moisture content of 17.9 per cent., but for grain of 14.1 and 22.0 per cent. moisture, a comparable degree of control of the pathogen was associated with a similar reduction in plant survival. The three results upon which this conclusion is largely based are:- grain of 14.1 per cent. moisture - 24,000 mg.h./l. (within 20 hours), 17.9 per cent. - 2,300 mg.h./l., and 22.0 per cent. - 275 mg.h./l., between which three treatments, there was no significant (P = 0.05) difference in percentage plant survival.

The smut infection values given in Table 13 were obtained by a transformation of the treatment means from angles back to percentages. At values below about 1.0 per cent., this estimate was smaller by about 0.1 per cent. (in these instances) than the percentage of infected plants determined directly, using the data of Appendix Table A.9. For example, in the treatments of 14.1 per cent. grain at 24,000 mg.h./l. for 40 and 80 hours, the percentages obtained by back-transformation (angles) were 0.89 and 0.06 per cent., whereas the average infections determined from the sums of the three replicates (Appendix Table A.9), were 1.01 and 0.19 per cent. respectively. The significant differences apply to the values given in Table 13.

Summary of results.

Loose smut was significantly reduced by fumigation of grain of 14.1, 17.9 and 22.0 per cent. moisture content at C.T. products of 12,000, 2,300 and 325 mg.h./l. respectively. However these treatments were also phytotoxic and reduced plant survival by at least 14 per cent.

At comparable levels of phytotoxicity, the disease was more easily controlled at 17.9 than at 14.1 and 22.0 per cent. moisture contents, but the improvement though statistically significant ($P = 0.05$), was very slight.

With grain of 14.1 per cent. moisture, 80 hr. fumigations at relatively low concentrations gave a slightly superior control of loose smut, to 20 hr. fumigations at relatively high concentrations, where the C.T. product in both instances was 24,000 mg.h./l.

The grain of both Arès and Rika barley varieties showed a similar order of tolerance to methyl bromide at 14 per cent. moisture content. Comparison at the higher moisture contents was vitiated by the more rapid deterioration of Rika grain.

Percentage plant survival did not differ by more than 8.2 per cent. from field emergence, and showed a fair agreement with the percentage of normal seedlings in a sand test, conducted at approximately the same time.

E.4. The fumigation of grain of Edda II with methanol and chloropicrin to control loose smut.

4.1. Introduction.

Following the unsuccessful attempt to control loose smut in Rika grain with several fumigants (Section E.2), this experiment was designed to ascertain the efficacy of methanol for this purpose. This alcohol has been used effectively by Wagner (1958 - 1963) as a concentrated aqueous solution for treating barley grain, the details of which were considered in Section C.2. It was thought, however, that the use of a vapour treatment might minimize the absorption of the material, and thus reduce the degree of phytotoxicity.

Only a small quantity of grain was available, so that the trial was restricted to only a few treatments. Grain lots were also fumigated with chloropicrin to facilitate comparison with the results of the other trials.

Materials and methods.

The grain sample was adjusted to 14.0 per cent. moisture content, and fumigated in an atmosphere saturated with the vapour of either fumigant, in 4.3 l. desiccators (Sections D.9 and D.7 respectively). Five fumigation treatments, the durations of which are given in Table 14, were each separately repeated twice, totalling 15 lots, which were compared with 3 unfumigated lots. From each of these, 100 grains were randomly selected and tested for viability in the 20^{o}C. sand test, and a further

600 grains were field-sown in plots, of five rows 7 ft. long, arranged in a randomized design. The trial was sown in early July 1963, and the plants were dug up, classed and counted in early October.

Results.

The results of the laboratory and field tests are given in Appendix Table A.12 and are summarized in Table 14. The field results were examined for significant differences by two separate analyses of variance upon angular transformations of results expressed as percentages. These analyses are summarized in Appendix Tables A.13 and A.14.

The survival of plants from unfumigated grain was lower than was expected from the frequency of normal seedlings in the sand test. This was partly accounted for by the late sowing of this trial and the severe infestations of stem-boring insects and Erysiphe graminis, both of which were also troublesome in the 1962 Rika trial (Section E.2). In addition, the grain showed a high proportion, of about 10 per cent., of sprouted grains (Section D.1, Table 4), which indicated that the grain sample as a whole, was probably of a low quality and of low germination vigour, from which a lower emergence in the field than in a sterile sand test, would normally be expected.

The summarized results of the field trial are given in Table 14, together with indications of the significance (P = 0.05) of the differences between means.

A very satisfactory and highly significant control of loose smut

Table 14. The influence of the fumigation of Edda II grain, of 14.0 per cent. moisture content, with methanol and chloropicrin, upon plant survival and loose smut infection.

Treatment: Fumigant	Treatment: Fumigation Duration (Hours)	20°C. Sand Test: Per Cent. Seedlings from 300 Grains: Emerged	20°C. Sand Test: Per Cent. Seedlings from 300 Grains: Normal	Field Test*: Per Cent. Survived (1800 Grains)	Field Test*: Plants Smutted (Survived Plants)
Unfumigated check	0	86.0	80.0	57.0 a...	5.54 e...
Methanol	1.25	79.0	70.0	44.3 ..cd	1.00 ..g.
	2.5	66.7	61.0	42.0 ...d	0.08 ...h
Chloropicrin	3.0	61.3	58.7	51.0 .b..	3.12 .f..
	24.0	57.0	50.7	48.3 .bc.	4.22 ef..
	46.0	56.3	52.0	49.1 .bc.	2.94 .f..
				For comparison:	0.00 ...h

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

from 5.5 to 0.1 per cent., was obtained with a 2.5 hr. fumigation with methanol, which also reduced plant survival by 15 per cent. Chloropicrin was less effective, since a 46 hr. fumigation reduced the smut infection to only 2.9 per cent. (significant at P = 0.05), with an associated reduction in plant survival of 7.9 per cent.

Methanol was shown to be superior to chloropicrin for controlling loose smut. A 1.25 hr. fumigation with methanol and fumigations of 24 and 46 hours with chloropicrin, resulted in plant survival values which were not significantly different (P = 0.05) from one another. However, the frequency of loose smut in the methanol treatments was significantly lower than in the chloropicrin treatments, showing that methanol was not only the quicker acting, but also the more effective of the two fumigants.

The percentage of plant survival from grain fumigated with chloropicrin, showed a closer agreement with the percentage of normal seedlings in the sand test, than did the results for the methanol-fumigated, and unfumigated grain.

Summary of results.

Loose smut was almost completely controlled by a 2.5 hr. fumigation with methanol, which reduced plant survival by 15 per cent. A 46 hr. fumigation with chloropicrin was less phytotoxic, but failed to control the disease to the same extent.

At comparable levels of phytotoxicity, methanol controlled loose smut more effectively than chloropicrin, and did so more rapidly.

The percentage of plant survival from unfumigated grain was only 57 per cent. This low value was largely explained by the poor quality of the grain, indicated by the high frequency of sprouted grains, and also by heavy infestations of insects and Erysiphe graminis, which weakened, and probably killed some of the smaller plants.

E.5. The fumigation of grain of variety Donaria with methanol and chloropicrin.

5.1. Introduction.

This trial was conducted at the same time as the previous experiment with Edda II grain (Section E.4), and was designed for the same purpose of comparing the effectiveness of methanol with that of chloropicrin, for controlling loose smut, when applied to grain with a moisture content of 14.0 per cent. In addition, grain samples of 10.2 and 18.0 per cent. moisture content were also fumigated with methanol, to give some information upon the susceptibility of the grain to fumigation injury, and the ease of control of the disease, at these moisture levels.

The grain sample was received in early 1962 from Prof. Böning (München, Germany), and was probably of similar quality to the samples of Donaria used by Dr. F. Wagner, who worked in the same Institute (Wagner, 1960a, 1961a, b). Aqueous solutions of methanol, applied to grain as a liquid, have been shown by Wagner (1958 - 1963) to be as effective as the hot water treatment for controlling loose smut in barley. In this experiment, the feasibility of using a fumigation treatment for applying methanol to grain, was investigated.

5.2. Materials and methods.

The details of the Donaria grain sample are given in Table 4 (Section D.1). Quantities of grain were adjusted to 10.2, 14.0 and 18.0 per cent. moisture content (Section D.9), and subsamples from each were

fumigated at saturation vapour concentrations (20°C.) of the two fumigants, in 4.3 l. desiccators (Section D.7). Because of the low level of embryo infection, of about 1.0 per cent., approximately 2200 grains weighing about 85 g. were used for each fumigation. The durations of the 15 treatments, including the 3 unfumigated checks, are given in Table 15. Each treatment was separately repeated twice, making a total of 45 lots, from each of which 100 grains were sampled and tested for viability in the 20°C. sand test. A further 500 and 1500 grains were randomly selected and field sown in mid-July 1963, in adjacent subplots in a design in which the plots were arranged in a randomized manner.

Seedling emergence in the field was evaluated from the subplots sown with 500 grains, whereas survival and smut infection results, were derived from the plant population in the whole plot, sown with the total of 2000 grains.

Artificial illumination was used in the field to hasten the emergence of the inflorescence from the flag leaf, with the object of improving the ease of identification of smutted plants (Section D.13).

The durations of the fumigations were chosen, so as not to reduce plant survival by more than 20 per cent., on the basis of preliminary phytotoxicity tests, in which the grain was fumigated in desiccators and germinated on tension plates at 20°C. (Section D.11.1).

The quantities of fumigant sorbed by the grain samples in three of these fumigation treatments, was determined in a subsequent experiment, which is described in Section G.

5.3. Results.

The results of the laboratory and field tests are given in Appendix Table A.15, and are summarized as percentages in Table 15. For each replicate, the percentage plant survival and the percentage of plants infected by a smut fungus, were each transformed to angles, and examined for significant treatment differences by two separate analyses of variance. As explained in Section E.2.3, the treatments in which all the replicates showed zero smut infection, were excluded from the analysis of smut frequency, so that the treatment and error variances would not be reduced, inappropriately, by virtue of the greater number of degrees of freedom. In this way the significance of treatment differences was not overestimated. Summaries of these two analyses of variance are given in Appendix Tables A.16 and A.17, together with the least significant differences.

Loose smut was significantly ($P = 0.05$) controlled by methanol, with associated reductions in plant survival of 8.6 - 16.3 per cent., but it was not controlled in fumigations with chloropicrin which reduced plant survival by as much as 31.1 per cent. Methanol was therefore markedly superior to chloropicrin, in this particular experiment.

Significant ($P = 0.05$) reductions in plant survival were associated with significant ($P = 0.05$) control of loose smut. For grain of 10.2 per cent. moisture, the reduction was 12.3 per cent., for 18.0 per cent. grain it was 16.3 per cent., but for grain of 14.0 per cent. moisture the reduction in plant survival was only 8.6 per cent.

Loose smut was most easily controlled at a grain moisture content

Table 15. The influence of the fumigation of grain of Donaria with methanol and chloropicrin, upon seedling development, plant survival and loose smut infection.

Treatment: Fumigant		20°C. Sand Test: Percentage Seedlings from 300 Grains		Field Results*		
Per Cent. Moisture Content	Fumigation Duration (Hours)	Emerged	Normal	Per Cent. Emergence (1500 Grains)	Per Cent. Survival (6000 Grains)	Per Cent. Smut (Survived Plants)
Methanol						
10.2	0	98.3	96.3	88.7	84.5 ab....	1.02 g...
	18.0	84.3	83.7	75.6	72.2 ..c...	0.16 .hi.
	48.0	70.7	69.0	65.9	62.2 ....ef	0.00 ...j
14.0	0	99.3	96.7	90.7	88.3 a.....	1.07 g...
	6.0	90.3	87.7	82.7	79.7 .b....	0.04 ..ij
	14.0	77.3	76.7	69.9	69.4 ..cd..	0.00 ...j
	21.5	55.7	53.3	55.0	-	-
	25.0	56.3	54.3	49.5	-	-
18.0	0	95.0	89.3	83.5	81.6 .b....	1.06 g...
	0.25	95.3	88.0	82.1	79.8 .b....	1.00 g...
	0.5	83.7	57.0	68.5	65.3 ...de.	0.30 .h..
Chloropicrin						
14.0	7.5	79.0	74.7	70.7	66.5 ..cde.	1.08 g...
	36.0	75.3	68.0	64.0	-	-
	54.0	74.0	60.7	60.3	57.2 .....f	1.15 g...

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

of 14.0 per cent., and least easily at 18.0 per cent., as indicated in the preceding paragraph. The evidence for the former point is grained from a comparison between the 14 hr. fumigation of grain of 14.0 per cent. moisture, and the 18 hr. fumigation of grain of 10.2 per cent. The percentage plant survival values were not significantly different (P = 0.05), but the control of loose smut in the 14.0 per cent. grain was significantly superior (Table 15). Likewise, the 14 hr. fumigation of 14.0 per cent. grain resulted in an insignificantly different plant survival from the 0.5 hr. fumigation of 18.0 per cent. moisture grain, and again, the disease control in the 14.0 per cent. grain was superior. The greater ease of control of loose smut in grain at 10.2 per cent. moisture compared with 18.0 per cent., is substantiated by a comparison of the results of the 48 hr. fumigation at the low moisture with those of the 0.5 hr. fumigation at the high moisture. For these two treatments, plant survival values were not significantly different (P = 0.05), but the disease control in the 10.2 per cent. moisture grain, showed a significant improvement.

Very few (2 - 3 per cent.) abnormal seedlings developed in the 20°C. sand test from grain fumigated at moisture contents of 10.2 and 14.0 per cent., even though at the latter moisture, the fumigations of over 20 hours duration reduced seedling emergence to less than 60 per cent. This was in contrast to the large number (over 26 per cent.) of abnormal seedlings that emerged, in the sand test, from grain fumigated at 18.0 per cent. moisture, even though the emergence was more than 80 per cent.

In general agreement with the previous trials, the percentages

of seedling emergence and plant survival in the field, showed a closer numerical proximity to the percentage seedling normality, rather than seedling emergence, in the sand test.

5.4. Summary of results.

Methanol was shown to control loose smut in a shorter time and with less associated phytotoxicity than chloropicrin.

Loose smut was controlled with the least reduction in plant survival at 14.0 per cent. moisture, followed by 10.2 per cent., while the greatest reduction occurred with grain of 18.0 per cent. moisture.

In the sand test, greater numbers of abnormal seedlings developed from grain fumigated at 18.0 per cent. moisture, than at either 10.2 or 14.0 per cent., indicating some major change in the physiology of the grain affecting its susceptibility to fumigant injury, between 14 and 18 per cent. moisture contents.

F. THE FUMIGATION OF WHEAT GRAIN TO CONTROL USTILAGO TRITICI.

F.1. The fumigation of infected grain of variety Fylgia II with methyl bromide and hydrogen cyanide.

1.1. Introduction.

Both hydrogen cyanide and methyl bromide have been used extensively to control insect pests in stored grain, at treatment levels which were generally non-toxic to the grain. It was therefore of interest to enquire to what extent these fumigants would also control seed-borne pathogenic fungi, and in particular Ustilago tritici in wheat grain.

The fungicidal properties of methyl bromide were considered in Section E.3, where it was also observed that wheat was more liable to fumigation injury than barley. Lubatti and Blackith (1957) showed that with a fumigation of 1200 mg.h./l., grain of 14.0 per cent. moisture was severely reduced in germination, in contrast to the greater tolerance of grain of about 11.0 per cent. moisture. A fumigation of 600 mg.h./l., however, resulted in virtually no loss of viability of grain of 14.0 per cent. moisture. Whitney et al. (1958) found that a C.T. product of 200 - 400 mg.h./l., reduced the viability of 14 per cent. moisture grain by 10 - 20 per cent., whereas at the other extreme of tolerance, Strong and Lindgren (1959a) showed that when grain with the same moisture content received a fumigation treatment of 1728 mg.h./l. the number of normal seedlings was reduced by only about 50 per cent. Concentration-time

products of 500 and 1000 mg.h./l. were therefore chosen for this preliminary investigation.

Hydrogen cyanide has been reported to be less fungicidal than ethylene oxide, chloropicrin and formaldehyde (Dalton and Hurwitz, 1948), nevertheless satisfactory toxic effects have been demonstrated at C.T. products as extreme as 2.5 mg.h./l. (Sibilia, 1927) and about 1500 mg.h./l. (Polunin, 1942), when agar cultures of the fungi were exposed to the fumigant. However van de Pol (1964) found that Ustilago nuda was not appreciably controlled when infected barley (?) grain of 12 per cent. moisture, was fumigated with hydrogen cyanide (max. C.T.P. 1536 mg.h./l.) at non-phytotoxic levels.

Strong and Lindgren (1959a) showed that hydrogen cyanide was not appreciably phytotoxic to wheat, since grain of 14 per cent. moisture fumigated at 21°C. for 72 hours at 2.5 lb./1000 cu. ft. (max. C.T.P. 3280 mg.h./l.) was only slightly reduced in viability. Furthermore, a repeat fumigation did not increase this degree of phytotoxicity.

1.2. Materials and methods.

In the summer of 1961, a large number of ears of Fylgia II wheat were inoculated, at anthesis, with an aqueous suspension of spores of Ustilago tritici, by the partial vacuum method (Moore, 1936). The ripened grain was carefully harvested and subsequently adjusted to 14.0 per cent. moisture content, fumigated, and sown in a field trial in late July 1962. Uninfected seed lots of three other varieties of wheat, Hybrid 46, Capelle desprez and Svenno, obtained from commercial sources,

were similarly adjusted to 14 per cent. moisture and replicates, separately contained in muslin bags, were fumigated simultaneously with samples of Fylgia II grain in the same fumigation bins. The total quantity of grain used in each fumigation was about 200 g.

The fumigation bins were of 20 l. capacity and their operation has been described in Section D.5 (see Plate 1). Quantities of both fumigants sufficient to give the desired initial concentration were introduced by fracturing glass ampoules. The concentration of methyl bromide was determined with a thermal conductivity meter, and the duration of the fumigation was adjusted to give the required C.T. product. Fumigations of 250 mg.h./l. lasted about 11 hours, and those of 500 and 1000 mg.h./l. were of 19 - 25 hr. duration.

The concentration of hydrogen cyanide was not determined, but the duration of the fumigation was adjusted according to the quantity of fumigant introduced, and was about 23 hours for 500 and 1000 mg.h./l. and about 45 hours for the fumigations with a C.T. product of 2000 mg.h./l. (Section D.6).

Each fumigation was separately repeated once, and the temperature during fumigation was maintained at 20°C.

Samples of 500 grains were randomly selected from each of the eight fumigated and two unfumigated lots of Fylgia II, and field-sown in plots arranged in a randomized design, adjacent to the trial of fumigated Rika barley (Section E.2). Artificial illumination (Section D.13) was used to hasten the emergence of the inflorescence.

From each of the other lots of fumigated grain, 250 grains were randomly selected and sown in a separate unilluminated trial. Seedling emergence was recorded five weeks after sowing. The Fylgia II trial was dug up at anthesis and the plants counted for a measure of plant survival.

1.3. Results.

Concentration-time products of 250 mg.h./l. of methyl bromide and 2000 mg.h./l. of hydrogen cyanide reduced seedling emergence by 7 - 34 per cent. in three of the wheat varieties (Table 16). Grain of Fylgia II was more tolerant and sufficient plants survived to anthesis, to enable a reliable measure of loose smut infection to be made.

Table 16. Percentage field emergence of seedlings from grain of 14.0 per cent. moisture content, of 4 wheat varieties, fumigated with methyl bromide and hydrogen cyanide.

Treatment		Percentage Field Emergence			
Fumigant	C.T.P. (mg.h./l.)	From 1000 Grains	From 500 Grains		
		Fylgia II	Hybrid 46	Capelle Desprez	Svenno
Check	0	64.7	67.8	75.0	68.4
Methyl bromide					
	250	-	33.4	46.4	39.6
	500	43.0	7.2	4.2	19.6
	1000	38.6	3.2	1.0	9.4
Hydrogen cyanide					
	500	-	67.0	73.6	73.8
	1000	69.0	66.2	71.0	59.0
	2000	73.2	60.4	58.8	57.6

The results of the trial of Fylgia II grain are presented in Appendix Table A.18, and are summarized as percentages in Table 17. Two separate analyses of variance were performed on percentage plant survival and percentage of smutted plants, as angles, and these are summarized in Appendix Tables A.19 and A.20.

Table 17. The influence of methyl bromide and hydrogen cyanide fumigations of Fylgia II grain of 14.0 per cent. moisture content, upon seedling emergence, plant survival and loose smut infection.

Treatment Fumigant	C.T.P. (mg.h./l.)	Percentage Emergence (1000 Grains)	Percentage* Survival (1000 Grains)	Percentage* Loose Smut (Survived Plants)
Check	0	64.7	55.2 a.	5.86 c
Methyl bromide				
	500	43.0	39.8 .b	7.54 c
	1000	38.6	36.6 .b	8.37 c
Hydrogen cyanide				
	1000	69.0	63.1 a.	6.71 c
	2000	73.2	63.4 a.	6.94 c

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

Loose smut was not significantly controlled, even by the methyl bromide fumigations which significantly reduced percentage survival by over 15 per cent.

The percentage plant survival from unfumigated grain in the 1962

trial was only 55.2 per cent. This is significantly (P = 0.05) lower than the value in the repeat trial conducted the following year, when it was 85.9 per cent. (Section H.4). The low frequency is probably accounted for by the death of small plants, resulting from heavy infestations of stem-boring larvae of insects, and Erysiphe graminis, which were favoured by the late sowing of this trial.

F.2. The treatment of grain of variety Fylgia II with methanol, hot water and (trichlorophenoxythio) trichloromethane.

2.1. Introduction.

In the trial of fumigated grain of the barley variety Donaria (Section E.5), methanol was shown to control loose smut with only a slight reduction in plant survival. This experiment was designed to compare the efficacy of methanol fumigations with the hot water treatment for controlling Ustilago tritici in wheat grain.

Additional dust treatments of the grain with the slightly volatile (2:4:5-trichlorophenoxythio) trichloromethane, were included because this compound has been used as a fungicidal fumigant to control Penicillium italicum on oranges and Gloeosporium spp. on apples (Fawcett et al., 1958; Spencer and Wain, 1961). Pea seeds heavily infected with Ascochyta spp. and Mycosphaerella sp. were also treated with this compound as a 10 or 20 per cent. dust, but showed only a slight increase in germination (Spencer and Wain, 1961).

2.2. Materials and methods.

The stock of Fylgia II grain used in this experiment was of the same origin as that used in the fumigations described in Section F.1. However, prior to its use in this trial, the lot was sieved to remove the small shrivelled grains, and the details of the resulting grain stock are given in Table 4 (Section D.1).

The grain was adjusted to 13.0 per cent. moisture content (Section D.9) and small quantities of it were fumigated with methanol at the saturation vapour concentration at 20°C., in 4.3 l. desiccators for 1 or 3 hours.

The hot water treatment consisted of a presoak at 34°C. for 4 hours, a preheat at 50°C. for 1 minute and a final treatment at 53°C. for 5 or 8 minutes, after which the grain was rapidly cooled in water to 20°C. The grain was dried at 27°C. for 1 day, and sown in moist soil. Samples of 400 - 500 grains were contained in numbered muslin bags to facilitate handling.

The (2:4:5-trichlorophenoxythio) trichloromethane (sometimes abbreviated as TCPTCM in this study) was received as a crystalline solid (melting point 46 - 47°C.). A small quantity was intimately mixed with twice its weight of talc and the minimum of acetone necessary to dissolve the compound, and the mixture was stirred until the acetone had evaporated. The resulting impregnated talc was dusted on to 15 g. quantities of grain of 13.0 per cent. moisture, at the rate of 1 g. of active ingredient per kilogram of grain. The dusted grain was sealed in airtight, screw-top

glass jars of 160 ml. capacity, and stored at 34°C. for one or two weeks, with vigorous agitation every third day. A talc check consisting of a similar quantity of grain dusted with talc only, was stored in jars at 34°C. for two weeks.

Preliminary experiments with weighed crystals of the compound, in jars containing 15 g. of grain, exposed at 34°C. for 2 weeks, with agitation, showed that the reduction in weight of the crystal was only about 2 mg. Therefore the 15 mg. of active ingredient added in the impregnated talc treatments, was sufficient to maintain a saturation vapour concentration over the two week period, despite losses through sorption.

The eight treatments were separately repeated twice totalling 24 lots, from each of which 100 grains were tested for viability in the 20°C. sand test, and a further 300 grains were sown in soil in the glasshouse in a randomized design (Section D.12).

2.3. Results.

The laboratory and field results are given in Appendix Table A.21, and are summarized as percentages in Tables 18 and 19. Percentage plant survival and loose smut infection, transformed to angles, were examined for significant treatment differences by two separate analyses of variance, which are summarized in Appendix Tables A.22 and A.23.

Seedling emergence in soil under glasshouse conditions was lower than in the 20°C. sand test, and in some treatments the discrepancy between the percentage of normal seedlings in the sand test and the percentage

of seedling emergence in soil was about 20 per cent., as for example with the hot water treatments and with the 2 week treatment with (trichlorophenoxythio) trichloromethane.

A feature which was not noticed in the earlier field trials was the higher value for percentage plant survival than for percentage normality in the soil test. This indicated that a number of abnormally developed seedlings subsequently recovered, and developed into plants of normal appearance.

Table 18. The results of laboratory and glasshouse tests of the viability of grain treated with methanol, (2:4:5-trichlorophenoxythio) trichloromethane, and hot water.

Treatment	20°C. Sand Test from 300 Grains Per Cent. Seedlings		Glasshouse Trial from 900 Grains Per Cent. Plants		
	Emerged	Normal	Emerged	Normal	Survived
No treatment-check:	97.3	95.7	88.2	86.2	87.1
Hot water:					
5 min.	94.7	89.7	71.2	63.4	66.8
8 min.	94.0	82.0	57.2	50.2	54.7
Methanol fumigation:					
1 hr.	80.0	66.0	56.1	52.2	55.3
3 hr.	56.7	54.7	46.6	43.7	46.4
Talc check:	95.3	91.7	77.8	73.2	77.3
'TCPTCM' -					
1 week	-	-	66.8	63.8	66.5
2 weeks	90.3	81.3	55.9	52.2	55.3

Loose smut was significantly reduced by a 3 hr. fumigation with methanol, and a 2 week fumigation with (trichlorophenoxythio) trichloromethane and by both hot water treatments. However severe reductions of plant survival of more than 20 per cent. were associated with these degrees of control.

At comparable levels of plant survival of 54.7 - 55.3 per cent. (having the letter 'd' in common) the disease control resulting from the hot water treatment was significantly (P = 0.05) superior to that obtained with either methanol (1 hour) or (trichlorophenoxythio) trichloromethane (2 weeks). The values of per cent. loose smut associated with these latter treatments were not significantly different (P = 0.05).

Table 19. The influence of grain treatment upon percentage plant survival and loose smut infection in Fylgia II wheat.

Treatment	Per Cent. Survival* (900 Grains)	Per Cent. Loose Smut* (Survived Plants)
No treatment-check:	87.1 a....	9.22 f...
Hot water: 5 min.	66.8 ..c..	0.00 ...i
8 min.	54.7 ...d.	0.00 ...i
Methanol fumigation: 1 hr.	55.3 ...d.	6.50 fg..
3 hr.	46.4 ....e	1.38 ..h.
Talc check:	77.3 .b...	6.56 fg..
'TCPTCM' - 1 week	66.5 ..c..	8.31 fg..
2 weeks	55.3 ...d.	5.61 .g..

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

Methanol was markedly less effective than the hot water treatment for controlling Ustilago tritici, and a significant reduction in the frequency of loose smut by a 3 hr. methanol fumigation, was associated with a reduction in plant survival of 40.7 per cent. This ineffectiveness of methanol for controlling this disease contrasts sharply with its successful use in the control of U. nuda in Donaria barley, where the associated reduction in plant survival was as little as 8.6 per cent.

G. THE SORPTION OF METHANOL BY BARLEY GRAIN AND ITS ASSOCIATED PHYTOTOXIC EFFECT

1.1. Introduction.

In the trial of Donaria grain fumigated with methanol (Section E.5), loose smut was significantly controlled at grain moisture contents of 10.2, 14.0 and 18.0 per cent., by fumigations of 18.0, 6.0 and 0.5 hr. duration, respectively. In an endeavour to explain why short fumigations at high moisture contents were almost as effective as long fumigations at low moisture contents, observations were made upon the quantity of fumigant sorbed by the grain, during a separate set of fumigations. These results were compared with both the sorption of methanol by, and its effect upon seedling emergence under field conditions of, Arès grain, also with a moisture content of 14.0 per cent.

1.2. Materials and methods.

Subsequent to the fumigations described in Section E.5, a small quantity of Donaria grain was adjusted to 10.2, 14.0 and 18.0 per cent. moisture contents, and 30 g. samples were fumigated in 4.3 l. desiccators for the respective periods of 18.0, 6.0 and 0.5 hours. Prior to fumigation each sample was accurately weighed in an airtight tin, and afterwards rapidly transferred to the grain carrier which was placed in the desiccator, simultaneously with the commencement of the fumigation (Section D.7). Upon completion of the fumigation, the grain was quantitatively returned to the tin, through a funnel, and the change of

weight ascertained.

Samples weighing 30 g. of Arès grain, with a moisture content of 14.0 per cent., were fumigated for 4, 10 and 24 hours. The weight of the sorbed fumigant was measured, and the samples were aired for 3 days at 20°C. and 500 grains were sown in a field trial of randomized design, for observations of seedling emergence.

For comparative purposes, 30 - 40 g. samples from the same lot of Arès grain, were weighed into glass jars of 160 ml. capacity, fitted with metal screw tops with rubber gas-tight seals. Liquid methanol was added from a burette at the rate of 0.80, 1.59 and 3.18 g. per 100 g. of grain (equivalent to 1.0, 2.0 and 4.0 ml./100 g.). The jars were immediately closed and the contents were shaken to distribute the methanol amongst the grain. The treated grain samples were maintained at 20°C. for 24 hours and then aired for 3 days in muslin bags at 20°C. in a ventilated room. From each lot, 500 grains were sampled and field-sown in the same trial as the grain fumigated with methanol vapour, in desiccators.

1.3. Results.

The results for the sorption of methanol on Donaria grain are compared in Table 20 with the levels of phytotoxicity observed in the earlier field trial of Donaria grain which had received similar fumigation treatments, as described in Section E.5. The rate of sorption by grain of 18 per cent. moisture was about 20 times more rapid than by grain of 10 per cent. However, the quantity of sorbed fumigant was not positively

Table 20. The sorption of methanol by grain of the barley variety Donaria, at three moisture contents, associated with comparable degrees of phytotoxicity.

Per Cent. Moisture Content	Duration Fumigation (Hours)	Per Cent. Plant Survival (6000 Grains)	Sorption g./100g. of Grain	Mean Sorption g./100g.	Rate of Sorption g./100g./hr.
10.2	0	84.5	0		
	18.0	72.2	1.745	1.769	0.10
		reduction=12.3	1.779		
			1.782		
14.0	0	88.3	0		
	6.0	79.7	2.778	2.468	0.41
		reduction=8.6	2.255		
			2.372		
18.0	0	81.6	0		
	0.5	65.3	1.226	1.116	2.23
		reduction=16.3	1.064		
			1.059		

correlated with the reduction in plant survival, since the least reduction in plant survival, of 8.6 per cent., was associated with the greatest sorption of fumigant of 2.5 per cent. by weight of the grain. This apparent anomaly may be partially explained if most of the fumigant remained sorbed in the outer parts of the grain, and indeed during the fumigations, the grains did assume a "water-soaked" appearance, while others became moist by the condensation of fumigant. This process of condensation, which was most rapid on grain of 18 per cent. moisture, but was scarcely noticeable on grain of 10 per cent. moisture, was a conspicuous feature of methanol fumigations but was not observed to the same extent

with other fumigants used under similar conditions (Section D.7).

In this experiment the amount of methanol sorbed by grain, during fumigations which had satisfactorily controlled loose smut, was 1.1 - 2.5 g. per 100 g. of grain, corresponding to reductions in plant survival of 8.6 - 16.3 per cent.

Arès grain of 14 per cent. moisture fumigated for 4 and 10 hours showed a reduction in seedling emergence of 5.1 and 9.6 per cent., which was associated with fumigant sorption of 3.6 and 6.0 g. per 100 g. of grain respectively (Table 21). The quantity of fumigant sorbed by Arès grain was therefore about 1.5 - 2 times greater than that by Donaria of the same moisture content.

These results with Arès grain are in sharp contrast with those obtained when grain was treated with liquid methanol (Table 22), where the seedling emergence of grain treated for 24 hours with as little as 1.59 g. methanol/100 g. grain, was reduced by more than 10 per cent.

These results tend to support the contention that fumigant, sorbed slowly from the vapour phase, remained associated with the outer structures of the grain, and had little immediate effect upon the viability of the embryonic tissues. However, this does not explain why smaller quantities of fumigant, added as a liquid at the commencement of the treatment, should prove more phytotoxic.

The quantity of methanol required to effect a control of loose smut in Donaria was in excess of 1.0 g./100 g. of grain, but since the saturation vapour concentration at 20°C. is only 0.166 g./l. (Table 5,

Table 21. The relationship between the sorption of methanol by grain of Arês barley, of 14 per cent. moisture content, and seedling emergence in soil.

Duration Fumigation (Hours)	Methanol Sorbed g./100g.Grain	Rate of Sorption g./100g.Grain/hr.	Per Cent. Seedling Emergence (1500 Grains)
0	0	0	84.2
4	3.556	0.89	79.1
10	5.976	0.60	74.6
24	11.571	0.48	59.7

Table 22. The treatment of Arês grain, of 14 per cent. moisture, with known weights of methanol for 24 hours, and its effect upon seedling emergence in soil.

Treatment Duration (Hours)	Weight of Added Methanol g./100g. Grain	Per Cent. Seedling Emergence (1500 Grains)
0	0	84.2
24	0.80	85.1
24	1.59	69.9
24	3.18	46.8

Section D.8), the treatment of grain with methanol in the vapour phase, does not appear to be a practical proposition, on a large scale. Instead, the wetting or drenching of grain with liquid methanol would seem to be a more convenient, quicker and probably a more precise method of application.

It was shown in Table 22 that the addition of methanol at a rate of 1.6 per cent. or more, by weight of the grain, resulted in an appreciable reduction in seedling emergence. This value may be compared with the rates of application of about 2.0 - 2.5 per cent. by weight of grain, of the early methanol formulations used by Wagner (1958, 1960a, 1961b).

H. THE INFLUENCE OF POST-FUMIGATION STORAGE UPON THE VIABILITY OF GRAIN, AND OF THE LOOSE SMUT FUNGI.

H.1. The influence of storage upon the viability of Arès grain fumigated with methanol.

1.1. Introduction.

The viability of cereal grains stored at low moisture contents declines slowly, and the same appears to be broadly true of fumigated grain. However, at high moisture contents, deterioration of quality and loss of viability is often rapid, with some of the largest reductions being attributable to interaction effects between the moisture content and the fumigation treatment, as demonstrated in rye and maize by Lubatti and Blackith (1957). Lucerne seed was also shown to lose viability if stored at high moisture contents for 6 months at 22°C. (Isaac and Heale, 1961), but in this case, the fumigated seed showed a similar behaviour with no evidence of a pronounced loss of viability due to a moisture-fumigation interaction.

In the present study, observations were made on the viability of barley grain, fumigated with methanol, over a period of 6 - 7 months, for comparison with the field results of other trials of stored grain, considered later.

1.2. Materials and methods.

Grain of the barley variety Arès of 14.0 per cent. moisture was fumigated in 4.3 l. desiccators for 25 hours to reduce seedling normality

to about 60 per cent., and was subsequently aired for 2 days at 20°C. in a ventilated room, during which time the moisture content of the grain fell to about 12.5 per cent. The samples were stored at this moisture content in perforated paper envelopes at 20°C. The fumigation treatment was conducted in triplicate, and the three lots of grain were stored separately under similar conditions.

Two hundred grains were randomly sampled from each replicate 0.4, 1.0, 3.5, 11.0, 30.0 and 208 days after the termination of the fumigation, and were tested for seedling emergence and normal development at 20°C. in the sand test. The final count of seedling normality was made 14 days after the commencement of the test.

1.3. Results.

The percentage emergence and seedling normality are shown in Table 23. Significant treatment effects were determined by an analysis of variance upon angular transformations of percentage seedling normality (Appendix Table A.24).

The 25 hr. fumigation reduced seedling normality from 96.4 per cent. in the check to about 60 per cent. Grains aired for only 0.4 days germinated to yield 62.2 per cent. normal seedlings, and this frequency was neither increased by further airing of the grain, nor decreased by a period of airing/storage of 30 days. After 6 - 7 months storage however, there was a significant reduction in percentage normality to 55.0 per cent.

Less than 2.0 per cent. of the emerged seedlings showed any abnormal development.

Table 23. The influence of the duration of post-fumigation storage upon the percentage of normal seedlings that developed from Arès grain fumigated with methanol.

Duration of Storage (Days)	Per Cent. Seedling Emergence (600 Grains)	Per Cent. Seedling Normality* (600 Grains)
0.4	62.8	62.2 .bc.
1.0	62.5	61.8 .bc.
3.5	61.3	61.0 .bc.
11.0	64.5	63.7 .b..
30.0	58.5	58.3 ..cd
208.0	55.5	55.0 ...d
Unfumigated Check:	97.8	96.4 a...

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

H.2. The effect of a 12 month storage of Arès grain, fumigated with chloropicrin, upon plant survival and the incidence of loose smut.

2.1. Introduction.

Russell (1961) showed that when grain stocks of barley infected by Ustilago nuda were stored for more than six years, the resulting percentage loose smut infection in the field trials, showed a steady decline with increasing length of the storage period. This was accounted for by the more rapid loss of viability by the infected grains than by the uninfected ones. This endorsed Russell's earlier conclusions (Russell, 1954) that infected grains were weaker than uninfected grains.

It may be suggested, therefore, that the fumigation of grain weakens the infected more than the uninfected grains, and further, that this effect might be more apparent after a storage period of several months, during which time any fumigant remaining sorbed by the grain, might continued to have this differential toxic effect. Therefore, according to this hypothesis loose smut is controlled by the elimination of infected grains, through their more rapid loss of viability.

An alternative hypothesis, is that the fumigant is more toxic to the fungus than to the host embryo. During the storage of fumigated grain it is possible that the loose smut mycelium is further reduced in viability by the small quantities of fumigant, or its reaction products, that remain in the grain.

Some support for this latter hypothesis is gained from the work of Mukkath (1961), reviewed in Section C.2 (Table 3). In this study celery seeds bearing pycnidia of Septoria apii were fumigated with chloropicrin and the spores within the pycnidia were afterwards recovered and examined for viability. Following a fumigation of 24 hr. duration, percentage spore germination remained as high as in the unfumigated check, but after 3 weeks storage it had declined from 18 to less than 1 per cent. It was suggested, in Section C.2, that this delayed toxic effect may have been due to the action of chloropicrin, released slowly from solution in the resins and oils contained within the "seed" pericarp. Since wheat and barley grains have only about 1.0 - 1.5 per cent. by weight, of oil (Blackith & Lubatti, 1960), such a pronounced effect was not expected in

barley grain, within such a short time. However, it was thought that the storage of fumigated grain for 12 months might result in some loss of viability of the fungus, without an associated reduction of grain viability.

The present experiment was not designed to test these hypotheses, but merely to observe whether or not a reduction in the frequency of loose smut infection resulted from 12 months storage.

2.2. Materials and methods.

Grain remaining from the April 1962 trial of Arès grain fumigated with chloropicrin (Section E.1), had been stored at 20°C. with a moisture content of about 12.5 per cent. 250 grains were sampled from each of the three replicates of three of the treatments involving grain of 13.9 per cent. moisture, namely the 6 and 24 hr. fumigations, and also the unfumigated check. These were field sown in plots arranged in a randomized design, in April 1963. In August the plants were dug up, classed as loose-smutted or otherwise, and counted.

2.3. Results.

The 1963 results of plant survival and loose smut infection are summarized as percentages in Table 24, where they are compared with the results of the 1962 field trial.

The plant survival in 1963 was between 15.5 and 23.3 per cent. lower than in 1962, which was partly accounted for by poor plant development due to incomplete vernalization during the warm spring.

Although the frequency of loose smut infection in 1963 was

Table 24. The influence of 12 months post-fumigation storage of Arès grain fumigated with chloropicrin.

Duration Fumigation (Hours)	Per Cent. Plant Survival		Per Cent. Loose Smut Infection of Survived Plants	
	1962 (1800 Grains)	1963 (750 Grains)	1962	1963
0	87.5	72.0	6.5	5.6
6.0	82.4	59.1	5.4	5.2
24.0	77.3	55.3	6.2	5.5

slightly less than in the 1962 trial, there was no evidence of any further control being attributable to an interaction between the fumigation and storage treatments.

H.3. The effect of the storage of fumigated barley grain for 10 months upon plant survival and loose smut infection.

3.1. Introduction.

It was expected that the several compounds used in the fumigations of infected Rika grain described in Section E.2, were sorbed and subsequently desorbed, by the grain, to varying extents, and very probably had different toxic effects, in view of the diversity of their physical and chemical properties. Accordingly, it was reasoned that the interaction effects between the storage and fumigation treatments, might vary with the fumigant. Therefore, a large part of the field trial described in Section E.2, was

repeated, but using only half the previous number of grains per plot, in an endeavour to demonstrate any gross effects of a 10 month period of storage.

In the storage trial, particular attention was paid to the level of infection in those treatments which had not resulted in a significant control of the disease in the earlier field test.

3.2. Material and methods.

Grain samples remaining from the 1962 field trial of Rika grain, treated with nine fumigants, were stored in perforated paper envelopes at 20°C. at a moisture content of about 12.5 per cent. for 44 weeks. From 18 of the fumigation treatments, 250 grains were sampled from both replicates, together with four, 250 grain lots of unfumigated grain, and field-sown in plots of 2 rows, 7 ft. long, arranged in one large randomized design. The trial was established in early May 1963, and the plants were dug up, assessed for loose smut infection and counted in August.

3.3. Results.

The numbers of survived and infected plants are recorded in Appendix Table A.6, together with the 1962 results, with the figures for the corresponding replicates falling on the same horizontal line. The individual values were calculated as percentages, transformed to angles, and the survival and smut infection figures were separately examined by the analysis of variance test for significant effects of the fumigation, storage and interaction effects (Appendix Tables A.25 and A.26). The treatment means with indications of the significance of their differences,

are presented in the corresponding Tables 25 and 26, in the text.

The percentage survival values in 1963 were appreciably higher than in 1962, which resulted from the superior growing conditions, and particularly from the reduced seriousness of infestations by the stem-boring larvae of insects, and of Erysiphe graminis. As a result, there was a significant (P = 0.01) interaction effect, with respect to which the storage treatment was shown to be highly significant (P = 0.01). Statistically significant (P = 0.05) increases of percentage survival were demonstrated in the following treatments: acetic acid (1.5 hr.); allyl alcohol (2.0 hr.); allyl chloride (3.0 hr.) and all three of the allyl isothiocyanate fumigations.

Turning to the loose smut infection results, and to the analysis of variance (Appendix Table A.26), one degree of freedom was subtracted from the error term only, to make allowance for the absence of any within-treatment variance in the 2.0 hr. fumigation with allyl alcohol (1963 results). The results of acetic acid (1.5 hr.) and allyl alcohol (6.0 hr.) fumigations were not included in this calculation because they were all zero.

There was a highly significant (P = 0.001) reduction of loose smut in the 1963 trial compared with that of 1962 due to the interaction of the fumigation and storage treatments. However, with respect to the interaction effect, neither of the factors could be shown to have a separate (and significant) influence upon the control of the disease.

Table 25. A comparison of percentage survival of plants from grain of Rika barley fumigated in 1962 and stored for 2 and 44 weeks.

Treatment: Fumigant	Fumigation Duration (Hours)	1962 Trial* 2 Weeks Storage Per Cent. Survival from 1000 Grains[a].		1963 Trial* 44 Weeks Storage Per Cent. Survival from 500 Grains[b].	
Check	0	69.4	bc	72.2	b
Acetic acid	0.5	58.9	cdefghi	63.1	bcdefg
	1.5	32.9	opqr	54.2	fghijk
Acrylonitrile	1.5	65.8	bcdef	58.4	cdefghi
Allyl alcohol	2.0	38.2	lmnop	59.0	cdefghi
	6.0	23.8	r	32.9	opqr
Allyl bromide	3.0	33.1	nopqr	44.2	klmno
Allyl chloride	3.0	55.6	fghijk	83.5	a
	6.0	43.0	klmno	48.8	hijklm
	12.0	24.8	qr	23.3	r
Allyl isothiocyanate	2.0	60.1	cdefgh	86.5	a
	8.0	46.6	hijklmn	65.4	bcdef
	36.0	39.6	lmnop	58.0	cdefghij
n-Butanethiol	2.0	70.3	b.d	70.1	b.de
	8.0	65.5	bcdef	56.8	efghijk
	36.0	59.3	cdefghi	49.8	ghijklm
Chloropicrin	3.0	42.9	klmno	51.6	ghijkl
	12.0	33.3	nopqr	45.8	ijklmno
	48.0	27.4	qr	37.4	mnopq

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

a: 2000 grains in unfumigated check.

b: 1000 grains in unfumigated check.

Table 26. A comparison of percentage loose smut infection of plants from grain of Rika barley fumigated in 1962 and stored for 2 and 44 weeks.

Treatment: Fumigant	Fumigation Duration (Hours)	1962 Trial* Percentage of Survived Plants Smutted	1963 Trial* Percentage of Survived Plants Smutted
Check	0	5.75 ab	3.90 abc
Acetic acid	0.5	2.90 abcdefghij	0.35 jk
	1.5	0.00 k	0.00 k
Acrylonitrile	1.5	5.42 ab.defg	1.52 c..fghijk
Allyl alcohol	2.0	0.53 ijk	0.00 k
	6.0	0.00 k	0.00 k
Allyl bromide	3.0	1.49 c..fghijk	1.70 cdefghij
Allyl chloride	3.0	4.46 ab.defgh	3.80 abcdefghi
	6.0	5.08 ab.defg	0.82 c....hijk
	12.0	2.73 abcdefghij	0.51 ijk
Allyl isothiocyanate	2.0	6.12 a..de	3.69 abcdefghi
	8.0	5.93 a..def	2.29 abcdefghij
	36.0	1.66 c.efghij	0.36 jk
n-Butanethiol	2.0	5.13 ab.defg	6.53 a
	8.0	6.86 a	5.30 ab.defg
	36.0	6.42 a..d	3.00 abcdefghij
Chloropicrin	3.0	5.36 ab.defg	3.48 abcdefghi
	12.0	3.08 abcdefghij	1.20 c...ghijk
	48.0	0.74 ijk	0.26 jk

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

Only one treatment showed a significant (P = 0.05) reduction in loose smut frequency as a result of the storage period, namely the 6 hr. fumigation with allyl chloride, with a control from 5.08 to 0.82 per cent. However, because of the large error variance, neither value was significantly different from that of the respective check.

Of the 12 treatments included in this trial in which loose smut was not significantly controlled in 1962, only two showed a significant control in 1963. These were acetic acid (0.5 hr.) and allyl chloride (12 hr.). The frequency of loose smut in both cases, in the 1963 trial, was not significantly different (P = 0.05) from that resulting from the 48 hr. fumigation with chloropicrin, which had been used as a criterion of the control of disease in the 1962 trial (Table 11, Section E.2). However, the plant survival in 1963 associated with this control was 63.1 per cent. for acetic acid, and 23.3 per cent. for allyl chloride compared with 37.4 per cent. for the chloropicrin treatment. These differences were significant (P = 0.05).

3.4. Summary of results.

The higher percentage plant survival in the 1963 than in the 1962 trial, was interpreted as a confirmation of the supposition, that the growing conditions of the late-sown 1962 trial were very unfavourable. Consequently the 1962 survival values represent an overestimate of the degree of phytotoxicity associated with the various fumigation treatments.

There was a significant reduction in the frequency of loose smut

in the 1963 trial as a whole, as compared with the 1962 trial, due to an interaction between the storage and fumigation treatments. Even so, of the 12 treatments which did not control the disease in 1962, only 2 showed a significant control in 1963.

The storage trial has confirmed the effectiveness of fumigations with the water-soluble acetic acid and allyl alcohol, and indeed has shown these treatments to be less phytotoxic than those with chloropicrin. Allyl isothiocyanate (36 hr.) also resulted in a significant control of loose smut in both 1962 and 1963, with reductions in plant survival comparable with those resulting from the acetic acid and allyl alcohol fumigations. The degree of reduction with these three compounds, in the 1963 trial, was between 9.1 and 18.0 per cent. compared with 34.8 per cent. resulting from a 48 hr. fumigation with chloropicrin.

H.4. The influence of 10 months storage of Fylgia II wheat grain, fumigated with methyl bromide and hydrogen cyanide, upon plant survival and loose smut infection.

4.1. Introduction.

This was a repeat field trial of the wheat grain fumigated with methyl bromide and hydrogen cyanide in 1962 (Section F.1). The earlier trial was sown in July 1962, whereas this one was sown in May 1963 with the object of investigating the effect of the intervening 10 month period of storage, upon plant survival and loose smut infection.

4.2. Materials and methods.

Grain samples remaining from the 1962 trial were stored at a moisture content of 12.5 per cent., in perforated paper envelopes at 20°C. for 44 weeks. From each replicate, 250 grains were sampled and field-sown in plots of 2 rows, 7 ft. long each, in a trial of randomized design. The trial was sown in May 1963 and the plants were dug up, classed and counted in August.

4.3. Results.

The numbers of survived and infected plants are recorded in Appendix Table A. 18, and the treatment means in both trials are expressed as percentages in Table 27. The percentage plant survival in both trials was examined by a single analysis of variance, and the percentage smut infection data were similarly treated (Appendix Tables A.27 and A.28).

The percentage plant survival in the 1963 trial was appreciably higher than in the 1962 trial, and, as in Section H.3, this has been interpreted as a demonstration that the conditions under which the 1962 trial developed were particularly adverse, and severely reduced the number of plants that survived to anthesis.

The interaction between the fumigation and storage treatments, upon plant survival, was not statistically significant ($P = 0.05$), and neither was the interaction with respect to percentage loose smut infection, significant ($P = 0.05$).

Neither the hydrogen cyanide nor the methyl bromide fumigations

Table 27. The influence of post-fumigation storage of Fylgia II grain, upon percentage plant survival and loose smut infection.

Treatment Fumigant	C.T.P. (mg.h./l.)	Per Cent. Survival* 2 Weeks Storage 1962 Trial	Per Cent. Survival* 44 Weeks Storage 1963 Trial	Per Cent. Smut* 2 Weeks Storage 1962 Trial	Per Cent. Smut* 44 Weeks Storage 1963 Trial
Check:	0	55.2 .bc..	85.9 a....	5.86 f	8.08 f
Methyl bromide					
	500	39.8 ...de	51.6 .bcd.	7.54 f	7.67 f
	1000	36.6 ....e	46.0 ..cde	8.37 f	3.82 f
Hydrogen cyanide					
	1000	63.1 .b...	83.5 a....	6.71 f	6.54 f
	2000	63.4 .b...	83.7 a....	6.94 f	6.94 f

* Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

resulted in a significant (P = 0.05) control of Ustilago tritici, despite reductions of plant survival of 34.3 - 39.9 per cent. in the 1963 trial. In view of this, and the fact that the higher concentration-time products severely reduced the percentage seedling emergence from the grain of three other varieties of wheat, in the 1962 trial, the possible development of a satisfactory treatment involving either of these fumigants, for controlling the loose smut fungus in wheat grain, appears remote.

I. OBSERVATIONS OF THE TYPES AND FREQUENCY OF ABNORMAL SEEDLINGS

1.1. Introduction.

Very few critical studies have been made on the morphological development of seedlings arising from fumigated seeds, although there are numerous reports of the effects of fumigation treatments upon seed germination.

The delay in the germination of fumigated seeds has been widely observed (Cobb, 1956, 1958; Lubatti & Blackith, 1957; Blackith and Lubatti, 1960) and has been shown to be greatest for oily seeds (Blackith & Lubatti, 1960). Germination was improved in the case of fumigated oily seeds, by several days airing prior to the test (Plaut, 1957; Lachover, et al., 1958). Airing periods of 5 - 12 days were also shown to increase the germination of cereal grains fumigated with hydrogen sulphide (Lobik, 1937).

Cobb (1958) has illustrated the irregular germination of sorghum grain fumigated with methyl bromide, and the weak appearance of seedlings. In an earlier publication (Cobb, 1956) it was noted that no diagnostic structural abnormalities in germinating seedlings could be found, except a slight discolouration in some samples. Discolouration of the radicles of methyl bromide fumigated groundnuts (Somade, 1955) and of peas and beans (Lubatti & Blackith, 1956b) have also been observed, and Lubatti & Blackith (1956b) have drawn attention to the regenerative properties of the root systems of such damaged seedlings.

As regards the latter development of seedlings, Lubatti & Blackith (1957) concluded from an experiment with maize that once the plant was

established, its previous treatment had little effect upon its subsequent development.

The hot water treatment of grain, also delays germination (Tapke, 1924; Batts, 1956), and by contrast with the experience with methyl bromide, it also results in a variety of deformed seedlings, examples of which, have been figured by Tapke (1924).

1.2. The effect of treatment of Arès grain of 14.0 per cent. moisture, with methyl bromide, methanol, chloropicrin and hot water, upon the rate of germination and seedling abnormality.

In order to investigate the influence of some of the treatments described in this study, upon seedling development, samples of Arès grain of 14.0 per cent. moisture were treated in 5 ways:-

1. Untreated check.
2. 24 hr. fumigation with methanol, at the saturation vapour concentration.
3. 48 hr. fumigation with chloropicrin at the saturation vapour concentration.
4. 30 min. hot water treatment at 51.5°C., following a presoak of 6 hours at 20°C., and a preheat of 2 min. at 49°C.
5. Methyl bromide fumigation of 20 hr. duration, C.T.P. 24,000 mg.h./l.

These treatments were chosen to reduce the percentage of normal seedlings in the 20°C. sand test from 95 per cent. in the check, to about 60 - 70 per cent. The rate of germination in the sand test was recorded for two weeks, at the end of which the seedlings were examined for morphological details.

The rate of development of normal seedlings in the 20°C. sand test was expressed as a percentage, relative to the number of normal seedlings recorded on the 14th day, and are presented in Table 28. There was a close agreement between percentage emergence and percentage normality in the unfumigated check, and in the lots fumigated with methanol and methyl bromide, but there was a difference of 13.4 and 19.4 per cent. in the hot water and chloropicrin treatments respectively. The rate of development of normal seedlings from grain fumigated with chloropicrin was comparable with the check, but with grain treated with methanol and methyl bromide there was evidence of a slight delay. However, delay in germination resulting from the hot water treatment was appreciably greater than that noticed with the fumigated grain.

The seedlings that developed in the sand test, were grown in darkness so as to encourage the maximum development of the etiolated structures. They were classed as normal or abnormal on the appearance of the shoot system only. The criteria used for judging an abnormal seedling were:-

1. coleoptile less than 2/3 the normal length.

2. coleoptile split more than 1 cm.

3. first vegetative leaf less than 2/3 the length of a normal one.

Seedlings classed as abnormal and normal were measured for coleoptile and first vegetative leaf length, the number of roots, and the percentage of seedlings having coleoptiles split for more than half their length. These summarized observations are presented in Table 29.

Table 28. The rate of development of normal seedlings in the 20°C. sand test.

Treatment	Per Cent. Relative Seedling Normality						Result on 14th Day from 300 Grains	
	Days from Commencement of Test							
	4	5	6	7	10	14	Per Cent. Normal	Per Cent. Emerg.
1. Check.	100	100.4	100.7	101.0	101.0	100	95.0	97.7
2. Methanol.	93.1	96.5	99.5	100.5	100.5	100	67.3	68.7
3. Chloropicrin	100.0	104.1	104.7	105.2	104.1	100	64.3	83.7
4. Hot water.	29.9	78.1	101.1	106.4	112.3	100	62.3	75.7
5. Methyl bromide	95.3	101.3	100.9	100.4	101.3	100	77.3	80.3

Table 29. The morphological details of normal and abnormal seedlings developed in the sand test from treated grain.

Treatment	Normal or Abnormal Seedlings	Number of Seedlings	No. Roots /Seedling	Coleoptile Length (cm.)	First Leaf Length (cm.)	Per Cent. ½-1 Split Coleoptiles
Unfumigated:	Normal	40	5.0	6.7	19.7	0
Fumigated:	Normal					
2. Methanol.		40	5.0	6.2	19.8	0
3. Chloropicrin.		40	approximately the same			
4. Hot water.		40	5.2	6.9	21.7	0
5. Methyl bromide.		27	5.2	6.4	21.5	0
	Abnormal					
2. Methanol.		3	4.0	3.7	10.8	0
3. Chloropicrin.		40	5.1	3.5	12.7	72.5
4. Hot water.		40	3.2	5.0	10.1	2.5
5. Methyl bromide.		3	5.0	3.0	13.5	0

The root and shoot development of normal seedlings from treated grain, was comparable with that of seedlings from untreated grain. By contrast, the abnormal seedlings showed two interesting features which have been repeatedly observed. The first was the considerable reduction in coleoptile length and also the high frequency of severely split coleoptiles, in seedlings developed from grain fumigated with chloropicrin. However, the chloropicrin treatment did not reduce the average number of viable seminal roots per seedling. The second feature concerned grain treated with hot water in which the frequency of viable seminal roots was markedly reduced, and in contrast to the chloropicrin effect, this was not associated with any pronounced abnormality of the coleoptile.

In considering the effects of these three fumigants upon grain of 14 per cent. moisture, the reduced length and splitting of the coleoptile (Plates 4 and 5) was diagnostic of the chloropicrin treatment. But when grain of about 18 per cent. moisture content was fumigated with the other two fumigants, comparable types of abnormality were observed. Indeed at the lower moistures, abnormal seedlings were infrequent, and most of the grains either developed normally, or showed no development and were considered inviable. The reasons for supposing inviability rather than induced dormancy, were based upon the fairly close agreement between percentage seedling normality in the sand test and seedling emergence in the field, in the methyl bromide and methanol trials (Tables 12 and 15).

The coleoptile serves to direct as well as to protect the more delicate vegetative leaves, during the early passage of the shoot through

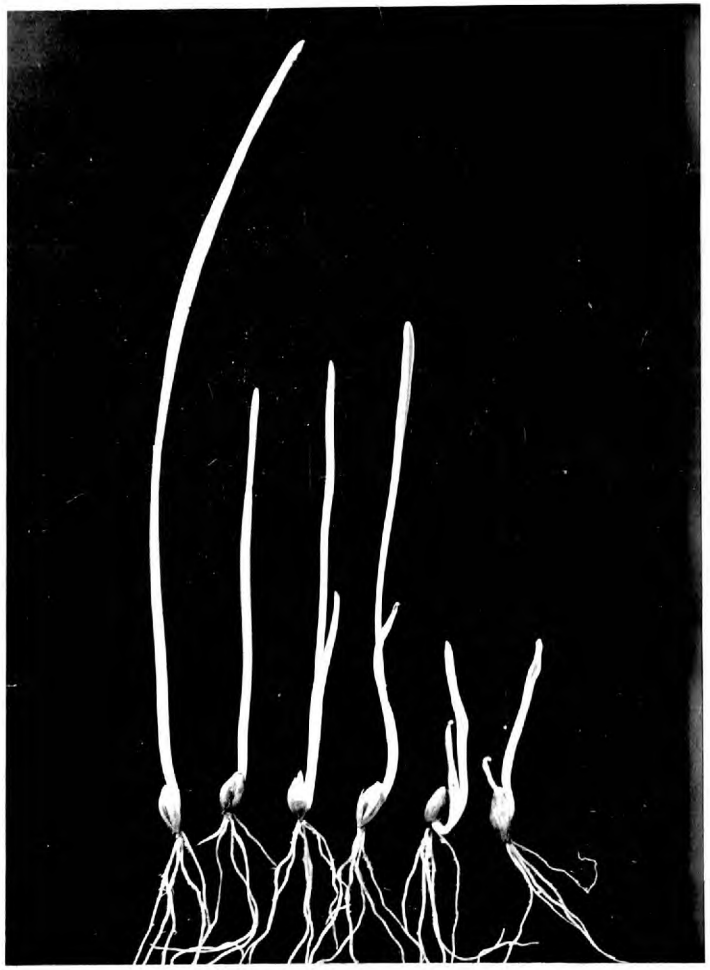


Plate 4. Examples of abnormal seedlings, developed in the sand test from grain fumigated with chloropicrin.

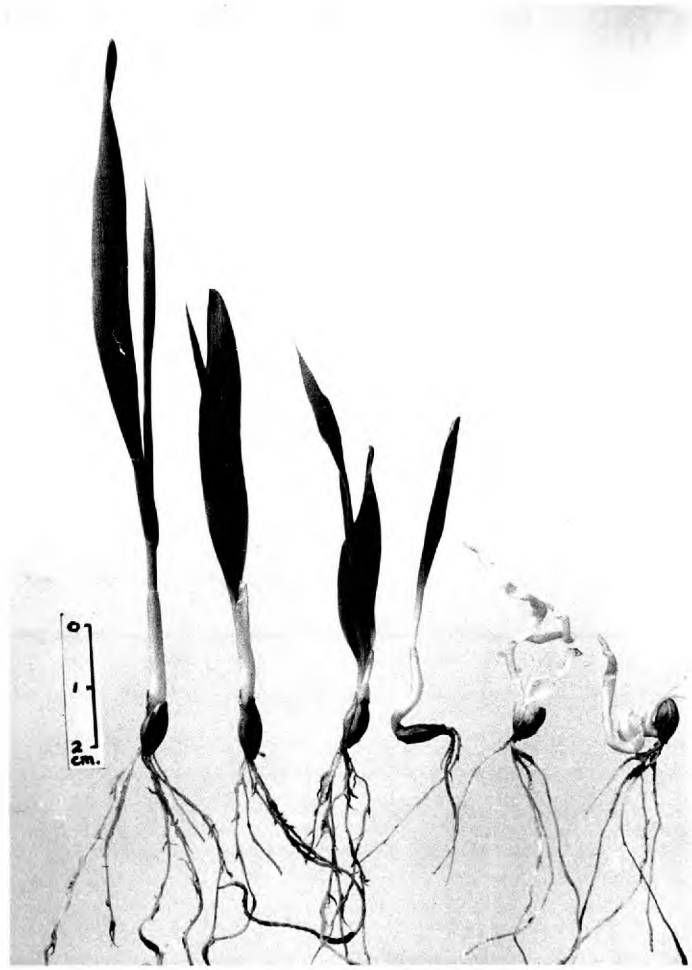


Plate 5. Examples of abnormal seedlings, developed in a glasshouse test from grain fumigated with chloropicrin.

the soil. However, when the coleoptile is damaged as a result of a fumigation treatment, the vegetative leaves cease to have any orientation, and often fail to emerge above the soil surface. The two etiolated and stunted seedlings on the right of Plate 5 are examples of this type, originating from grain fumigated for 48 hours with chloropicrin.

All of the seedlings shown in Plates 4 and 5 were selected from tests with grain fumigated with chloropicrin for 48 hours, and give an idea of the range of suceptibility of different grains within the same seed sample, to fumigant toxicity.

1.3. The frequency of abnormal and wilted seedlings in the field trial with fumigated Rika grain.

In the trial of Rika grain treated with several fumigants, (Section E.2) seedling emergence was recorded five weeks after the trial was sown, and at the same time the numbers of abnormal and wilted seedlings, and those that showed a combination of these conditions, were counted and separately recorded. These results were expressed as percentages of the numbers of emerged seedlings, and are presented in Appendix Table A.4. The details of the allyl alcohol fumigations are given in Table 30.

Since the percentage seedling emergence was appreciably reduced by the fumigation treatments, it was expected that the surviving seedlings would also have been weakened, and were thus more likely to be infected by soil-borne wilt pathogens. This was expected to be particularly true for the abnormally developed seedlings.

Table 30. The percentage of abnormal and/or wilted seedlings in the field trial of Rika grain fumigated with allyl alcohol.

Duration Fumigation (Hours)	Per Cent. Emerg. (1000 Grains)	Per Cent. Survival (1000 Grains)	Per Cent. Emerged Seedlings affected by		
			Abnormal Development	Wilt	Abn.Dev. + Wilt
0	87.3*	69.1*	2.8	3.7	0.0
2	47.8	38.2	4.6	2.3	0.0
6	29.9	24.0	8.4	1.0	0.0
12	7.4	4.9	20.3	0.0	0.0

* Based on 2000 grains.

The results do not agree with this hypothesis.

Field observations showed that wilted seedlings occurred in widely spaced groups of 1 - 12 seedlings (most of which were of normal development), which indicated that there were, in all probability, relatively few wilt pathogens in the soil.

Therefore, because of the supposed scarcity of wilt organisms in the soil, this particular hypothesis could not be reliably tested.

Evidence is advanced in Section F.2, that some abnormal seedlings, subsequently recover and develop to the stage of anthesis, quite normally.

J. THE INFLUENCE OF THE SIZE, AND DEGREE OF PHYSICAL DAMAGE OF GRAINS, UPON THEIR SUSCEPTIBILITY TO FUMIGANT INJURY, AND THE CONTROL OF LOOSE SMUT.

J.1. The relationship between grain size and loose smut infection.

1.1. Introduction.

Several workers have demonstrated for composite samples of infected barley grain, a higher frequency of infected grains amongst the light weight fractions, than amongst those composed of heavy grains. McFadden et al. (1960), showed that when a stock of Montcalm barley, with 12.5 per cent. of the embryos infected, was separated into 5 grades by sieving, the level of infection increased from 2.7 to 27.0 and 23.0 per cent., as the 1000 kernel weight was reduced from 46.0 to 23.2 g. The percentage infection in a growth test showed a close agreement with this trend. These findings were confirmed by Demirlicakmak and Kaufmann (1963) for several other 6-rowed varieties of barley, and it was further shown, for these varieties, that the lateral grains were lighter, and a higher percentage of them were infected, than were the central grains. Taylor and Harlan (1943) had also found that a higher percentage of infected ears developed from lateral than from central kernels, and offered an explanation for it in relation to the time that the floral parts open and the consequent likelihood of infection at anthesis.

McFadden et al. (1960) made some additional observations on the variety Rex, a 2-rowed barley, and showed that the relationship between

grain size and infection was similar to that in the 6-rowed varieties. More recently, Doling (1964) has shown this correlation to be true for the 2-rowed variety Rika.

In the studies described in the literature, the grain stock was usually divided into fractions by sieving, and the details of the sieve and/or the 1000 kernel weight of the resulting fractions, were given. The weighing of individual grains, in an attempt to improve the grading (with respect to the variation of individual kernel weight in the various fractions) does not appear to have been described.

This experiment was designed to investigate the possibility of effecting a superior separation of the infected from the uninfected grains, by segregating the heavy from the light grains on the basis of weight, rather than upon a correlated feature such as grain length or thickness, as is done when grain is sieved.

The degree of correlation between grain weight and the three dimensions, length, breadth and thickness, of grain of Rika barley, was examined by partial correlation.

1.2. Materials and methods.

From an untreated grain stock of Arès (a 6-rowed barley), 1200 grains were sampled and allowed to equilibrate with the relative humidity of the laboratory air, resulting in a moisture content of about 12.5 per cent. The grains were individually placed in small numbered holes, drilled in a block of wood, and separately weighed to the nearest 0.1 mg.

When the frequency distribution of grain weights was known, the 400 lightest kernels, weighing 39.2 mg. or less, were picked out and the first hundred in the numerical sequence was designated the first replicate. The second hundred was designated the second replicate, and so on for the third and fourth replicates. The 400 heaviest grains, with kernel weights of 47.6 mg. or more, were divided in the same manner, and so too were the grains in the intermediate category with grain weights of 39.3 - 47.5 mg.

A sample of 960 grains of Rika (a 2-rowed barley variety) was similarly divided according to weight, into three fractions consisting of four replicates, each of 80 grains, but with the modification that the intermediate category was further divided into medium-light and medium-heavy fractions, with replicates of only 40 grains. This permitted a grouping of the results into either two or three weight categories. The subsequent analysis was performed using only two categories, light and heavy grains, and this served as a preliminary enquiry for the experiment described in Section J.3.

The levels of embryo infection in the grain stocks were low (Table 4, Section D.1), and the numbers of infected embryos were expected to be small. For this reason, and from the fact that the number of treatments and the degree of replication were both small, these tests were expected to demonstrate only gross effects. The embryos were extracted from the grains using sodium hydroxide solution, and were examined for loose smut infection by the methods described in Section D.10.

A sample of 50 randomly selected grains of Rika barley were

equilibrated to about 12.5 per cent. moisture content, and weighed to the nearest 0.1 mg. The length (L), breadth (B) and thickness (T) of each grain was measured to the nearest 0.1 mm. with a calliper square.

1.3. Results.

The results of the examination of Arès barley embryos for the presence of Ustilago nuda, are shown in Table 31, and correspond to percentages.

Table 31. The frequency of infected embryos in light, medium and heavy fractions of Arès grain.

	Number Infected Embryos per 100 Grains		
Weight Grade:	Light	Medium	Heavy
Limits (mg.):	39.2 and less	39.3-47.5	47.6 and more
10^3 Kernel Wt. (g.):	33.97	43.12	53.55
Replicate 1.	8	10	9
2.	14	8	9
3.	9	9	6
4.	10	10	8
Per Cent. Embryo Infection (400 Grains)	10.3	9.3	8.0

The values were transformed to angles and examined by an analysis of variance (Appendix Table A.29) for any association between grain weight and infection. No significant (P = 0.05) effect was however evident, so that with this small sample, there was no difference between the frequency

of infected embryos in the light, medium and heavy grains.

With the examination of Rika grain, the results were more of the type expected (Table 32).

Table 32. The relation between grain weight and the frequency of embryo infection in Rika grain.

Weight Grade:	Number of Grains infected by Ustilago nuda			
	Light	Medium-Light	Medium-Heavy	Heavy
Limits (mg.):	37.8 and less	37.9-41.1	41.2-44.1	44.2 and more
10^3 Kernel Wt.(g.):	32.3	39.7	42.7	48.3
No.Grains/Replicate	80	40	40	80
Replicate 1.	11	1	1	3
2.	8	3	4	1
3.	8	4	4	1
4.	9	3	3	7
Total Wt. of 4 Replicates (g.)	10.3207	6.3440	6.8394	15.4472
10^3 Kernel Wt.(g.)	34.7		46.4	
Per Cent. Embryos Infected (480 Grains)	9.8		5.0	

When the results were condensed into two grain-weight categories, the light fraction with a 10^3 kernel weight of 34.7 g. and 9.8 per cent. of the embryos infected, contrasted with the heavy fraction having a 10^3 kernel weight of 46.4 g. and 5.0 per cent. of the embryos infected. An analysis of variance test (Appendix Table A.30) showed that the difference between the

frequencies of infected embryos in the two weight categories, was highly significant (P = 0.01).

Turning to the correlation studies, the values for the weight, length, breadth and thickness of 50 grains of Rika barley are presented in Appendix Table A.31. For a clarification of the terms "breadth" and "thickness", reference may be made to Plate 6. The distance 'c' in grain 3 has been termed "breadth" and is the distance between the "shoulders" of the grain (a term used in the milling trade). For all of the examined grains, the length was always the longest, and the thickness always the shortest of the three measurements.

The correlation coefficients between weight and each of the three dimensions of the barley grain, were calculated by the methods of the analysis of correlation and partial correlation. The accounts of these methods in the book by Bailey (1959), were found to be particularly helpful. The numerical suffixes used to denote the quantities being examined, are as follows:-

Quantity	Suffix	Letter in Plate 6
Weight W	1	-
Length L	2	a
Breadth B	3	c
Thickness T	4	b

The total correlation coefficients involving weight as one of the quantities, are of primary interest and have been evaluated as:-

weight - length	r_{12}	= 0.662
weight - breadth	r_{13}	= 0.944
weight - thickness	r_{14}	= 0.924

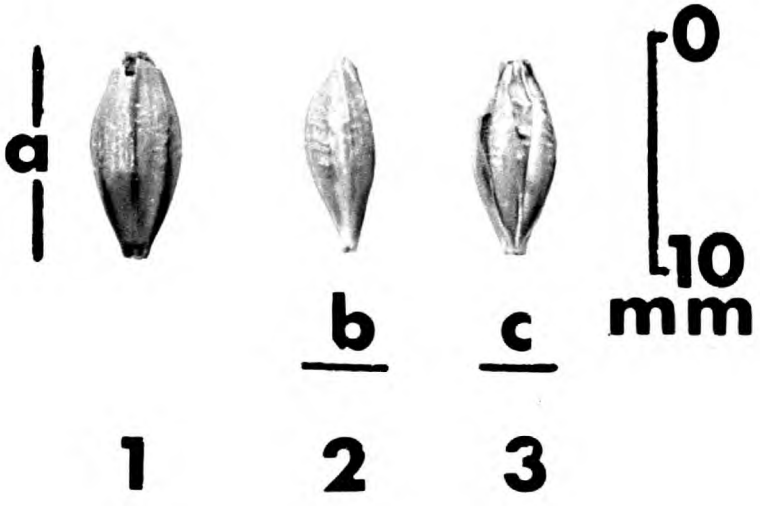


Plate 6. Details of the gross structure of hulled barley grains of variety Rika.

Meaning of letters: a - length of grain.
b - thickness of grain.
c - breadth of grain.

Grain 1. Embryo side of the grain covered by the lemma.

Grain 2. Side view; embryo is on the right hand side.

Grain 3. "Groove" side of the grain, protected by the palea, the margins of which are overlapped by the lemma.

The three remaining total coefficients were: $r_{23} = 0.526$, $r_{24} = 0.517$, $r_{34} = 0.894$ of which the high order of positive correlation between breadth and thickness (r_{34}) is of particular interest.

Using a z-transformation of r, the coefficients for weight - breadth (r_{13}) and weight - thickness (r_{14}), were shown to be highly significantly different ($P < 0.001$) from the correlation coefficient of weight - length (r_{12}), but not significantly ($P = 0.05$) different from one another. The correlation coefficient of weight - length (r_{12}) was shown to be highly significantly different from zero ($P < 0.001$).

In order to eliminate the influence of various other correlated factors, six partial correlation coefficients with one factor excluded were calculated and have the following values:-

$r_{12.3}$	0.588	$r_{13.4}$	0.689
$r_{12.4}$	0.563	$r_{24.3}$	0.123
$r_{14.3}$	0.541	$r_{23.4}$	0.167

These partial correlations were substituted in formulae of the type:

$$r_{12.34} = \frac{r_{12.4} - r_{13.4} r_{23.4}}{\sqrt{(1-r^2_{13.4})(1-r^2_{23.4})}}$$

in order to obtain partial correlations with both of the remaining factors eliminated (which are indicated as the two numerical suffixes after the stop). The values for three such partial coefficients which are of interest are:-

weight - length:	$r_{12.34}$	= 0.624
weight - breadth:	$r_{13.24}$	= 0.730
weight - thickness:	$r_{14.23}$	= 0.584

Once again using a z-transformation, these three partial coefficients were shown to be not significantly ($P = 0.05$) different from one another, but they were all highly significantly different from zero ($P < 0.001$).

In conclusion it may be noted that on the basis of the total coefficients, there was a significantly ($P < 0.001$) closer correlation between breadth or thickness of the grain, with its weight, than there was between length and weight. This information could be (and, in agricultural practice, almost certainly is) used to advantage, for the rapid grading of grains by means of sieves, if the mesh were chosen to select grains for their breadth and thickness, rather than for their length.

J.2. The relation between the physical damage of barley grains and their susceptibility to fumigant injury.

2.1. Introduction.

The embryos of barley are protected by the compressed remains of the testa, the pericarp and, in the case of hulled grains, by the lemma which is usually firmly attached to the surface of the pericarp. The scutellar surface of the embryo abuts indirectly upon the endosperm. Removal or splitting of the lemma and chipping of the pericarp allows materials applied to the surface of the grain, to pass more rapidly into the tissues of the endosperm and embryo. Jones et al.(1955) found that serious mercury poisoning of wheat embryos was attributable to small breaks in the tissues overlying the seminal roots. The hot water treatment was also more injurious to physically damaged wheat (Tapke, 1924) and barley (Russell, 1950) grains.

It was therefore of interest to compare the effect of fumigation upon the viability of damaged and undamaged barley grains.

2.2. Materials and methods.

Samples of 450 grains of variety Rika, were randomly selected from two grain lots fumigated in 1962, one with allyl alcohol for 2 hours and the other with acetic acid for 1.5 hours. These were compared with a similar number of untreated grains, initially from the same unfumigated stock. The fumigated grain remained from lots from which grain had been sown in the 1962 and 1963 Rika trials (Sections E.2 and H.3), and in which

a satisfactory control of the loose smut fungus had been demonstrated.

Grains were individually weighed, and, in the manner described in Section J.1, each sample was ultimately divided into a light and a heavy, grain-weight fraction, composed of three replicates each of 75 grains.

The grains in each replicate were examined and classed according to the extent of any physical damage.

Four grades of physical injury were distinguished:

1. Intact. Lemma entire and uncracked.

2a. Lemma damaged, with more than $\frac{1}{4}$ of the embryo region of the pericarp visible.

2b. Lemma split in the region of the embryo.

2c. Lemma loose, but intact, over the region of the embryo.

Examples of the types of physical damage are shown in Plate 7. The grain at the bottom right hand side of each block of four grains is orientated to give a side view. The breaking and splitting of the lemma of the types 2a and 2b, probably results from mechanical damage during harvesting, while the loose lemma is thought, by the author, to result from the grain ripening under damp climatic conditions. The latter association was observed for a late-harvested lot of Arès barley grown in 1962.

The number of grains in each of these four categories of physical injury were recorded, and the grains were then grouped as either intact or damaged and sown on tension plates (Section D.11). The germination test continued for 7 days but many of the normal and abnormal seedlings were

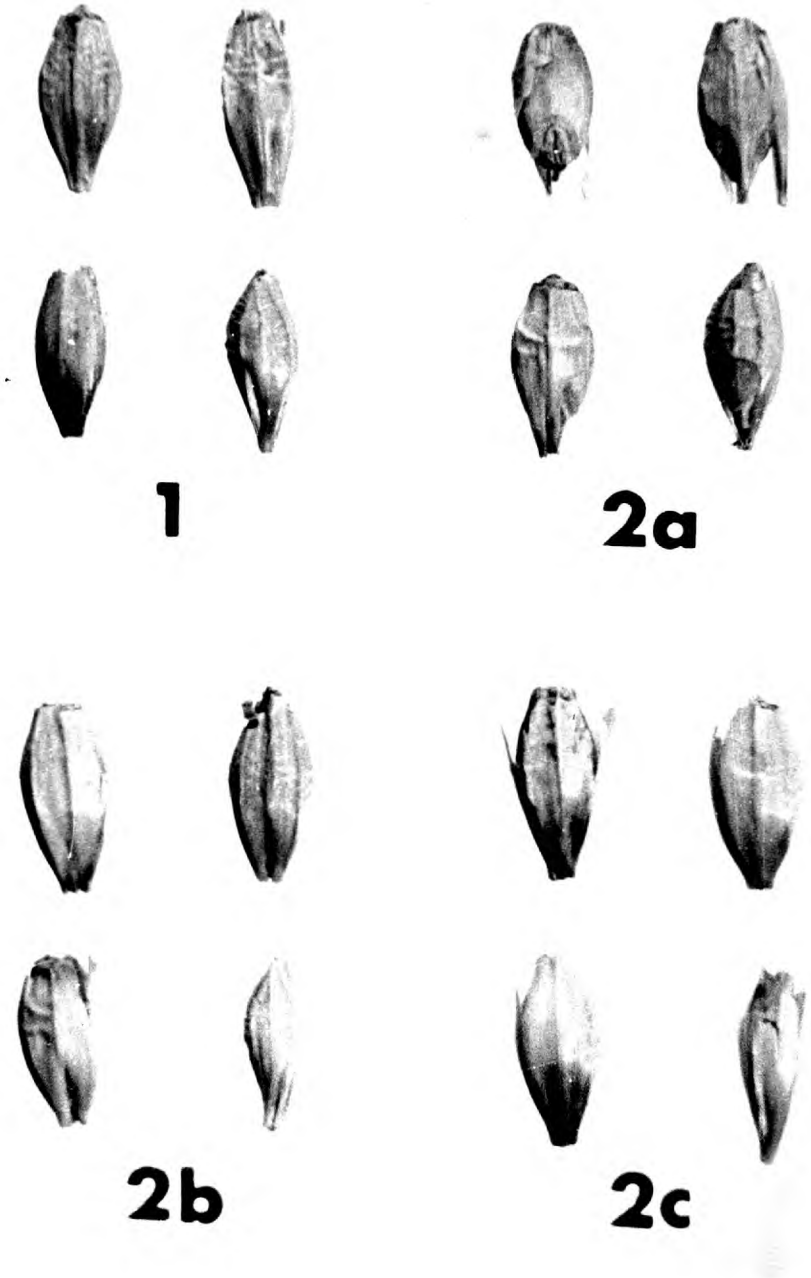


Plate 7. Various types of physical damage exhibited by barley grains. (For a description refer to the text).

removed before its conclusion, and were dried and preserved for a subsequent examination of loose smut infection (Section J.3).

2.3. Results.

The numbers of grains showing each type of physical injury, were arranged according to grain weight, and the results are presented in Table 33, as percentages of 225 grains.

Table 33. The frequency of the types of physical injury in fractions of light and heavy grains of Rika barley.

Grain Weight Category / Fumigation Treatment	Per Cent. Frequency of 225 Grains			
	Lemma Intact 1	Lemma Partially Missing 2a	Lemma Cracked 2b	Lemma Loose 2c
Light Grains				
Check	66.2	8.0	18.7	7.1
Allyl alcohol	64.9	8.9	19.1	7.1
Acetic acid	62.2	9.3	20.0	8.4
Average	64.4	8.7	19.3	7.6
Heavy Grains				
Check	69.3	9.8	16.4	4.4
Allyl alcohol	60.4	8.9	28.4	2.2
Acetic acid	65.8	7.6	24.4	2.2
Average	65.2	8.7	23.1	3.0

Approximately 35 per cent. of the grains showed some pronounced type of physical injury, and it is perhaps surprising that this frequency was similar for both the light and heavy grains. The most frequent type of injury was that in which the lemma was split over the region of the

embryo (type 2b). The observation that there were similar proportions of damaged grains in both the fumigated and unfumigated lots of grain, indicates that the fumigation treatments had little or no effect upon the further splitting or loosening of the lemma.

The results of the germination tests have been summed across the effect of grain weight, which will be considered in Section J.3, and are presented in Table 34 as percentages of relative frequency.

Table 34. The development of seedlings from intact and damaged grains, fumigated with allyl alcohol and acetic acid.

Treatment	No. Seedlings Examined	Per Cent. Relative Frequency of Seedlings		
		Inviable	Abnormal	Normal
Intact grains				
Check	305	13.1	29.8	57.0 (1.00)
Allyl alcohol	282	19.5	34.4	46.1 (0.81)
Acetic acid	288	19.1	36.8	44.1 (0.77)
Damaged grains				
Check.	145	24.1	44.8	31.0 (1.00)
Allyl alcohol	168	28.0	46.4	25.6 (0.83)
Acetic acid	162	43.8	34.0	22.2 (0.72)

Fewer normal seedlings and a correspondingly larger proportion of abnormal seedlings developed from lots of physically damaged grains than from undamaged grains. The fumigation of grain with allyl alcohol or acetic acid reduced the proportion of normal seedlings, but surprisingly, the reduction for both the intact and the damaged grains was comparable. The relative reduction (figures given in parenthesis in Table 34) in the

number of normal seedlings from intact grain was 0.81 and 0.77, as against 0.83 and 0.72 for damaged grain.

This experiment has demonstrated two unexpected features. Firstly, the similarity of the number and types of damaged grains in the fractions of light and heavy grains, and secondly a comparable degree of tolerance of both intact and damaged grains to fumigation treatments.

J.3. The influence of the size of grains upon their susceptibility to fumigant injury, and its relation to the control of loose smut.

3.1. Introduction.

In Section H.2, the concept was introduced that loose smut might be controlled by the elimination of infected grains, which have been shown to lose their viability more quickly than uninfected ones (Russell, 1961). Evidence was presented in Section J.1 that infected grains were more numerous in the light grain fractions than in the heavy grain fractions, and this was shown to be true of the barley variety Rika.

This experiment was designed to investigate the effect of a 2hr. fumigation with allyl alcohol upon the viability of light and heavy grains of Rika barley, and to establish whether or not the infected grains were more liable to be injured or killed by fumigation than the uninfected grains.

3.2. Materials and methods.

The details of this experiment are the same as those given in Section J.2, because the germination results and the individual seedlings were utilised in this enquiry. The inviable grains, abnormal and normal seedlings were dried and stored separately, and subsequently examined for evidence of infection of the scutellum by Ustilago nuda. The seedlings that developed from grain fumigated with acetic acid were not examined for loose smut infection. The method of separating the seedling from the remainder of the grain, is given in Section D.10.2.

3.3. Results.

The results of the germination test were summed across the effect of physical damage of the grain upon viability, and are presented in Table 35 as percentages of 225 grains. In the two lots of fumigated grain, the proportion of normal seedlings that developed from light grains was less than that from heavy grains. When these values were expressed relative to the proportions in the check (figures given in parenthesis), it was evident that the fumigation treatments had reduced the proportions of normal seedlings in both grain weight categories alike, i.e. 0.84 and 0.76 with allyl alcohol and 0.72 and 0.76 with the acetic acid treatment. There was therefore, no evidence that the light grains were more susceptible to a loss of viability as a result of fumigation.

The numbers of seedlings or inviable grains with scutella infected by Ustilago nuda, are shown in Table 36. Within each replicate, these numbers (obtained by summing across the influences of physical injury and

Table 35. The percentage frequency of the types of seedling development from fumigated grain of Rika barley.

Fumigation Treatment	Grain Weight Category	Percentage Frequency of Seedlings from 225 Grains (in Classes 1 and 2) or from 450 Grains (in Class 3 - Bulk)		
		Inviable	Abnormal	Normal
Check	1.Heavy	11.1	28.4	60.4 (1.00)
	2.Light	22.2	40.9	36.9 (1.00)
	3.Bulk	16.7	34.7	48.7 (1.00)
Allyl alcohol	1.Heavy	12.9	41.3	45.8 (0.76)
	2.Light	32.4	36.4	31.1 (0.84)
	3.Bulk	22.7	38.9	38.4 (0.79)
Acetic acid	1.Heavy	18.3	36.0	45.8 (0.76)
	2.Light	37.8	35.6	26.7 (0.72)
	3.Bulk	28.0	35.8	36.2 (0.74)

Table 36. The effect of a 2 hr. fumigation of grain with allyl alcohol upon the numbers and relative frequencies of inviable grains and seedlings with scutella infected by U. nuda.

Treatment	Types of Seedling Development					
	Inviable		Abnormal		Normal	
Replicate	Number	Per Cent. Freq.	Number	Per Cent. Freq.	Number	Per Cent. Freq.
Check 1.	4	44.4	3	33.3	2	22.2
2.	3	27.3	6	54.5	2	18.2
3.	8	50.0	8	50.0	0	0.0
Calculated* mean		40.3		45.8		9.3
Significance**		ab..		ab..		..c.
Allyl alcohol 1.	5	71.4	2	28.6	0	0.0
2.	7	58.3	5	41.7	0	0.0
3.	4	66.7	2	33.3	0	0.0
Calculated* mean		65.6		34.4		0.0
Significance**		a...		.b..		...d

* from the angular transformations of the individual values.
** Treatment means with one or more letters in common are not significantly different, but those with no letters in common are significantly different (P = 0.05).

weight of grains), were expressed as percentage frequencies. These values were transformed to angles and the results, as a whole, were examined by an analysis of variance (Appendix Table A.32). Reference to the entries in Table 35 for the frequency of seedling types that developed from the bulked samples (class 3), shows that the 2 hr. allyl alcohol fumigation did not reduce the proportion of normal seedlings to less than 79 per cent. of the check. So that, as an approximation, the proportions of types of seedlings that developed from both fumigated and unfumigated grain, may be considered comparable. Any reduction in the proportion of infected scutella in the normal or abnormal seedling categories therefore reflected the differential elimination of infected grains. The absence of this demonstration might indicate that the control of U. nuda had been effected by killing the pathogen in grains from which seedlings subsequently developed.

The numbers of infected scutella were very small, because the level of infection in the initial grain stock was only about 7.4 per cent. (Table 4, column 6), and the investigation was accordingly regarded as preliminary.

The analysis (Appendix Table A.32) revealed a significant ($P = 0.05$) interaction between the fumigation treatment and the frequency of seedlings with infected scutella, and reference to Table 36 shows that this was in the direction of a reduction in the number of infected grains in the normal seedling category, and a corresponding increase in the inviable grain category.

The results are only of a preliminary nature, but are taken to endorse the hypothesis advanced in Section H.2.1, that infected grains are weaker, and possibly, are more readily killed by a fumigation treatment, than uninfected grains.

Infected grains also tend to be lighter and smaller than uninfected grains, but their greater susceptibility to fumigant injury could not be wholely explained on this basis, because light grains were shown (Table 35) to exhibit a similar degree of tolerance to fumigation treatment as the heavier grains.

K. DISCUSSION

In the experimental part of this study an endeavour has been made to control the embryo-infecting loose smut fungi of barley and wheat (*Ustilago nuda* and *U. tritici*, respectively) by fumigation of the grain. At the present time these two fungi are fairly adequately controlled in Great Britain, and the number of infected grain stocks in circulation would appear to be appreciably fewer than six or nine years ago (Batts, 1956; Marshall, 1959). This reduction has resulted from the use of therapeutic measures such as the hot water treatment, but more particularly from the introduction of more resistant varieties, and the establishment, under the Comprehensive Certification Scheme and the Cereal Field Approval Scheme, of low tolerance levels of these diseases in crops intended for seed multiplication.

The frequency of infection in either the standing crop or the resulting grain, that is tolerated in Certified barley stocks, varies from 0.01 - 0.1 per cent. by field assessment in England, Wales, Holland and Norway, to 0.1 - 1.0 per cent. by grain examination in Scotland, and by field assessment in Denmark and the United States (standards of the International Crop Improvement Association) (Kelly, 1964). The tolerated frequency of infected embryos in Canadian Number 1 seed barley has recently been raised from 0.5 to 4.0 per cent. (Russell, 1962).

The levels of infection tolerated in a standing crop intended for seed production are of interest because they indicate the level to which a

therapeutic method must reduce the infection for the treatment to be commercially acceptable. However, in this study, because the number of grains used in each treatment replicate was generally only 500 - 600 (2000 grains in the Donaria trial, Section E.5), and the treatments were replicated only two or three times, levels of infection lower than about 0.5 - 0.2 per cent. could not be investigated, with the exception of the trial of Donaria grain (Section E.5). Nevertheless, a very effective control of barley loose smut was achieved in several of the trials (Table 38).

Despite the failure to demonstrate the presence of Ustilago nigra in the several field trials, there remained the possibility that the observed reduction of loose smut infection was due not to the control of U. nuda, but to that of U. nigra, situated within the superficial tissues of the grain. It was explained in Section D.3 that, because of its more exposed situation, U. nigra was expected to be more easily controlled than U. nuda, so that a proportion of the initial reduction in the frequency of loose smut might, therefore, be wrongly attributed to the control of U. nuda. As a measure of this possible error, the Percentage Probability of U. nigra (Table 4, Column 9) was expressed as a percentage of the average (in the trials where grain fumigated at several moisture contents was evaluated) least significant (P = 0.05) reduction of loose smut infection (Table 37).

In the right hand column of Table 37, the low percentage error for Arès barley of 6.1 per cent. is very satisfactory. However, the other values indicate that for the remaining three barley varieties there must remain a large element of uncertainty as to the proportions of the reduction

Table 37. A measure of the proportion of the control of loose smut, possibly attributable to Ustilago nigra.

Barley Variety and Trial (Section of Thesis)	1. Least Significant Reduction in Per Cent. Loose Smut	2. Per Cent. Probability of U. nigra (Table 4, Col. 9)	Possible Error $\frac{1}{2}$ x 100 Per Cent.
Arès - 1962 (E.1)	2.64	0.16	6.1
Rika - 1962 (E.2)	3.56	1.19	33.4
Edda - 1963 (E.4)	2.21	0.93	42.1
Donaria - 1963 (E.5)	0.47	0.09	19.0

in loose smut that was due to these two loose smut fungi.

There was, however, a strong possibility that most of the observed reduction in loose smut was due to the elimination of Ustilago nuda, because in all four varieties the frequency of embryos infected by Ustilago nuda was high, and similar to the frequency of infected plants (Table 4, columns 6 and 8).

To facilitate a comparison of the effectiveness of the various fumigation treatments, a criterion was established with respect to which, each treatment was assessed as either satisfactory or otherwise. The chosen criterion was the reduction in the frequency of smut-infected plants to less than 0.5 per cent., with a reduction in plant survival of not more than 20 per cent. (relative to the number of grains from which these plants developed). On this basis the treatments presented in Table 38, were judged satisfactory.

Table 38. The details of fumigation treatments at 20°C. of barley grain of 14.0 - 15.4 per cent. moisture content, which satisfactorily controlled the loose smut infection of plants to 0.5 per cent., with reductions in plant survival of less than 20 per cent.

Fumigant	Reference to Table No.in Text	Fumigation Duration (Hours)	C.T.P. Product (mM.h./l.)	Reduction in Plant Survival Per Cent.
Methanol	15	6.0	31.2	8.6
Methanol	14	2.5	13.0	15.0
Allyl alcohol	25 & 26	2.0	2.0	13.2
Allyl isothiocyanate	25 & 26	36.0	7.1	14.2
Methyl bromide	13	80.0	252.8	16.2
Acetic acid	25 & 26	1.5	1.0	18.0

In terms of the duration of the fumigation treatments, the three most effective fumigants were methanol, acetic acid and allyl alcohol, all three of which are water-soluble, which indicates that this property may influence the rate of penetration of these compounds into the grain. Allyl isothiocyanate and methyl bromide are only slightly soluble in water (Table 5, column 10), nevertheless, methyl bromide normally penetrates tissues very rapidly.

Methyl bromide and methanol were however not so effective in controlling Ustilago tritici (Sections F.1 and F.2), in wheat grain, where the frequency of loose smutted plants was not reduced below 1.0 per cent., despite reductions in plant survival of about 40 per cent.

Two compounds, acrylonitrile and diethyl ether gave a degree of control comparable to allyl alcohol (2.0 hr.) in the 1962 trial (Section E.2), but were not assessed the following year after 10 months storage. Allyl bromide gave a satisfactory control of the disease in the 1962 trial (Section E.2), but not in the 1963 trial, in which the plant survival did not increase significantly under the improved growing conditions.

In addition to allyl bromide, the other fumigants, evaluated in this study, which were found unsatisfactory, included allyl chloride (Sections E.2 and H.3), chloropicrin (Sections E.1, E.2, E.4, E.5, H.2 and H.3) and n-butanethiol (Sections E.2 and H.3) tested upon barley grain, and hydrogen cyanide (Sections F.1 and H.4) upon wheat grain.

Only a slight improvement in the control of loose smut in fumigated grain, was obtained by storing it at the relatively low moisture content of 12.5 per cent., for 10 - 12 months, and this effect was small compared with the degree of control effected by the initial, fumigation treatment.

In order to decide whether or not these fumigation treatments were superior to the existing hot water, anaerobic or cold water soak methods, used for controlling these internal pathogens of wheat and barley, a comparison was made between them, on the basis of the reduction in plant survival associated with a satisfactory control of the disease.

A precise comparison is vitiated by the considerable variability of the tolerance of different grain samples to similar treatments. High quality samples, ripened under dry conditions, and showing a minimum of mechanical damage, exhibit only slight reductions in survival, associated

with satisfactory disease control, but the converse is true of cereal grains of poor quality.

Schafer and Hansing (1950) demonstrated reductions in the germination of barley of 7 - 33 per cent., following a 5 hr. presoak, and a 13 min. treatment at 126°F. The long presoak stage was necessitated by the failure of a 3 hr. presoak to effect a satisfactory control of the pathogen. Russell (1950, 1962) has also demonstrated reductions of 35 and 10 - 25 per cent. in the germination of hot-water treated barley. Large varietal differences were apparent in the evaluation, by Batts (1956), of the hot-water method for barley grain. The reduction in laboratory germination of grain, which when sown in the field showed an absence of loose smut infection, was as little as 0 - 7 per cent. with variety Carlsberg, but as much as 40 per cent. in Herta.

In most of these investigations, the assessment of germination reductions was made on the basis of a laboratory test, usually involving sand. In the author's experience with hot-water treated wheat (Section F.2), the emergence of seedlings in soil may be as much as 20 per cent. lower than in the sand test, so that many of the published results may well be underestimates of the severity of the reduction of seedling emergence under field conditions. In agricultural practice this reduction in viability is minimized in economic importance, by sowing the grain at one and a half times, or twice the normal rate.

The anaerobic method of treating moistened barley grain has been shown to be very much less phytotoxic than the hot-water treatment. Tandon

and Hansing (1957) showed that whereas the reduction in seedling emergence in the field resulting from a water-soak treatment was 1 - 12 per cent., the reduction resulting from an anaerobic treatment, which controlled the disease with equal effectiveness, was only 0 - 4 per cent. Vary favourable results were obtained by Weihing and Daly (1957) and also by Zemánek and Bartoš (1962), who showed a comparable high order of tolerance for 20 Czechoslovak barley varieties.

The treatment of barley and wheat grain by chemicals has most usually been effected by dissolving the compound in water, and then exposing the grain to a long soak for 24 - 72 hours. The reductions in germination resulting from such treatments are somewhat intermediate between those associated with the anaerobic and the hot-water methods. A wide variety of compounds both inorganic and organic (Hanna & Popp, 1933; Tyner, 1951, 1953; Zemánek & Bartoš, 1956; Russell & Chinn, 1958; Bartoš et al., 1962) have been evaluated but offer relatively little advantage over the confirmed effectiveness of the anaerobic method, originally described by Hebert (1955).

Considerable interest in the group of quinone compounds, was aroused following the demonstration by Tyner (1951, 1953) of the effectiveness of tetrachloro-p-benzoquinone (Spergon, chloranil), (c.f. the works by Bartoš and by Zemánek). However, Batts (1956) found this compound to be too phytotoxic for treating English-grown barley grain.

These various methods all involve water, and during the treatment the grain may absorb at least 10 - 15 per cent. of its weight of water, which must be subsequently removed by drying if the grain is to be stored

for more than one day before sowing so as to avoid deterioration. For this reason and for several others, such as the need for a precise control of the temperature in the hot water treatment, an alternative method for controlling these pathogens would be a welcome development. The fumigation of grain for the control of such internal pathogens, is therefore seen as a convenient method which has yielded some very promising results.

One of the chief disadvantages of the fumigation method, is the considerable difference in the susceptibility of grains of different moisture contents to fumigation injury and loss of viability. This is not an important variable in the hot-water treatment, but in this study (Section E.3) an increase in the susceptibility of barley to methyl bromide toxicity was shown to be as great as 100 times, for grain of 22 per cent. moisture content compared with that of 14 per cent. moisture.

In practice, differences of moisture content may not be as great as this, since barley grain at more than 18 per cent., and wheat grain at more than 14 - 15 per cent. moisture content, show signs of deterioration when stored at 20°C. and are accordingly dried to lower values.

The observation that most control measures result in some loss of viability has been widely interpreted as an indication that the treatment must be sufficiently phytotoxic to reduce the viability of the infected grains, which are thought to be weaker than the uninfected ones. In confirmation of this view, Weihing and Daly (1957) produced evidence which indicated that the control of loose smut by the anaerobic method resulted from the loss of viability by the infected grains. Wells and Platt (1949)

also showed that infected grains were of a lower viability, and Russell (1961) has confirmed this in studies of the effect of aging upon infected grain.

The results obtained in Section J.3, though only preliminary, indicate that the control of loose smut by the fumigation of the grain with allyl alcohol can also be explained by the greater susceptibility of the infected grain. Although the infected grains are on average smaller than the uninfected ones, this difference in size (or weight) did not have a direct influence upon their greater susceptibility to fumigant injury.

The use of methanol also resulted in some loss of viability, but in contrast to allyl alcohol or acetic acid, methanol is not very fungitoxic (Baechler, 1939), nor is it very phytotoxic (Siegel & Halpern, 1964). It is therefore suggested, by the author, that an alternative mode of action may be involved. Since, the control of loose smut in Donaria barley (Section E.5), was shown to be associated with a sorption of methanol in excess of 1 g./100 g. of grain (Section G), it is possible that the methanol within the grain may influence the availability and toxicity of fungicidal materials already present in the grain. The possibility that quinones may be involved is perhaps worth considering further, since Mace and Hebert (1963) found that during an anaerobic or a long water-soak treatment of barley and wheat grain, the quantity of 2-methoxy-hydroquinone-glucoside within the grain, was reduced by 22 - 28 per cent. with a resultant appearance of the very fungicidal, 2-methoxy-hydroquinone.

This interpretation could be tested by applying these materials

to the grains, not in aqueous solutions because this has been tried and found phytotoxic, but in organic solvents. This, and comparable approaches using other organic solvents, may well enable a number of deep-seated pathogens to be reached and killed without increasing the degree of hydration of the host tissue.

The work of Milborrow (1963) is of interest in this connection since cereal grains were shown to have a high tolerance towards long soak treatments in acetone, which completely penetrated the grain. This particular compound, has been used by Wagner (1958) and by Lewis (1962), but was not found to be effective in controlling loose smut of barley. Lewis (1962) also investigated the possibility of using an aqueous solution of acetone as a solvent for an organomercury compound (ethyl mercury-p-toluene sulfonanilide), which was applied to barley seed with a mercury-damage inhibitor, as a slurry. The results of these experiments were, unfortunately, not very encouraging. It may be mentioned that sym-dichlorotetrafluoroacetone has recently been shown by Allen and Freiberg (1964) to be effective as a rust chemotherapeutant, although it shows only poor direct action upon the uredospores when tested *in vitro*. This compound might be very suitable for treating infected cereal grains to control the loose smut pathogens.

In view of the large quantity of methanol sorbed by Donaria grain (Section G) during the fumigation exposure, it is thought that a more convenient and precise method of application would be the treatment of the grain with the compound in the liquid form. This conclusion therefore

endorses the techniques used by Wagner (1961b) in which methanol formulations were sprayed on to the grain, which was then stored under airtight conditions, in some instances in polythene bags, for periods ranging up to one day, depending upon the quantity of material used, its composition and the temperature of the grain (Wagner, 1963a). Recently, Wagner has increased the volume of the treatment fluid from 2 - 3 l. to 5 l. applied to 100 kg. of grain. The phytotoxicity resulting from the use of methanol was alleviated by dissolving ammonium nitrate in the slightly aqueous solution of methanol (Wagner, 1960b).

The more volatile compounds which would appear to be of value for controlling superficial, seed-borne pathogens are ethylene oxide, nitrogen peroxide and possibly methyl bromide. Further development of seed treatment methods with these fumigants appears to be warranted. It is possible that an improved control of these microorganisms might be obtained by the use of combinations of fumigants (Richardson & Monro, 1962), but little work has been performed along these lines.

Many seed-borne pathogens, including viruses, are very inadequately controlled, at the present time, for technical reasons such as the small treatment latitude between adequate fungitoxicity and an inadmissible degree of phytotoxicity, and partly for economic or administrative reasons. It is felt that several new approaches to this subject could be made along the lines suggested in this discussion.

L. SUMMARY.

1. A survey of the literature relating to the use of volatile compounds to control seed-borne fungi (Section C.2), revealed that hydrogen sulphide and chlorine, which had been evaluated chiefly for the control of pathogens situated superficially on the seed, in general, proved to be very slow acting, and phytotoxic, respectively, in treatments which were satisfactorily fungicidal. Nitrogen peroxide, ethylene oxide, formaldehyde, allyl alcohol, allyl bromide and chloropicrin, appeared to give an improved control of superficial pathogens with a reduced risk of a loss of seed viability. Fumigants evaluated for their efficacy in controlling pathogenic fungi situated within the seed, include hydrogen sulphide, ethylene oxide, chloropicrin and volatile formulations of acetone and methanol. Of these five compounds, chloropicrin and methanol were sufficiently fungicidal and relatively non-phytotoxic to warrant their further assessment.

2. Four barley varieties of different types and provenance and with differing proportions of embryos infected by Ustilago nuda, were used in variously designed experiments involving eleven fumigants. Using as a criterion, the reduction in the frequency of plants infected by loose smut (predominately Ustilago nuda) to less than 0.5 per cent. with an associated reduction in plant survival of not more than 20 per cent. (relative to the number of grains sown), the following compounds were found to give a satisfactory degree of control:

Fumigant	Per Cent. Reduction in Plant Survival	Effect Demonstrated in Thesis Section:
Methanol	8.6 (& 15.0)	E.5 (& E.4)
Acetic acid	18.0	H.3
Allyl alcohol	13.2	H.3
Allyl isothiocyanate	14.2	H.3
Methyl bromide	16.2	E.3

Acrylonitrile and diethyl ether were as effective as allyl alcohol in one experiment (Section E.2), which warrants their further evaluation. In these experiments, allyl bromide (c.f. Section H.3), allyl chloride, chloropicrin (c.f. Section H.3 and Sections E.1 and E.5), and n-butanethiol were less effective and failed to satisfy the criterion.

3. Infected grain of one variety of wheat, Fylgia II, was fumigated with methanol, methyl bromide and hydrogen cyanide, but the frequency of plants infected by Ustilago tritici, was not reduced below 1.0 per cent., by treatments which reduced plant survival by 40 per cent. The treatment of grain at 34°C. for 2 weeks with a dust application of the slightly volatile, fungicidal compound (2:4:5-trichlorophenoxythio) trichloromethane, failed to effect an appreciable control. The hot water treatment satisfactorily controlled the pathogen, but the treatment was severely phytotoxic, due probably, to the high temperature of the pre-soak stage (Sections F.1, F.2 and H.4).

4. Laboratory and field tests confirmed that fumigation treatments

more readily injured grain of a high than of a low moisture content. In one experiment (Section E.3) with methyl bromide, fumigation treatments, of grain of 14.1, 17.9 and 22.0 per cent. moisture content, which reduced plant survival to 60 per cent., were equivalent to concentration-time products, expressed approximately, in the ratio 100:10:1. Methanol fumigations of a given duration, were also appreciably more phytotoxic to grain of a high, than of a low, moisture content (Section E.5). Ustilago nuda was most easily controlled by methyl bromide in grain of 17.9 per cent. (Section E.3) and by methanol in grain of 14.0 per cent. moisture content (Section E.5). These effects were significant ($P = 0.05$), but the degree of improvement appeared to be slight.

5. The storage of fumigated grain with a moisture content of about 12.5 per cent., at 20°C. for 10 - 12 months (Section H.), did not result in any marked improvement in the control of the loose smut pathogens of either barley or wheat, when compared with that observed, when the grain was field-sown immediately after the fumigation treatment.

Small reductions in plant survival as a result of the storage treatment were anticipated, but could not be demonstrated in the field because the growing conditions of the repeat trials were superior, and resulted in an increase in plant survival (Sections H.3 and H.4).

6. The agreement between the percentage of normal seedlings in the laboratory sand test and the percentage emergence of seedlings in the field was generally good (Sections E.1, E.3 and E.5). Accordingly, the laboratory tests were widely employed in preliminary phytotoxicity studies, prior to

the final fumigation treatments reported in the thesis.

7. Abnormal seedlings more frequently developed from grain fumigated with methanol and methyl bromide at moisture contents of 18 and 22 per cent. than at 10 and 14 per cent. By contrast, the fumigation of grain of 14 per cent. moisture content with chloropicrin, resulted in a high proportion of abnormal seedlings, with shortened and split coleoptiles. This combination of features was therefore diagnostic for grain of 14 per cent. moisture content fumigated with chloropicrin. Types of seedling abnormality similar to this, did result, however, from the fumigation of grain at moisture contents of 18 per cent. or higher, with methanol, methyl bromide and chloropicrin (Section I).

The abnormal seedlings that developed from fumigated grain, exhibited severe deformity of the coleoptile and vegetative leaves, but root development was usually normal. This was in contrast to the effect of the hot water treatment which markedly reduced the development of seminal roots and vegetative leaves, but had only a slight damaging effect upon the coleoptile. These observations were confined to one variety of barley and may not have a general validity.

Delays in germination due to fumigation treatments were noticed, but were shown to be small compared with the effect of the hot water treatment (Section I). In general, abnormal seedlings emerged later than normal seedlings in the sand test.

In a glasshouse experiment, a proportion of the abnormal seedlings were shown to recover from the effects of the treatment, and to develop

apparently normally, to anthesis (Section F.2).

No persistent plant abnormalities attributable to the fumigation treatments were noticed in the several field trials, nor was there any evidence of chlorophyll mutations etc., although these were scarcely expected.

8. Observations with a quantity of grain of Rika barley revealed that similar proportions of light and heavy grains exhibited similar types of physical damage, principally to the part of the lemma overlying the embryo. The damaged grains were not found to be more susceptible to fumigant injury than the undamaged ones (Section J.2).

9. In Rika though not in Arès barley, the infected grains were shown to be more numerous in the light-weight grain fraction, than in the heavy weight fraction. The light grains were, however, shown to have a similar tolerance of fumigation treatments as the heavy grains (Section J.1).

10. The control of loose smut by a 2 hr. fumigation of Rika grain with allyl alcohol was shown to be accounted for by the greater loss of viability by the infected rather than the uninfected grains (Section J.3).

11. An examination of the relation between the weight and the length, breadth and thickness of Rika grains, demonstrated that on the basis of the total correlations, breadth and thickness were significantly more closely correlated with weight than was the length of the grain. However, when the various partial correlation effects were taken into account, the partial coefficients of weight with respect to each of the three dimensions, were not significantly different from one another, but they were all significantly different from zero (Section J.1).

12. Observations of the sorption of methanol by grain of barley varieties Donaria and Arès, showed that the sorption of quantities greater than 1 g./100 g. of grain were associated with an effective reduction in loose smut infection. The rate of sorption by Arès grain was greater by a factor of 1.5 - 2, and with Donaria grain the rate of sorption of methanol was about 20 times more rapid in grain of 18.0 per cent. than that of 10.2 per cent. moisture content (Section G).

13. There is ample scope for the development of new techniques for the control of seed-borne pathogens and two approaches are thought to be worthy of immediate attention.

Firstly, as regards the control of superficial pathogens, fumigation techniques which might have a more specialized use than the methods involving dusts or small volumes of concentrated solutions, chiefly of organomercury compounds, which are in current use, might be developed using ethylene oxide, nitrogen peroxide or possibly methyl bromide. Other more fungicidal yet less phytotoxic fumigants may well exist, and their further evaluation is required.

The use of combinations of fumigants might result in an improved kill of superficial pathogens, but this approach appears to have received very little attention.

In the second approach, different methods are suggested for the control of deep-seated infections of seeds. Reasoning from the demonstrated effectiveness of the water-soluble compounds, acetic acid, allyl alcohol and particularly of methanol, and from the fact that large quantities of methanol

need to be sorbed by the grain to effect this degree of control, it is proposed that these or similar, water-soluble compounds such as alcohols, should be added to the grain in liquid form, in a manner comparable with that developed by Wagner in Germany (considered in Section C.2).

The volatility of these water-soluble compounds is thought to be an important factor influencing the rate of penetration of the compound into the seed.

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N. APPENDIX I.

In the appendix several symbols have been used in the analysis of variance tables, which represent the following quantities.

S.S.	Sums of squares.
D.F.	Degrees of freedom.
M.S.	Mean square.
F.	Variance ratio.
*	Significant difference, with a fiducial probability P = 0.05 or 5 per cent.
**	Significant difference - P = 0.01 or 1 per cent.
***	Significant difference - P = 0.001 or 0.1 per cent.
n.s.	No significant difference - P = 0.05 or 5 per cent.

Table A.5 has been omitted.

Table A.1. The influence of moisture content of Arès barley grain, and the duration of fumigation with saturation concentration of chloropicrin, at 20°C., upon emergence, survival and the frequency of loose smut.

Per cent. Moisture Content	Duration of Fumigations (hrs.)	20°C. Sand Test No. Seedlings from 200 Grains		Field Test No. Plants from 600 Grains		
		Emerged	Normal	Emerged	Survived	Smutted
10.0	0	199	198	526	488	41
		198	190	526	530	29
		195	194	506	532	32
	1.5	190	189	529	484	37
		199	194	516	508	32
		194	191	498	514	35
	6.0	189	181	477	468	28
		190	187	514	499	38
		192	188	452	489	28
	24.0	171	165	487	478	23
		171	168	473	483	35
		178	173	483	467	32
13.9	0	197	192	534	545	39
		194	193	510	523	38
		198	197	521	507	25
	1.5	195	191	501	470	36
		193	193	492	493	30
		195	193	523	505	43
	6.0	168	167	495	491	25
		185	182	485	489	31
		175	170	501	504	24
	24.0	171	150	476	474	32
		171	160	464	457	21
		177	167	468	460	33

Table A.1 continued.

Per cent. Moisture Content	Duration of Fumigations (hrs.)	20°C. Sand Test No. Seedlings from 200 Grains		Field Test No. Plants from 600 Grains		
		Emerged	Normal	Emerged	Survived	Smutted
17.9	0	194	194	542	508	34
		195	195	549	526	31
		196	196	530	525	31
	1.5	181	180	491	448	41
		182	178	513	469	35
		181	177	492	489	24
	6.0	178	175	482	439	22
		172	170	481	472	26
		170	167	489	493	29
	24.0	173	111	461	417	15
		175	149	455	450	20
		163	110	467	437	18
21.5	0	196	196	540	454	30
		196	196	532	503	33
		197	196	549	526	40
	1.5	185	149	400	404	11
		174	134	427	399	13
		178	137	409	392	14
	6.0	158	47	233	202	1
		139	57	233	221	2
		156	52	247	212	6
	24.0	0	0	0	0	0
		39	0	35	32	1
		21	0	14	15	0

Table A.2. Analysis of variance table of percentage loose smut (in angles) in the 1962 trial of Arès grain fumigated with chloropicrin (derived from Table A.1).

Source of variation	S.S.	D.F.	M.S.	F.
Treatments:				
a Moisture	283.609	3	94.536	a/c 5.59 *
b Duration	116.964	3	38.988	b/c 2.31 n.s.
c Interaction	152.112	9	16.901	c/d 4.04 **
d Error	133.897	32	4.184	a/d 22.59 ***
Total	686.582	47		b/d 9.32 ***

Least significant difference (percentage smut)

L.S.D. between two treatment means each of three items:

for P = 0.05, $L.S.D._{3:3}$ = 3.41 angles.

Table A.3. Analysis of variance table of percentage survival (in angles) in the 1962 trial of Arès grain fumigated with chloropicrin, (derived from Table A.1).

Source of variation	S.S.	D.F.	M.S.	F.
Treatments:				
a Moisture	5,015.57	3	1,671.86	a/c 4.40 *
b Duration	2,655.34	3	885.11	b/c 2.33 n.s.
c Interaction	3,419.36	9	379.93	c/d 49.16 ***
d Error	247.34	32	7.729	a/d 216.3 ***
Total	11,337.61	47		b/d 114.5 ***

Least significant difference (percentage survival)

L.S.D. between two treatment means each of three items:

for P = 0.05, $L.S.D._{3:3}$ = 4.64 angles.

Table A.4. The effect of fumigation upon the frequency of emergence, abnormal development and wilt of seedlings, and of their survival to anthesis in barley variety Rika (1962 trial).

Fumigant	Duration of Fumigation (hrs.)	Per cent. Emergence from 1000 Grains	Per cent. Survival from 1000 Grains	Per cent. of Emerged Seedlings affected by		
				Abnormal Development	Wilt	Abn.Dev. + Wilt
Unfumigated Check (2000 Grains)	0	87.3	69.1	2.8	3.7	0.0
Chloropicrin	3	46.3	42.9	1.7	2.2	0.0
	12	35.1	33.3	1.1	0.3	0.3
	48	31.1	27.4	1.6	2.9	0.0
Allyl Alcohol	2	47.8	38.2	4.6	2.3	0.0
	6	29.9	24.0	8.4	1.0	0.0
	12	7.4	4.9	20.3	0.0	0.0
Allyl Bromide	3	44.7	33.1	3.4	6.0	0.0
	12	43.1	33.3	3.0	5.6	0.0
	48	25.4	20.8	3.9	4.7	0.0
Allyl Chloride	3	70.1	55.6	3.3	4.9	0.3
	6	52.1	43.0	3.8	3.1	0.2
	12	32.8	25.0	6.4	9.2	0.0
Allyl Isothiocyanate	0.5	79.8	70.2	1.8	5.1	0.1
	2	68.5	60.1	4.1	4.2	0.0
	8	50.8	46.6	3.0	0.4	0.0
	36	45.9	39.6	4.8	5.2	0.0
Acrylonitrile	1.5	80.1	65.8	3.9	2.9	0.0
	6	35.5	28.4	5.4	4.2	0.0
	24	0.9	0.7	33.3	0.0	0.0

Table A.4 continued

Fumigant	Duration of Fumigation (hrs.)	Per cent. Emergence from 1000 Grains	Per cent. Survival from 1000 Grains	Per cent. of Emerged Seedlings affected by		
				Abnormal Development	Wilt	Abn.Dev. + Wilt
Acetic Acid						
	0.5	76.1	58.9	3.8	4.7	0.0
	1.5	45.2	32.9	2.7	5.5	0.2
	4.5	0.0	0.0	0.0	0.0	0.0
Diethyl Ether						
	24	50.8	46.7	2.2	2.4	0.0
	96	36.1	32.8	1.1	2.5	0.0
n-Butanethiol						
	0.5	67.1	64.4	0.8	0.6	0.0
	2	81.0	70.3	2.6	6.7	0.0
	8	79.3	65.5	2.3	7.8	0.0
	36	65.1	59.3	4.6	1.7	0.0

Table A.6. The influence of fumigation, and of a 10 month post-fumigation storage period, upon the number of plants that survived to anthesis, and the number of these that were infected with loose smut (U. nuda) in the barley variety Rika (1962, 1963 trials).

Fumigant	Duration of Fumigation (hrs.)	1962: Grain stored 2 weeks		1963: Grain stored 44 weeks	
		No. Plants from 500 Grains		No. Plants from 250 Grains	
		Survived	Smutted	Survived	Smutted
Unfumigated (check)		289	22	167	10
		409	21	197	10
		348	21	168	5
		335	15	188	4
Chloropicrin	3.0	215	11	126	4
		214	12	132	5
	12.0	153	3	127	6
		180	8	102	0
	48.0	152	1	90	0
		122	1	97	1
Allyl bromide	3.0	177	3	111	1
		154	2	110	3
	12.0	168	0	-	-
		165	1	-	-
	48.0	100	0	-	-
		108	0	-	-

Table A.6 continued

Fumigant	Duration of Fumigation (hrs.)	1962: Grain stored 2 weeks		1963: Grain stored 44 weeks	
		No. Plants from 500 Grains		No. Plants from 250 Grains	
		Survived	Smutted	Survived	Smutted
Acrylonitrile	1.5	346	13	150	9
		312	23	142	0
	6.0	150	0	-	-
		134	1	-	-
	24.0	5	0	-	-
		2	0	-	-
Allyl chloride	3.0	237	10	215	6
		319	15	202	10
	6.0	214	5	131	1
		216	19	113	1
	12.0	97	6	67	0
		153	1	50	1
Allyl alcohol	2.0	165	1	145	0
		217	1	150	0
	6.0	149	0	94	0
		91	0	71	0
	12.0	26	1	-	-
		23	0	-	-
Acetic acid	0.5	279	9	171	0
		310	8	144	2
	1.5	163	0	144	0
		166	0	127	0
	4.5	0	0	-	-
		0	0	-	-

Table A.6 continued

Fumigant	Duration of Fumigation (hrs.)	1962: Grain stored 2 weeks		1963: Grain stored 44 weeks	
		No. Plants from 500 Grains		No. Plants from 250 Grains	
		Survived	Smutted	Survived	Smutted
Diethyl ether	24.0	223	18	-	-
		244	10	-	-
	96.0	173	0	-	-
		155	0	-	-
Allyl isothiocyanate	0.5	362	21	-	-
		340	21	-	-
	2.0	304	16	205	10
		297	21	226	6
	8.0	236	17	125	4
		230	11	198	3
	36.0	176	2	152	0
		220	5	138	2
n-Butanethiol	0.5	340	22	-	-
		304	13	-	-
	2.0	337	20	188	13
		366	16	162	10
	8.0	320	21	130	9
		335	24	154	6
	36.0	301	19	126	6
		292	19	123	2

Table A.7. Analysis of variance table of percentage smut (in angles) in the 1962 trial of fumigated grain, of the variety Rika (derived from Table A.6).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	813.615	22	36.983	4.08 ***
Error	226.638	25	9.066	
Total	1040.253	47		

Least significant difference (percentage smut, 1962).

$L.S.D._{4:2}$ between two treatments of 4 and 2 items respectively:

for P=0.05, $L.S.D._{4:2}$ = 5.37 angles

$L.S.D._{2:2}$ between treatments of 2 items each:

for P=0.05, $L.S.D._{2:2}$ = 6.20 angles

Table A.8. Analysis of variance table of percentage survival (in angles) of plants in the 1962 trial of fumigated grain of the variety Rika (derived from Table A.6).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	9,466.17	27	350.60	32.8 ***
Error	320.41	30	10.68	
Total	9,786.58	57		

Least significant difference (percentage survival, 1962).

$L.S.D._{4:2}$ between two treatments of 4 and 2 items respectively:

for P=0.05, $L.S.D._{4:2}$ = 5.78 angles

$L.S.D._{2:2}$ between treatments of 2 items each:

for P=0.05, $L.S.D._{2:2}$ = 6.67 angles

Table A.9. Details of laboratory and field tests with grain of varieties Rika and Arès, fumigated at three moisture contents with methyl bromide.

Per cent. Moisture Content	C.T.P. (mgh/l)	20°C.Sand Test(3wks) 1964 (100 Grains)			Field Emergence Arès		Field Results Arès, 1964.	
		Rika	Arès		1963 (600 Grains)	1964 (500 Grains)	No.Plants from 500 Grains	
		Norm.	Emerg.	Norm.			Survived	Smutted
14.1	0	80	98	96	557	452	438	20
		73	99	98	536	429	414	22
		71	99	98	528	459	423	24
	2,300	63	93	92	467	407	387	25
		74	90	87	420	406	363	19
		60	88	86	482	391	344	18
	12,000	58	82	81	500	398	375	9
		60	79	79	466	396	365	9
		61	87	86	447	366	321	5
	18,000	65	88	86	416	393	-	-
		66	80	79	474	366	-	-
		68	83	80	459	382	-	-
	24,000 (20h.)	58	85	84	457	327	290	6
		60	84	81	449	375	339	3
		60	82	82	466	383	359	4
	24,000 (40h.)	59	78	75	478	366	357	2
		52	82	80	464	388	377	2
		60	90	85	483	375	331	6
	24,000 (80h.)	61	81	80	478	394	381	2
		63	80	77	435	373	329	0
		48	78	78	506	361	322	0
17.9	0	56	98	97	556	404	365	21
		51	98	98	561	452	419	27
		54	97	97	513	449	396	19

Table A.9 continued

Per cent. Moisture Content	C.T.P. (mgh/l)	20°C.Sand Test(3wks) 1964 (100 Grains)			Field Emergence Arès		Field Results Arès, 1964.	
		Rika	Arès		1963 (600 Grains)	1964 (500 Grains)	No.Plants from 500 Grains	
		Norm.	Emerg.	Norm.			Survived	Smutted
17.9	2,300	32	83	70	443	335	327	0
		39	76	60	392	310	262	1
		36	73	63	391	300	262	1
	6,500	9	34	19	186	129	120	0
		6	35	26	200	130	118	0
		10	35	16	192	155	146	0
	8,900	7	36	25	161	113	-	-
		13	36	19	186	127	-	-
		14	41	23	162	120	-	-
	12,000	9	23	12	123	81	-	-
		6	23	14	117	61	-	-
		10	35	22	109	75	-	-
22.0	0	32	90	90	497	392	353	9
		34	93	92	498	419	383	8
		43	91	89	502	388	358	10
	175	15	85	84	473	401	380	5
		3	84	81	429	386	376	6
		8	92	92	509	402	372	3
	275	1	85	66	453	365	318	7
		0	85	60	492	362	332	4
		0	72	18	422	223	198	1
	325	0	71	27	434	283	245	0
		0	65	14	342	196	176	1
		0	59	6	264	109	92	1
	375	0	38	3	113	43	-	-
		0	23	0	141	25	-	-
		0	25	2	150	54	-	-

Table A.10. Analysis of variance table of percentage loose smut (in angles) in the 1964 trial of grain of variety Arès fumigated with methyl bromide (derived from Table A.9).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	603.6513	11	54.877	16.93 ***
Blocks	0.1528	2	0.076	0.02 n.s.
Error	71.2944	22	3.241	
Total	675.0985	35		

Least significant difference (percentage smut)

L.S.D. between two treatment means each of 3 items:

for P=0.05, $L.S.D._{3:3}$ = 3.05 angles.

Table A. 11. Analysis of variance table of percentage plant survival (in angles) in the 1964 trial of grain of variety Arès fumigated with methyl bromide (derived from Table A.9).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	3,940.17	12	328.348	17.97 ***
Blocks	110.50	2	55.250	3.02 n.s.
Error	438.54	24	18.273	
Total	4,489.21	38		

Least significant difference (percentage survival)

L.S.D. between two treatment means each of 3 items

for P=0.05, $L.S.D._{3:3}$ = 7.20 angles.

Table A.12. The results of laboratory and field tests of grain of variety Edda II, fumigated with methanol and chloropicrin.

Duration of Fumigation (hrs.)	20°C. Sand Test (2 wks) No. Seedlings from 100 Grains		Field Test No. Plants from 600 Grains	
	Emerged	Normal	Survived	Smutted
0 (Check)	81	77	321	20
	85	78	359	15
	92	85	346	22
Methanol				
1.25	75	69	279	3
	80	67	262	3
	82	74	257	2
2.5	67	62	250	0
	68	62	293	2
	65	59	213	0
Chloropicrin				
3.0	63	61	303	12
	63	61	316	10
	58	54	298	7
24.0	59	52	277	7
	60	56	293	14
	52	44	299	17
46.0	50	45	300	10
	58	53	301	8
	61	58	283	8

Table A.13. Analysis of variance table of percentage loose smut (in angles) in the 1963 trial of grain of variety Edda II fumigated with methanol and chloropicrin (derived from Table A.12).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	291.8762	5	58.375	19.19 ***
Error	36.5073	12	3.042	
Total	328.3835	17		

Least significant difference (percentage smut)

L.S.D. between two treatment means of 3 items each:

for P=0.05, $L.S.D._{3:3}$ = 3.10 angles.

Table A. 14. Analysis of variance table of percentage plant survival (in angles), in the 1963 trial of grain of variety Edda II fumigated with methanol and chloropicrin (derived from Table A. 12).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	138.08	5	27.616	7.36 **
Error	45.04	12	3.753	
Total	183.12	17		

Least significant difference (percentage survival)

L.S.D. between two treatment means of 3 items each:

for P= 0.05, $L.S.D._{3:3}$ = 3.45 angles.

Table A.15. The results of laboratory and field tests with grain of variety Donaria, fumigated with methanol and chloropicrin.

Per cent. Moisture Content	Fumigation Duration (hrs.)	20°C.Sand Test(2 wks) No. Seedlings from 100 Grains		Field Results		
				No. Seedlings from 500 Grains	No. Plants from 2000 Grains	
		Emerged	Normal	Emerged	Survived	Smutted
Methanol						
10.2	0	97	95	417	1735	12
		99	97	433	1634	21
		99	97	480	1698	19
	18.0	81	81	383	1462	1
		83	82	360	1390	3
		89	88	391	1480	3
	48.0	68	67	322	1260	0
		73	71	307	1229	0
		71	69	360	1241	0
14.0	0	99	98	441	1613	17
		99	97	455	1826	24
		100	95	465	1832	16
	6.0	91	88	427	1622	2
		96	94	405	1607	0
		84	81	408	1549	1
	14.0	77	76	343	1375	0
		75	75	346	1400	0
		80	79	360	1386	0
	21.5	50	49	270	-	-
		55	53	304	-	-
		62	58	251	-	-
	25.0	54	52	264	-	-
		60	59	232	-	-
		55	52	247	-	-

Table A. 15 continued.

Per cent. Moisture Content	Fumigation Duration (hrs.)	20°C. Sand Test (2wks) No. Seedlings from 100 Grains		Field Results		
		Emerged	Normal	No. Seedlings from 500 Grains Emerged	No. Plants from 2000 Grains Survived	Smutted
18.0	0	95	88	409	1577	14
		95	91	409	1627	18
		95	89	434	1684	20
	0.25	96	90	396	1599	14
		96	91	419	1606	20
		94	83	416	1582	14
	0.5	82	50	332	1316	4
		81	57	332	1226	6
		88	64	364	1373	2
Chloropicrin						
14.0	0	as above		as above		
	7.5	79	77	337	1248	9
		78	74	358	1337	13
		80	73	366	1397	23
	36.0	73	68	326	-	-
		75	67	334	-	-
		78	69	300	-	-
	54.0	79	62	312	1156	10
		79	59	285	1113	19
		73	61	308	1164	11

Table A.16. Analysis of variance table of percentage loose smut (in angles) in the 1963 trial of grain of variety Donaria, fumigated with methanol and chloropicrin (derived from Table A. 15).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	90.4142	8	11.302	14.38 ***
Error	14.1501	18	0.786	
Total	104.5643	26		

Least significant difference (percentage smut)

L.S.D. between two treatment means each of 3 items:

for P=0.05, $L.S.D._{3:3}$ = 1.52 angles

Table A.17. Analysis of variance table of percentage plant survival (in angles) in the 1963 trial of grain of variety Donaria, fumigated with methanol and chloropicrin (derived from Table A.15).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	1337.38	10	133.74	28.93 ***
Error	101.71	22	4.62	
Total	1439.09	32		

Least significant difference (percentage survival)

L.S.D. between two treatment means each of 3 items:

for P=0.05, $L.S.D._{3:3}$ = 3.64 angles

Table A.18. The detailed results of field trials with grain of Fylgia II wheat, fumigated at a moisture content of 14.0 per cent., with methyl bromide and hydrogen cyanide, and stored for 2 weeks (1962 trial) and 44 weeks (1963 trial).

Treatment		1962 Trial Grain stored 2 Weeks No. Plants from 500 Grains			1963 Trial Grain stored 44 Weeks No. Plants from 250 Grains	
Fumigant	C.T.P. (mg.h./l.)	Emerged	Survived	Smutted	Survived	Smutted
Unfumigated check	0	331	309	17	207	22
		316	242	15	222	13
Methyl bromide	500	216	197	10	134	12
		214	201	21	124	8
	1000	191	184	19	117	3
		195	182	12	113	6
Hydrogen cyanide	1000	328	286	21	221	21
		362	344	21	195	8
	2000	392	325	29	204	14
		340	309	16	214	15

Table A.19. Analysis of variance table of percentage loose smut in plants developed from grain of Fylgia II wheat, fumigated with methyl bromide and hydrogen cyanide (derived from Table A.18).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatment	8.9043	4	2.226	0.32 n.s.
Error	34.8657	5	6.973	
Total	43.7700	9		

Table A.20. Analysis of variance table of percentage survival of plants from grain of Fylgia II wheat fumigated with methyl bromide and hydrogen cyanide (derived from Table A.18).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatment	435.03	4	108.76	9.75 *
Error	55.78	5	11.16	
Total	490.81	9		

Least significant difference (percentage survival).

L.S.D. between two treatment means of two items each:

for P = 0.05, $L.S.D._{2:2}$ = 8.58 angles.

Table A.21. The results of laboratory and glasshouse trials of treated grain of Fylgia II wheat.

1. Treatment	2. 20°C. Sand Test No. Seedlings from 100 Grains	3.	4.	5.	6. Glasshouse Trial No. Plants from 300 Grains	7. No. Survived Plants
	Emerged	Normal	Emerged	Normal	Survived	Smutted
No treatment - check	97	94	258	250	249	19
	98	97	271	265	271	30
	97	96	265	261	263	24
Hot water: 53°Cx5 min.	95	89	214	192	200	0
	97	92	206	184	197	0
	92	88	221	195	204	0
53°Cx8 min.	93	78	162	132	156	0
	98	88	172	156	162	0
	91	80	181	164	174	0
Methanol fumigation: 1.0 hr.	80	64	163	157	163	7
	81	69	165	150	161	15
	79	65	177	163	174	11
3.0 hr.	57	55	126	109	125	2
	54	51	147	144	146	3
	59	58	146	140	146	1
Talc check: 2g./kg. grain. 34°Cx2 weeks	96	92	227	209	225	14
	95	92	226	216	223	13
	95	91	247	234	247	19
'TCPTCM', 1g.+2g.talc/ kg.grain. 34°Cx1 week	-	-	204	196	203	16
	-	-	203	192	203	20
	-	-	194	186	192	14
'TCPTCM', 1g.+2g.talc/ kg.grain. 34°Cx2 weeks	90	80	177	161	173	11
	90	82	161	152	159	9
	91	82	165	157	165	8

Table A.22. Analysis of variance table of percentage loose smut in the 1964 glasshouse trial of treated grain of Fylgia II wheat (derived from Table A.21).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatment	225.453	5	45.091	14.49 ***
Error	37.334	12	3.111	
Total	262.787	17		

Least significant difference (percentage loose smut).

L.S.D. between two treatment means of three items each,

for P = 0.05, $L.S.D._{3:3}$ = 3.14 angles.

Table A.23. Analysis of variance table of percentage plant survival in the 1964 glasshouse trial of treated grain of Fylgia II wheat (derived from Table A.21).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatment	1540.18	7	220.026	52.2 ***
Error	67.40	16	4.213	
Total	1607.58	23		

Least significant difference (percentage plant survival).

L.S.D. between two treatment means of three items each,

for P = 0.05, $L.S.D._{3:3}$ = 3.55 angles.

Table A.24. Analysis of variance table of the influence of storage upon the percentage of normal seedlings that develop from Arès grain fumigated with methanol.

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	2069.50	6	344.92	151.9 ***
Error	31.78	14	2.27	
Total	2101.28	20		

Least significant difference.

L.S.D. between two treatment means of three items each:

for P = 0.05, $L.S.D._{3:3}$ = 2.64 angles.

Table A.25. Analysis of variance table of percentage plant survival (in angles) from fumigated grain of Rika barley, stored for 2 and 44 weeks (derived from Table A.6).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments				
a. Fumigation	6161.13	18	342.29	a/c 6.89 ***
b. Storage	555.40	1	555.40	b/c 11.18 **
c. Interaction	894.32	18	49.68	c/d 3.16 **
				a/d 21.77 ***
d. Error	660.39	42	15.72	b/d 35.33 ***
Total	8271.24	79		

Least significant differences (percentage plant survival).

1. Between two treatment means of four items each:

   for P = 0.05, $L.S.D._{4:4}$ = 5.67 angles.

2. Between two treatment means of four and two items:

   for P = 0.05, $L.S.D._{4:2}$ = 6.94 angles.

3. Between two treatment means of two items each:

   for P = 0.05, $L.S.D._{2:2}$ = 8.01 angles.

Table A.26. Analysis of variance table of percentage loose smut (in angles) in trials of fumigated grain of Rika barley, stored for 2 and 44 weeks (derived from Table A.6).

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments				
a. Fumigation	883.519	16	55.220	a/c 0.86 n.s.
b. Storage	211.357	1	211.357	b/c 3.31 n.s.
c. Interaction	102.258	16	63.911	c/d 5.04 ***
				a/d 4.36 ***
d. Error	468.932	38-1=37	12.674	b/d 16.68 ***
Total	1666.066	71-1=70		

Least significant differences (percentage loose smut).

1. Between two treatment means of four items each:

   for P = 0.05, $L.S.D._{4:4}$ = 5.09 angles.

2. Between two treatment means of four and two items:

   for P = 0.05, $L.S.D._{4:2}$ = 6.23 angles.

3. Between two treatment means of two items each:

   for P = 0.05, $L.S.D._{2:2}$ = 7.19 angles.

Table A.27. Analysis of variance table of percentage loose smut in the 1962 and 1963 trials of fumigated Fylgia II wheat grain, stored for 2 and 44 weeks, respectively (derived from Table A.18).

Source of Variation		S.S.	D.F.	M.S.	F.
	Treatments				
a.	Fumigation	8.0970	4	2.024	a/d 0.26 n.s.
b.	Storage	1.9096	1	1.910	b/d 0.24 n.s.
c.	Interaction	35.3480	4	8.837	c/d 1.12 n.s.
d.	Error	79.2549	10	7.925	
	Total	124.6095	19		

Least significant difference (percentage loose smut)

L.S.D. between two treatment means of two items each:

for P = 0.05, $L.S.D._{2:2}$ = 6.27 angles.

Table A.28. Analysis of variance table of percentage plant survival in the 1962 and 1963 trials of fumigated Fylgia II wheat grain, stored for 2 and 44 weeks, respectively, (derived from Table A.18).

Source of Variance		S.S.	D.F.	M.S.	F.
	Treatments				
a.	Fumigation	1518.13	4	379.53	a/c 11.02 *
b.	Storage	699.51	1	699.51	b/c 20.31 *
c.	Interaction	137.76	4	34.44	c/d 3.18 n.s.
					a/d 35.02 ***
d.	Error	108.36	10	10.84	b/d 64.55 ***
	Total	2463.76	19		

Least significant difference (percentage plant survival).

L.S.D. between two treatment means of two items each:

for P = 0.05, $L.S.D._{2:2}$ = 7.34 angles.

Table A.29. Analysis of variance table of percentage (in angles) of embryos infected by Ustilago nuda in light, medium and heavy grains of Arès barley.

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	9.735	2	4.868	1.60 n.s.
Error	27.330	9	3.037	
Total	37.065	11		

Table A.30. Analysis of variance table of the percentage (in angles) of embryos infected by Ustilago nuda in light and heavy grains of Rika barley.

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	61.107	1	61.107	15.76 **
Error	23.264	6	3.877	
Total	84.371	7		

Table A.31. The correlation between weight (W), length (L), breadth (B), and thickness (T) of individual grains of barley, variety Rika.

No.	W (mg)	L (mm)	B (mm)	T (mm)	No.	W (mg)	L (mm)	B (mm)	T (mm)
1	33.4	7.8	3.2	2.5	26	29.6	7.2	3.3	2.5
2	39.8	8.5	3.6	2.8	27	41.3	7.7	3.6	2.8
3	48.6	7.9	3.8	3.1	28	35.6	7.5	3.5	2.8
4	38.8	8.2	3.5	2.8	29	40.5	8.3	3.6	2.8
5	41.1	7.9	3.7	2.7	30	33.2	7.6	3.3	2.6
6	43.4	8.2	3.6	2.7	31	38.8	8.1	3.6	2.8
7	42.4	8.1	3.6	2.6	32	51.2	8.3	3.9	3.1
8	24.0	7.9	2.9	2.2	33	49.3	8.3	3.8	3.0
9	31.6	7.6	3.3	2.6	34	39.2	7.4	3.7	2.8
10	37.6	7.7	3.5	2.8	35	44.0	8.4	3.7	2.8
11	37.8	8.4	3.4	2.7	36	56.0	8.8	4.0	3.1
12	30.2	8.1	3.2	2.4	37	44.0	8.4	3.7	2.8
13	49.0	8.5	3.8	2.9	38	28.4	7.6	3.0	2.4
14	35.2	7.6	3.7	2.4	39	30.0	7.0	3.4	2.6
15	49.4	8.0	3.8	3.1	40	43.3	8.0	3.6	2.8
16	25.2	7.6	3.1	2.2	41	49.6	8.4	3.9	3.1
17	29.7	7.9	3.2	2.3	42	29.3	7.6	3.2	2.4
18	33.2	7.6	3.3	2.5	43	39.8	7.2	3.6	2.7
19	47.3	8.2	3.8	3.0	44	38.4	8.1	3.4	2.8
20	50.3	8.1	3.9	3.0	45	30.6	8.0	3.1	2.4
21	47.7	8.5	3.8	2.9	46	32.9	7.4	3.5	2.5
22	48.0	8.3	3.8	2.9	47	40.1	8.4	3.7	2.8
23	30.3	7.0	3.3	2.6	48	26.6	7.6	3.0	2.4
24	47.0	8.6	3.7	2.8	49	42.2	8.3	3.7	2.8
25	31.4	7.8	3.3	2.6	50	52.6	8.0	3.9	3.1

Table A.32. Analysis of variance table of the percentage frequency (in angles) of Ustilago nuda in fumigated and unfumigated grains, graded according to the type of seedling development.

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments				
a. Seedlings	4823.18	2	2411.59	a/c 5.92 n.s.
b. Fumigation	48.21	1	48.21	b/c 0.12 n.s.
c. Interaction	814.85	2	407.43	c/d 5.61 *
				a/d 33.20 ***
d. Error	726.48	12-2=10	72.65	b/d 0.66 n.s.
Total	6412.72	17-2=15		

Least significant difference

L.S.D. between treatment means each of three items

for P = 0.05, $L.S.D._{3:3}$ = 15.51 angles.

APPENDIX II.

N.II. The influence of the circulation of chloropicrin vapour upon its phytotoxicity to wheat grain.

1.1. Introduction.

In an investigation of the phytotoxicity of chloropicrin to the grain of Hybrid 46 wheat, the effect of fumigant circulation through a column of grain upon the subsequent seedling emergence was compared with that of grain fumigated in desiccators, by diffusion processes.

1.2. Materials and methods.

Grain of 14.0 per cent. moisture content was used in lots of 40 g. and fumigated at 20°C. for 3 hours, so as to reduce seedling emergence from 95 per cent. in the unfumigated check, to 50 - 60 per cent. The diffusion studies were conducted in 4.3 l. desiccators as described in Section D.7.

In the circulation investigations, a 120 l. bin internally protected from corrosion by enamel and epoxy-resin paint, was used as a reservoir, and the air within it was saturated with chloropicrin vapour at 20°C. The fumigant-air mixture was pumped through a circuit of brass tubing of ½ in. internal diameter, at the rate of 50 l./min., and returned to the reservoir. Fumigant was admitted to a second circuit at controlled rates by using gate-valves, and its rate of circulation was measured with a flowmeter of the Rotameter type. The fumigant was passed downwards through a column of grain contained in a copper tube 12 in. long and

approximately ~~1·25~~ 1·0 in. internal diameter. The flowrates shown in Table A.33 were calculated from a knowledge of the volume of fumigant-air mixture passed per unit time, and the internal diameter of the grain chamber, and refers to the empty tube. The flowrates through the grain were probably 3 - 4 times faster, and the airflow would have been fully turbulent. The fumigated grain was aired for 2 days at 20°C., and from each replicate 150 grains were sown in moist sand, for a measure of seedling emergence.

1.3. Results.

The emergence values are expressed as percentages in Table A.33, and were transformed to angles and examined by an analysis of variance (Table A.34).

In comparison with the grain fumigated by diffusion processes in the desiccator, the circulation of the fumigant did not increase the degree of phytotoxicity. It would have been of interest to have related this effect to the sorption of fumigant, but this was not attempted.

Kennedy (1959) found that chloropicrin was very slowly sorbed by pea seeds, and it is probable that in this experiment the rate of diffusion of chloropicrin in the seed tissues was rate limiting, and not the concentration of the fumigant in the vapour phase at the seed surface. Studies with sub-saturation concentrations of methanol, which has been shown to be sorbed very rapidly by barley grain (Section G) might reveal a very different effect of fumigant circulation.

Table A.33. The influence of the circulation of chloropicrin vapour upon its phytotoxicity to wheat grain.

Flow Rate through Empty Grain Chamber		Per Cent. Seedling Emergence			
		Replicates			Mean
litres/min.	cm./sec.	1	2	3	
0	0	51.3	60.7	62.7	58.2
2	6.6	60.7	52.7	60.7	58.0
6	19.7	48.0	51.3	52.7	50.7
15	49.3	54.7	74.7	-	64.7

Table A.34. The analysis of variance table of percentage seedling emergence in the fumigant circulation studies with wheat grain.

Source of Variation	S.S.	D.F.	M.S.	F.
Treatments	86.591	3	28.864	1.74 n.s.
Error	116.200	7	16.600	
Total	202.791	10		