International Biodeterioration

A combined primary and abstract journal covering the fields of biodeterioration and biodegradation-biotechnology.
International Biodeterioration

International Biodeterioration is a quarterly journal of news, reviews, original papers and abstracts from the CAB ABSTRACTS database covering the whole field of biodeterioration and biodegradation. It succeeds the International Biodeterioration Bulletin, Biodeterioration Research Titles and Waste Materials Biodegradation Research Titles and is owned by Bioquest Ltd.

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NOTES FOR INTENDING CONTRIBUTORS

Articles submitted for publication in *International Biodeterioration* must report original research in biodeterioration, i.e. deterioration of materials, artefacts or facilities of economic importance by living organisms, including microorganisms, insects, rodents, birds, higher plants, etc. Articles on biodegradation, i.e. conversion of materials to less objectionable, more easily disposable, or higher value products by living organisms will also be considered.

Review articles on these subjects may also be submitted, but are normally invited by the Editor.

Two copies of each paper, including an abstract, which must be in English and must not have been published or accepted for publication elsewhere, should be sent to:

Dr. H.O.W. Eggins,
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St. Peter's College,
College Road,
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Birmingham B8 3TE, UK.

Papers will normally be published in order of their receipt in definitive form.

All articles will be submitted by the Editor to one or more independent referees for advice on their clarity, originality, and general suitability for publication, but the final decision whether or not to publish an article rests with the Editor. If articles are rejected, the substance of the referee's report will usually be communicated to the author and in suitable cases the Editor will be pleased to help authors to improve their papers with a view to possible publication.

A current issue of *International Biodeterioration* should be studied before a manuscript is submitted, so as to ascertain the style adopted by the journal, especially for references, bibliography and section headings.

Papers should be typewritten, with double spacing, on one side only of A4 (21 x 29.5 cm) paper with a margin of at least 2.5 cm at the top, bottom and sides.

Tables should be reduced to the simplest form and should not be used where text or illustrations give the same information. Omit vertical rules. Tables should be submitted on separate sheets at the end of the article.

Illustrations: submit originals of all line drawings. Letters and captions should not be marked in; these should be shown on a photocopy or overlay so that they can be set by the printer.

Names of organisms should follow accepted checklists wherever possible, and must conform to the most recent appropriate International Code of Nomenclature. It is recommended that authors check with appropriate specialists that they are using the correct scientific names for organisms mentioned in their papers. Where living cultures used have not already been obtained from a major public culture collection, representative isolates of the strains used should be deposited in such a collection (e.g. the Commonwealth Mycological Institute in the case of fungi) to ensure their continued availability to future workers.

Manuscripts chosen to be refereed, but which do not conform to journal presentation, will be returned to the author for modification.
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**Contact:**
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For further details please contact:

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NEWS ITEMS

Biodeterioration Society

The Proceedings of the autumn 1985 meeting of the Society on the Biodeterioration and Biodegradation of Plastics and Polymers is now available. The contents cover biodegradable plastics, waste disposal of plastics, rubber and polyurethanes, preservation of plastics and biological testing of plastics. The proceedings are available from Dr. Ken Seal, Biotechnology Centre, Cranfield Institute of Technology, Bedford MK43 0AL, UK, price £15 plus £1.80 UK and Europe or £3.80 USA and rest of world to cover postage (post-free to Society and PABS members).

The spring 1986 meeting of the Society was held at the University of Surrey, Guildford in March 1986. The subject of this meeting was the Spoilage of Cereals and other Stored Products. The meeting covered fungal development in cereals, water activity, mycotoxin production, storage pests, pest control, fungal identification, cytotoxicity testing and immunological testing of mycotoxins.

The summer 1986 meeting of the Society will be held at TNO, Delft, Holland on September 18-19, 1986. The theme of the meeting is the Biodeterioration of Constructional Materials, and further details can be obtained from Dr. L.H.G. Morton, School of Applied Biology, Lancashire Polytechnic, Corporation Street, Preston PR1 2TQ, UK.

Seventh International Biodeterioration Symposium

The 7th International Biodeterioration Symposium will be held at Emmanuel College, Cambridge from 6-11 September, 1987. There is a call for offered papers and posters on all aspects of the biodeterioration of materials of economic importance and the biodegradation of waste materials. For further details please contact the Secretary of the Organising Committee, Mrs. Christine Gaylarde, Department of Biological Sciences, City of London Polytechnic, Old Castle Street, London E1 7NT.

PABS

The first meeting of the Pan-American Biodeterioration Society will be held at the George Washington University, Washington DC, from 17-19 July 1986. For information please contact Dr. Charles E. O'Rear, Department of Forensic Sciences, The George Washington University, Washington DC 20052.

Biodeterioration Standards

British Standard No. 2494 1976 - Material for Elastomeric Joint Rings for Pipework and Pipelines - is at present being revised. The revision will contain a biodeterioration test devised by the Water Research Centre. The test, in its present form, involves the immersion of rubber test strips in a tank continuously drip fed with river or borehole water which contains an inoculum of rubber scaling ring-degrading organisms. The current test procedure specifies six-monthly inspections of the samples for up to two years immersion. Further details may be found in the WRC Engineering Centre's Information and Guidance Sheet No. 4-40-01 (address: P.O. Box 85, Frankland Road, Blagrove, Swindon, Wilts. SN5 8YR).

British Standard No. 1982 - Fungal Resistance of Manufactured Building Materials - has now been revised. All sections should be available for public comment early in 1986.

If any members have news of new or revised national standards please contact the News Editor or Dr. Ken Seal at Cranfield Institute of Technology, Bedford MK43 0AL, UK.

Useful Addresses

For information regarding the safe disposal of waste materials, including special or difficult wastes contact Mr. K.R. Miller, Confederation of British Industry, Arndale House, Crossgates, Leeds LS15 8EU, UK.

Please send items for inclusion in NEWS ITEMS to:
Dr. John Mills
Editor, News Items
Department of Biological Sciences
Sheffield City Polytechnic
Pond Street
Sheffield S1 1WB, UK.
FORTHCOMING MEETINGS


Microbe 86: International Congress of Microbiology, Manchester, UK, 7-13 September 1986. Symposia, poster sessions, workshops and roundtables on all aspects of microbiology. For details contact The Secretary, Dr. J.A. Cole, Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, UK.

Biodeterioration Society summer meeting and AGM, 18-19 September 1986, Holland (see above for details).

Stored Products: 4th International Working Conference on Stored Product Protection, Tel Aviv, Israel, 21-26 September 1986. For details contact Mrs. S.J. Rhodes, VIP International Conference Services Ltd., 42 North Audley Street, London W1A 4PY.


AWRA: American Water Resources Association International Symposium on the Biofouled Aquifer, Atlanta, Georgia, 13-14 November 1986. Sessions, workshops, poster presentations on biofouling in wells and groundwater systems. For details contact Dr. Leslie Mack, Water Resources Research Centre, University of Arkansas, Fayetteville, Arkansas 72701, USA.

ASPERGILLUS CLAVATUS – AN ALLERGENIC, TOXIGENIC DETERIOGEN OF CEREALS AND CEREAL PRODUCTS

BRIAN FLANNIGAN

Abstract: The cosmopolitan soil fungus, Aspergillus clavatus, is found on a wide range of fruits and seeds and other stored products. It is associated with spoilage of cereals, causing extensive degradation of rice, and appears to be particularly well adapted for growth during malting and hydroponic production of cereal leasage for cattle feed. A. clavatus is the causative agent of an extrinsic allergic alveolitis, malt worker's lung. In addition to being rich in allergenic substances, the spores have shown mutagenic and tumourigenic properties in experimental studies. The fungus has been implicated in a number of mycotoxicoses, such as 'malt germ toxicosis': disease symptoms have ranged from hyperkeratosis to neurological and respiratory disturbances, and deaths have occurred in cattle. In culture, various strains isolated from toxic foods or feedstuffs produce ascladiol, patulin, tryptoquivaline, tryptoquivalone and cytchalasins E and R.

Introduction

Aspergillus clavatus Desm. is the causative agent of an occupational respiratory disease, malt worker's lung (Riddle et al. 1968), and has been recognised for some thirty years as a toxigenic organism (Forgacs et al. 1954). Together with A. giganteus Wehmer and A. clavotonanicus Batista, Maia & Alecrim, it forms a group within the genus Aspergillus which is characterised by its large blue-green conidial heads. These are claveate when very young, but rapidly split into divergent columns of compacted conidial chains. The smooth, relatively thick-walled, uninucleate, elliptical conidia (mostly 3.0–4.5 x 2.5–3.5 µm) are produced prolifically by a single series of phialides covering the entire surface of clavate vesicles, which arise from conidiophores 1.5–3.0 mm in length (Raper & Fennell 1965). Albino, tan and buff mutants are known (Raper & Fennell 1965; Varshney & Sarbhoy 1972), albino strains being repeatedly and abundantly isolated from soil in Kerala (Varshney & Sarbhoy 1980). Heterokaryosis has been noted in A. clavatus, and heads bearing a mixture of white and pigmented conidial chains were observed when a wild-type strain was paired with an albino mutant Raper & Fennell (1953).

Natural Occurrence of Aspergillus clavatus

Soil

Raper & Fennell (1965) have indicated that members of the A. clavatus group are common in soils and decomposing materials. They suggest that the occurrence of these fungi in soil may be associated with faecal material, since they commonly grow on the dung of a variety of animals, e.g. on the droppings of pigeons (Hubalek 1974) and chickens (Thom & Raper 1945). In reviewing the literature on A. clavatus (sensu stricto) as a soil organism, Domsch et al. (1980) cite reports that it has been isolated from soils in India, Bangladesh, Sri Lanka, Hong Kong, Japan, USA, Costa Rica, Jamaica, Brazil, Argentina, South Africa, Ivory Coast, Libya, Egypt, Turkey, Greece, Italy, Czechoslovakia and USSR. Nigeria (Commonwealth Mycological Institute 1982) and Saudi Arabia (Abdel-Hafez 1982a) can be added to this list. Domsch and his colleagues suggest that this species is almost entirely tropical, subtropical and Mediterranean in its distribution. However, its isolation from a variety of organic substrates in Canada (Wallace 1973), Great Britain (Riddle et al. 1968), France (Moreau & Moss 1979), German Democratic Republic (Abadjieff et al. 1966), Rumania (Stankevich 1969), Bulgaria (Tomov 1965) and Yugoslavia (Pepelnjak & Cvetnic 1984), as well as Thailand (Angsubhakorn 1972), South Korea (Lee et al. 1968, 1976) and New Guinea (Commonwealth Mycological Institute 1982), and from house dust in Surinam (von Bronswijk 1972), indicates that A. clavatus is probably cosmopolitan.

Although most reports concern its presence in cultivated soil, A. clavatus has been found in barren natural (Naim 1967) or desert soil (Ali et al. 1975, Abdel-Hafez 1982a), in clay and on rocks in carst caves (Lisinsu-Kulik 1968), in stratigraphic drilling samples (Sugiyma 1967), and in soil in teak forests (Bhargava & Bhargava 1971) and burnt steppes (Rambelli et al. 1973). The assertion of Raper & Fennell (1965) that the ability of A. clavatus to withstand strongly alkaline conditions enables it to act as an agent of decomposition in situations where almost all other fungi are eliminated is supported by observations of Kamal & Kumar (1982). These workers found that, although the levels of organic matter, nitrogen and particularly available phosphate were lower, counts of A. clavatus in saline (surcl) soil with a pH of 8.7 were considerably higher than in grassland, forest and cultivated soils at pH 6.8–7.0. It has been isolated from soils under crops of cotton (Kamysko 1968), legumes (Dayal & Lailal 1971), potatoes (Kamysko 1968), rice (Montemartini-Corte 1972) and sugar-cane (Robison 1968), and specifically from the rhizosphere in banana (Goos 1960), basil (Afifi 1975), clover (Lugauskas & Grybauskiene 1970), French bean (Afifi 1975), groundnut (Horie et al. 1977), rice (Jaluluddin 1975), and wheat (Jooste 1966, Abdel-Hafez 1982b).

The nature of the growing crop or its residues may have a profound influence on A. clavatus in cultivated soils. Williams & Schmitthenner (1960) found that growth of wheat and/or the addition of its residues had a considerably greater effect on counts in soil three months after afterwards than similar treatments with maize, oats, soybeans or alfalfa (lucerne). Subsequently, Jooste (1966) found that rotational crop sequence affected its incidence in the rhizosphere of wheat. Although, for example, Cladosporium sp. was more common when wheat was preceded by oats, clover or lucerne, A. clavatus was mainly isolated from the rhizosphere where there was a continuous wheat regime. Both percentage germination of spores and the rate of germ-tube growth were increased by the addition of powdered roots of cotton, tomato and clover to soil (El-Abyad & Ismail 1984). The role of the living plant is indicated by the stimulation of spore germination in A. clavatus by root exudates of basil, French bean and marjoram (Afifi 1975).

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Nitrogen availability in the soil can also be expected to affect the incidence of A. clavatus. Jooste (1966) noted its abundance in the rhizosphere after the addition of nitrogenous fertilizer to supplement the crop residues in wheat plots. Similarly, NPK fertilizer was found by Jalaluddin (1975) to increase the counts of A. clavatus in soil round the roots of rice. However, in laboratory experiments, unsupplemented wheat straw has been found to be an adequate substrate for growth and production of clavacin (patulin) by A. clavatus, as have chopped timothy grass and beet pulp (Grossbard 1952). Its ability to grow on such plant residues may account for its presence in stable manure (Waksman et al. 1942), composted municipal waste (von Klopotek 1962) and silage made from maize, sorghum, rye and pasture grasses (Escoula 1975).

The atmosphere

Although A. clavatus sporulates profusely (M. Moreau & C. Moreau 1960) and its spores are well known to be readily dispersed, there are few references to its incidence in the atmosphere. However, it has been isolated outdoors from air in urban Barcelona (Calvo et al. 1980), and in rural Uttar Pradesh (Bhati & Gaur 1979), where it was found in ten months of the year and formed nearly 2% of the total mould count on settle plates. Indoors, A. clavatus was found more frequently in the air of a centrally heated flat in Stockholm than A. candidus Link:Fr. and A. terreus Thom, but less frequently than A. niger v. Tieghem and A. repens (Corda) Sacc. (Ripe 1962), although the Aspergillii were rare relative to the principal components of the air mycoflora, Cladosporium spp. and Penicillium spp. In an industrial context, A. clavatus has been noted in the air of compartments for drying pasta products (Moreau & Moreau 1959), a storage hall for the ripening of bananas (Joly 1961) and a mallings (Riddle et al. 1968).

Plants in the field

In addition to isolating A. clavatus from the air around the herb Ocimum basilicum (basil) in the field, Afifi (1975) cultured the organism from the leaves and considered it to be a component of the phyllosphere microflora. Emanations from crushed leaves of this herb stimulated in vitro germination of A. clavatus spores, but those of Origanum majorana L. (marjoram), from the leaves of which the fungus was not isolated, inhibited spore germination (Afifi 1975). Sainger & Garg (1977) isolated A. clavatus from healthy leaves of the hoop pine, Araucaria cunninghamii D. Don. However, from what is known of the physiology of the organism, it seems more likely that it was present as a casual contaminant on the leaves of basil and the hoop pine, rather than as an actively growing component of the phyllosphere. Although it has been isolated from a range of fruits and seeds, there is little published evidence which suggests that in the field A. clavatus is any more than an uncommon superficial contaminant of these organs. Typically, Mehrotra & Dwivedi (1977) were able to isolate it from 5.7% of stored grains after surface-disinfection, but could not detect it in wheat gathered immediately before harvest and similarly disinfected. Rather, it appears to be associated with poor drying and storage. Lindenfelser et al. (1978), for example, found that A. clavatus was the predominant species in commercially cured and dried wild rice, Zizania aquatica L., which had become damp and mouldy through accident.

Stored products

A. clavatus has been isolated from a range of leguminous seeds (Gill et al. 1983) in Nigeria, and Hikokoto et al. (1981) have reported its presence at very low levels in broad and Lima beans in Japan. It is not normally associated with spices, but Hadlok & Tourné (1973) recorded 1800 propagules/g in samples of unsterilised commercial spices and 1400/g in 'sterilised' spices, and Jacquet & Tcherani (1974) commented that it was one of the Aspergillus spp. present in pepper which might possibly cause spoilage and produce toxins in meat products seasoned with that spice. A. clavatus was found to be present randomly among samples of both commercially packed nuts and dried fruits and those rejected for packing in South Africa (Wehner & Rabie 1970). An investigation in USA by Hanlin & Blanchard (1974) indicated that A. clavatus was a rather minor component of the mould flora of pecan fruits. However, although Chipley & Heaton (1971) found that A. clavatus could be detected in only 4% of samples of meat from pecans which they sheltered aseptically, the spread of inoculum during commercial processing was indicated by the presence of the mould in 15% of the samples of meat from commercially shelled pecans. In addition to being isolated from deteriorating tomatoes in USA (Ragheb & Fabian 1955), A. clavatus has been found in internally rotted pomegranates in India, where Kanwar & Thakur (1972) demonstrated experimentally that it could cause post-harvest decay if introduced via wounds in the fruit. It has also been associated with internal mouldiness of cocoa beans in Nigeria (Olutola 1977).

Many reports of A. clavatus in foods and feedstuffs concern cereals, however. In investigating the self-heating of stored wheat in USA, Carter & Young (1950) found that in experimental farm-type bins A. clavatus was less frequent on kernels than other storage fungi, being intermediate between Alternaria spp. and species of Fusarium, Cladosporium and Helminthosporium, which are all field fungi. It did not appear to be involved in the heating of the grain. Nikov et al. (1977) reported that in southwestern Khazakstan A. clavatus was one of twelve Aspergillus spp. prevalent in wheat. Surveys of stored wheat in India revealed that approx. 5% of samples were contaminated by this mould, but the percentage contaminated by many other Aspergilli, including members of the A. glaucus group, A. candidus, A. flavus Link.:Fr., A. nidulans (Eidam) Winter, A. niger and A. oryzae (Ahlburg) Cohn, was much higher (Mehrotra 1976, Mehrotra & Basu 1976, Mehrotra & Dwivedi 1977). A. clavatus has been isolated from barley in stores in Canada (Wallace 1973) and Egypt (Abdel-Kader et al. 1979), as well as India (Mehrotra 1976), where nearly one-third of the samples of stored grain were contaminated by this species (Table 1). In Rumania, however, Stankushev (1969) found that only 1% of samples collected from barley stored for up to two years in sheds for use as winter fodder yielded A. clavatus, the mean percentage of kernels so contaminated being only 0.1%. The corresponding values in oats were 2.8% (samples) and 0.3% (kernels).

In USA, Marasas & Smalley (1972) detected A. clavatus in meal prepared from mouldy crib-stored maize, but, as was the case with wheat (Carter & Young 1950), it appeared not to have proliferated when the maize had heated spontaneously. A. clavatus was infrequently encountered in stored Egyptian maize by Hassan & Selim (1982). However, Wallace & Sinha (1975) found that, over a nine-month period during 1966-67, 1.3% of all maize and sorghum kernels received from USA at one scaport in Japan were contaminated by A. clavatus at the time of import. Burroughs & Sauer (1971) also isolated A. clavatus from sorghum, but found that in lots with a moisture content of 25 or 29% stored at 15 and 25°C for up to 72 days the number of kernels contaminated by the mould were small. In India, rice and various millets (Table 1) have also been found to be contaminated by A. clavatus, and in USSR it has been found in both rice and millet, as well as wheat (Nikov et al. 1977). In Yugoslavia, A. clavatus was less frequently encountered in wheat stored in lofts and attics of houses than in maize, but more frequently than in beans (Pepeljnjak & Cvetnić 1984). There was little difference between households where families were affected by endemic nephropathy and those not so. A. clavatus appeared to be a relatively minor component of the mycoflora; A. flavus, A. niger and A. ochraceus Wilhelm were isolated much more
frequently. Yamazaki (1971) recorded that <0.02% of kernels from 634 samples of rice grown in Japan were contaminated by *A. clavatus*, but Tsunoda (1970) and Kurata (1978) have both stated that this species was frequently present in imported rice, the degree of contamination varying with the country of origin. In rice kernels it has been observed to cause marked damage (Tsunoda 1970), the texture of the kernels becoming chalky (Moreau & Moss 1979). In Surinam, von Bronswijk (1972) suggested that the presence of *A. clavatus* in house dust might have been due to the habit of storing rice in households.

In addition to being found in whole cereals, *A. clavatus* has been detected in various milled products. In India, Mehrotra & Basu (1976) observed that 8.4% of milled wheat fractions – bran, semolina and fine flour – were contaminated by this species, but only 4.6% of samples of whole wheat. In addition, it was detected in flours prepared from barley, rice and pearl millet (Table 1). Graves & Hesseltine (1966) also found it in flour wheat in USA, and Nikov et al. (1977) in flour and meal, as well as whole wheat, in USSR. Udagawa et al. (1970) stated that *A. clavatus* was present in 8% of wheat flour samples from markets surveyed in Japan, and in 7% of rice flour samples. *A. clavatus* has been isolated from spoiled refrigerated dough products (Graves & Hesseltine 1966), bean paste (Lee et al. 1968) and dried milk (Kurata 1978). This mould has also been noted in a range of other foodstuffs in both Japan (Kurata et al. 1971) and Thailand (Aukarahanont & et al. 1984), although in Thai markets <1% of samples were contaminated by it. It has also been detected in animal feeds. Forcades et al. (1954) isolated the mould from feed pellets (composed largely of maize and alfalfa) which were associated with toxic symptoms in calves in USA. In Poland, Gladoch (1974) noted the dominance of *A. clavatus, A. flavus* and *A. fumigatus* in poultry feed-mixes and their components, e.g. maize from USA and groundnut meal from India.

Table 1. Frequency of occurrence of *A. clavatus* in Indian grain and flour, as percentage of samples contaminated (Mehrotra 1976)

<table>
<thead>
<tr>
<th>Cereal*</th>
<th>Grain</th>
<th>Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>4.6</td>
<td>8.3</td>
</tr>
<tr>
<td>Rice</td>
<td>8.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Barley</td>
<td>32.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Great millet <em>(Sorghum bicolor)</em></td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pearl Millet <em>(Pennisetum typhoides)</em></td>
<td>23.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Finger Millet <em>(Eleusine coracana)</em></td>
<td>18.1</td>
<td>—</td>
</tr>
<tr>
<td>Italian Millet <em>(Setaria italic</em>a)*</td>
<td>20.0</td>
<td>—</td>
</tr>
</tbody>
</table>

*, not examined.

Water relations

As to the circumstances under which *A. clavatus* invades stored products, Christensen (1975) has stated that the organism only appears in the final stages of decay and is not a ‘storage' fungus in the sense that the term is most often used, i.e. it is not strongly xerophilic. The minimum relative humidity (R.H.) for its growth was found by Panasenko (1944, 1967) to be 88%, and the optimum 98%, whilst the corresponding R.H. for production of conidia were 90 and 98–100%, respectively. In examining the tolerance of Aspergilli to sodium chloride, Tresner & Hayes (1971) found that the maximum salt concentration permitting growth of this species was either 15 (2 strains) or 20% (14 strains), which according to the tables of Robinson & Stokes (1955) approximates to a water activity (\(a_w\)) of 0.91 or 0.87, respectively, and is in broad agreement with the minimum R.H. quoted by Panasenko (1944, 1967). More recently, Manabe & Matsura (1979) found that there was good growth in koji medium containing 12% NaCl (\(a_w\) approx. 0.93) or less, but progressively poorer growth at levels up to 18% (\(a_w\) approx. 0.89) and none at 21% (\(a_w\) <0.87). The toxicity to chick embryos of the koji medium in which the organism had grown declined in a similar manner. Mehrotra (1976) earlier reported that the minimum R.H. at which spores would germinate was 85% and the optimum 100% R.H. The values quoted in these various reports would place the organism in the ‘slightly xerophilic' category of Lacey et al. (1980). It should, however, be emphasised that monocultural studies may not accurately represent how the organism behaves in substrates where it is only one member of the mycflora. Flannigan et al. (1985) agreed with the minimum value for growth reported by Panasenko (1944, 1967), and found that the organism grew well on agar medium in equilibrium with an atmosphere of >93% R.H. However, when in competition with other more xerophilic Aspergilli (A. candidus and members of the A. glaucus group), *A. clavatus* on contaminated malt only increased in numbers when the R.H. of the storage atmosphere was >96.6%. Sto!off et al. (1978) found that, when in competition with other toxigenic Aspergilli and Penicillia in a mixture of spores dusted on extruded pasta dough, *A. clavatus* apparently did not grow, even when the dough was held at 95% R.H.

Temperature relations

The cardinal temperatures listed by Panasenko (1944, 1967) for growth of *A. clavatus* are: minimum, 5–6°; optimum, 20–25°; maximum, 42°. Moreau & Moss (1979) suggested that its ability to grow well at 37° might account for the presence of the fungus in the atmosphere where pasta was dried at 30–35° in compartments with a R.H. around 80% (Moreau & Moreau 1959). These authors stated that the conidiophores produced at 20° were markedly longer than those at 30°, and also noted that the optimum temperature for growth was 23–26. However, Varshney & Sarbhoy (1972) observed maximum increase in biomass of a tan mutant at 25–30° and of albino and buff strains at 30°, whilst Mehrotra (1976) reported that, irrespective of R.H., germination of *A. clavatus* conidia was most rapid at 28°.

pH of substrate

Although the ability of *A. clavatus* to survive and grow in alkaline conditions (Raper & Fennell 1965, Kamal & Kumar 1982) was mentioned earlier, Ragheb & Fabian (1955) found in their study of fungi associated with deterioration of tomatoes that the optimum pH for growth of *A. clavatus* in tomato juice was 4.5–6.0. Varshney & Sarbhoy (1972) reported that sporulation was greatest at pH 6.5–7.5.
Substrate utilisation

The utilisation of different sugars by A. clavatus has been examined by Varshney (1981), who found that for four out of five grains the pentoses, arabinose and xylose, were better as sole carbon source than the hexoses, glucose and fructose. The disaccharides, sucrose and lactose, and the trisaccharide, raffinose, also supported good growth and sporulation, but the best carbon source appeared to be mannitol. Agnihotri (1963) and Varshney (1981) both found that A. clavatus could utilize starch as sole carbon source, and the latter also reported that dextrin, glycogen and inulin were utilized, although fructose was observed to be a better carbon source than any of the polysaccharides or glucose. In both of these studies the organism grew with sodium nitrate as the source of nitrogen. Robinson et al. (1974) found that ammonia, urea and glutamate could be used as sole nitrogen source by germinating conidia, with ammonia being used preferentially in the presence of either of the others.

Few studies have been made of the extracellular enzymatic activity of A. clavatus. Although it may be associated with starchy cereal grains, Vojtkova-Lepikova & Knockov-Kratochvilova (1968) found that, in relation to amylolytic species of Candida and Cryptococcus, A. clavatus showed low extracellular activity against starch. Siu (1951) indicated that A. clavatus could be isolated from exposed cotton and yet was non-cellulolytic. Marsh et al. (1949) reported, however, that more than one-half of the strains that they examined showed definite cellulolytic activity. Under laboratory conditions, the most active strain reduced the tensile strength of 8 oz cotton duck by 80% in 2 weeks. Nevertheless, Reese & Downing (1951) found that, although their strains were active against carboxymethyl cellulose (CMC), they caused strength losses in cotton sheeting and duck well below the arbitrary lower limit of 15% these authors had set for a cellulolytic mould. Fractionation of culture filtrates of A. clavatus grown with insoluble cellulose or CMC as sole carbon source indicated that there was a cellulolytic enzyme system consisting of four components of differing specificities with respect to CMC and cellulobiose breakdown (Olutofa 1977). Ragheb & Fabian (1955) found that A. clavatus grown in tomato juice had pronounced pectinolytic activity. Muruta et al. (1981) deduced from observations on lignin levels in straw exposed to the organism in both liquid and soil culture that it was lignoclastic, and Bandoni & Towers (1967) presented evidence that it degraded usnic acid, a phenolic carboxylic acid pigment present in lichens.

As judged by loss of tensile strength in wool, Reese & Downing (1951) found that A. clavatus was keratinolytic, and extracellular proteolytic activity was demonstrated when it was grown on wheat bran (Koch & Dedic 1957) or in glucose-salts medium (Cohen 1981). Although the related A. giganteus produced an acid protease, A. clavatus was found by Cohen (1981) to be one of 14 Aspergillus spp. releasing neutral or alkaline proteases, production of which was not induced but was controlled by derepression. An extracellular RNase was investigated by Bezborodova et al. (1969). Although there are no reports of lipolytic activity in A. clavatus, Coursey & Eddings (1961) found that palm oil was hydrolysed by the related species A. giganteus. However, in this laboratory, plate and tube tests indicated that lipolytic activity was less common among isolates of A. clavatus than anylase, pectinase, cellulase, protease, amylase, RNase, DNase or phosphatase (Gilliam L. Shiel, unpublished results). Phosphatase activity was first reported by Jacquet et al. (1958), who indicated that both alkaline and acid phosphatases were present. Morozova & Bezborodova (1972) reported that, in addition to an acid phosphodiesterase, A. clavatus produced a thermolabile acid monophosphoesterase, with an optimum temperature of 55° and optimum pH of 5.5-6.5.

A. clavatus as a respiratory pathogen

The potential respiratory hazard to those handling and processing stored grain or other material heavily contaminated by A. clavatus is evident when the occupational disease malt worker's lung is considered. Although the prevalence and severity of chronic respiratory diseases, such as bronchitis and emphysema, in malt workers was noted more than 50 years ago by Bridge (1932), maltworker's lung was not described until 1968 (Riddle et al. 1968). The disease is caused by exposure to high concentrations of the spores of A. clavatus, and apparently also A. fumigatus Fres. Fungal spores which are >10 μm in diameter are likely to be deposited predominantly on the mucus membranes of the naso-pharynx, and those which are in the size range 4-10 μm are mainly deposited in the bronchi and larger bronchioles. The spores of A. clavatus, however, fall into the category <4 μm in diameter — which are deposited in greatest numbers on the respiratory surface of the terminal bronchioles and alveoli. Although the larger spores can provoke an allergic response, it is a Type I reaction of a rhinitis (hay-fever) or asthma type, depending on the site of deposition of the spores. In the case of A. clavatus the response is in the alveoli and is a Type III reaction. The spore walls are particularly rich in allergenic substances (Blyth 1978), and the condition which develops is described as an extrinsic allergic alveolitis.

Unlike the immediate, relatively short-lived, overt symptoms of a Type I reaction, those of the alveolitis do not develop for some hours and continue for 48h or more. The characteristic fever, malaise, dry cough and breathlessness may not develop until after completion of work for the day, so that the sufferer often fails to associate them with earlier exposure to the high concentrations of A. clavatus spores which have caused them. Being a Type III reaction, the disease is mediated by precipitating antibodies, so that the pulmonary tissue is damaged: there is perivascular inflammation, granuloma formation, and with repeated attacks the lung becomes progressively fibrosed. It is permanently damaged and consequently its efficiency is considerably impaired, so that there is increasing dyspnoea. It has been suggested that a Type I reaction provoked by allergens in the spores might also be a component of the disease (Grant et al. 1976). Provocation of both Type I and Type III responses in the same individual is relatively rare, but should it occur the respiratory distress will be extreme.

The reasons for the occurrence of this extrinsic allergic alveolitis in maltworkers can be appreciated when the malting process is examined. In traditional floor malting, the first stage, which may take 2-3 days, involves steeping dry barley until the moisture content (M.C.) rises from an initial 10-12% to 43-47%. In order to accelerate subsequent germination, steeping is interrupted by drained air rests, the length and number of which are dependent on a number of factors including the characteristics of the barley and the temperature of the steep water. After a final draining, the barley is cast onto a malting floor where it is spread out in a layer 10-15 cm deep to germinate — in United Kingdom usually at an ambient temperature of 13-16°. To control the rising temperature of the bed of germinating barley on the floor, to promote more even germination by ensuring adequate aeration and to prevent the rootlets becoming matted, the barley is turned periodically. This involves maltmen throwing the barley ahead of them by shovel as they advance along the floor, and also raking the bed of grain. Again depending on the nature of the barley and the ambient temperature, this germination to produce a 'green malt' at an appropriate stage of enzyme development and endosperm modification may take 7-12 days. The green malt is then kilned at 40-60° for up to 24 h, and finally cured briefly at 70-80°, the final M.C. being 3-4%. However, the temperature and duration of kilning depend on the exact type of malt to be produced. Coleoptiles, rootlets and any fragmented material are then screened off mechanically for incorporation into animal feed.
A. clavatus can grow and sporulate on the germinating barley on the malting floor, so that it is when the barley is being turned that malt workers are likely to inhale large numbers of spores. In extreme cases, sporulation may be so profuse that visibility across the malting floor is reduced by thick clouds of spores rising from the green malt as it is being turned (Riddle et al. 1968). The dispersal of spores into the atmosphere during the loading and stripping of kilns also presents a hazard, but the practice of turning malt manually in kilns to promote uniform drying has virtually disappeared.

In many areas, floor maltings have been superseded by box (Saladin) maltings, in which the depth of the layer of barley can be as much as 2 m and the germinating grain is turned mechanically by helical screws. Other modern developments have included germination vessels, rotating drums and Domalt continuous malting plant (Briggs et al. 1981). Although all have reduced the time required for germination, the high level of moisture availability and the presence of solubilised and partially solubilised nutrients still favour microbial growth. After the first reports of maltworker’s lung among workers in floor maltings (Riddle et al. 1968, Channell et al. 1969), Blyn et al. (1977) surveyed 56 Scottish maltings of various types and found A. clavatus in 12. Among 711 maltworkers in the 56 maltings (Grant et al. 1976), 5.2% had maltworker’s lung. The incidence was only 1.1% among those working where modern mechanical systems were employed, e.g. where the germinating barley was enclosed in rotating drums, but almost as many in box maltings (6.2%) had the disease as in floor maltings (6.8%). Grant et al. (1976) concluded that the prevalence of the disease was likely to diminish rapidly with increasing mechanisation of malting. However, the present author is aware that A. clavatus has been found in at least one automated plant in Europe.

A. clavatus is also associated with the malting of sorghum. In surveying foodstuffs in Eastern Transvaal and Swaziland, Martin et al. (1971) found that A. clavatus was one of a small group of moulds which proliferated and balanced the decline in the numbers of other species during the malting of sorghum. More recently, Rabie & Lübben (1984) reported that in South Africa it can be one of the main components of the mycoflora of dried sorghum malt. They reported that A. clavatus was among the dominant species in 45% of malts from industrial Saladin-type maltings and 9% from outdoor commercial floor maltings.

Since A. clavatus appears to be rather uncommon on dry barley, it is not clear how malting premises become contaminated by the organism in the first instance. Channell et al. (1969) suggested that the fungus might have been introduced with a particular consignment of grain in one maltings. Previously, Riddle et al. (1968) suggested that the source in another maltings might have been feral pigeons which had free access to the grain store. The mould was isolated from pigeon droppings in both this investigation and that of Channell et al. (1969). In surveying free-living birds, Hubulek (1974) found that the mould was characteristic of the droppings of feral pigeons, where it was found much more frequently than in those of other birds. No matter the means of introduction, it is perfectly clear that once A. clavatus is present in a maltings it is provided with extremely favourable conditions for its growth and sporulation, i.e. a fully hydrated substratum and a high R.H. Although it only grows on the green malt, and possibly during the early part of kilning, its spores are readily dispersed throughout the premises. As well as detecting A. clavatus in all parts of a distillery maltings, Channel et al. (1969) isolated the mould from the spumt of employees in the distillery who did not work as maltmen and seldom entered the maltings. The fact that the spores are spread with ease makes the organism particularly difficult to control. Dry grain stored on the premises becomes contaminated by aerially dispersed spores, and addition of hypochlorite to the steep water at the concentrations frequently employed is not effective in killing these spores (Flannigan et al. 1984). Although Riddle et al. (1968) suggested that clidine hypochlorite might actually stimulate growth, it seems more probably that it encourages development of the mould by reducing the numbers of competing species.

A factor which may lead to particularly profuse growth on the green barley malt is the presence of cracked kernels (Riddle et al. 1968), pregerminated grain or even accidentally introduced finished malt (Flannigan et al. 1984). These present easily colonised substrata from which contamination may spread. However, the growth of the mould on the germinating grain is often associated with elevated temperatures, e.g. in U.K. during warm weather when there is no adequate means of preventing the malting temperature exceeding the normal 16°C (Flannigan et al. 1984), or when it is purposely raised to accelerate the process (Riddle et al. 1968). Recalling that the optimum temperature for the growth of A. clavatus is c. 25°C, it was not unexpected that raising the temperature for preparation of experimental malts from 16°C to 25°C would result in a x10000 increase in the viable count of the mould (Flannigan et al. 1984). Similarly, it is also not surprising that A. clavatus is so frequently isolated from sorghum malt (Rabie & Lübben 1984) in South Africa, since the temperature of malting may be as high as 28°C.

**Mycotoxicoses associated with A. clavatus**

It is not only during malting that A. clavatus develops on germinating grain: symptoms of disease attributable to the presence of the mould on cereals sprouted hydropionically for use as animal feed were described in 1960 (C. Moreau and M. Moreau 1960). As Moreau & Moss (1979) have pointed out, the hydropionic production of cereal shoots is not without controversy, but during the very dry summer of 1959 many livestock farmers in France adopted hydropionic methods to provide forage for cattle. When C. Moreau & M. Moreau (1960) examined one farm where cattle had died after feeding on such forage, they were able to isolate A. clavatus not only from the forage but also from the walls of the premises where the wheat was sprouted (for 7 days) and from the circulating water system, steep tanks and germination trays. In a subsequent investigation of a dairy farm (Moreau & Moreau 1961), the same authors again found that the organism was widely distributed within the premises: it was isolated from the air and walls of the germination room and from most other areas. They observed that A. clavatus appeared to be in competition with Geotrichum candidum Link, a species which also appears to be well adapted for growth during malting (Flannigan et al. 1984). The reason for the distribution of A. clavatus throughout such premises became obvious when they examined sporulation under conditions simulating hydropionic production of cereal shoots; after 7 days at 25°C, a single germinated wheat kernel could yield up to 6.5 x 10⁸ spores and a barley kernel as many as 1.2 x 10⁹ (M. Moreau & C. Moreau 1960).

Neurological and other disorders in cattle fed on cereal shoots produced hydropionically were attributed by C. Moreau & M. Moreau (1960) to production of patulin (Fig. 1) by A. clavatus. Again in France, Jacquet et al. (1965) also noted the development of neurological symptoms in cattle fed on wheat germinated for 6 days to produce winter feed, and Nikov et al. (1965) reported mass poisoning of Bulgarian cattle fed on barley sprouts from which A. clavatus had been isolated, in company with A. fumigatus, A. flavus, and various Penicillia and Fusaria. Interestingly, 25 years after the hazards associated with hydropionically grown cereal shoots were discovered in France, toxicological problems have been encountered in U.K. since hydropionic production of 'barley grass' has been increasingly adopted. Again, Geotrichum candidum has been one of the predominant fungi. However, most disease episodes appear to have been associated with Fusarium spp., although in one case a strain of A. clavatus producing cytochalasin E...
Aspergillus Clavatus - an allergenic, toxicogenic danger of cereals......

(Fig. 1) was implicated (Miss J. Robb, personal communication). In addition, Kellerman et al. (1984) have recently reported that sprouted maize contaminated with A. clavatus was the cause of an outbreak of fatal tremorgenic mycotoxicosis among dairy cattle in South Africa.

In Bulgaria, Tomov (1965) noted toxicosis in >250 dairy cows fed on 'malt germs'. Although most died, the remainder were culled because of neurological disorders, respiratory disturbance or decreased lactation. These symptoms were reproduced when a mixture of barley and oats inoculated with A. clavatus was fed to cattle. A 'malt germ intoxication' characterised by disturbances in coordination was recorded by Abadjeff et al. (1966) when cattle were fed on culms from malting plants in the German Democratic Republic. The symptoms were attributed to two strains of A. clavatus which were isolated from the culms and shown to be toxic in skin tests and by their effect on Paramaecium (Fritzsche & Abadjeff 1967). In further investigations, it was found that during malting up to 230 mg of a fraction toxic to experimental mice could be produced per kg of finished malt and culms (Schultz 1968), and that the intoxication could be reproduced by feeding cattle on maize inoculated with the mould (Schultz et al. 1969). Subsequently, Schultz & Motz (1973) described a routine method for extraction of the toxic principle and advocated that malt culms should be examined for both the organism and toxicity before release for animal feed. In experimental malting in this author's laboratory, it was found that, on a dry weight basis, culms were nearly three times as toxic as the corresponding finished malt (R. Apta, unpublished results). Although conidia of A. clavatus are inactivated by exposure to dry heat at 120° for 60 min and to moist heat at 80° for 30 min (Panasenko 1944), it is clear that, as well as toxic components, allergens and inoculum also survive kilning of barley malt. Riddle (1974) reported that maltworkers showed marked respiratory symptoms after cleaning culms out of storage bins. Apta et al. (1984) found that after kilning experimental green malt, 25% of the viable inoculum present survived in finished malt, and 5-7% in culms. This has obvious implications for storage of both finished malt and its by-product. Flannigan et al. (1985) indicated that during transhipment to, and storage in, tropical regions uptake of moisture by dried barley malt might lead to the resumption of growth of A. clavatus surviving on this hygroscopic substratum and thereby increase the potential respiratory and mycotoxic hazards.

Although Rabie et al. (1983) found mycotoxins produced by other fungi in sorghum malt, there did not appear to be any originating from A. clavatus. However, some isolates of A. clavatus produced cytochalasins E and K in culture. Feeding on residues from sorghum beer production which were contaminated with A. clavatus led to fatal tremorgenic disease among cattle (Kellerman et al. 1976). The practice of spreading out the residue on a floor to dry was considered to be conducive to the growth of the mould, and another factor contributing to the mycotoxicosis was thought to be the use of even obviously mouldy residue for feed.

Among experimental studies on the effects of A. clavatus on animal health there is some contradiction as far as weight gain in animals in concerned. Semeniuk et al. (1971) found that when wheat inoculated with the mould was supplied to chicks and mice there was low feed consumption and stunting of growth. Scott (1965) also noted reduced weight gain in mice, but Chah et al. (1975) noted improved weight gain in broiler chicks when fermented soya-bean feed contaminated with the mould was used as rations. The causal relationship between A. clavatus and hyperkeratosis first demonstrated in calves by Forgacs et al. (1954) was also observed in mice which were fed contaminated rations (Blyth & Lloyd 1971). Angushakorn et al. (1977) found that extracts of A. clavatus caused haemorrhage and congestion in rats, in addition to other symptoms previously noted in calves (Forgacs et al. 1954). Among other effects which have been recorded is a remarkable proliferation of tubular epithelial cells, fibroblasts and macrophages in the kidneys of mice fed on maize-malt cultures of A. clavatus (van Rensburg et al. 1971). Saito et al. (1971) reported that both culture filtrates and mycelial extracts caused 'mitosis injury' in mouse tissue cultures, and Blyth & Hardy (1982) found that alkaline hydrolysates of the mould were mutagenic in Salmonella typhimurium (Loeffler) Castellani & Chalmers. These last authors also observed development of pulmonary tumours in mice after nasal administration of spores.

**Mycotoxins produced by A. clavatus**

The best-known of the mycotoxins produced by A. clavatus is patulin (Fig. 1), which is produced by various other Aspergilli and species of Penicillium. Its discovery, synonymy, antibiotic properties and earlier use as a therapeutic agent are well-documented and have been reviewed by, for example, Moreau & Moss (1979). Zamir (1980) has reviewed the biosynthesis of this unsaturated lactone, which shows acute toxicity towards laboratory rodents. Lovett & Thompson (1978) found that production of patulin by A. clavatus increased with temperature between 1.7 and 12.8°, but Northolt et al. (1978) reported that the toxin was elaborated between 12 and 14°, the optimum being 16°. It does not appear to be produced on the substrate at <0.99 a w (Northolt et al. 1978). Some strains produce relatively large quantities of the toxin: cherries canned in water contained >100 μg patulin/ml after growth: of one strain for 6-8 days (Loevelt et al. 1974). The possible involvement of patulin in the poisoning of cattle by A. clavatus present in hydroponically grown forage is supported by the demonstration of some of the disease symptoms in mice injected intravenously with the toxin (Capitaine & Balouet 1974). Concern has been expressed over the possible presence in food of a mycotoxin reported to be a carcinogen. However, notwithstanding its carcinogenicity in subcutaneously injected rats, Beechi et al. (1981) did not detect tumours after oral administration of patulin to rats.

The formation of ascladiol (Fig. 1) by a strain of A. clavatus isolated from wheat flour was reported by Tanabe & Suzuki (1970) and Suzuki et al. (1971), who noted that the toxin had an acute toxicity to mice approx. one-quarter of that of patulin. Although it is structurally similar to patulin, Zamir (1980) considers that ascladiol is an end product, the formation of which is unrelated to patulin biosynthesis.

After Blyth & Lloyd (1971) reported that intraperitoneal injection of acid hydrolysates of A. clavatus produced tremors and loss of co-ordination in mice, two tremorgens, tryptoquivaline and tryptoquilone (Fig. 1), were isolated and purified by Glinsukon et al. (1974). With cytochalasin E (Fig. 1), they were extracted from glutinous rice inoculated with a strain of A. clavatus originally detected together with other Aspergilli in a sample of leftover rice implicated in a case of Reye's syndrome in Thailand. Nothing is known of the effect of the tremorgens on humans, but they caused persistent tremors and hypersensitivity in intraperitoneally injected rats, which died after 8 days without showing any specific histopathological changes in the major organs (Glinsukon et al. 1974). In addition to tryptoquivaline and tryptoquilone (Clardy et al. 1975), four related compounds have been described (Büchi et al. 1977, Springer 1979); these are nortryptoquinaline and the secondary amines, deoxytryptoquinaline, deoxytryptoquilone and deoxytryptoquilone. Cytochalasin E (Büchi et al. 1973), like the other cytochalasins, inhibits cytoplasmic cleavage in tissue
Fig. 1 Mycotoxins synthesised by *A. clavatus*: (a) tryptoquivaline, (b) tryptoquivalone, (c) cytochalasin E, (d) cytochalasin K, (e) patulin and (f) ascladiol.
cultures, and Glinsukon et al. (1974) reported that in rats it caused death within 2–18 h of intraperitoneal administration. Post-mortem examination revealed severe congestion of the liver and other organs, pulmonary haemorrhage and cerebral oedema. Further work confirmed that the symptoms were replicated on oral administration, and indicated that death was from shock caused by injury to vascular walls and resultant effusion of plasma fluid, albumin and globulins (Glinsukon et al. 1975a, 1975b, Glinsukon & Lektau 1979). Trirawatanapong et al. (1980) have since suggested that symptoms such as oedema of the brain and congestion in other organs are secondary to vascular damage and hypovolemic shock. In addition to these symptoms, rapid depletion of liver glycogen in rats (Glinsukon et al. 1975b) and inhibition of glucose uptake from the jejunum in mice (Glinsukon et al. 1983) have been observed. Vesely et al. (1984) observed that cytochalasin E was highly toxic to chick embryos, but only one-third of 22 A. clavatus strains isolated from grain and various feeds produced the toxin. The first report of the natural occurrence and structure of cytochalasin K (Fig. 1) was made by Steyn et al. (1982). They noted that production of both cytochalasin E and K accounted for the toxicity of a strain of A. clavatus from sorghum malt.

Some isolates of A. clavatus are also known to synthesise kojic acid (Parrish et al. 1966), a compound produced by a wide range of moulds. However, although this metabolite is known to be toxic to laboratory rodents (Carlton & Szczec 1978), there are no reports of human or animal disease which can be attributed to ingestion of food or feedstuffs contaminated by it.

Conclusion

The paragraphs above illustrate that A. clavatus belongs to that category of deteriogens (Eggins 1984) which will ‘interact first with a material, multiplying on it and subsequently causing disease by the inhalation or ingestion of spores or toxic compounds’, and is therefore the ‘proper concern of those involved in the study of biodeterioration’. The possibility that the inhalation of high concentrations of spores by maltworkers and others handling materials heavily contaminated by A. clavatus might lead to pulmonary mycotoxicosis and tumourigenesis, in addition to allergic respiratory disease, should heighten that concern.

The author is grateful to Miss Jean Robb, East of Scotland College of Agriculture, Edinburgh, for information given.

References


International Biodeterioration 1986 Vol. 22 No.2


Manabe, M.; Masuura, S. (1979) [Studies on the fluorescent compounds in fermented foods. Part 5. The behaviour of fungi during miso and shoyu fermentation and aflatoxin inspection of commercial shoyu]. Shokukin Sogo Kenkyusho Kenkyu Hokoku 34, 79-83.


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Received November 1985
MICROORGANISMS ASSOCIATED WITH PAINT FILMS IN COLD ROOMS AND AIR-CONDITIONING VENT PANELS IN NIGERIA

S. AYO ODUNFA

Abstract: The predominant organisms isolated from discoloured paints in cold rooms in Nigeria were Cladosporium cladosporioides and Phialophora verrucosa. Those on the humid walls at the entrance of cold rooms and on panels around air conditioning vents are C. cladosporioides, C. sphaerospermum and C. oxysporum. All the isolates were capable of growing at low temperatures and preferred high humidity for growth. Their occurrence and predominance of the Cladosporium spp. was ascribed to their prevalence in the air spora in Nigeria, tolerance of cold temperatures and heavy metals.

Introduction

Microorganisms, especially moulds, are commonly known to discolour paint film surfaces of both gloss and emulsion paints. They are generally favoured by high humidity and high temperature. Fissures in the paint also predispose paint films to microbial attack (Ross & Hollis 1976). The high humidity and high temperatures make the tropical environment especially favourable for paint biodeterioration (Whiteley 1966). The solution to this problem in the tropics was the incorporation of appropriate antimicrobial agents in the paints (Ross & Hollis 1976).

In Nigeria, the newer problem is microbial attack on painted films on the walls around the entrance of cold rooms, panels around central air conditioning vents and on the walls inside the cold rooms. This problem is arousing more concern, especially with the increasing use of central air conditioning plants in many offices and of cold rooms for commercial food storage. These environments are characterized by cool temperature and at times high humidity. The cool air emanating from the vents or escaping from the cold rooms would cause condensation of water thus creating a highly humid environment for microbial growth. The walls are stained with black spots and at times may be covered with slime.

Previous records of paint microorganisms in Nigeria have been made at a mesophilic temperature range (Whiteley 1966, MacNulty 1966). Information on the occurrence of microorganisms on cool walls in the tropics would provide new and helpful information in developing special paints for use in these areas. This information is even more desirable since paint microflora is markedly influenced by geographical location (Ross & Hollis 1976).

Materials and methods

Sites

The cold rooms and air-conditioning vent panels used were at three locations in Ibadan including the University of Ibadan. The temperature of the cold rooms varied from 5°-8°C.

Isolation of microorganisms

Scrapings were made from the discoloured walls and collected in Petri dishes. Some of these scrapings were plated directly on agar plates. Others were ground to a fine powder in a mortar previously sterilized with 10% sodium hypochlorite solution and one gram of the powder suspended in sterile water. The suspension was serially diluted and one tenth of a millilitre of 10^-4 dilution was spread on plates of nutrient agar to isolate the bacteria, and acidified Czapek Dox agar (Oxoid) to isolate the fungi. The jelly-like slime discolouration on the interior walls of the cold room was streaked directly on plates containing the above agar media.

All the plates were incubated at 5°, 10°C and 15°C for 1 to 2 weeks for the bacteria and for 2 to 5 weeks for the fungi.

Identification of the isolates

The fungal cultures were sent to the Commonwealth Mycological Institute, Kew for identification. The bacterial cultures were identified using biochemical and physiological tests outlined by Conn et al (1957). The identification to species level was done by reference to Buchanan and Gibbons (1974).

Effect of temperature on microbial growth

The fungal isolates were incubated on potato dextrose agar plates. The bacteria were inoculated in nutrient broth (Oxoid). The cultures were incubated at 0°, 10°, 20°, 30° and 40°C. Growth was rated visually.

Measurement of growth by mycelial extension in liquid medium was not practicable for many of the fungal isolates, because of their growth habit (see Fig. 1).

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**Microorganisms associated with paint films in cold rooms**

**Results**

The most frequently isolated fungus species on the cool paints were *Cladosporium* (Table 1). Three species of this were isolated, viz.: *C. cladosporioides* (Fres.) de Vries, *C. sphaerospermum* Penz. and *C. oxysporum* Berk. & M.A. Curtis. *C. sphaerospermum* was not isolated on the interior of the cold room.

*Phialophora verrucosa* Medlar was also frequently isolated on the paints on the interior of the cold room. It was absent on the ‘exterior’ paints.

Two pigmented bacteria were found on the paint film (Table 2). These were *Flavobacterium ferrugineum* Sickles & Shaw and *F. intescens* (Migula) Bergey, they have orange and yellow pigments respectively.

The other unpigmented bacterium species were *Bacillus* spp. The identification of the bacterial isolates are shown in Table 3. Most of the microbial isolates found predominantly grew at all the temperatures tested (Table 3). The *Cladosporium* species were the only ones that grew at 0°C; growth at this temperature was very slow.

**Table 1. Frequency (%) distribution of the major fungi obtained from discoloured paints.**

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Walls around cold rooms and air-con. vents.</th>
<th>Cold room wall</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladosporium cladosporioides</em></td>
<td>47.7</td>
<td>62.5</td>
</tr>
<tr>
<td><em>C. sphaerospermum</em></td>
<td>20.5</td>
<td>–</td>
</tr>
<tr>
<td><em>C. oxysporum</em></td>
<td>11.4</td>
<td>6.9</td>
</tr>
<tr>
<td><em>Phialophora verrucosa</em></td>
<td>–</td>
<td>19.4</td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>9.0</td>
<td>–</td>
</tr>
<tr>
<td><em>Penicillium</em> spp.</td>
<td>2.3</td>
<td>–</td>
</tr>
<tr>
<td>Others</td>
<td>9.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Total no. of colonies</td>
<td>88</td>
<td>72</td>
</tr>
</tbody>
</table>
Table 2 Physiological and biochemical properties isolated from coloured slimes on painted walls of cold rooms.

<table>
<thead>
<tr>
<th>Laboratory ref. No.</th>
<th>P01</th>
<th>P02</th>
<th>P03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cell shape</td>
<td>rods</td>
<td>cocobacilli</td>
<td>rods</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxygen relationship</td>
<td>fac. anaerobe</td>
<td>fac. aerobic</td>
<td>aerobic</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Endospore</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methylene red</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>white</td>
<td>orange</td>
<td>yellow</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 10°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid from</td>
<td>Sucrose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Manitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mannose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Trehalose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sorbose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Probable genus</td>
<td>Bacillus sp.</td>
<td>Flavobacterium ferrugineum</td>
<td>F. intecens</td>
</tr>
</tbody>
</table>

+= positive result
-= negative result

Table 3. Growth of the predominant microbial isolates at various temperatures.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladosporium sphaerospermum</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>C. cladosporioides</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Phialophora verrucosa</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Flavobacterium ferrugineum</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F. intecens</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

-= No growth
+= Scanty growth
++= Moderate growth
+++ = Good growth

Discussion

From the extent of discolouration of the paint films, the fungi are more important than the bacteria. The pigmented bacteria were restricted to the interior of the cold rooms. Of the fungi isolated, species of Cladosporium have often been isolated from discoloured paint films (Winters et al. 1975; Ross & Hollis 1976; Barry et al. 1977, Smith 1977a).

The predominance of Cladosporium species on paint films is most probably due to their prevalence in the air spora and their tolerance of cold temperatures. Cladosporum species have been found to be most predominant in the air spora in Nigeria (Dransfield 1966) especially in the humid southern Nigeria (Cammach 1955; Ogunlana 1975; Ayanru 1980). Various species of Cladosporium were isolated by these workers although they were not identified beyond the genus.

Although many other paint infesting fungi such as Aureobasidium sp. and Alternaria sp. were predominant in the air spora and have been isolated from paint films, they were not recorded in this study. The ability of Cladosporium species to grow, though slowly, at low temperatures possibly accounts for its frequent isolation in this study. Strains of C. cladosporioides were also found to grow on meat at sub-zero temperatures (Gill & Lowry 1982).
Microorganisms associated with paint films in cold rooms

*Philophora verrucosa*, which was isolated from the cold room wall, has not been previously recorded from paint films. Its restriction to the cold wall probably reflects its preference for a less humid environment. The source of *P. verrucosa* is problematic, as it has not been recorded in air spora although it has been frequently isolated from soil and other natural habitats (Gezuele et al. 1972; Smith 1977b). It has been found to be a human pathogen (Medlar 1915; Isawton & Miyaji 1978), and has also been found in association with *C. cladosporioides* to cause disease of the human eye (Palack et al. 1976).

Another feature of Cladosporium spp. and *Phialophora* sp. is their ability to tolerate heavy metals such as lead, zinc and aluminium (Smith 1977b), these are common constituents of many paints.

Species of *Flavobacterium* and *Bacillus* have been reported on paint films at mesophilic temperature (Ross & Hollis 1976). This study shows that they can still be a problem at low temperature. The significance of the *Flavobacterium* spp. isolated in this study is the discolouration by the reddish pigmentation while the *Bacillus* sp. is significant in slime formation.

References


Polack, F. M.; Siverio, C.; Brecky, R. H. (1976) Corneal chromomycopsis: double infection by *Phialophora verrucosa* (Medlar) and *Cladosporium cladosporioides* (Fres.) *Annales de Ophthalmologie* 8, 139–144.


Received September 1984
Abstract: The present paper deals with the isolation of fungi from miniature paper paintings and lithographs of the State Museum, Lucknow, India. Twenty three species of fungi were isolated which belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Paecilomyces*, *Alternaria*, *Cephalosporium*, *Chaetomium*, *Cladosporium* and *Trichoderma*.

Percentage relative humidity and temperature of an almirah of reserve collection was recorded throughout the year 1981-82. The peak of average percentage RH (minimum and maximum) 42-78% was recorded in the month of June and temperature 33-35°C (minimum-maximum) in the month of July.

Introduction

Many Indian museums possess a valuable heritage of miniature paintings, lithographs and manuscripts. These works of art are mostly done on paper. The various basic components of paper are cellulose of cotton, linen, straw, wood pulp, waste paper etc. Besides cellulose, paper contains non-fibrous raw materials like starches, pigments and sizes. All these materials are nutrients for the growth of fungi. Being hygroscopic in nature, paper provides suitable conditions for biological attack. In museums a number of miniature paper paintings and lithographs are being deteriorated day by day, due to the long term storage of these paper materials under unsuitable conditions i.e. dust, dirt, optimum temperature and high humidity for the development of fungi.

The problem of micro-biodeterioration is a serious problem in countries with a tropical humid climate (Nair 1972, Agrawal 1977). In spite of the significant artistic and historical value of these paper objects, unfortunately the micro-biodeterioration aspects could not receive adequate attention in India. A research programme was initiated for a 'Survey of fungal flora growing on the works of art in Indian museums', the aim being to have an idea of the various fungal species which we could normally expect to come across in the normal Indian museums. Only when we have this knowledge could we think of evaluating the efficacy of control measures.

For the study of the above project, preliminary studies were initiated in the reserve collection of the State Museum, Lucknow. The Museum has a collection of about 1750 miniatures and about 100 lithographs.

The paper presented here deals with the isolation and identification of fungi and the possible micro-environmental variables of steel almirahs surrounding the objects. Through the study of these factors, the necessary action for the preservation of long term conditions may be improved.

Materials and Methods

About 800 miniature paper paintings and 20 lithographs were examined carefully with the help of a magnifying glass. Samples were gathered from 20 objects.

Isolation and Identification

The traditional methods used in microbiology for isolation of fungi are not appropriate for museum objects because they require the destruction of the object. Therefore a non-destructive method of isolation was used.

One cm small discs of Whatman filter paper No. 40 were sterilized, the filter paper disc was pressed over the affected area and then placed in 5 ml sterilized distilled water, left for two hours and shaken well. Care was taken in sampling that two discs contaminated with the same area or of a different place, should not be used in the same water. 0.5 ml - 1 ml of solution were poured in sterilized petri-dishes and then 10-15 ml of nutritive agar media of Czapek's Dox Agar, Potato-dextrose agar, Oat-meal agar, malt extract agar were poured separately with a small amount of an antibiotic (dicrycistine). Plates were incubated at 28±1°C and fungal growth was observed at different intervals. Pure isolations were carried out on different nutritive agar slants.

Fungal species were identified on the basis of growth of colour, texture and morphology with the help of work done by taxonomists (Raper & Fennell 1965, Pitt 1980, Onions et al. 1981).

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Measurement of humidity and temperature
To have an approximate idea of the micro-environment under which the collection is stored a thermohygrograph was placed in one of the almirahs of the store room and graphs were collected at weekly intervals. The data was recorded throughout the year and an average data of maximum and minimum results of the month was calculated and plotted on a graph (Fig. 1).

Fig. 1. Percentage relative humidity and temperature throughout the year (1981-82) inside the almirah of reserve collection.

Results

During the present study a number of fungal forms were isolated namely:

1. *Aspergillus clavati*ri (Mangin) Thom & Church
2. *A. flavus* Link
3. *A. melleus* Yukawa=A. *queratus* (Bainier) Thom & Church
4. *A. nidulans* (Eidam) Winter
5. *A. niger* v. Tieghem
6. *A. stellatus* Curti
7. *A. sydowii* (Bainier & Sartory) Thom & Church
8. *A. terreus* Thom
9. *A. usus* (Bainier) Thom & Church
10. *A. versicolor* (Vuill.) Tiraboschi
11. *A. wentii* Wehmer
12. *Alternaria alternata* (Fr.) Keissler
13. *Cephalosporium acremonium* Corda=Acremonium strictum W. Gams
15. *Cladosporium cladosporioides* (Fr.) de vries
16. * Fusarium oxysporum* Schlecht.
17. *Paecilomyces variotii* Bainier
18. *Penicillium chermesinum* Biourge
19. *P. chrysogenum* Thom
20. *P. citrinnum* Thom
21. *P. corylophilum* Dierckx
22. *P. frequentans* Westling

Table 1 shows the detailed description of the fungal flora isolated from different objects. It has been observed that among all the isolated fungi the species of *Aspergillus* and *Penicillium* were isolated from almost all the objects.

It will be seen from Fig. 1 that there was a wide variation in the maximum and minimum RH during the year while the fluctuation in the temperature range was not so wide. The peak temperature 33°C-35°C (minimum and maximum) was recorded in the month of July and RH percentage in June was 42-78% (minimum and maximum).
Discussion

The miniature paper paintings generally consist of support, ground and paint layer. It has also been noticed that some paintings were fixed on cardboard with the help of gum or glue. Thus all these layers represent a nutritive medium favourable in the setting of saprophytic fungi particularly under favourable conditions.

The Indian miniature paintings were executed with paint prepared with pigment mostly mineral and glue or gum (Agrawal 1984). Furthermore a coating of sizing material, namely starch solution, was applied to the surface of the paper, which could also act as a nutrient for fungi. For the preparation of thicker papers (known as wasli) several layers of paper were pasted together. The chances of deterioration of paintings are mainly due to the binding material, which serves as a food for fungi. Deterioration of water based poster colour have recently been demonstrated by Dholokia and Chhalpar (1980). Rosa and Strezelecky (1979) demonstrated that the development of micro-organisms first takes place through the natural glues (e.g. starch paste flour, paste, animal glue) and then also cellulose. However, during the present study the fungal attack was observed to take place from the back towards the painted upper surface.

The utilization of cellulose in the nutrition of fungi has been well known for a long time. Eggins and Pugh (1962) have demonstrated the utilization of cellulose by using cellulose agar media. Pietrykowska Le'znicka (1979) found a considerable reduction in the alpha-cellulose content in paper due to the action of fungi and bacteria.

The lithographs studied were covered with glass for their protection. In this case, fungal growth appeared to have generally developed due to the absorption of moisture from the back of the lithographs, which are on cardboard. Penetration of moisture and absorption on cardboard probably takes place from moist walls or from a damp environment. Gradually it comes towards the surface. Thus the microclimate formed in between the lithograph and the glass may have a higher humidity than the ambient humidity which in turn favours the development of various fungal species.

During the present study a number of fungal forms were isolated (Table 1) which belong to the genera Aspergillus, Penicillium, Fusarium, Paecilomyces, Alternaria, Cephalosporium, Cladosporium, Chaetomium, and Trichoderma. All these fungi form colonies of different shades i.e. brown, black, green, pink etc. which gradually extend in area and produce spores, sclerotia and cleistothecia in huge quantities on paintings in the layers of support etc., thus masking the design of the picture (Figs 2-4). One can observe these coloured areas of growth in the monsoon season very easily. Otherwise dried brown spots (like foxed spots) or brown, black dust of spores.

Under optimum environmental conditions, these produce coloured exudation which diffuses into the substratum (Kathpalia 1969, Nuksha 1960 and Kowalik 1980) induce stains difficult and sometimes impossible to remove by the usual restoration methods. The spores of fungi are ubiquitous and they await only the proper conditions of moisture and temperature to grow and reproduce. Kowalik (1980) has mentioned that some species of common fungi are able to germinate at 70% or even lower RH (63-65%).

<table>
<thead>
<tr>
<th>Name of Fungus</th>
<th>Percentage incidence of fungus on miniature paper paintings</th>
<th>Percentage incidence of fungus on lithographs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus Chevalieri</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>A. flavus</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>A. melleus</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>A. niger</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>A. stellatus</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>A. sydowii</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>A. terreus</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>A. usatus</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>A. wentii</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>Cephalosporium acremonium</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Cladosporium cladosporoides</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>Paecilomyces varioti</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>P. citrinum</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>P. coryophilum</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>P. frequentans</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>25</td>
<td>3</td>
</tr>
</tbody>
</table>

Authors are thankful to Dr. R.C. Sharma, Director, State Museum, Lucknow for permitting them to study the objects and the scientists of C.M.I., London for identification of fungi.
Pitt (1975) demonstrated, although in the field of the food industry, that some species of *Aspergillus*, *Penicillium* and *Paecilomyces* need very low water activity (below 0.85) to grow and he referred to these fungi as 'xerophiles'. These studies seem to be equally important in the field of conservation of museum objects and also in the support of our observations. It is quite clear from Fig. 1 that there is a great variation in percentage RH (maximum and minimum) as compared to the temperature during the particular year. The variations in humidity with dust encourages the growth of fungi. Maximum humidity 78% was recorded in the month of June and the temperature was between 30-34°C which seems very alarming. In conditions of RH close to 70%, the process of intense deterioration of materials does not appear but even short periods of more than 70% RH may accelerate the germination of fungal spores.

The present study encourages us to extend our investigations with a view to prevention and control.

Fig. 2 A paper miniature painting affected by fungi

Fig. 3 The detail of a miniature painting showing fungal growth
Fig. 4 Fungal growth in the layers of support of a miniature paper painting

References

Agrawal, O.P. (1977) Care and preservation of museum objects, Published by N.R.L.C., New Delhi at present situated at Lucknow (Government of India Publication).


Received May 1985.
THE ECOLOGY OF PASTEURIZED METALWORKING FLUIDS

R. ELSMORE and E.C. HILL

Abstract: The effect of continuous and batch thermal pasteurization on the aerobic bacterial population of several metalworking fluids is examined.

Batch pasteurization appears to select for certain organisms namely those which are Gram positive, sporing and thermophilic. In the continuously pasteurized system studied these organisms were not selected for in the final sump population, suggesting that they are not competitive with the sump flora although they are more resistant to pasteurization.

Pasteurization may cause an increase in lag period of growth of the surviving population but it did not select for a more thermotolerant population in the example studied. The flora which became established in the continuously pasteurized system lost the ability to degrade hexadecane.

Introduction

The problems caused by microbial contamination and degradation of aqueous metalworking fluids has been well publicised and reviewed by several workers (Bennett 1972, Genner & Hill 1981). In order to control these microbial problems conventional approaches have relied on a combination of good housekeeping and the use of chemical biocides (Hill 1982, Oates 1982). Recently however, due to an increased awareness of the dangers associated with certain biocides, namely toxicity to workers and environmental risk, there has been a tendency to look for safer alternative control measures.

Many alternatives to the use of biocides have been examined in the past. These include Gamma irradiation (Rossmoore & Brazin 1969, Mixer et al. 1969), filtration (Symes & Cowap 1975), centrifugation (Cook 1977), ultrasound (Rossmoore 1974, Wort & Lloyd 1979), aeration (Abu Shaqra 1983) and pasteurization (Christiansen 1979, Rowe 1983, Rowe & Guest 1985, Elsmore 1985, Hill & Elsmore 1983). Of these physical methods of microbial control continuous pasteurization appears to be the most promising.

Continuous pasteurization involves the removal and heat treatment of a slipstream of fluid from the machine sump. The heat treated fluid is then returned to the sump. In such a system for effective microbial control to occur, the net kill must exceed the net growth (Elsmore 1983). The heat treatment will exert a selection pressure in favour of any thermotolerant or sporing organisms that may be present in the fluid and as such should select for a more thermo resistant population. However this does not appear to occur in marine lubricating oil pasteurizers (Hill & Genner 1981).

The typical spoilage flora found in metalworking fluids is dominated by Gram negative rods, particularly Pseudomonas spp. and sulphate reducing bacteria, typically Desulfovibrio spp. Yeast and filamentous fungi may also be significant spoilage organisms, particularly in synthetic, semi-synthetic and biostable fluids. These spoilage organisms may be antagonistic towards certain organisms entering the fluid (Rossmoore & Williams 1967) and in fact, many Gram positive organisms are incapable of surviving in the metalworking fluid environment (Bennett & Wheeler 1954). Thermally selected surviving organisms from the pasteurization process would have to be capable of surviving in and utilizing the bulk fluid when returned to the sump in order to produce significant change in the dominant population of the fluid.

This paper examines the effect of pasteurization on the bacterial flora of two metalworking fluids - one undergoing batch pasteurization treatment and the other having been continuously pasteurized for a period of time. The aim was to determine if a thermotolerant population was selected and if so whether the selected organisms became dominant in the infected fluid sump. A numerical scoring method was used to characterise isolates because it allows incorporation of all isolates into the analysis, unlike conventional identification methods in which many isolates might be excluded because they were not identifiable (Griffiths & Lovitt 1980, Hill 1983). The differentiation of isolates was on the pattern of positive test results. Four sets of tests were used giving a 4 digit number or profile. This is the same method as is used in the API identification system (API systems S.A., Balme Les Grottes, 38190 Montaleau Vercieu, France). Test results were analysed by comparing percentage positive test results for each sample. Each sort was compared to every other sort and the number of character differences calculated to indicate the similarities between the different sorts in each sample. The effect of pasteurization on growth rate and thermal resistance was also examined, to determine if the surviving population differed significantly from the original population.

*Isolates labelled with numerical profiles.

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Materials and Methods

Batch pasteurized sample
A sample of an infected metalworking fluid (5% v/v oil emulsion concentrate in 120 ppm hardness water) was heat treated at 70°C for 5 seconds using a capillary tube technique (Hill & Elsmore 1983). The heat treated sample and a non-treated control were plated on Tryptose blood agar base (TBAB, Oxoid) using the Spiral Platemaker (Spiral Systems, Ohio) using the method of Gilchrist et al. (1973). The plates were incubated at 25°C for 5 days.

Continuously pasteurized samples
The sump of the slipstream pasteurization unit (Fig. 1) was filled with 3% v/v metalworking fluid (a semisynthetic fluid made up in tap water) and inoculated with a heavily infected industrial sample of the same fluid. The first sample was removed after a period of ten days during which time no heat treatment of the fluid had taken place. The second sample was taken immediately after the pasteurization unit was turned on. It was taken between the steam heater and cooler and had been heat treated at 70°C for approximately 15 seconds. The third sample was taken from the sump after 4 months of pasteurization for 8 hours a day (Monday to Friday). All three samples were plated onto TBAB plates using the Spiral plate maker and incubated at 25°C for 5 days.

Selection of isolates
After incubation, colonies were removed from the outermost edges of the plates. This region of the plates represented the highest dilution areas and contained the dominant organisms (Hill 1983). The colonies were transferred to TBAB plates and checked for purity by microscopic examination after 5 days incubation at 25°C. Further subculturing was carried out where necessary until pure cultures of each isolate were obtained.

Characters
Twelve characters were used to describe each isolate. The characters were chosen because they were not mutually exclusive, provided variety and could be conveniently and easily assessed. The ability to grow at 50°C and the presence of spores were thought to be important to determine whether selection of thermophilic or sporing organisms had occurred in the pasteurization process. The test characters used are listed in Table 1.

Spore test
A 2 ml sample of a 3 day nutrient broth (Difco) culture of the isolate in a sterile thin-walled test tube was immersed in a water bath at 80°C for 10 minutes. A 1 ml aliquot of the heat treated suspension was then spread on a TBAB plate using a flamed glass spreader. The plates were incubated at 25°C for 5 days before being examined for growth. Growth was also examined after a 5 day period at 37°C and 50°C. In the isolates at 25°C the presence of a non-diffusible pigment was noted.

Hexadecane utilization
Isolates were streaked onto the surface of Bushnell-Hass agar plates. This was prepared from Bushnell-Hass broth (Difco) to which Noble agar (Difco) had been added (1.5% v/v). The sole carbon source was supplied by n-Hexadecane (BDH Chemicals Ltd.) which was present as a vapour. This was achieved by soaking a sterile 9 cm filter paper (Whatman, Ltd. U.K.) with 1 ml of Hexadecane in the petri dish lid. The plates were incubated in the inverted position for 21 days at 25°C.

The following tests were carried out according to the methods of Cowan and Steel (1974).

- Gram stain – (using Hillie’s (1928) modification)
- Motility – (using the hanging drop method)
- Catalase – (on TBAB grown colony)
- Oxidase – (using Kovacs (1956) method)
- Gelatin liquefaction (Nutrient gelatin method)
- Oxidation and fermentation of glucose. (Hugh and Leifson (1953) method)

Fig. 1 Diagram of continuous pasteurization unit showing steam heated plate heat exchanger (a) and water cooled heat exchanger (b)
Differentiation and isolates

The isolates were differentiated on the basis of the presence or absence of the twelve characters. The characters were arranged into four groups of three (Table 1) and scores of 1, 2 and 4 given to each character according to their position in the table. Each isolate was assigned a four digit number or profile which represented the total scores for each set of characters. These profiles were unique for each combination of positive characters. It is important to note that negative results were not recorded in the final numerical profile. Isolates labelled this way are referred to as sorts.

Table 1 Characters used in the differentiation of isolates.

<table>
<thead>
<tr>
<th>SCORE</th>
<th>1st Digit</th>
<th>2nd Digit</th>
<th>3rd Digit</th>
<th>4th Digit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 or 1</td>
<td>Gram positive</td>
<td>Rod Shaped</td>
<td>Growth at 37°C</td>
<td>Oxidative (H+L)</td>
</tr>
<tr>
<td>0 or 2</td>
<td>Catalase</td>
<td>Oxidase</td>
<td>Growth at 50°C</td>
<td>Non-diffusible pigment</td>
</tr>
<tr>
<td>0 or 4</td>
<td>Motility</td>
<td>Hexadecane utilized</td>
<td>Gelatin liquefied</td>
<td>Spore</td>
</tr>
</tbody>
</table>

As an example of this approach, if an isolate gave the following positive characters it would generate a profile of 6751 thus:

Characterization | Total Scores
--- | ---
Gram negative | 6
Catalase positive | 
Motile | 
Rod shaped | 7
Oxidase positive | 
Utilized hexadecane | 
Growth at 37°C on TBAB | 
No growth at 50°C on TBAB | 
Liquefied gelatin | 5
Oxidative | 
No pigment | 
No spore | 1

Effect of pasteurization on growth rate

One of a pair of flasks containing sterile metalworking fluid was inoculated with a heat treated (65°C for 10 seconds) sample of infected metalworking fluid. The other flask was inoculated with the same infected metalworking fluid which had not been heat treated. Both fluids were then incubated at 25°C in an orbital shaker at 100 rpm for up to 90 hours. The number of viable bacteria per ml was assessed using a spiral plate technique. Two metalworking fluids A and B (both soluble oil emulsion fluids) were subjected to this test.

Effect of pasteurization on thermal resistance

A heat treated sample of infected metalworking fluid (pasteurized at 70°C for 15 seconds) was used to inoculate a 500 ml flask of sterile nutrient broth (Difco). The flask was incubated for 48 hours at 30°C on an orbital shaker (100 rpm). Of this broth 0.1 ml was used to inoculate a 500 ml flask containing 200 ml of sterile metalworking fluid. The flask was then incubated on an orbital shaker under the same conditions as above for 5 days. A capillary tube technique (Hill & Elmore 1983) was then used to determine the thermal resistance of these recovered organisms. A non-pasteurized sample of the same metalworking fluid was also tested to determine if any selection of thermotolerant organisms had occurred on pasteurization.

Results

A total of 439 bacteria were isolated from the samples of which 95 unique 4 digit sorts were generated. Table 2 shows the occurrence of the most common 4 digit sorts. The two fluids contained populations composed of differing sorts with only a small overlap of profiles between one fluid and the other. In the batch pasteurized sample a change in dominant populations can be seen between the treated and untreated sample. In the unpasteurized sample sorts 6740 at 50% and 7850 at 28% were the most abundant profiles isolated. After pasteurization at 70°C for 5 seconds these values fell to 8% and 9.5% respectively and sort 6710 became the most dominant profile at 34%. In the continuously pasteurized fluid samples the sort with the highest occurrence in the original sample was 6550 (with a frequency of 34%) and 6150 (15%).

These sorts were not detected in the sample which had been treated at 70°C for 15 seconds but 6150 contributed 11% of the dominant flora in the sump sample that had been continuously pasteurized for 4 months. In the heat treated sample, 6110 had the highest occurrence (41%) with a significant increase in its frequency compared with the original sump sample of which it represented only 2% of the flora. In the continuously pasteurized sump, sort 6111 had the highest occurrence in which it represented 29% of the isolates.

The changes in proportions of positive reactions to the tests applied can be seen in Table 3. In the sample batch pasteurized at 70°C for 5 seconds an increase could be seen in the percentage positives for Gram positive, growth at 37°C, growth at 50°C and spore test. A drop in percentage positives for gelatin liquefaction could be seen. In the case of the continuously pasteurized metalworking fluid 2, when sorts from the sample heat treated at 70°C for 15 seconds are compared to those of the original sump sample positive test results can be seen as growth at 37°C and 50°C and increase in Gram and spore test results. This was accompanied by a decrease in gelatin liquefaction and hexadecane utilization.
Differences could be seen between the sorts from the continuously pasteurized sump and the original sump. In the pasteurized sump a greater proportion of percentage positive test results occur for glucose, growth at 37°C and the oxidase test, while a decrease was seen in gelatin liquefaction and hexadecane utilization. It is significant that an increase of Gram positive sporforming organisms or organisms capable of growing at 50°C was not detected. Each sort in a sample was compared to every sort and the number of character differences calculated; similarities between the different sorts can thus be shown. Fig. 2 shows the percentage occurrence of sorts with 0-8 character differences for the first MWF samples studied. The pasteurized samples showed a shift towards more test differences suggesting that the survivors of the heat treatment are a more heterogeneous group than the original microbial population of the fluid. The original fluid population appears to be a homogeneous community of similar types (the mode being two test differences). In the case of the second metalworking fluid samples (Fig. 3) the initial sump fluid sample showed a narrow span of only 4 test differences between sorts with a mode of only two test differences. This suggests that the population was composed of a relatively homogeneous community of similar types.

After heat treatment a shift to the right was seen implying that a more heterogeneous population has been selected by the heat treatment. This population did not appear to be a highly structured community but was a random assemblage of organisms that had survived the pasteurization process. The continuously pasteurized sample appeared to be more heterogeneous than the original sample but did not show as wide a span of test differences as the first heat treated sample.

Fig. 4 shows test differences against percentage of comparison for a combination of the sump flora initially and after heat treatment (a), and a combination of heat treated sample flora and final sump flora (b). It can be seen that the final sump flora had fewer test differences when compared to the initial populations than when compared to the heat treated sample. This suggests that the final populations were more closely related to the initial sump population than to the survivors of the heat treatment.

The effect of heat treatment on growth rate is seen in Fig. 5. It can be seen that with the heat treated inoculum a longer lag period occurred. The growth rate in the second metalworking fluid B appeared to be slower after heat treatment. Fig. 6 shows the effect of pasteurization on the thermal resistance of the microbial population of a heat treated and non heat treated metalworking fluid. The results suggest that in this case the survivors were more sensitive to heat than the original population.

Discussion

The numerical profile system used allowed all of the organisms isolated to be incorporated into an analysis of the effect of heat on the microbial population. It also allowed detection of subtle changes of only one test difference which might be thought of as inconsistence in a conventional identification scheme. The susceptibility of such a system to test errors should be noted as reproducibility of test results can vary (Lapage et al. 1973). However, such errors can be expected in any identification scheme.

It is interesting to note that relatively few single differences could be seen when the profiles were compared (Figs 2, 3 and 4) but many of the dominant organisms showed single test differences from each other. It is possible that changes in profiles may not necessarily be due to changes in the dominant population, particularly where single test differences are concerned but may be due to loss of a particular attribute of character due to the heat treatment.

The batch pasteurization experiment showed that a change in the dominant bacterial population of the metalworking fluid occurred in heat treatment. The heat treatment also caused an increased in diversity compared to the no heat treated sample. When the similarities between the sorts was examined it revealed that the normal fluid population was composed of a homogeneous community of similar types. The heat treated sample, however, appeared to be a more heterogeneous assemblage of organisms. The heat treatment seemed to select for certain types of organisms e.g. Gram positive and sparing organisms.

In the continuously pasteurized sample a significant change can be seen between samples following heat treatment. Again an increase in diversity was seen with the sorts selected being less similar to each other than the sorts in the original population. The original population appeared to be composed of a homogeneous community of similar types, the heat treated sample showing no such community structure.

The continuously pasteurized sump sample showed a smaller increase in diversity than the heat treated sample. The sorts were less similar to each other than the sorts from the original sump sample but were more homogeneous than the heat treated sample, suggesting that some community structure was present. An unexpected result was the complete disappearance of hexadecane utilizers. This implies that the new sump flora were not oil degraders.

Changes in the relative numbers of certain sorts could be seen, their numbers altering between the initial and final sample. Certain sorts present in the original sample were not detected in the final sample whilst others not found in the original sample had become dominant. This implies that some succession of the dominant sorts had occurred.

It is significant to note that certain characters selected for in the heat treatment process, that is Gram positive, spore formation and the ability to grow at 50°C, were not seen in the final sump population. This suggests that these organisms, having survived the heat treatment process, were not competitive with the sump spoilage flora. They may originally have been present at transient environmental contaminants rather than true spoilage organisms.

The use of differing holding times in the batch and continuous treatment experiments may have been an additional factor affecting the results. The continuously treated sample received a more severe treatment 70°C for 15 seconds rather than 70°C for 5 seconds in the batch experiment and thus received a greater selective pressure.

The heat treatment of the infected metalworking fluid increased the lag period of the fluid flora when compared to the original population. This phenomenon has been seen in other heat treated cultures (Beuchat & Lechowich 1968, Kaufmann et al. 1959).

The results of the capillary tube heat treatment of the recovered survivors of the pasteurization process suggest that selection of thermotrophic organisms does not occur as the recovered microorganisms appeared more heat sensitive than the original population. The reason for this may be due to incomplete recovery of the heat treated cells causing them to be more susceptible to environmental stress (Harris 1963).

Both authors acknowledge with thanks the technical support of Esso Research Centre, Abingdon and APV in Crawley for the loan of equipment. Richard Elsmore also acknowledges with thanks the financial sponsorship for his post-graduate studentship by Esso Research Centre, Abingdon.
Table 2: Table of occurrence of most common sorts in the batch and continuously pasteurized metalworking fluids.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Batch experiment</th>
<th>Continuous experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MWF 1</td>
<td>MWF 2</td>
</tr>
<tr>
<td></td>
<td>Pasturized at 70°C for 5 sec</td>
<td>Sump</td>
</tr>
<tr>
<td>4110</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4111</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6108</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6104</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>6141</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6150</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>6151</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>6340</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>6350</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>6550</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

No. of isolates in sample: 105 105 91 37 101
No. of sorts: 15 32 14 12 22
MWF = metalworking fluid

Table 3. Percentage of total isolates positive for each of the 12 characters used to differentiate the isolates.

<table>
<thead>
<tr>
<th>Character</th>
<th>Initial Population</th>
<th>Sample after pasteurization at 70°C for 5 seconds</th>
<th>Initial population in sump</th>
<th>Sample after pasteurization at 70°C for 15 seconds</th>
<th>Sump population after 4 months of continuous pasteurization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td>1</td>
<td>7</td>
<td>6.6</td>
<td>11</td>
<td>3.9</td>
</tr>
<tr>
<td>Catalase</td>
<td>100</td>
<td>95</td>
<td>100</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>Motility</td>
<td>96</td>
<td>91</td>
<td>100</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Rod shaped</td>
<td>97</td>
<td>95</td>
<td>100</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Oxidase</td>
<td>97</td>
<td>87</td>
<td>13</td>
<td>14</td>
<td>83</td>
</tr>
<tr>
<td>Hexadecane utilized</td>
<td>92</td>
<td>94</td>
<td>47</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Growth at 37°C on TBAB</td>
<td>33</td>
<td>79</td>
<td>29</td>
<td>70</td>
<td>89</td>
</tr>
<tr>
<td>Growth at 50°C on TBAB</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>100</td>
<td>28</td>
<td>96</td>
<td>19</td>
<td>36</td>
</tr>
<tr>
<td>Oxidative (H+L)</td>
<td>0</td>
<td>2</td>
<td>18</td>
<td>8</td>
<td>68</td>
</tr>
<tr>
<td>Pigment</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Spore</td>
<td>10</td>
<td>20</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of isolates in each sample: 105 105 91 37 101

References

The ecology of pasteurized metalworking fluids

Fig. 2 Character test differences between profiles as a percentage of the total number of comparisons for the batch pasteurization experiment.
(a) Sample 1 non-heat treated - 15 profiles (105 comparisons)
(b) Sample 1 heat treated at 70°C for 5 seconds - 32 profiles (496 comparisons).


Received April 1985.
Fig. 3 Character test differences between profiles as a percentage of total number of comparisons for the continuous pasteurization experiment.

a) Original sump sample – 14 profiles (91 comparisons).

b) Sample heat treated at 70°C for 15 seconds – 12 profiles (66 comparisons).

c) Sample from sump after 4 months of continuous pasteurization – 22 profiles (231 comparisons).
The ecology of pasteurized metalworking fluids

Fig. 4 Character test differences between profiles as a percentage of the total number of comparisons for the continuous pasteurization experiment.

- a) Comparison of original sump and continuously pasteurized sump profiles (435 comparisons).
- b) Comparison of heat treated sample and continuously pasteurized sump sample profiles (378 comparisons).

Fig. 5 Comparison of the growth of heat treated and non heat treated microorganisms in two metalworking fluids, indicating the effect of heat treatment on growth rate. Note the increased lag period of the heat treated inocula. (15% coefficient of variation).

- a - Growth in 5% MWF A at 25°C.
- ▲ - Non heat inoculum - log number of organisms per mL.
- △ - Heat treated inoculum - log number of organisms per mL.
- b - Growth in 2% MWF B at 25°C.
- ■ - Non heat treated inoculum - log number of organisms per mL.
- ○ - Heat treated inoculum - log number of organisms per mL.
Fig. 6  Survival curves for recovered survivors of a pasteurization process (b) compared to survival curves for a non-pasteurized sample of the same fluid (a) showing the increased susceptibility of the recovered organisms to heat treatment. (15% coefficient of variation)

- Effect of 50°C for a period of 90 seconds on log number of viable organisms per ml.
- Effect of 60°C for a period of 30 seconds on log number of viable organisms per ml.
- Effect of 70°C for a period of 30 seconds on log number of viable organisms per ml.
- Effect of 80°C for a period of 30 seconds on log number of viable organisms per ml.

Open symbols are for non-heat treated controls.
FUNGAL DETERIORATION OF DRIED BARLEY MALT IN INTERNATIONAL TRADE

R. N. OKAGBUE

Abstract: All the malt samples supplied by Nigerian Breweries Ltd were contaminated by fungi. The two species Aspergillus fumigatus and A. versicolor were the only ones found in relatively high frequencies. However, A. versicolor was probably located superficially on the grain. The results supplement the findings of other workers and suggest that measures should be taken to control these species.

Introduction

Unmalted dried barley is usually stored at a moisture content of 13% or below. In that condition, its heterogeneous microflora (Flannigan 1969) cannot proliferate and spoilage does not occur. During malting, the grains are steeped in several changes of water and their moisture content increases to up to 45%. In consequence, some fungi (the hydrophilic category) multiply for some time (Flannigan et al. 1982). Their growth stops when the malt is dried (by kilning) to a moisture content of approximately 4%.

Although Flannigan et al. (1982) showed that the microflora of finished malt could grow and cause spoilage during storage in the laboratory for 9 weeks at relative humidities 79-100%, cases of microbial spoilage of malt in international trade seem to be rarely reported. Apparently, spoilage is limited by the low moisture content of malt and by the relatively good storage and packaging facilities in the malt-producing countries (usually European and American). Another possibility is that many developing countries may ignore defects in malt imported from advanced countries because facilities for reporting the defects may not exist. It is clear, however, that genuine reports would be beneficial to all parties interested in the quality of malt used in breweries and other food and beverage industries. This paper reports an investigation of a case of mouldiness in malt imported by a Nigerian brewery.

Materials and Methods

Description and source of the mouldy malt: The sample of malt analysed in this study was supplied to our laboratory by H. Otter, the Technological Controller of Nigerian Breweries Limited, Kaduna, Nigeria. It was contained in a fresh plastic bag and was in the form of 'cake' in which individual kernels were apparently glued together by fungal mycelia. Mouldiness was obvious as a brownish powdery coating with some bluish-green patches on the sample. According to the supplier, many similar 'cakes' of malt were seen when a consignment of imported malt arrived at the brewery (six days earlier) from the sea-port. Neither the country of origin nor the date of arrival at the port was reported. The sample was kept at 10°C and analysed after one week. Before the analysis, malt kernels were carefully dislodged into sterile petri dishes by using a sterile spatula.

Mycological analysis: The agar plate test (Flannigan 1969) was used to isolate the fungi associated with the mouldy kernels. Before plating, one batch of the kernels was surface-disinfected by soaking for 10 minutes in a 3% (w/v) dilution of Milton (Richardson-Vicks Ltd., Egham, Surrey, U.K.), followed by rinsing four times with cold sterile tap water. The surface-disinfected and the non-disinfected lots of malt were then plated (10 kernels in each of ten 9.0 cm petri dishes) on Czapek's agar media containing 3% and 20% sucrose, the latter for isolation of osmophilic fungi (Smith 1969, Mehrotra & Basu 1975). Flame-sterilised forceps were used for plating the kernels, the forceps being allowed to cool between transfers of individual kernels. The test agar plates were incubated at room temperature (approximately 25-27°C) for 6 days. Isolates were purified on media similar to those of isolation and were identified by reference to Smith (1969) and to Gilman (1957).

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Results and Discussion

The percentage of malt kernels yielding specific microorganisms when incubated at 25°C on Czapek agar containing 3% and 20% sucrose are presented in Table 1.

<table>
<thead>
<tr>
<th>Contamination %</th>
<th>Czapek’s agar*</th>
<th>Czapek’s agar + 20% sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus fumigatus Fres.</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A. versicolor (Vuill.) Tiraboschi</td>
<td>4</td>
<td>92</td>
</tr>
<tr>
<td>A. glaucus (group)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* = contains 3% sucrose; s.d. = surface disinfected; n.d. = non disinfected.

Preliminary studies with non disinfected kernels showed that bacterial contamination of the spoiled malt was negligible. Thus, no antibacterial agent was incorporated into the growth media. Some surface disinfected kernels yielded a Bacillus sp. which formed large mucoid colonies in the high sugar medium.

Fungi were the predominant isolates from the sample under test. A preliminary study, in which dust and debris from the malt were sprinkled randomly on the agar plates, revealed presence of Rhizopus stolonifer (Ehrenb.: Fr.) Lind and Aspergillus candidus Link: Fr. The latter seemed to grow appreciably well under high and low sugar conditions but R. stolonifer was severely inhibited by 20% sucrose. The two fungi were not observed subsequently when kernels were plated out on the media, suggesting that they were probably chance or superficial contaminants of the spoiled malt. In contrast, A. glaucus group and Cladosporium sp. which were not observed on agar plates containing the sprinkled materials, were isolated infrequently from plated kernels (Table 1). A. fumigatus and A. versicolor were the only fungi which occurred in relatively high frequencies on or in plated kernels (Table 1) as well as on fragments and debris from the spoiled material. The reduction in recorded frequency of A. versicolor, caused by surface disinfection of the kernels showed that the fungus was probably superficially located on most of the kernels. It was also apparent that the fungus was osmotolerant (shown by its relative luxuriant growth in the high sugar medium). Indeed, growth of A. versicolor in the presence of 20% sucrose often suppressed the appearance and colony-size of A. fumigatus. This fact probably explains the low recorded frequency of the latter fungus on non-disinfected kernels plated on the high sugar-containing medium.

Fungi similar to those observed in this study were isolated from dried barley grain (Flannigan 1969). Thus the present isolates were probably survivors of the malting process. The occurrence in finished malt of the A. glaucus group of fungi and A. fumigatus was shown by Flannigan et al. (1982) and by Gyllang and Martinson (1976); the latter group of workers also implicated A. fumigatus in gushing in beer. Flannigan and co-workers also showed that A. versicolor and A. glaucus group, among others, could proliferate and cause spoilage of malt under experimental storage conditions. Since A. glaucus was isolated only infrequently in this study, it probably played a negligible role in the spoilage of the malt. As finished malt is strongly hygroscopic, it is probable that the moisture content was raised by exposure to moist conditions before or during transoceanic transportation or at the port of arrival in Nigeria before delivery to the brewery, and facilitated spoilage of the malt by A. fumigatus and A. versicolor. In general, the findings of this study supplement previous observations (Gyllang & Martinson 1976, Flannigan et al. 1982) on contamination and spoilage of finished malt by the two fungi and show that the later phenomenon can occur under commercial conditions.

Although the extent of economic loss caused by fungal deterioration of malt imported by developing countries is uncertain, the case described in this paper suggests that suitable sanitary measures should be intensified in maltings to minimise fungal populations in finished malt. It is also clear that suitable precautions should be taken during packaging, shipment and storage at the port of arrival to protect malt from high humidity and other conditions which might cause proliferation of the fungi carried on the kernels.

I am grateful to M.H. Gumel of the Department of Crop Protection, Ahmadu Bello University, Zaria for confirming the identity of isolates of A. fumigatus and A. versicolor.

References


Received May 1985
BIODEGRADATION TEST FOR MICROBICIDES*

J. DE WAART1 and M.M. VAN DER MOST1

Abstract: The closed-bottle-test was modified for determining the degradability of microbicides with widely differing chemical and physical properties by introduction of the following: 1. fixing test concentration at 7 mg COD/litre 2. standardizing the inoculum (1.5 mg dry matter sludge/litre); (sieving and aerating sludge) 3. keeping endogenous oxygen consumption low (2 mg O2/litre) by using nitrate in the medium

The degradability of eleven different microbicides from five different groups was determined: quats, phenols, chloramides, ampho-tensids and dialdehydes. The method is reproducible. The ampho-tensid which is difficult to degrade could be broken down with adapted sludge. Radiochemical investigation demonstrated that two microbicides when degraded, had been mineralized.

Introduction

Several biodegradation tests for organic compounds have already been developed (Gerike & Fischer 1979, 1981). In these methods a test concentration is used of 10 mg to over 50 mg chemical oxygen demand (COD) per litre (Pauli & Franke 1972, Pitter 1976, Bolk 1979, Means & Anderson 1981). These microbicide concentrations are toxic to the bacteria in the test. In official application for approval of pesticides a biodegradation test is suggested using radioactive labelled compounds (Commission Admission Pesticides 1981). However, this is expensive and requires high concentrations.

The present research is based on the closed-bottle-test according to Fischer, a biodegradation test applied to low concentrated chemical compounds, among others microbicides. The Fischer test was developed for saponaceous compounds. The closed-bottle-test including an inhibition test is described in detail in publication sheet L251 of the Commission of the European Communities (EEG-L251, 1984). Biological oxygen demand (BOD) is used as a criterion for biodegradation which can be determined according to ISO regulations. As the closed-bottle-test itself is not suitable to follow the degradation of microbicides, a modification was sought. The test had to be modified in such a way that it would be carried out with microbicides below a concentration of 10 mg COD per litre.

An explanation concerning the correlation between COD and weight for several microbicides had been added to Table 2. In most cases these low concentrations of microbicides are non-toxic to bacteria. The limitation of the new test however is that it can only be used for compounds soluble in water. The most important modifications of the closed-bottle-test introduced in this research (EC-L215 '84) on the detection of biodegradability of microbicides are:

1. determination of a maximum test concentration
2. standardizing the inoculum
3. reducing the endogenous respiration

Industry and public authorities need a rapid standardized and reproducible test for the determination of the degradation of microbicides in connection with the admission examination of new microbicides.

In this paper a description is given of the reproducibility and accuracy of the method developed using six out of the twelve microbicides. Also suggestions are made for a possible further simplification of the test.

Methods and Materials

I. Old method

In an early stage of the research a lower concentration of dry matter in the inoculum was used. Furthermore the old inoculum consisted of three components and, because of filtration through glasswool, showed variation in the sludge content, i.e. bacterium content.

The inoculum is prepared as follows: 30-minute stirring of a mixture of: 50 ml fresh river water, 10 ml active sludge from sewage purification plant, 40 ml tap water and 400 mg garden soil. This suspension is filtered through glasswool. The final concentration of the sludge is 1.7 g ammonium chloride. This produced a too high endogenous oxygen consumption as a result of nitrification.

II. Modified method

The biodegradation test with sludge lasts four weeks. Simultaneously a one-week inhibition test is carried out. (See appendix for details of nutrient solution, inoculum and inhibition test). This test can be used to indicate toxicity in case the compound is not broken down. In the test the microbicide serves as the only carbon source. Consequently, the oxygen consumption is a measure of the degradation of the compound. Every week the oxygen is measured three times. Sodium acetate is incorporated in the test as an easily degradable compound, as a reference.

The water soluble active component of a microbicide (7 mg COD/litre) is added to an oxygen-saturated (9.2 mg O2/litre) nutrient solution together with activated sludge, and incubated in closed-bottles in the dark at 20°C for four weeks. The concentration of the microbicide to be examined is expressed in COD. The inhibition test is started with sodium acetate, so that any inhibition of the biodegradation by this compound is tested with a concentration range of the microbicide. The degradation and inhibition test have controls of sodium acetate without microbicide and blanks with sludge in nutrient solution and with nutrient solution only.

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2 As the term disinfectant does not completely cover the compounds investigated, we will use the word microbicide instead.

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Every week the oxygen content is measured with an oxygenmeter and the degradation and inhibition percentages are calculated.

Apparatus:
A well ventilated incubator of 20°C ± 2°C.
An oxygenmeter for in situ measurement.
63 Narrow-necked brown stoppered flasks of 250–300 ml the stoppers of which are greased with silicone.

The microbicides tested in this research belong to six different groups: quaternary ammonium compounds; phenols; chloroamides; ampho-tensids; chlorhexidincs and dialdehydes. Altogether twelve different microbicides have been examined of which those marked with * were tested according to the old method.

**scientific name** | **trivial name**
--- | ---
- *didecyldimethyl ammonium chloride | quat 2
- *dodecyldimethyl ammonium chloride | quat 3
- *-4-chlorophenol | chlorophenol
- *2-hydroxy-5-chlorodiphenylmethane | hydroxyphenol
- *N-methyl chloroacetamide | chloroamid-2

*quat 4*, an C14 labelled microbicide decylbenzyldimethyl ammonium bromide, had not been included in the reproducibility experiment. With the following microbicides the modified closed-bottle-test is examined for its reproducibility.

**scientific name** | **trivial name**
--- | ---
- alkylbenzyldimethyl ammonium chloride | quat 1
- sodium salt of N-chloro-4-toluenesulfonamide | chloroamid-1
- dodecylamine propylaminoo acetic acid | ampho-tensid
- penteniald | dialdehyde
- 4-chlorophenol | chlorophenol
- 1,8-di(4-Chlorophenyldiguanido) hexane | chlorhexidine

(i) Degradability percentage

The oxygen consumption is a measure of bacterial activity and consequently an indication of biodegradability, because the microbicide is present in the bottle as the only carbon source. The endogenous respiration in the control is obtained by subtracting the oxygen content of the inoculated control from that of the non-inoculated control (C1).

The equation \( C_{to} - C_{t} \times 100\% \) is used for the calculation of the degradability percentage of the microbicide in which:

- \( C_{to} \) = oxygen content in mg O2/litre in bottle of the inoculated blank after time t (in weeks)
- \( C_{t} \) = oxygen content in mg O2/litre after time t (weeks) in bottles with microbicide
- \( \text{COD}_{0} \) = quantity of microbicide in mg COD per litre in bottle at the beginning of the test

(ii) Calculation of inhibition percentage

The inhibition test determines the inhibition of a series of microbicide concentrations on the biodegradability of sodium-acetate after one week. The calculation is done to the formula:

\[ \frac{C_{to} - C_{t}}{C_{to}} \times 100\% \]

of which

- \( C_{to} \) = oxygen content in mg O2/litre in bottle without microbicide and with sodium acetate after time (t)
- \( C_{t} \) = oxygen content in mg O2/litre in bottle with sodium-acetate and a certain microbicide concentration after time (t).

### III. Radiochemical method

The radiochemical research has been carried out according to the slightly modified MT-TNO method (Compan 1977). The research was executed at TNO-CIVO Toxicology and Nutrition Institute by Dr. H. van den Berg. C14 labelled quat 4 and chlorophenol have both been synthesized by MT-TNO Delft and obtained through the mediation of Dr. J.F. de Kreuk. Measurements of the blanks and of the complete experiments have been carried out in three and six fold respectively.

### IV. Adaptation or sludge

The construction of the diminutive sewage purifying plant was performed according to the instruction in the EC publication sheet (EEG-L347 1973).

By increasing the concentration of ampho-tensid by 0.5 COD/litre every 2.5 days equilibrium of sludge to ampho-tensid was reached in 35 days at 7 mg COD per litre influent. The installation was inoculated with OECD-medium, the components of which were added aseptically with a retention time of 3 hours. The sludge was returned every 5 minutes, instead of continuously. Previous to the application of the adapted sludge in the degradation test the installation was supplied continuously for 9 days with influent containing 7 mg COD ampho-tensid per litre.
Results

1. Comparison of degradation percentages of four microbicides according to the old - and final modified method

The old method gives a wide spread of results between the triplicate measurements of the percentage degradation of chloroamide-1,quat 1 and ampho-tensid with a range of at least 70% (Table 1). The middle value of a series of measurements has been taken as the final percentage of degradation or inhibition. With the old method the break down occurs in the 3rd and 4th week and with quat 1 (13% versus 79%) and ampho-tensid (0 versus 36%) reaches a percentage degradation three times as slow as determined by the final modified method after four weeks. Even chloroamide-1 is hardly degradable. Although the percentage degradation of chlorophenol is lower with the old method (71% versus 93%) and starts a week later, a reasonable degradation percentage with chlorophenol is reached with only 7% variation between the triplicate measurements. The modified closed-bottle-test indicates a regularly increasing degradation percentage with small variations (0–20%) in the measurements for three of the four microbicides examined. With this method the degradability of ampho-tensid in the 3rd and 4th week is also demonstrable, although at a low value (36%) and showing marked variations in the results. Besides for chlorophenol (Table 1) the percentage degradation could still be determined satisfactorily with the old method for quat 2 (60% ± 2.9); quat 3 (63% ± 25.5); chloroamide-2 (32% ± 6.7*); 2-hydroxyphenol (75% ± 1.2) and 2-hydroxy-5-chlorodiphenylmethane (69% ± 3.1). Ampho-tensid cannot be easily tested for biodegradability by the new method. However, the experiment with adapted sludge shows a degradation percentage of 82%, as indicated in Table 5.

*See discussion.

2. Reproducibility of the degradation test for microbicides

Table 2 gives a survey of the reproducibility of the degradation test for the following microbicides: quat 1, chlorophenol, chloroamidine, ampho-tensid, dialdehyde and chloroamide-1. The results indicate that the oxygen consumption by the endogenous respiration in the experiment with 6 replicates yield corresponding figures under 1.5 mg oxygen per litre. In the concentration tested chloroamine appears not to be degradable. Table 3 shows that chloroamine still produces an inhibition of 35% and 47% at the lowest test concentration (2.2 mg). As indicated in Table 2, the degradability percentages of chlorophenol, quat 1 (series 1 and 3) and chloroamide-1 (series 1) correspond in the 3rd and 4th week with those of sodium acetate and in the 4th week of the second series with ampho-tensid with adapted sludge (Table 5). Dialdehyde remains below the percentage degradability of sodium acetate in the 4th week with a score of 61% to 64%. The relevant figures for ampho-tensid are still lower: 14% to 36%. Table 4 shows that ampho-tensid can be toxic in the test concentration of 7 mg COD per litre and reaches an inhibition percentage of 56% as the middle value of the three measurements. Apart from the deviation of the figures in the second series of chloroamide-1 in the 3rd week and the irregular breakdown design of ampho-tensid, the degradability percentages of each of the five measured microbicides are close (Table 2). In comparison with the 5.5% standard deviation of sodium acetate the variations in the measurements of series 2 and 3 of chlorophenol and of all three series of ampho-tensid are large in the 4th week: 15% and 16 to 100% respectively. As the degradation of a microbicide is calculated from the results of the measurements in the 4th week, the reproducibility of the degradation tests of the five microbicides mentioned in Table 2 are considered to be satisfactory. The variation between the series of all microbicide lies within 20%.

3. Radiochemical research

A degradability of 82% was reached in four weeks with C14 labelled radioactive chlorophenol. Furthermore, it has been found that the degraded microbicide is completely converted to carbon dioxide gas. The mass-balance showed that hardly any chlorophenol is absorbed by the bacteria.

After 7 weeks C14 labelled radioactive quat 4 is degraded by 92%. Even after three weeks one of the three bottles showed a breakdown of 51%. As with chlorophenol this percentage degradability confirmed earlier results. The mass balance of each of these two labelled microbicides was found to be in good order. The completely degraded quat 4 is converted to carbon dioxide. Furthermore, a very small quantity adheres to the bacteria and remains on the filter (Compaan 1977).

4. Influence of nitrogen in the medium on degradability in the biodegradation test

Table 6 shows the influence of the nitrogen source on the degradability of three microbicides: quat 1, chlorophenol and ampho-tensid. The endogenous oxygen consumption is favourable and remains under 1.5 mg oxygen per litre with and without nitrogen in the medium. In the 4th week the percentage degradation with and without nitrogen of quat 1, chlorophenol and ampho-tensid differs only by 2%, 8% and 10% respectively. It is noticeable that the standard deviation for the four successive measurements per microbicide in one month is larger without nitrogen than with nitrogen in the medium. This difference is also observed with sodium acetate and may be as great as a factor of 8.

It is evident that in the medium without nitrogen the breakdown of chlorophenol is retarded in the 2nd and 3rd week (Table 6). However, after 4 weeks the degradability of sodium acetate is again approaching that obtained in the nitrogen enriched media. The standard deviation of the measurements for quat 1 (under 8%) are even lower than for sodium acetate (6 to 15%) in the 3rd and 4th week. The maximum variation coefficient in the measurements without nitrogen in the medium for chlorophenol is 24% and for ampho-tensid it fluctuates between 36% and 100%, in the 3rd week.
Discussion

The test developed for detecting the degradability of microbicides demonstrates that for five microbicides with sludge from a large sewage works plant reproducible values can be obtained (Table 2). Chlorhexidine appeared not to be degradable. Concerning the remaining six microbicides the degradability was determined according to the old method. The old method described above already deviates in various respects from the closed-bottle-test published in the EC-publication sheet 1984: i.e. a higher dry matter content, a different composition of the inoculum and a difference in the execution of the inhibition test. The final modified test is based on a test-concentration of 7 mg COD microbicide per litre and on an increased sludge content as high as 1.5 mg dry matter. This appeared to be possible only if the endogenous oxygen consumption was decreased at the same time. This is effected by previously aerating the sludge for at least 30 minutes and largely eliminating nitrification by replacing ammonium in the medium by nitrate. In order to standardize the inoculum it was necessary to sieve the sludge through aseptic gauze instead of glasswool. This inoculum can be increased if it is taken into account that 1.5 mg dry matter per litre leads to an endogenous respiration of only 1.4 mg oxygen per litre. This means that the chance of degradability of recalcitrant microbicides can still be increased (Table 2 and 6). In order to control the endogenous oxygen consumption a non-inoculated blank was incorporated as a control. The difference in the oxygen consumption of the inoculated and non-inoculated blank must be under 2 mg O₂ per litre. With an endogenous oxygen consumption of 2 mg O₂ per litre sufficient oxygen is still left in the test bottle to measure the decrease in oxygen as a result of the bacterial activity during the breakdown of the microbicide.

At the beginning of the test the oxygen saturation concentration in the medium in the bottle is 9.2 mg O₂/litre. Variation in the measurements determining the endogenous respiration is small: 1.1 to 1.7 (Table 2 and 6). The variation coefficient lies between 7% and 23%.

The degradability for three series per microbicide in the 4th week varies as follows: under 10% with dialdehde and quat 1, 25% with chlorophenol, under 16% with chloroamidc-1 and 54% with chlorophenol.

Variation in degradability of ampho-tensid is only 5% if adapted sludge is used (Table 5).

Chloroamide-1 releases chlorine. Chlorine is an oxidizing agent responsible for the antimicrobial effectiveness. Chlorine is very quickly consumed by organic material occurring in an inoculum. Therefore the biodegradation of chloroamide-1 is to be ascribed to 4-toluenesulfonamide. Chloroamide-2 releases 24% formaldehyde which corresponds to 40% of the COD of chloroamide-2. The 32% ± 6.7 biodegradation of chloroamide-2 has consequently to be attributed to the easily biodegradable formaldehyde. The irregularities in determining the degradability of chloroamide-1 was probably caused by leaking stoppers.

Presumably, because of its toxicity in the applied concentration of 7 mg COD/litre it appeared that the degradability of chlorhexidine cannot be tested (Table 2). The irregular breakdown of the ampho-tensid is probably also due to its toxicity, as appeared from the results of the inhibition test given in Table 4. With the old method which has a lower degradation-potency caused by a 10 x lower dry matter content of the inoculum, the degradability of the following microbicides could still be determined: 2-hydroxyphenol; 2-hydroxy-5-chlorodiphenylmethane; quat 2; quat 3 and chloroamide-2. The literature on this subject demonstrates the importance of quality and quantity of the inoculum in biodegradation tests (Blok 1984). Better results are obtained in determining degradability with the modified method (Table 1).

Of the twelve microbicides examined only chlorhexidine appeared not to be degradable while ampho-tensid was difficult to degrade. However, if ampho-tensid adapted sludge is used in the test, the degradability is comparable with that of sodium acetate (Table 5). From this it is evident that in cases of microbicides that are difficult to degrade, the test with adapted sludge is applicable. Introduction of an inhibition test may be a suitable means to find out whether toxicity is the reason that a compound is not broken down.

The radiochemical experiment with C₁₄ radioactive labelled quat and chlorophenol was carried out in the present investigation to follow the occurrence of mineralisation of microbicides. The experiment showed that the carbon skeleton of two microbicides is broken down to CO₂ gas. The calculated degradation values for C₁₄ labelled quat 4 and chlorophenol are 92% and 82% respectively, confirming previously established values.

In order to achieve further simplification of the experimental design, investigations have been carried out in which nitrogen addition was omitted (Table 6). The difference in degradability of the microbicides examined with and without nitrogen appeared to be 10% at most. Only with chlorophenol does retardation of breakdown occur as a result of nitrogen shortage (Table 6). Without the standard deviation of the degradation values is in many cases a multiple of that obtained with nitrogen in the medium. A possible simplification of the test can be reached by dropping the nitrification inhibitor allylthiourea from the medium. Potassium nitrate that has been introduced in the final modified medium probably makes this addition superfluous.

Table 1: Comparison of percentage biodegradability of four microbicides obtained according to the old and the modified closed bottle test

<table>
<thead>
<tr>
<th>% biodegradation according to the old method of the closed bottle test</th>
<th>Week</th>
<th>chloroamide-1 measurement</th>
<th>quat-1 measurement</th>
<th>chlorophenol measurement</th>
<th>ampho-tensid measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0</td>
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<tr>
<td>3</td>
<td>0</td>
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<td>9</td>
<td>19</td>
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<td>0</td>
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</table>

% biodegradation according to the modified method of the closed bottle test

<table>
<thead>
<tr>
<th>% biodegradation according to the modified method of the closed bottle test</th>
<th>Week</th>
<th>chloroamide-1 measurement</th>
<th>quat-1 measurement</th>
<th>chlorophenol measurement</th>
<th>ampho-tensid measurement</th>
</tr>
</thead>
<tbody>
<tr>
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<td>67</td>
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<td>66</td>
<td>73</td>
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<tr>
<td>3</td>
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<td>91</td>
<td>94</td>
<td>94</td>
<td>78</td>
<td>79</td>
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Table 2. Reproducibility of the biodegradation test for six microbicides based on oxygen consumption

<table>
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<th>3</th>
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<th>5</th>
<th>6</th>
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<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
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<td>1.2</td>
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Endogenous oxygen consumption in mg per litre

<table>
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<th>3</th>
<th>4</th>
<th>5</th>
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% degradation Na-acetate

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<tbody>
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<tr>
<td>4</td>
<td>72</td>
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<td>77</td>
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</table>

% degradation quat-1

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<td>58</td>
</tr>
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<tr>
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<td>76</td>
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</table>

% degradation Na-acetate

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<th>3</th>
</tr>
</thead>
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<td>0</td>
</tr>
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<tr>
<td>4</td>
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% degradation ammob-tensid

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</tr>
</thead>
<tbody>
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</tr>
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<td>36</td>
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% degradation chlorophenol

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<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
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<td>67</td>
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<td>74</td>
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<td>75</td>
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<td>3</td>
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<td>81</td>
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<td>4</td>
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% degradation chloroamide-1

<table>
<thead>
<tr>
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<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
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% degradation dialdehyde

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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
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</tr>
<tr>
<td>2</td>
<td>54</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
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<td>64</td>
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% degradation chlorohexidine

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<tr>
<th>Week</th>
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<th>2</th>
<th>3</th>
</tr>
</thead>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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1-6 inclusive: days on which sludge of municipal sewage works in Zcist was taken in the period of 6th up to and including 23rd September

Concentrations of chemicals in the bottles of these experiments:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>mg/litre</th>
<th>mg COD/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate</td>
<td>14.55</td>
<td>6.91</td>
</tr>
<tr>
<td>Quat-1</td>
<td>5.51</td>
<td>7.00</td>
</tr>
<tr>
<td>Chlorophenol</td>
<td>4.43</td>
<td>6.96</td>
</tr>
<tr>
<td>Chloroamide-1</td>
<td>6.34</td>
<td>6.97</td>
</tr>
<tr>
<td>Amphotensid</td>
<td>9.91</td>
<td>6.94</td>
</tr>
<tr>
<td>Dialdehyde</td>
<td>7.13</td>
<td>6.94</td>
</tr>
<tr>
<td>Chlorohexidine</td>
<td>5.35</td>
<td>6.85</td>
</tr>
</tbody>
</table>
Table 3  *Inhibition of the biodegradation of sodium acetate by increasing concentrations of chlorhexidine*  

<table>
<thead>
<tr>
<th>Measurement after Week</th>
<th>Concentration chlorhexidine mg COD per litre</th>
<th>% inhibition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>1</td>
<td>3.8</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td>1</td>
<td>6.9</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>1</td>
<td>12.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>21.8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>35</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>39</td>
<td>42</td>
</tr>
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<td>2</td>
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<td>49</td>
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<tr>
<td>2</td>
<td>12.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>21.8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>3.8</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>6.9</td>
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<td>3</td>
<td>21.8</td>
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<td>2.2</td>
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<td>12.3</td>
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</tr>
<tr>
<td>4</td>
<td>21.8</td>
<td>54</td>
<td>65</td>
</tr>
</tbody>
</table>

N.B. Calculation of this % inhibition according the formula:

\[
\text{oxygen content in inhibition test} - \text{oxygen content in control sodium acetate} \times 100\% \\
\text{oxygen content in blank} - \text{oxygen content in control sodium acetate}
\]

Table 4  *Inhibition of bacterial degradation of sodium acetate by increasing concentrations of ampho-tensid*  

<table>
<thead>
<tr>
<th>Week of measurement</th>
<th>ampho-tensid 6.9 mg COD/litre</th>
<th>ampho-tensid 6.9 mg COD/litre</th>
<th>ampho-tensid 12.3 mg COD/litre</th>
<th>ampho-tensid 12.3 mg COD/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 26 32</td>
<td>1 21 26</td>
<td>29 29 29</td>
<td>0 21 24</td>
</tr>
<tr>
<td>2</td>
<td>25 26 32</td>
<td>0 24 44</td>
<td>6 9 15</td>
<td>0 0 22</td>
</tr>
<tr>
<td>3</td>
<td>- 5 10</td>
<td>0 19 22</td>
<td>0 8 9</td>
<td>0 20 24</td>
</tr>
<tr>
<td>4</td>
<td>0 56 88</td>
<td>0 0 6</td>
<td>0 35 51</td>
<td>29 30 34</td>
</tr>
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</table>

N.B. For calculation of percentage inhibition see legend Table 3

Table 5  *Bacterial degradation of ampho-tensid with adapted sludge in the biodegradation test for microbicides*  

<table>
<thead>
<tr>
<th>Week of measurement</th>
<th>% of degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 30 30</td>
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<tr>
<td>2</td>
<td>48 51 58</td>
</tr>
<tr>
<td>3</td>
<td>37 80 80</td>
</tr>
<tr>
<td>4</td>
<td>79 82 83</td>
</tr>
</tbody>
</table>

N.B. Inoculum: 1,5 mg dry matter sludge per litre
Adapted sludge was obtained from a laboratory activated sludge system
Table 6  Influence of nitrogen source on biodegradability of three microbicides

<table>
<thead>
<tr>
<th>Week of measurement</th>
<th>Endogenous respiration sodium acetate</th>
<th>quat I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in mg oxygen per litre</td>
<td>% degradation</td>
</tr>
<tr>
<td></td>
<td>with N</td>
<td>without N</td>
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<tr>
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<td>1,1</td>
<td>1,1</td>
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<table>
<thead>
<tr>
<th>Week of measurement</th>
<th>chlorophenol</th>
<th>amphotensid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% degradation</td>
<td>% degradation</td>
</tr>
<tr>
<td></td>
<td>with N</td>
<td>without N</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
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</table>

Conclusion

A simple and reproducible screening test was obtained by modification of the closed-bottle-test for the determination of the biodegradability of water soluble microbicides. In many cases microbicides that are difficult to degrade appear to be degradable if adapted sludge from a diminutive sewage purifying plant is used.

References

Europese Gemeenschappen (19.9.84) Publikatieblad L 251, 27e jaargang ISSN 0378-7087. Richtlijn van de Commissie van de Raad betreffende de aanpassing van de wettelijke en bestuursrechtelijke bepalingen inzake de indeling, de verpakking en het kenmerken van gevaarlijke stoffen. Bijlage deel C: Methoden voor de bepaling van de ecotoxieiteit, C.6. Degradatie-biologische afbraak; gesloten besproei, p.188.

Received June 1985.
Appendix 1 – Protocol for the degradation test for microbicides

Application
For water soluble microbicides with a final concentration of 7 mg COD per litre.

Material
Incubator of 20°C ± 2°C. Oxygen metre. 63 Narrow-necked brown stopper flasks of 250–300 ml of which 48 are for the degradability test and 15 for the inhibition test.

Dilution liquid
The nutrient solution consists of four components:
1. Phosphate buffer pH = 7
   - Dissolve in circa 500 ml water: 8.5 g KH₂PO₄; 28.4 g K₂HPO₄·3H₂O; 22.2 g Na₂HPO₄·2H₂O and 3.2 g KNO₃ (potassium nitrate).
   - Fill up to 1 litre
2. Solution of magnesium sulphate
   - Dissolve in water 22.5 g MgSO₄·7H₂O and fill up to one litre
3. Solution of calcium chloride
   - Dissolve in water 22.5 g CaCl₂ and fill up to 1 litre
4. Solution of ferric chloride
   - Dissolve in water 0.25 g FeCl₃·6H₂O and fill up to one litre
Demineralized or distilled water of ca 20°C is aerated to oxygen saturation, i.e. 9.2 mg oxygen per litre. Add per litre water 1 ml of each of the above mentioned solutions.

Nitrification inhibitor
Add 0.5 mg allylthiourea to 1 litre dilution liquid.

Inoculum
Take active sludge from the aeration tank of a sewage purification plant of a city with chemical industries. Condition the sludge by aerating and stirring for at least 30 minutes. Sieve the sludge through an aseptic gauze with mesh width 1 x 1 mm. Determine the dry matter of the sludge as follows: filter 10 ml sludge through a glassfibre filter (Whatman GF/C) that has been prewashed, then pre-dried for 30 minutes at 105°C and finally dried again for 30 minutes at 105°C. Weigh the filter with and without dried sludge and calculate the dry weight. Homogenize circa 100 ml sludge with a magnetic stirrer for 30 minutes. Add to the dilution liquid a quantity of sludge corresponding to circa 1.5 mg dry matter per litre. In general this agrees with 1.75 mg oxygen consumption. Although no test condition, the measure of degradability is more reliable if the oxygen consumption of the sludge remains under 2 mg per litre.

Procedure
Set in a degradation/inhibition test, starting with 22 litres air-saturated dilution liquid with allylthiourea. Divide the 22 litres as follows:
1. for non-inoculated blanks: fill twelve 300 ml bottles with 4 litres of the liquid.
   - Add the inoculum to the remaining 18 litres to a concentration of 1.75 mg COD/litre and divide this volume in three portions of 4 litres and one of 3 litres.
2. for inoculated blanks: fill twelve 300 ml bottles with 4 litres of the liquid
3. for control on sludge activity: fill twelve 300 ml bottles with 4 litres of the liquid to which 7 mg COD sodium acetate/litre has been added
4. for the microbicide to be examined for degradability: fill twelve 300 ml bottles, to which 7 mg COD microbicide per litre has been added
For the inhibition test, 7 mg COD sodium acetate was added to 5 litres dilution liquid plus allylthiourea, which are divided in five portions of 1 litre.
5. inhibition test: 1 litre containing 2.2 mg COD microbicide/litre is divided between three bottles
6. inhibition test: 1 litre containing 4.0 mg COD microbicide/litre is divided between three bottles
7. inhibition test: 1 litre containing 7.0 mg COD microbicide/litre is divided between three bottles
8. inhibition test: 1 litre containing 12.7 mg COD microbicide/litre is divided between three bottles
9. inhibition test: 1 litre containing 22.5 mg COD microbicide/litre is divided between three bottles

Formulæ stated in the paragraph “methods and materials” are used for the calculation of the degradability percentage and the inhibition percentage.
ABSTRACTS

BIODETERIORATION - GENERAL

Legislation and Standardization


Experimental procedures, given in the standard, for the determination of resistance to mould growth (Aspergillus niger) of ceramic tile adhesives are assessed and suggested amendments are made.

Health, Hygiene and Pathogens

See also abs 395, 542, 592, 596-597, 602-604, 610


Evidence implicating Aeromonas hydrophila and A. sobria as agents of human gastroenteritis is reviewed under headings of: taxonomy, epidemiology, toxicology, ecology, and isolation and enumeration of foodborne aeromonads. These psychrotrophs are common contaminants of refrigerated animal products e.g. meat, poultry and raw milk.


Facecal samples from patients with diarrhoea (1050), poultry meat from an abattoir and restaurants (70), swabs from the environment of this abattoir (93) and washings of chicken organs and seawage water (15) from the abattoir were examined by direct seeding on a solid selective medium and immersion in a broth enrichment medium (BEM). Of 112 positive samples, 81 (72.3%) were identified by this method and 31 (27.7%) were positive only in BEM. Improvements in isolation rate were greater for faecal (40.0%) and meat (36.6%) samples than for swabs (13.9%) and effluents (20.0%). Campylobacter isolates could be preserved for at least 2 months at 4°C in BEM without antibiotics.


Data on foodborne disease in Canada in 1978 are compared with data for 1977. A total of 836 incidents, comprising 642 outbreaks and 194 single cases, causing illness in 5960 persons was reported for 1978. The number of incidents and cases decreased by 1.3% and 7.7%, respectively, from 1978 to 1979. Like the previous year, Salmonella spp. were responsible for more incidents (67) and cases (2171) than any other agent. Other incidents were caused by Staphylococcus aureus (29), suspect mould and yeast (18), Bacillus cereus (16), Clostridium perfringens (11), B. subtilis (1) and Hafnia alvei (1). No C. botulinum cases were reported. The deaths of 3 persons were attributed to salmonellosis and probable mushroom poisoning. About 23% of foodborne incidents (197) and 38% of cases were associated with meat and poultry. Vegetables, fruits, bakery products and marine products were also important vehicles in causing foodborne disease. Mishandling of food took place mainly in foodservice establishments (38.9% of incidents, 59.3% of cases) and homes (13.3% of incidents, 7.0% of cases). However, mishandling by manufacturers caused some problems including salmonellosis from a cake and staphylococcal intoxication from canned fish and sausages. Narrative reports of selected foodborne incidents are presented.


One hundred and fourteen strains of Bacillus cereus were isolated during presumptive plate-counts from 18 groups of industrialized, non-industrialized, crude or cooked food, belonging to 19 separate classes. Specific presumptive counts ranged from 105 to 6 X 10^2 g or ml. Among these isolates, 13 strains were derived from 3 outbreaks of food poisoning (involving a minimum of 57 people), as determined by the assayed bacteriological quality of the ingested foods. Results suggest that the recommended number of B. cereus per g or ml of food sample should be re-evaluated in Brazil. Furthermore, a wider range of food should be brought under bacteriological sanitary control for this species.
Analysis and Sampling

See also abs. 407, 680

395 Humphrey, T. J.; Cruickshank, J. G. Food surveillance in England and Wales: a role for the PHLS. *PHLS Microbiology Digest* (1985) 2 (1) 16-19 [En, 9 ref.] Public Health Lab., Church Lane, Exeter EX2 5AD, UK.

This article discusses food surveillance by the Public Health Laboratory Service (PHLS) which generally examines food samples at the behest of environmental health staff. Since 1973 the number of food samples tested by the PHLS has decreased while the number of reported cases of food poisoning has recently been on the increase. One possible reason for this is the change in eating patterns with more consumption of fast foods, reheated foods and prepackaged foods. The majority of instances of food poisoning are due to failures in storage, cooking and handling of the food in the home, restaurant or institution. Examination of some foods, prior to their sale to the consumer, would be ineffective in reducing food-borne diseases. It is suggested that a food surveillance program should concentrate on foods that have a high risk of becoming contaminated with pathogens, e.g. cooked meat and poultry, shellfish and raw milk. The high proportion of heated milk products and ice cream samples tested could be reduced. Tests should include detection of pathogens, faecal contamination and total bacterial counts.

**FOODSTUFFS - GENERAL**

See also abs. 824

Spoilage and Infestation

See also abs. 390-391, 686, 833, 836-837

Storage

See also abs. 465, 493, 499, 501, 508, 512-513, 515, 519, 529, 533, 548, 553, 557, 563, 585, 587-588, 600, 624

Techniques

See also abs. 389, 491, 543, 545, 555-556, 561, 565, 572-577, 590, 594, 598, 601, 618, 620


Radioimmunometric and enzyme-immunometric assays were developed for detection of salmonellae in pure and mixed cultures as well as in food samples [soft cheeses, dried milk, chicken livers, chicken carcasses and egg products]. The performance of titanous hydroxide suspension and microtiter plates as the solid phase for the immobilization of microorganisms were compared in these immunosassays. Good correlation existed between results of a standard cultural method for the detection of salmonellae in foods and those obtained from radioimmunometric and enzyme-immunometric assays utilizing titanous hydroxide. However, a high incidence of false-positive and false-negative results with food samples occurred with the enzyme-immunometric assay utilizing microtiter plates. The results provided strong evidence for the merits of substituting titanous hydroxide for microtiter plates as the solid phase for the immobilization of salmonellae for their detection by immunosassays. The immunosassays were rapid and enabled the analysis of a large number of selective enrichment cultures of food samples for salmonellae within 8 h.


Thermonuclease was detected by toluidine blue and DNA agar diffusion techniques. A cured pasteurized milk that had been artificially contaminated with *Staphylococcus aureus* 100 and inoculated with *Streptococcus lactis* + *St. cremoris* starter, and in buttermilk prepared by churning 50 g curd with 70-100 ml water, and in milk prepared from contaminated butter. Thermonuclease was detected in curd and butter­milk containing 1.4 X 10^5 and 1.7 X 10^5 S. aureus/ml, resp., but was not detected in pasteurized buttermilk (pH 4.6) inoculated with 8.8 X 10^5 S. aureus/ml, either initially or after 24 h, by which time the S. aureus count had fallen to 2.6 X 10^5/ml.


The use of a selective medium which permits the enumera­tion of psychrotrophic pseudomonads responsible for the spoil­age of chilled, proteinaceous foods, enables counts to be obtained relatively rapidly from the processed product and thus facilitates the assessment of potential shelf life and the influence of processing procedures on product contamination. Most of the existing selective media effectively suppress Gram-positive bac­teria but vary in their ability to inhibit unwanted Gram-negative psychrophilts. This problem has been largely overcome by the development of CFC medium which is based on heart infusion agar supplemented with 0.5% fucidin, 0.25% cephaloridine, fucidin and cetrimide to give final concn. of 50, 10 and 10 μg/ml, resp. The medium permits quantitative recovery of both pigmented and non-pigmented pseudomonads whilst inhibiting most other organisms. Although designed for use in relation to poultry meat, the medium has also been applied successfully to other food products, including pork, pork sau­ sage, egg products and Cottage cheeses.


Different media for enumeration of microflora in animal products were examined using 53 strains of 40 spp. of bacteria isolated from milk and meat products. For Gram-positive bac­teria, plate count agar containing 0.3% β-phenethyl alcohol was highly selective and could detect low numbers of these types of organisms in refrigerated milk in which Gram-negative bacteria predominate. Desoxycholate agar is recommended for deter­mining Gram-negative bacteria in raw milk and fresh meat; ammonium lactate agar containing 10 μl penicillin/ml was satis­factory for enumerating pseudomonads. For selective enumer­ation of faecal coliforms (Escherichia coli), desoxycholate agar was used with overlaid pour plates and incubation at 45°C for 24 h. A number of selective media, already developed for identi­fication of different types of Gram-positive bacteria (e.g. faecal streptococci, lactobacilli) were also found useful. For yeast and mould counts, potato dextrose agar acidified with tartaric acid, and yeast glucose agar containing chloramphenicol were satisfac­tory for the 65 representative strains tested.


Modifications are described to a previously published tech­nique for quantitative determination of mite populations in animal feedstuffs. In the modified method, the oil phase is kerosene instead of a mixture of kerosene and isopentane (1 + 1), and aqueous industrial methylated spirit is substituted for aqueous ethanol in the aqueous phase. Emulsion formation at the inter­face is considerably reduced by incorporating a pre-extraction stage. Examination of mites on the filter paper is made easier and quicker by staining them with Phenothin B. Trials carried out
with known numbers of mites in samples of dairy feed concentrates indicate mean recoveries of 84–96% throughout the range tested. The results show that the accuracy of the technique is not affected by the level of infestation.


After pre-enrichment in buffered peptone water, 376 samples from chicken carcasses, minced meat, pork sausages, faces of healthy pigs and sewage-polluted seawater were enriched in Rappaport—Vassiliadis medium prepared either 4 d or 6-7 months before use. It was observed that the two media were equally effective for detecting Salmonella spp. (82 positive samples with each medium) and inhibiting competing organisms.


A naturally contaminated dried sample was reconstituted with 3 different methods (1:1 swirl, 1:9 soak and 1:9 rapid rehydration and analyzed for enterococci on m-Enterococcus agar and aerobic mesophilic plate count on plate count agar. The enterococcal counts obtained by the 1:1 swirl and the 1:9 soak methods were 41.6% and 26.5%, respectively, higher than the commonly used 1:9 rapid rehydration method. The aerobic mesophilic plate counts for the 3 systems were not significantly different.


An ELISA technique using a horseradish peroxidase-protein A-S-peroxidase A complexes was developed for the detection of Salmonella colonies on membrane filters. In pure culture, 64 S. spp. tested gave a positive reaction (purple stain). In 22 naturally contaminated food samples, there was an exact correlation between the A and blotter D filter procedure and the ELISA technique (40.9% positives). This technique is simple, requires little equipment and can be completed in less than 2.5 h, thus allowing the detection of S. spp. in foods within 48 h from initiation of sampling.


Three media for isolation of B. cereus from foods were compared: mannitol-egg yolk-polyvinyl (MYP) agar, polyvinyl pyravate-egg yolk-mannitol-bromothymol blue agar (PMBA) and non-selective blood agar. Twenty-six of 45 samples of different reconstituted and incubated dry food products and 18 of 29 samples of milk and cream (incubated overnight) contained B. cereus. None of the media performed significantly better than the others as regards quantitative recovery or selectivity.


Various factors that affect the recovery of S. spp., which include: (a) sample rehydration; (b) period of preenrichment; (c) incubation in both aerobic and anaerobic environments; (d) media composition; and (e) the relative merits of preenrichment and direct selective enrichment are reviewed. Because resuscitation of injured S. cells does not occur during the selective enrichment step and beyond, the effect and interaction of these factors are considered primarily for the preenrichment step of the isolation procedure for S. Five methods recently developed for recovery of coliforms, including: (a) hydrophobic grid membrane filtration; (b) radiometry; (c) electrical impedance; (d) fluorogenic assay; and (e) the Petriell system are also reviewed. Each of these methods may incorporate a step for resuscitation of injured organisms.

Preparation

See also absbs. 494, 510, 534, 541, 551-552, 554, 580, 586, 611


Germination and outgrowth of 3 str. of Clostridium botulinum in PYEG medium were measured by phase contrast microscopy. Reduction in pH from 7 to 5.5 completely inhibited germination of str. 12885A, reduced the extent of germination of str. 62A and had no effect on the extent of germination of str. 53B. At pH 5.5, 225 mg/litre of undissociated sorbic acid had no effect on the germination of str. 53B, while at pH 6.5, 225 mg/litre of undissociated sorbic acid completely inhibited germination of str. 62A and 12885A.

Bacterial toxins

See also absbs. 534, 541, 554, 559


An enzyme linked immunoasorbent assay using a monoclonal antibody (BA11), prepared against C. botulinum type A neurotoxin by fusion of myeloma cells (P3 NS1/1-Ag4-1) with spleen cells from BALB/c mice immunized with the neurotoxin, detected 5 to 10 mouse 50% lethal doses (MLD50) of purified toxin. The antibody was specific for neurotoxin A, did not bind to sodium dodecyl sulphate denatured toxin and bound only weakly to the separate heavy and light subunits of the neurotoxin, suggesting a conformational requirement for the antigenic determinant. The assay was equally sensitive for crude toxin in food. The minimum detectable level was 9 ± 3.1 MLD50/ml in tinned salmon and corned beef.


The preparation of specimens for the detection of thermostable (STa) enterotoxin of E. coli by 73 St3a str., using pieces of agar punched off from the proximity of their growth on Biken medium followed by elution of STa in phosphate buffered saline, revealed that the STa produced is either insufficient or is not eluted in appropriate quantities to be detected by the infant mouse test. The use of other culture media (such as MacConkey, solid-CAYE and nutrient agar) did not improve the efficiency of above method.
Sinica aflatoxin significance. 92.4-95.8% for the alternative procedure. Results from the 95616, major metabolite of aflatoxin 413 456-458 [En, 9 ref., 2 fig., 3 tab.] California AOAC mycotoxins by TLC, GLC and ynivalenol (all eluates were concentrated and spotted on a high performance thin layer chromatographic plate which was then developed in chloroform-acetone (9:1) and/or ether-methanol-water (94:4:5:1) or chloroform-acetone (85:5:1:4). Each aflatoxin was quantitatively determined by densitometry. Recoveries were much lower for the AOAC BF method compared with this simple method and the AOAC CB method.


A method is described for simple and rapid determination of aflatoxins in maize, buckwheat, groundnuts and cheese. Aflatoxin B1 was extracted with chloroform-water and was purified by a Florisil column chromatographic procedure. Column eluates were concentrated and spotted on a high performance thin layer chromatographic plate which was then developed in chloroform-acetone (9:1) and/or ether-methanol-water (94:4:5:1) or chloroform-acetone (85:5:1:4). Each aflatoxin was quantitatively determined by densitometry. Recoveries were much lower for the AOAC BF method compared with this simple method and the AOAC CB method.


The method is based on the AOAC extraction procedure, cleanup of the extract on a silica cartridge, and LC quantitation. Alternatively, a rapid column cleanup procedure can be used. Milk artificially spiked with aflatoxin M1 at 0.05, 0.1 and 0.5 ppm was analyzed using the new approach as well as an AOAC method coupled with LC for quantitation of the toxin. The AOAC method gave lower recovery (85.6-90.7%) of aflatoxin M1 compared with 93.4-99.1% for the new method and 92.4-95.8% for the alternative procedure. Results from the AOAC method had a somewhat smaller standard deviation for replicate analyses than did results of the new method.


The milk collected in the first 24h following low dosing of 14C-aflatoxin B1 contained radioactivity equivalent to 0.45-1.1% of the dose given. The radioactivity in each sample was partitioned into 4 fractions: ether, protein, dichloromethane and water-alcohol. Over 80% of the radioactivity was detected in the dichloromethane fraction, of which over 95% was attributable to aflatoxin M1. No aflatoxin B1 or other known aflatoxin metabolites were detected in any fraction. Of the aflatoxin M1, the major metabolite of aflatoxin B1 in goat milk is aflatoxin M1, and that other metabolites, including conjugates, are of minor significance.


The 174 F. isolates from rice, maize, sugarcane and field soils comprised F. moniliforme [Gibberella fujikuroi] (31%), F. oxysporum [29.3%], F. roseum 'Graminearum' [G. zeae] (26.4%) and F. solani (13.2%). Of the 61 examined for mycotoxins by TLC, GLC and HPLC, 0.21% produced zearalenone, especially F. oxysporum and G. zeae; 3% produced deoxynivalenol (all G. zeae). Only 1 G. zeae isolate (PKH 5-1) produced T-2 toxin at 2.8 mg/kg. Two G. fujikuroi isolates produced moniliformin. Zearalenone-producing isolates were widespread among the crop plants and field soil but only synthesized small amounts of this mycotoxin.


The synthesis of 4-deoxynivalenol (DON) with acetic anhydride/pyridine gave mixtures of di- and tricetly derivatives, which on further refluxing with acetic anhydride were transformed into an isomer. On hydrolysis, it gave a compound isomeric with DON. Samples of the wholemeal baked from fortified and naturally DON contaminated wheat analyzed by gas chromatography/single ion monitoring showed that iso-DON was formed to the extent of 3-13% of the DON present; levels were higher in the crust than in the crumbs. No iso-DON was found in commercially processed wheat-based breakfast cereals made from grain, naturally contaminated with DON at levels ranging from 0.35 to 0.75 mg/kg DON.


Details were performed with 3 aflatoxin-forming isolates of A. flavus from formic acid-treated materials containing aflatoxin, 1 A. flavus isolate from mouldy barley kept for 2 months in an anerobic jar and 1 non-toxic A. flavus str. from the culture collection at the Department. The non-toxic str. and one aflatoxin producer were cultured in salts-sugar-asparagine substrate (SLM) for aflatoxin production and in a specially prepared grass substrate (GS). Formic acid and ammonium formate were added to both substrates, and sucrose in a low amount was added to the grass substrate. The aflatoxin-forming isolate segregated on the grass substrate into 2 different lines, one with high aflatoxin production and one with very low aflatoxin-forming ability, higher growth rate and reduced sporulation, on the SLM substrate. When exposed to sucrose in grass substrate, and formic acid in SLM, the aflatoxin-forming str. were provoked to increased aflatoxin formation. The A. flavus isolate from the anaerobic jar also segregated on the grass substrate, and these segregants were more sensitive to a high dose of formic acid. It is concluded that in these A. flavus strains there seemed to exist different lines, depending on cultivation conditions. In the remaining 2 aflatoxin-forming isolates such segregation tendencies were not very marked on any substrate.


Data on the fungal flora of 6676 samples of food, classified into 26 types of food, are presented. From the data, indicator sets of fungi are proposed for the assessment of fungal deterioration and mycotoxicogic risk.


Zearalenone was converted to zearalenone-6'-carboxymethylxylidine and conjugated to bovine serum albumin and poly-L-lysine for use as immunogen and solid-phase marker, respectively. Immunization of rabbits with the bovine serum albumin conjugate resulted in zearalenone antibody titres of 108% produced moniliformin. Zearalenone-producing isolates were widespread among the crop plants and field soil but only synthesized small amounts of this mycotoxin.
lysine solid phase and then determining the bound rabbit immu
moglobulin with goat anti-rabbit peroxidase conjugate. Reac
tivity range for zearalenone in the resulting competition
curve was between 1 and 50 ng/ml. Reactivities of this antize
rum for α-zearalenol, β-zearalenol, α-zearalenol and β-zearala
nol were, respectively, 50, 12, 6 and 3% of that found for
zearalenone. By using the competitive indirect ELISA, zeara
lene was identified in four of six egg samples that were
analyzed.

418 HITOKOTO, H.; MOROZUMI, S.; WAKUE, T.; KURATA, H.
Distribution of mycotoxin-producing fungi in marketing foods
in Japan. In Toxigenic fungi — their toxins and health hazard
[edited by Kurata, H.; Ueno, Y.]. Tokyo, Japan; Kodansha
Ltd.; Amsterdam, Netherlands; Elsevier (1984) 15-23 [En, 2
ref., 5 fig., 1 tab.] Tokyo Metropolitan Res. Lab. Public Health,
Tokyo 160, Japan.

The mycological examination of 6676 food samples over 6
years led to the creation of 3 sets of mycological indicators for
the evaluation of fungal deterioration and mycotoxin contami
nation in marketed foods.

419 WICKLOW, D. T. Ecological approaches to the study of
mycotoxicogenic fungi. In Toxigenic fungi — their toxins and health hazard
[edited by Kurata, H.; Ueno, Y.]. Tokyo, Japan; Kodansha
Ltd.; Amsterdam, Netherlands; Elsevier (1984) 33-43 [En, 2
ref.]

The author reviews his studies, including some not yet
published, on (1) the role of the sclerotium as primary inoculum
in the life cycle (colonization of pre-harvest cereals) of Asper
gillus flavus and Eupenicillium ochroalsonemum; (2) an
attempt to identify biological and nonbiological environmental
factors determining whether a fungus (A. flavus) invades
the developing seed and contaminates its tissues with mycotoxins;
(3) the geographical variation and infraguild distribution of
mycotoxins from the evolutionary ecologists' perspective of
selective forces shaping fungal chemical defense systems; and
(4) below ground storage of seed by desert rodent granivores
compared with man managed seed stores.

420 LEISTNER, L. Toxigenic penicillia occurring in feeds and
foods. In Toxigenic fungi — their toxins and health hazard
[edited by Kurata, H.; Ueno, Y.]. Tokyo, Japan; Kodansha
Ltd.; Amsterdam, Netherlands; Elsevier (1984) 162-171 [En,
30 ref., 6 tab.] Federal Cent. Meat Res., 8560 Kulmbach, Ger
man Federal Republic.

Data from a study of 1451 Penicillium isolates from foods
and feeds are summarized. The isolates represented 42 spp.
and 1011 isolates were shown to be toxigenic in brine shrimping
and chemical assays. Investigations by the author and co-workers on
an outbreak of suspected Penicillium toxiocosis in sows, the presen
ce of ochratoxin A in blood and kidneys from healthy pigs
slaughtered in W. Germany, the production of mycotoxins in
salmi and raw ham and by isolates of Penicillium roquefortii and
P. camembertii used for cheese manufacture are also summarized.

421 NISHIJIMA, M. Survey for mycotoxins in commercial
foods. In Toxigenic fungi — their toxins and health hazard
[edited by Kurata, H.; Ueno, Y.]. Tokyo, Japan; Kodansha
Ltd.; Amsterdam, Netherlands; Elsevier (1984) 172-181 [En, 2
fig., 13 tab.] Metropolitan Res. Lab. Public Health, Tokyo 160,
Japan.

During Nov. 1977-June 1982 approx. 1500 food samples
were examined for mycotoxins. Aflatoxins were detected in 11
of 610 samples of beans, aflatoxins and chlorin were found in
several samples of maize (imported from Thailand or Burma),
aflatoxins and aflatoxicol were found in 6 of 54 samples of
pistachios, ochratoxin A was found in several samples of rye
flour (all of one brand) and aflatoxin M1 was found in 17 of 79
natural cheese samples (of 281 dairy product samples)
examined.

422 POMLAND, A.; THORPE, G.; SPAIN, J. The analytical
chemistry of deoxynivalenol. In Toxigenic fungi — their toxins
and health hazard [edited by Kurata, H.; Ueno, Y.]. Tokyo,
Japan; Kodansha Ltd.; Amsterdam, Netherlands; Elsevier
FDA, Washington, DC 20204, USA.

Methods for the detection and estimation of deoxynivalenol
are outlined. The increasing need for inexpensive screening
procedures is stressed. Approaches which show promise are the use of
tandem mass spectrometry and an enzyme immunoassay.

423 CHU, F. S. Immunochemical studies on mycotoxins. In
Toxigenic fungi — their toxins and health hazard [edited by
Kurata, H.; Ueno, Y.]. Tokyo, Japan; Kodansha Ltd.; Amster
dam, Netherlands; Elsevier (1984) 234-244 [En, 57 ref., 3 tab.]
Dep. Food Microbiol. Toxicol., Univ. Wisconsin-Madison,
Madison, WI 53706, USA.

The development of radioimmunoassay and enzyme-linked
immunosorbent assay techniques for the estimation of aflatoxin,
kojic acid, ochratoxin A, rubratoxin B, sterigmatocystin, T-
2 toxin and zearalenone in grains and biological fluids is
reviewed.

424 WHEATLEY, C.; COCK, J. Methods of aflatoxin analysis
— with particular reference to cassava samples. [Correspondence.
International Journal of Epidemiology (1985) 14 (1) 158-165] [En, 8 ref.]

With reference to a paper by J. Bulatao-Jayme et al. (Emph,
Institutul de Igiene, Bucuresti, Romania (1984) 16, 89-95
Tygerberg 7505, South Africa.

The procedure described proved to be extremely sensitive and
reproducible. Chromatograms of extracts from maize, groundnut butter, sorghum malt and duckling mash are
presented illustrating the value of the procedure for confirming
the presence of aflatoxins B1 and G1.

425 GIMENO, A.; MARTINS, H. M.; OUKAININ, J. S.
[Research on mycotoxins in baby foods.] Pesquisa de microtox
inos em produtos para alimentação infantil. Repositório de
Trabalhos do Laboratório Nacional de Investigação Veteriná

Mycotoxins were not detected in any of the 172 samples of
baby food examined by thin layer chromatography.

426 THIEL, P. G.; STOCKENSTRÖM, S.; GATHERCOLE, P. S.
Aflatoxin analysis by reverse phase HPLC using post-column
derivatization for enhancement of fluorescence. Journal of Liq
uid Chromatography (1985) 8 (1-10) 303-310 [En, 37 ref., 3 tab.
Tygerberg 7505, South Africa.

The technique has a detection limit of 0.03 mg/kg for T-2
and T-1 toxin in maize.
The use of a number of trimethylsilylating and trifluoroacetylamidation agents for gas chromatographic analysis of mycotoxins. A C18 LC column interfaced with a TCDS (trifluoroacetyltrimethylsilyl ether, neocitralanil and diacetoxyscirpenol) and type B (nivalenol, deoxynivalenol and fusarenon X) trichothecenes were compared. The application of trifluoroacetic anhydride, in comb with sodium bicarbonate, was found to be the best choice.


Optimum conditions for the gas chromatographic (GC) separation of these derivatives and their analysis by negative ion chemical ionization (NICI) mass spectrometry were determined. Characteristic ions for the specific detection and accurate quantification of these derivatives were chosen. Results indicated that pentfluoropropionyl (PF-P) derivatives are better suited for the analysis of simple trichothecenes using the GC-NICI-MS technique. Ultra trace (0.5-2 pg) amounts of these PFP derivatives were detected by the developed procedure.


A method which allows the sensitive analysis of the 2 mycotoxins in a single run after a simple acid-base partition clean-up is described. The detection limits were 10 ng/g for citrinin and 40 ng/g for ochratoxin A. The analysis time was less than 10 min.


Capillary gas chromatography, following the purification of extracts using Sephadex LH20, was more accurate and sensitive than packed column gas chromatography for the analysis of vomitoxin (deoxynivalenol).


Trichothecenes, including verrucaricin J and trimethylsilyl derivatives of verrucarin A, roridins A and E, saurotoxins G and H and baccharin B5 (a plant metabolite), were separated and identified by gas chromatography-mass spectrometry using a short capillary column with on-column injection.


Results of surveillance for deoxynivalenol contamination of home-grown and imported cereals during 1981-83 carried out in MAPP's own labs. and the philosophy behind the series of research projects which were initiated and contracted by MAPP with other research organisations are summarized.


The coefficient of variation of the ELISA method was determined on 6 replicate extracts of a barley sample and a compound feed from 3 lots of ochratoxin A and was found to be approx. 14% in both cases. The technique was compared with a qualitative TLC method and with a quantitative HPLC technique.


The time-sequence of formation of xanthomangin and violacein in Penicillium viridicatum and P. cyclopium isolates from a barley sample collected from a case of mycotoxic porcine nephropathy [RMVM 19, 396] was investigated.


An ELISA based technique for the rapid screening of aflatoxin B1 present in milk by using a monoclonal antibody specific for aflatoxins is described. Detection limits for aflatoxin M1 in milk were 0.1-20 ng/ml.


Biosynthesis of aflatoxins in the concentrate by Aspergillus flavus was max. at RH 85-95% 10°C. The accompanying microflora (staphylococcus, Bacillus cereus, Escherichia coli and Penicillium cyclopium) inhibited aflatoxin formation to some extent. It is concluded that Soviet standards for conserving the concentrates makes them harmless for the consumer.


Fusarium isolates obtained during a 1982 survey of wheat in UK were investigated for mycotoxic production (deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2 toxin (T-2), neosolaniol (NEO) and zearalenone (ZEA)) by thin layer chromatography and gas chromatography or high performance liquid chromatography. Technical DON by 8 F. culmorum isolates and 2 F. graminearum [Gibberella zeae]; ZEA, 25 F. culmorum, 3 C. zea; DAS, 1 G. zeae, 17 F. poae, 2 T. triticiun; T-2, 8 F. poae; NEO, 4 F. poae.


A 12.5 kg sample of whole grain maize and an 11 kg sample of soybean meal, both shown to be contaminated with approx. 50 µg/kg zearalenone, were used to demonstrate the distribution of the mycotoxin in a sample. Each sample was divided into 100 sub-samples and the level of zearalenone in each was measured. The distribution of zearalenone in 40 × 110 g samples of soybean meal was shown to be normal at a mean value of 50 µg/kg with a variance of 77. The distribution of zearalenone in...
100 x 125 g samples of maize was best described by a log-normal distribution when the mean value was 52 µg/kg with a variance of 141.26. It is concluded that for soybeans the proposed sampling technique of 3 x 4 kg aggregate samples each composed of 20 x 200 g incremental samples is adequate, but that for maize the 5 aggregate samples should be bulked together to give a 12 kg sample composed of 60 x 200 g incremental samples. For maximum accuracy more than one 12 kg aggregate sample should be used.


A bioassay based on the cytotoxic effects of mycotoxins on mammalian cultured cells is described. A range of mycotoxins was tested and the bioassay was found to be sensitive for trichothecces, up to a max. sensitivity of 0.003 µg/ml for roxidin A.


Fungi isolated from mould-spotted foods, including Aspergillus, Penicillium, Cladosporium, Rhizopus, Mucor, Alternaria and Wallemia spp., were screened for toxigenic activity by bioassays involving cell lines, brine shrimp, bacteria and pea seeds. Approx. 50% of the isolates were toxic by 3 or more bioassay techniques.


The antiserum was used in an enzyme linked immunoassay for the estimation of sterigmatocystin in barley.


The risks of obtaining cyclopiazonic acid (CPA) synthesis from heterokaryons between A. oryzae mutants affected in their ability to produce CPA are evaluated. These CPA-defective strains, widely used in the food industry, but it is concluded that the risks of producing the mycotoxin from 2 CPA-defective individuals are not high.


Crop mycotoxins found in Queensland are aflatoxins (A. flavus, A. parasiticus, A. ochraceus), zearalenone (Fusarium spp.), deoxynivalenol and other trichothecces, Alternaria metabolites, Diplodia metabolites, and those of Claviceps and Pithomyces; the mycotoxoces associated with these have not necessarily been seen in Australia. The influence of season, weather and geographical region on fungal and mycotoxin production is discussed.

ANIMAL FEEDS
See also abts. 400, 417, 420, 425, 435, 441, 589


A survey on aflatoxin contamination of some poultry feed ingredients, conducted in Andhra Pradesh during winter of 1981-82, indicated that groundnut cake (GNC 1st grade), contained more total aflatoxin, 587 µg/kg, than did maize 71, pearl millet 38, broken rice 43 and rice polish 23 µg/kg. The GNC samples collected from the coastal region had more aflatoxin, 974 µg/kg, than had those from Rayalaseema region, 573 µg/kg, and Telangana region, 215 µg/kg. Maize from Rayalaseema, the coast and Telangana had aflatoxin 99, 62 and 53 µg/kg, respectively. No consistent trend was observed in the other feedstuffs. The storage of samples for 2 months significantly increased the aflatoxin content in GNC and maize but not in the other ingredients.


On oats, barley and wheat grains stored for animals, F. poae predominated (1.11%), followed by F. graminearum [Gibberella zeae] (0.53%) and F. culmorum (0.18%). In 1984, it was very wet in late summer, F. avenaeomaceae predominated in some areas.


Aflatoxin B1 was detected in 66% of 101 samples of feeds and feed ingredients at up to 26.7 mg/kg. Of the 13 feed ingredients analysed, only groundnut oilcake contained aflatoxin B1, (0.33-26.7 mg/kg).


The use of high performance liquid chromatography or thin layer chromatography for the determination of ochratoxin A, citrinin and other mycotoxins in feeds are compared.


The risks of obtaining cyclopiazonic acid (CPA) synthesis from heterokaryons between A. oryzae mutants affected in their ability to produce CPA are evaluated. These CPA-defective strains, widely used in the food industry, but it is concluded that the risks of producing the mycotoxin from 2 CPA-defective individuals are not high.

GRAIN AND PULSES

See also abs. 431-432, 434, 441, 448, 714, 716, 740, 748-750, 752-754


Infestation of stored grain by insects and mites in the United Kingdom and their control are reviewed and discussed. The principal insect pests are Oryzaephilus surinamensis, Sitophilus oryzae, Tribolium castaneum, and the most harmful mite species are Glycophagus destructor [Lepidodiplosis destructor] and Acarus siro; however, these mites can only breed in grain with a moisture content of at least 14%. The topics dealt with are damage and loss, treatment with new chemicals (including chlorpyrifos-methyl, fenitrothion, methacrifos, cefitrothion and pirimiphos-methyl) in place of malathion, and the development of strains of insects resistant to the pesticides used.


The occurrence of insect pests in grain and seed stores was studied in the Wielkopolska region of Poland in 1976-81. The most frequently occurring pests were Tribolium confusum, Laemophloeus sp., Sitophilus granarius and Anagasta kuehniella [Ephestia kuehniella]. S. oryzae and T. confusum were found frequently, and a further 16 species occurred sporadically.

455 OSMAN, N. Assessment of damage by the rice moth, Corcyra cephalonica Scop., on different grains at four moisture contents. Pertanika (1984) 7 (3) 53-58 [En, my, 10 ref.] Department of Plant Protection, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor, Peninsular Malaysia.

Damage caused by Corcyra cephalonica on millet, sorghum, millet and rough rice stored at 50, 60, 70 or 80% RH in Peninsular Malaysia was determined on the basis of damaged kernels, percentage dry weight loss and dry weight of frass and webbing. The 4 rates of relative humidity did not affect any of these parameters. Rough rice was the least damaged of all, and few larvae survived. The percentage of larvae surviving to the adult stage increased between grain moisture contents of 11.1% and 13.2% but decreased at 14.1% in millet, sorghum and rough rice. In millet rice, the percentage of surviving larvae was significantly lower at the lowest than at the highest moisture content.

The development period of the insects in millet, sorghum and millet rice was significantly longer in grains with the lowest moisture content.


The grain mycoflora of sorghum, wheat, maize and barley from Giza, Shammar, El-Bahah, Zahran and Taif were investigated. At post-harvest a higher incidence of fungi was recorded for sound, non-disinfected grains than from grains disinfected with mercury chloride. In addition, problems with plant on PDA or on blotters at 23–25°C for 5-8 d, although percentages of isolation of associated fungi were approx. similar. Common genera (Alternaria, Aspergillus, Curvularia, Drechslera, Fusarium and Penicillium) varied in incidence on non-disinfected cereal grain samples. Spp. of Rhiotus, Cladosporium and Stemphylium were detected only infrequently. Curvularia spp. prevailed on discoloured white sorghum grains of high moisture content obtained from Giza. After 6 months' storage (at 22-25°C) of sound cereal grains from different localities Aspergillus, Fusarium, Drechslera and Penicillium spp. were frequently detected on disinfected grains. Alternaria alternata, Aspergillus flavus, A. niger, A. ochraceus, Curvularia lunata [Cochliobolus lunatus], F. moniliforme [Gibberella fujikuroi] and F. oxysporum constituted the most abundant spp., in comparison with unidentified spp., at post-harvest and after storage.


During 1980-82, deoxynivalenol (DON) was detected in the Ontario soft winter wheat crop (av. 0.5-3.1 p.p.m.), the Quebec hard spring wheat crop (av. 0.3-3 p.p.m.), and grains from the Maritime provinces (av. 0.13-0.47 p.p.m.), but there was no problem with Western Canadian wheat. New guidelines were established in 1983 for DON in soft wheat and derived non-staple food products. Mean concn of DON in domestic maize were 0.2-0.62 p.p.m.


Methods for the analysis of F. mycotoxins are appraised. Using multiple ion detection mass spectrometry, surveys for deoxynivalenol have been carried out in the United Kingdom and results are summarized for findings during 1980-83, for "home-grown" wheat, barley, oats and imported maize. Low levels were fairly common in home grown cereals, > 0.1 mg/kg being found in only 4% of all samples analysed. Higher levels and incidence were observed in EEC and N. American grains. Maize samples from S. Africa were remarkably free of contamination but maize from USA and Europe was universally contaminated with 0.1-1.4 mg/kg (av. 0.5 mg/kg). The fate of deoxynivalenol during milling and food processing is discussed.


Deoxynivalenol (DON) and nivalenol (NIV) were detected in 153 of 205 samples of wheat and barley samples examined (74.6%) and occurred sporadically at approx. equal levels in 61.5%, regardless of crop year, habitat, breed or variety. Levels of DON or NIV were > 2 p.p.m. in 36 (17.6%) of samples, > 1 p.p.m. in 57 (27.8%) and > 0.3 p.p.m. in 96 (46.8%) in barley. Zearealenone and bakuchiol were also observed. Commercial parched-barley flours were also contaminated with DON (27-85 p.p.b.) and NIV (37-190 p.p.b.).

Barley, Oats, Rye

See also abs. 435-436, 444

A simple and rapid radioimmunoassay for the quantitative determination of ochratoxin A in barley is described. ["C"]-Ochratoxin A, with a specific activity of 130 Ci/mmol, was used as the tracer. Toxic levels below 100 ng/ml required a cleanup step. Three methods (the Association of Official Analytical Chemists cleanup method, the solvent partition method and the Extremit 3 column cleanup method) were compared.


The development of the mycota of barley during commercial malting was monitored. Kilning of green malt reduced the viable inoculum. Plants of the toxigenic Fusarium spp. reached 8 x 10^7 propagules/kernel, and Geotrichum candidum and Aspergillus clavatus also developed rapidly during malting. The presence of damaged or pregerminated kernels and the incidental introduction of finished malt contributed to the growth of A. clavatus in green malt; elevated malting temp. led to the rapid development of A. clavatus.

Maize

See also abs. 409-416, 425, 428, 747


Seed germination, visible mould, number of fungal propagules, and ergosterol content were similar in undamaged and crowed-damaged maize kernels inoculated with 3 spp. of the A. flavus group. Cutting the pericarp over the embryo and, to a lesser extent, removing the pedicle significantly increased mould development and reduced germination, but the genotypes were affected differently. Kernels of two susceptible and two resistant genotypes inoculated with 3 spp. Maintained their differential responses whether undamaged or damaged with an abradmal cut. Excising the pedicle, cutting the pericarp over the embryo, or puncturing the embryo, respectively, resulted in increasing amounts of propagule development by P. and decreased seed germination. Despite the enhancement of mould development by seed damage, the resistant genotypes were more resistant than the susceptible genotypes. This differential response was associated with a second cut with P. in which the pedicle was excised or the hilum was pierced. The rankings of stored ground kernels, as determined by numbers of propagules of P. differed from the rankings of stored whole kernels.


Large quantities of ethanol are produced annually in USA from the ranks of stored whole kernels. Kernels from control and inoculated ears were removed from the rankings of stored whole kernels.


Depletion of sugar and starch carbon sources and concomitant formation of secondary fungal metabolites, aflatoxin and kojic acid, were examined in growing maize inoculated with A. flavus. Kernels from control and inoculated ears were removed and analyzed after 16, 24, 48, 96 and 168 h. Reducing sugars were not significantly different for inoculated and control non-inoculated samples, but after 168 h (7 days) starch content was 20% lower in inoculated than in control samples. Kojic acid was detected before aflatoxins formed. Kojic acid, the oxidized product of kojic acid and aflatoxin were all present in samples 2 days after inoculation. It is concluded that the formation of this oxidation product may influence toxin levels.

Rice


Since the rice harvesting was conducted manually during this survey in Mar.-June 1981, several factors such as lack of labour, delay in harvesting and threshing, improper threshing and handling, and the cultivar planted, were important in contributing to both harvesting and threshing losses (0.12-1.54% and 3.48-7.42% respectively). Losses during milling were mainly related to grain breakage. During the hulking process only 0.7% of the grains were lost. Storage losses were significantly higher in large mills (av. of 22.8%) than in small mills 2-5%. These differences were due to the amount, storage time and storage methods employed by the millers.

Wheat, Buckwheat

See also abs. 410, 414, 440, 480, 732-733, 746


A new approach to heat treatment of cereals and milling products for the elimination of insect pests using a hot-air fluidized bed or fluid lift is described on the basis of studies carried out in laboratory and pilot-scale tests showed that this process gave complete mortality of the species and stages tested (all stages of Sitophilus oryzae and S. granarius, larvae of Anagasta kuehniella [Ephestia kuehniella], Tenebrio molitor and Trogoderma granarium and adults of Orzyaeptihosphurea surinamensis) with an exposure of less than 1 min to air temperatures of 60-180°C. The temperature of the substrate during exposure did not exceed 72°C and was always followed by rapid cooling. Treatment did not adversely affect the baking quality of the flour obtained from treated or soft wheat or the technological properties of hard wheat or semolina, provided that the air temperature did not exceed 120°C. The cost of heat treatment was comparable with that of fumigation and was affected on cereals whenever insecticidal treatments are unsuitable.


Albumins, globulins and gliadins were extracted from field-sampled gms of wheat grain. The carcase fragments were freeze-dried and then added to the residue after protein extraction, or to the ground wheat grain. Adults of Tribolium confusum and larvae of T. confusum, Trogoderma granarium and Anagasta kuehniella [Ephestia kuehniella] were placed on the prepared food. The residue after protein extraction was inadequate for complete development of larvae of all species despite protein fractions being added. Larvae did not grow on such food although they remained alive for a very long time. Adults of Tribolium confusum lived shorter times and laid less eggs on the residue after extraction than on ground wheat.

468 Dunkel, F. V.; Pu, Z. L.; Chuan, L. Wheat grain storage by rural producers in southern China. Tropical Science (1985) 25 (2) 103-115 [En, 13 ref., 5 fig.] Department of Entomology, University of Minnesota, St. Paul, MN 55108, USA.
Twenty-one bulks of winter wheat, totalling about 87 tonnes, were surveyed in temporary storage before complete drying in southern China. The grain, in bamboo huts or baskets or on the floor of the granary, had less than 1% dockage and foreign matter but high levels (21%) of damaged kernels, mostly shrivelled. Live adult insects were present in about 75% of the samples taken, the most common species being Sitotroga cerealella, Sitophilus spp., Cryptolestes purpureus, Tychaena stercorea and Caropholis sp. were also quite common. Mites, but not psocids, were detected in more than 47% of the samples, and 3 of 4 bulks that were heating were heavily infested with mites. Insect traps (brass probes) were twice as effective as spear samples for the detection of infestation.

469 Nawrot, J. The susceptibility of grain various wheat varieties and cultivars to the post-harvest infestation by granary weevil (Sitophilus granarius L.). *Prace Naukowe Instytutu Ochrony Owocow* (1981, recd. 1985) 23 (2) 137-141 [En, pl, ru, 14 ref., 1 fig.]

The resistance of grain of 8 varieties and 10 clones of winter wheat and 5 varieties and 8 clones of spring wheat to damage by adults of *Sitophilus granarius* was studied in the laboratory and in the field. Significant differences in feeding activity, female fecundity and rate of progeny development on the various grains were observed. Grains with a resistance index higher than 7.5 were colonized and destroyed much more slowly than those with a resistance index lower than 6.0.


Embelin, an extract of the powdered berries of *Embelia ribes* (a shrub native to India) was mixed with wheat grain at concentrations of 0.1875-0.75%. It caused mortality to adults of *Tribolium* castaneum after treatment even at the lowest concentration. No chemosterilant action or contact toxicity against adults was found. Germination of the wheat was not significantly impaired even after treatment at the highest concentration.


Investigations into the losses of stored wheat caused by insects were carried out in central Anatolia, Turkey, in 1980-81 under natural conditions in different villages, for different kinds of storehouses (mud-brick or concrete) and for different periods of storage (8-11 months); wheat samples were kept outdoor in hard wheat and 0-105.5 insects/kg soft wheat. In the first year, loss of germinability averaged 23.8% after 8 months, and in the 2nd year it averaged 3.8% in hard wheat after 8 months and 5.5% in soft wheat after 9 months. Damaged grains averaged 9.6% after 9 months in the 1st year and 2.1% in hard and 2.6% in soft wheat after 11 months in the 2nd year. Weight losses after 9 months, calculated on the basis of 1000 grains, averaged 3.6-22.7% in the 1st year and 6% in hard and 1.9% in soft wheat in the 2nd year.

472 Chelkowski, J.; Visconti, A.; Goliński, P.; Kwaśna, H. [Vomitoxin and zearealenone production by Fusarium isolates from certain wheat grains.] *Zeszyty Naukowe WSI w Lublinie* (1981) 23 (2) 137-141 [En, pl, ru, 23 ref., 1 fig.]

During 1980-1983, 149 strains of *Fusarium* (16 species) were isolated from autoclaved wheat grains and examined for their ability to produce deoxynivalenol and zearealenone. Deoxynivalenol [up to 80 mg/kg of wheat grains], 3 acetyl-deoxynivalenol (up to 208 mg/kg) and zearealenone (up to 750 mg/kg) were produced by 43 strains of *F. culmorum* and 2 of *F. graminearum*.

Pulses

See also abts. 721, 738

473 Holloway, G. J.; Smith, R. H. Inheritance of the ability of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to feed and breed on yellow split-pea (*Pisum sativum*). *Bulletin of Entomological Research* (1965) 75 (2) 367-375 [En, 16 ref., 3 fig.] Population Biology Laboratory, Department of Pure and Applied Zoology, University of Reading, Whiteknights, Reading, Berks, RG6 2AJ, United Kingdom.

*Sitophilus oryzae* is a pest of stored cereals and, because the most populations are unable to survive on yellow split-pea and other legumes, admixture of legume with cereal has been suggested as a control method. However, some geographical strains have been found to be able to feed and breed on yellow split-pea. The ability is heritable and was found to be controlled in a strain by a single recessive, autosomal gene. The inheritance pattern of the ability was investigated in 3 different geographical strains, 2 of which could feed and breed on yellow split-pea and 1 which could not. The genetic mechanism controlling the character in these strains was not a single recessive allele, but differed between the 2 pea-breeding strains and was co-dominant with ability to feed on yellow split-pea in the non-pea-breeding strain. The results indicate there is considerable between-strain genetic variability for the character which may render cereal-legume mixture of little value as a control method against *S. oryzae*.


In studies in Taiwan, polyethylene bags containing 600 g of mung bean (*Vigna radiata*) or adzuki bean (*V. angularis*) seeds were treated with gamma-radiation at a dose of 10 krad for the control of infestations with *Callosobruchus chinensis* and subsequently stored for 3 months in an incubator at 25 ± 2°C. The numbers of weevils per bag, infested seeds per 100 seeds and eggs per 30 seeds following treatment were significantly lower than those in untreated bags. The cooking quality and sprouts produced from mung bean seeds was unaffected by the irradiation treatment.


Application of 20 g captan or 10 g thiourea/kg soybean seeds did not prevent the development of fungi under the seed coat of whole seeds or the increase in free fatty acids during storage at 85% R.H. At 65 and 75% R.H. the increase in free fatty acids and fungal development were lower than at 85%, but fungicide treatment was still ineffective. Fungicide application to split soybean seeds significantly reduced the free fatty acid content. The major fungi isolated from stored soybeans were *Aspergillus candidus, A. ruber, A. versicolor* and *Penicillium cyclopium*, all of which showed lipolytic activity. Incubation of intact seed with these fungi did not cause an increase in free fatty acids.


Current information is reviewed, with special reference to work on the mycflora of groundnuts and pecans and factors which favour invasion of seeds by Aspergillus flavus and the production of aflatoxin.


486 In olives and olive cakes, Penicillium spp. predominate, followed by Aspergillus, including A. flavus and A. ochraceus. Although some of the A. isolates are highly toxigenic on
favourable media, none of them has been found to secrete high concn of mycotoxins on olives. However, aflatoxin B1 or ochratoxin A have been detected in some samples of olives and crude olive oil.

COFFEE, COCOA AND TEA

Coffee


A mycological study was carried out on 11 samples of imported green coffee beans. The mould flora of the beans consisted mainly of Aspergillus flavus, A. fumigatus, A. niger, A. tamarii, Eurotium herbariae, Penicillium chrysogenum, and P. citrinum. Nine isolates of A. flavus were examined for aflatoxin production on rice and coffee bean media. Only one str. produced 1.1595 mg/kg and 58.69 µg/kg of aflatoxins on rice and coffee bean media, respectively. The existence of this coffee mycotoxicogenic str. indicates that green coffee beans are not always an unfavourable substrate for aflatoxin production.


The effect of incubation at 20%, 25%, 30%, and 37°C and an initial water content of 15, 20, 30 or 40% on growth of A. parasiticus and aflatoxin production on green (regular or decaffeineated) coffee beans was investigated. Caffeine inhibited synthesis of aflatoxins B1 and G1 but had practically no influence on fungal biomass (determined indirectly by measuring the chitin content). After incubation at 28°C for 35 days aflatoxin B1 concn was reduced by 10-14% and at 37°C by 15-22%, relative to peak levels obtained. Under the same conditions aflatoxin G1 concn was reduced by 12-18% at 28°C and 20-28% at 37°C.


The correlation of mycelial growth with accumulation of aflatoxins on decaffeinated and regular roasted coffee beans in stationary culture at 20-37°C and 15-40% water content was studied. The growth of biomass was monitored by measuring chitin concn and aflatoxins were measured with a Horoden­

BEVERAGES

See also abst. 463

491 Littel, K. J.; Larocco, K. A. Biomimetic standard curves for quantitative determination of yeast contaminants in carbonated beverages. Journal of Food Protection (1985) 48 (12) 1022-1024 [En, 8 ref., 1 fig., 2 tab.] Food Applications, Packard Instrument Co., Downers Grove, IL 60515, USA.

A simple model system is described for generating standard curves relating yeast ATP to conventional colony forming units (CFUs). Biomimetic standard curves were generated by spiking commercial cola or diet lemon-lime samples with Saccharomyces rouxii ATCC 36141. Yeast cells were concentrated onto filters under vacuum and ATP was subsequently extracted from the cells for analysis. Correlation coefficients for each S. rouxii standard curve indicated strong linear relationships between ATP and CFU levels (r=0.90). A composite standard curve (r=0.97) of data collected from all the S. rouxii-spiked studies predicted yeast levels from spiked cola samples in later experiments. When predicted yeast CFU values were plotted against conventional yeast CPU values for 3 different yeast types, a correlation coefficient of r=0.82 was obtained.


D. naardenensis is described to accommodate the ascogenous state of B. naardenensis ATCC 22075, the type culture of B. naardenensis which was isolated from aerated lemonade in the Netherlands, is designated as the type. The vitamin-enriched Endothia complete agar medium with a pH of 4.5 is most effective for inducing ascospore formation.

FRUIT AND VEGETABLE JUICES


The production of patulin by P. [Penicillium] expansum growing in apple juice was determined over a range of pH. Patulin was found to be produced in large quantities over a narrow range of pH from 3.2 to 3.8. The biomass produced increased with increasing pH. The changes in pH due to growth of the organism were found to be small.


Radiation inactivation of the ascospores of 3 str. (M 68-79, NRRL 1125 and NRRL 26414) of B. fulva suspended in apple juice was investigated. Whereas the ascospores of str. M
68-79 were significantly more sensitive to ionizing radiation, those of strs. NRRL 1125 and NRRL 2614 did not differ significantly from one another in this respect. High numbers of ascospores of the more resistant strs. required an absorbed dose of approx. 7.2 kGy (95% confidence interval 6.7 to 7.9 kGy) for inactivation; a decimal reduction dose of approx. 1.2 kGy was estimated for these strs. Ascospores of str. NRRL 2614 were confirmed as more radiation resistant when a small proportion survived an absorbed dose of 5 kGy and spoiled apple juice within a 3-month storage period. Although it was possible to inactivate B. fulva ascospores at absorbed doses of <10 kGy, it is probable that flavour impairment of apple juice, as well as cost currently limit the feasibility of this process.

**FRUIT AND VEGETABLES**

*See also abst. 697*


The following aspects are reviewed and discussed: production technology, quality at harvest; improved storage environment; chemical conservation; direct post-harvest application of nutrient solutions; irradiation; innovations in packaging and containerization; and newer processing technology.

**496** SOMMER, N. F.; MITCHELL, F. G. Irradiation as a possible treatment for fruits. *Citrograph* (1985) 70 (3) 56-59 [En] Department of Pomology, University of California, Davis, USA.

The potential of gamma-rays for controlling pests in fruit and vegetables in the USA, especially California, is discussed, and it is concluded that it is unlikely that either the capital investment or construction time would be available to permit rapid expansion of radiation facilities in a crisis.

**497** BROWN, R. E. Approved quarantine treatments: the Florida situation. *Citrograph* (1985) 70 (3) 64-65 [En] Department of Plant Industry, Florida Department of Agriculture, USA.

A table is presented showing the requirements for quarantine certification of a wide range of fruits and vegetables grown in Florida against various insect pests, together with the control measures on which such certification is based.


Regular surveys of the markets in 1980-84 revealed 78 postharvest fungal diseases on 36 vegetables. Of 23 gen. isolated, Alternaria and Fusarium spp. caused the most infections.


The suitability of packaging house operations on vegetables (*Brassica* spp., *Vigna sesquipedalis*, cabbage, Chinese cabbage and tomato) prior to storage in rooms of the Humifresh system were tested. Operations such as sorting, trimming, wiping with moisture cloth, washing, drip drying after washing and precleaning were very useful in reducing deterioration during storage. With proper packaging house operations, the above problems could be kept for 1, 2, 3 and 2 weeks, and c. 70% (w/w) of the produce being acceptable for human consumption. Losses were mainly due to water evaporation, physiological damage, wilting, yellowing, chilling injury, bacterial soft rot and anthracnose.

**500** SEALY, L. H. Post harvest losses — what are they? Are they important? How to prevent them — [An overview]. *Journal of the Agricultural Society of Trinidad & Tobago* (1985) 85 (2) 12-16 [En, 1 fig.]

The high losses sustained in the Caribbean region from post harvest disorders and diseases, estimated at 30-40% of annual output of fruits, vegetables, roots and tubers are emphasized and the necessity of introducing measures to reduce these losses is noted.

**Pome fruit**


Several growth regulators were applied on different dates to William's Bon Chretien and Duchesse d'Angouleme trees over a 4-year period. The fruits were stored at 0 to 1°C for 60 days. Treatment with Alar (diaminozide) at 1500-3000 p.p.m. 3 weeks after fruit set resulted in the least storage losses and good fruit quality. Treatments with ethephon (500 and 1000 p.p.m.), GA3 (100 and 200 p.p.m.) or 2,4-D (100 and 200 p.p.m.) gave unsatisfactory results.


In 2-year trials, Golden Delicious apples were dipped in several fungicide solutions and stored at -1°C and 85-90% RH for 6-7 months. Treatment with benomyl at 0.1% or thiabendazole at 0.2% reduced best the occurrence of *Penicillium expansum* and *Botrytis cinerea*. By the end of storage the residues of all fungicides had decreased to a level well below acceptable limits.

**503** MAHABIR SINGH *[Preliminary studies on toxtricid caterpillar Cacoecia epicyrtata (Meyr) feeding on apple fruit. Pesticides* (1984) 18 (11) 34 [En, 1 ref., 1 fig.] Regional Fruit Research Station, Mashobra, Simla-171 007, Himachal Pradesh, India.

The damage caused by the larvae of *Cacoecia epicyrta* (*Archips micaceus*) on apples in the field and in storage at Simla, India, is described. The infestation was found to increase from 5-6% in the field in August to about 40% in the stored fruits in October. Feeding damage began at the calyx end and was characterized by fine webbing and the removal of small patches of peel; at a later stage, larger portions of peel were consumed and the larvae entered the pulp. Preliminary spraying of trees in the field before harvest with malathion at 0.5% helped to prevent fruit infestation during storage.


The fungus was isolated from infected apples after harvest from the field and 24-48 h after removal from a cold store. Microsclerotina and chlamydospermae were observed in old, diseased cultures on 2% yeast extract medium.


The fungus (thiabendazole), Rosol (pentachlorophenone) and Baviastin (carbendazim) completely checked blue mould (*Penicillium expansum*) for up to 7 d, while Delon (*dithianon*), Diolatan (*capitox*), Rosol and thiabendazole were equally effective against *Rhizopus* sp. Black rot (*Sphaeropsis malorum* or *Botrytis cinerea obiusa*) was completely inhibited by all the test
fungicides except Sapro [triforine] up to 7 d. Thidiazonazole was the best treatment against all 3 rots and capitil the least effective.


Conidia of *Botrytis cinerea*, *Mucor piriformis*, *Penicillium expansum* or *Phialocephala maltorum* did not germinate after exposure to 54.4 °C for 25 min. Heating dump-tank water to 54.4 °C for 20 min in a packinghouse lowered populations of *M. piriformis* and *P. expansum*. Conidia in water from pear packinghouse dump tanks containing high populations were killed by heating to 54.4 °C for 25 min. Heating dump-tank water to 54.4 °C for 20 min in a packinghouse lowered populations of *M. piriformis* and *P. expansum*. Costs to heat were 77% less than costs to empty, clean and refill the 11 355 litre tank.


The populations were determined during 2 seasons in air and dump-tank water of 9 apple and pear packinghouses. Popu­lations of all fungi varied considerably among packinghouses. Spores of *B. cinerea* were more abundant in air and dump water than spores of *B. encrucii* or *M. piriformis*. Spores of *M. piriformis* and *P. expansum* have a greater potential for spreading than spores of *B. cinerea* in dump water, suggesting that decaying fruit stored in bins and processed later in the season increased propagule levels more than did debris brought into packinghouses from orchards early in the season. When selected isolates were characterized for pathogenicity and virulence on Anjou pear fruit and for toler­ance of benomyl, 60, 72 and 89% of *B. cinerea*, *P. expansum* and *M. piriformis* isolates, respectively, were pathogenic and the per­centage of pathogenic *P.* isolates tolerant of benomyl increased later in the season. Benomyl-tolerant isolates of *P. expansum* were less virulent than benomyl-sensitive isolates. The percentage of benomyl-tolerant *P.* isolates in the Mid-Columbia region of Oregon has not increased during the last 5 yr.


*Aspergillus niger*, *Rhizopus arrhizus* and *Penicillium digitatum* were responsible for most of the decay of apples in commercial market. Other isolates from rotting fruits included *Pythium ultimum*. Combined chemical treatment and storage in a cold room extended storage life, but wax coating of the fruits was ineffective.

509 GULLINO, M. L.; MEZZALAMA, M.; GARIBALDI, A. Activity of different fungicides against dicarboximide and/or benzimidazole resistant strains of *Penicillium expansum* and *Botrytis cinerea* on apple. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent (1985) 59 (3b) 1217-1225 [En, 8 ref., 2 fig., 1 tab.] Istituto di Patologia Vegetale, Univ. Torino, Italy.

The efficacy of 11 fungicides, employed as dipping treat­ments, alone or in combination, was evaluated on apple cv. Golden Delicious. Inoculated with spores of both fungi sensitive and resistant to dicarboximides and/or to benzimidazoles and kept at 25 °C or 3°C. Benzimidazole did not control benzimidazole-resistant strains, but of the dicarboximides tested, metconazole showed good activity against doubly resistant strains, of *B. cinerea*, the most important biocontrol antagonists of *P. expansum*, *stagonospora*, *DPX H6573* and imazalil gave the best control of sensitive and resistant strains of each pathogen.

Tropical and Subtropical fruit


Post-harvest treatments (ethephene or fungicides and/or storage conditions) accelerated ripening and colour development; and produced fruit of high quality as measured by various quality parameters. The treated fruits contained a higher level of carotenoids than tree-ripened fruits and were practically decay free. Lycopene accumulation was stimulated in tissue treated with chlorophenothio-triethylamine, Holding at 41°C and 90% ± 1% RH for 24 h before dipping in 230 p.p.m. ethephone for 1 minute and 27°C and 90% ± 1% RH, was the most efficient method of fruit ripening.


Fruits treated with calcium nitrate or calcium chloride were held at room temperature and 65% RH for up to 9 days. The least weight loss occurred in fruits treated with 1.5% cal­cium nitrate. The effect of treatments on fruit chemical composi­tion was also determined. The data are tabulated.


Changes in air composition of the storage atmosphere and of the internal atmosphere of the fruit as affected by the ventila­tion rate were studied with the grapefruit cv. Marsh and the orange cultivars Shamouit and Valencia. These changes were examined in relation to fruit weight loss, ethanol content of the juice and rot development during storage periods of up to 5 months. Rates of ventilation affected the CO₂ concentration in the atmosphere more than the O₂ levels of both the external and internal atmospheres of the fruit. In small-scale tests, ventilation rates as low as 10% h⁻¹ of the empty volume of the storage space did not cause major changes in the gas composition, nor did they affect fruit quality adversely. In commercial tests, however, an increased rate of ventilation (70 to 100% h⁻¹) was needed to effect similar results. A reduction in the ventilation rate in commercial citrus storage rooms from 150 or 200% h⁻¹ (the rate now commonly employed) to 100% h⁻¹ is recommended. It is suggested that the reduced ventilation rate will help lower the costs of refrigeration while maintaining good fruit quality.


Oranges (cv. Belladonna), sprayed post-harvest or immersed in 1500-3000 p.p.m. thidiazonazole for 3 min. *Penicillium* spp. control, had significantly higher residues of thidiazonazole in the peel than in the albedo. Immersion gave higher residues than spraying, but residue levels were less dependent on applica­tion rate and were similar whether the samples were taken immediately after treatment or 10 weeks later. All residue levels were below the critical limit allowed in Italy.

In saturation, the duration of pretreatment required was shorter than 20°C at 10°C. Loss of citric acid during storage was less after pretreatment at 20° than at 10°C, and although there was more decay at the beginning of the storage period in fruits pretreated at 20° there was less decay towards the end of the period. Peel colour was of a deeper orange colour with 20°C pretreatment and there was no loss of citric acid content. [For part I see Horticultural Abstracts 54, 5822]


All the oils tested were antifungal to Aspergillus niger. The best control was given by pure mustard oil (90.7%), followed by dudia (88.76%), groundnut oil (87.19%), coconut oil (82.33%) and linseed oil (74.04%). Fruits treated with coconut oil became discoloured within 24 h and started rotting.


Infected fruits showed a decrease in total soluble solids, ascorbic acids, percentage acidity and total phenols. The accumulated in infected tissue. Traces of alanine were found in of the 3 main fungal rots of kiwifruit in New Zealand is


Current knowledge of the symptoms, etiology, and control of the principal fungal rots of kiwifruit in New Zealand is reviewed. Field rot, caused by Sclerotinia sclerotiorum, affects immature fruits on the vines. Storage rot, caused by Botrytis cinerea, affects harvested fruits during cold storage. Ripe rot, caused by Botsryosphaeria dothidea, affects harvested fruits during post-storage ripening.


Problems in the continued use of fungicides for control of postharvest pathogens on citrus are discussed. A strategy for decay control tested and compared in 3 cent. California packhouses involved a multifaceted programme of packhouse redesign, daily sanitation and regular monitoring of P. spores in the atmosphere. Results indicate that the isolation of spores by segregation of decayed fruit can be more effective and economi­cally efficient than increased fungicide use or additional attention to cleansing and sanitation alone.


Among isolates from rotten fruits in Ahmadabad market, Aspergillus niger and Rhizopus arrhizus caused the most dam­age. Storage life was extended by wax coating of fruits and storing at low temp.
Grapes

See also abst. 510


Benomyl tolerant isolates of B. cinerea, G. [Monographella] nivalis and M. fructicola proved more sensitive to diphenylamine than benomyl-sensitive isolates. The diphenylamine EC50 values of the tolerant isolates decreased with increasing temp. Grapes treated with diphenylamine were susceptible to the benomyl-sensitive isolates of B. cinerea. Control of the disease induced by benomyl-tolerant isolates depended on the residue levels of diphenylamine on the grapes. Grapes treated with technical diphenylamine contained higher and organic acids in fruits of grapes than nonwrapped tomatoes. Decay development due to Alternaria rot depended on the residue levels of diphenylamine on the grapes. The effects of an imazalil-impregnated film with ethylene film depended on the residue levels of diphenylamine on the grapes. Storage losses, firmness, colour and decay development of dinosaurs of fruit rot diseases of Alternaria solani, Botryodiplodia theobromae and P. truncatus were individually wrapped in heat-shrinkable plastic films and stored for 1 or 2 weeks at 13°C, then stored at 21°C for 7 days. Wrapped tomatoes had significantly less weight loss and were firmer than nonwrapped tomatoes. Decay development due to alternaria rot [Alternaria solani or A. tomato], bacterial soft rot [Erwinia carotovora subsp. carotovora] and Monilinia fructicola [Oospora lactis=Geotrichum candidum] was similar for nonwrapped and wrapped tomatoes during storage and subsequent holding. External colour development was the same for nonwrapped and wrapped tomatoes, but internal colour development was greater for nonwrapped tomatoes than for wrapped tomatoes when stored for 1, 2 or 3 weeks at 13°C, or for 1 week at 13°C plus 7 days at 21°C. C2H4 and CO2 concentrations in the periphery of wraps were similar in the two films tested, Cysar EHC-SO-copolymer film and HDPE-100 density polyethylene film.


The peppers (Capsicum annum) were treated with a chlorine and imazalil dip, or either individually wrapped in an imazalil-impregnated heat-shrinkable plastic film or left unwrapped, then held in storage and examined for decay after 1, 2 and 3 wk at 7.2°C and a further 5 d at 15.6°C. Bacterial soft rot (BSR) was the most prevalent decay identified but fungal decay caused by Alternaria sp. and Botrytis sp. developed to a lesser extent following 3-wk storage. No treatment: effectively controlled the development of BSR. The imazalil film alone and imazalil dip (500 p.p.m.) alone significantly reduced the incidence of fungal rot but film wrapping increased the incidence of BSR compared with nonwrapping after 3 wk at 7.2°C + 5 d at 15.6°C. The imazalil film and imazalil dip in combination was more effective in reducing fungal decay than each. Colour development was slowed after 3 wk at 7.2°C + 5 d at 15.6°C and pod softening was consistently retarded in wrapped treatments.

Root vegetables

See also abst. 424


In 3-year trials with the cv. Giant der Stuttgarter the plants (from sets or seting) were grown at different NPK rates and after harvest the bulbs were stored at 1 to 10°C for up to 240 days. The least storage losses occurred in bulbs grown from seed and fertilized with N, P2O5 and K2O at 80:40:40 kg/ha.

530 HODGES, R. J.; MEIK, J.; DENTON, H. Infestation of dried cassava (Manihot esculenta Crantz) by Prostheosporus truncatus (Horn) (Coleotera: Bostrichidae). Journal of Stored Products Research (1985) 21 (2) 73-77 [En, 12 ref.] Tropical Development & Research Institute, Storage Department, Stieh, Berks, SL1 1HL, United Kingdom.

Stored dried cassava is known to become heavily infested by Prostheosporus truncatus. A field study was undertaken in Tanzania to determine the extent of losses that this pest could cause in fermented and unfermented dried cassava roots stored over a period of about 4 months. In fermented roots, the mean weight loss (+ s.d.) rose to 73.6 ± 25.9% over this period compared with 52.3 ± 12.0% in unfermented roots. At each time interval that roots were examined, the weight loss in fermented roots was significantly higher. In laboratory studies with P. truncatus developed at a similar rate in both fermented and unfermented roots, but the adults appeared to prefer the fermented cassava, possibly because it was easier to bore into. Although P. truncatus caused lower weight loss in the unfermented compared with the fermented roots, both were so heavily damaged that one could not be recommended for storage rather than the other. The role of cassava as an intermediate host for P. truncatus is discussed, and consideration is given to the need to control the beetle in cassava in order to reduce cross-infestation to maize.


A crude pectic enzyme preparation and the purified endo-polygalacturonase isozyme PG3 macerated and killed the cells of both yam and sweet potato slices, although the latter did so at a faster rate. Purified cellulase neither macerated nor killed cells from either plant. Although the action was delayed, the crude preparation of cellulase macerated slightly and uniformly damaged that one could not be recommended for storage. The role of cassava as an intermediate host for P. truncatus is discussed, and consideration is given to the need to control the beetle in cassava in order to reduce cross-infestation to maize.


Host specificity of 3 isolates of B. theobromae from yam, cassava and sweet potato is reported. Physiological differences
between the yam and sweet potato isolates were established in their growth on selected sugars, pattern of extracellular enzyme production and pathogenicity under different temperature and relative humidity regimes.

**Potato**

533 **Ojero, M. F. O.; Mueke, J. M.** Resistance of four potato varieties to the potato tuber moth, *Phthorimaea operculella* (Zell.) in storage. *Insect Science and its Application* (1985) 6 (2) 205-207 [En, 10 ref.] National Agricultural Laboratories, PO Box 14035, Nairobi, Kenya.

Insect-free tubers of 4 varieties of potatoes were exposed to adults of *Phthorimaea operculella* for 14 days in uncontrolled conditions of storage in Kenya and were examined after 16 days. No statistically significant differences in the percentage of potato infestation (62.50-86.25%) were found, but significant differences in the mean length of larval tunnels and the mean numbers of larvae surviving in 10 tubers were found. Mean tunnel lengths (cm) were 44.65 in Kenya Baraka, 95.33 in Anett, 88.33 in Kerrs Pink and 104.23 in Roslin Gucha. The corresponding mean numbers of larvae surviving were 6.73, 11.25, 15.75 and 18.00. Thus, Roslin Gucha was the least resistant and Kenya Baraka the most resistant. The mechanism of resistance was probably antibiotic.


Dipping potatoes in a solution of ascorbic and citric acid before vacuum-packing and cooking (50°C for 50 min) inhibited growth and toxin production by proteolytic *C. botulinum* type B at an incubation temp. of 15°C for 70 d and at 20°C for at least 14 d. This preservative treatment also resulted in an organoleptically acceptable product with a prolonged shelf life. Risk analyses showed that the presence of *C. botulinum* in vacuum-packed, cooked potatoes may be expected, i.e. 1 spore in each 1585 kg of product. A preservative treatment with a combination of ascorbic and citric acid will limit the public health risk even if the potato product is accidently stored for a short time at a temp. higher than 4°C.

**Green and salad vegetables**


Rutgers Beacon asparagus was pre-cooled and stored at 0±1°C in different controlled atmospheres for up to 6 weeks. Shelf life declined from 9 days for fresh spears to 2 days after 4 weeks' storage. The different atmospheres did not affect storage life. Spears stored in 15% CO₂, 10% O₂ for 2 weeks and recessed after 12 days' shelf life had slightly better flavour than those stored in air. Tip rot [unspecified] was a major storage problem.


In field trials with the cv. Licitrusca the plants were treated with several fungicides and the heads were stored at 0 to 1°C for 136 days. The lowest storage losses (3.1-3.5%) (due mainly to *Alternaria brassicae, Botrytis cinerea* and *Rhizopus stolonifer*) occurred after treatment with mancozeb (0.2%) or folpet (0.2%). Losses in the control were 28.4%.


The disease of stored Chinese cabbage was first observed in the GFR in 1983 and in Feb. 1985 further heavy damage was recorded. A short description is given of the disease symptoms, morphological and cultural characteristics of *Phytophthora parisi* Feoster and Kenya Baraka the most resistant. The mechanism of resistance was probably antibiotic.


Samples (250) of raw vegetables and salads, collected from hotels, restaurants, small foodservice shops, markets and street vendors, were tested. *Salmonella* was isolated from 2 samples of green leafy vegetables and 1 sample of mixed salad. *Shigella* was isolated from 1 sample of greens, 1 of parsley and 3 of mixed salads. Most samples of raw vegetables and salads were at either room or outside temp. just before sampling. Eighty percent of the samples had acrobolic colony counts > 10⁵ CFU/g. Three of 36 samples tested contained c. 1 X 10³ *Staphylococcus aureus* g⁻¹.

**FISH AND SEAFOOD**


*P. indicus*, collected live from Cochin backwater, were killed by shock treatment, thoroughly washed with sterile saline and stored at different temperatures. Samples were periodically drawn and analysed for spoilage. The presence of a high percentage of *Vibrio, Pseudomonas* and *Acinetobacter* in prawns during storage at various temperatures and the presence of the maximum percentage of trimethylamine oxide-trimethylamine reducers in these groups suggest their dominant role in the rapid deterioration of prawns and increase in the TMA content during storage.


Whitefish steaks were brined in NaCl, KCl or equimolar NaCl:KCl to contain similar chloride ion concn and inoculated intramuscularly with 10 or 100/g spores of *C. botulinum* type E. Steaks were then heated in a simulated hot-smoke process to internal temp. of 62.8°-76.7°C for the final 30 min of a 2- to 3-h process, packaged under vacuum in oxygen-impermeable film, and stored at 25°C. During 7 d of storage, toxin production was inhibited in steaks containing > 0.66 ionic strength NaCl, 0.64 KCl, or 0.71 equimolar NaCl:KCl. It is concluded that it is feasible to substitute KCl for NaCl in hot-process smoked fish for inhibition of outgrowth and toxin production by *C. botulinum* type E.

A comparison of the recoveries of *Clostridium perfringens*, *Escherichia coli* and *Streptococcus faecalis* from naturally and artificially contaminated mussels and oysters was made. Only *C. perfringens* was regularly recovered from naturally contaminated shellfish. Laboratory studies showed that this was due to *C. perfringens* spores retaining viability significantly longer than vegetative cells of the other organisms tested, under marine conditions. Over 97% of presumptive *C. perfringens* colonies were confirmed as positive. A survey of mussels at 24 sites, over c. 60 km of coastline, found *C. perfringens* at 23 but *E. coli* at only 2 of the sites. It is suggested, therefore, that enumeration of *C. perfringens* can indicate faecal pollution where enumeration of *E. coli* shows none. Also, confirmation of presumptive colonies may not be required, rendering enumeration more rapid. Despite the greater persistence of *C. perfringens* spores, studies in a commercial depuration tank showed that oysters were cleansed to an acceptable level using a standard 48-h immersion. Depuration was found to be essential because all 3 organisms tested survived for a considerable period of time in oysters stored dry at 4°C, which is normal commercial practice.

**MEAT**

*See also abs. 394, 399, 697*


University of *Y. enterocolitica* and *Y. pseudotuberculosis* in pork contaminated with 10³ to 10⁵ cells per g survived the direct KOH treatment and were never recovered by using KOH post-enrichment treatment. From 6 (4.8%) of 125 samples of retail ground pork, four biotype 4 serotype O3 and three biotype 3B serotype O3 strains of *Y. enterocolitica* and one *Y. pseudotuberculosis* serotype 4B strain were recovered by using direct KOH treatment without enrichment. As these isolations were attained without enrichment cultural procedures, they represent an important time-saving alternative to simplify and speed isolation of *Yersinia* spp. from meat.


Two wholesale cuts from 8 steers were used. Four steers were artificially stressed and the right side of all carcasses was depurated shellfish. Laboratory studies showed that this was due to *C. perfringens* spores retaining viability significantly longer than vegetative cells of the other organisms tested, under marine conditions. Over 97% of presumptive *C. perfringens* colonies were confirmed as positive. A survey of mussels at 24 sites, over c. 60 km of coastline, found *C. perfringens* at 23 but *E. coli* at only 2 of the sites. It is suggested, therefore, that enumeration of *C. perfringens* can indicate faecal pollution where enumeration of *E. coli* shows none. Also, confirmation of presumptive colonies may not be required, rendering enumeration more rapid. Despite the greater persistence of *C. perfringens* spores, studies in a commercial depuration tank showed that oysters were cleansed to an acceptable level using a standard 48-h immersion. Depuration was found to be essential because all 3 organisms tested survived for a considerable period of time in oysters stored dry at 4°C, which is normal commercial practice.


Petrifilm SM Plates (PSPM) were compared with the conventional aerobic plate count (APC) method using standard procedures (SMA). Total colony-forming units were determined in 119 fresh ground beef samples (29 extra-lean, 30 lean and 60 regular) purchased at 9 different retail markets over a period of 6 weeks. Linear regression analysis of PSM vs. APC counts gave a slope of -0.963, an intercept of -0.027, and a correlation coefficient of 0.951. Mean log₁₀ counts on PSM were 5.86 compared to 6.11 on SMA (P<0.01) or a mean log₁₀ difference of -0.25. It is suggested that the Petrifilm SM method would be a possible alternative for the aerobic plate count method.


Two S. str. showing resistance to multiple antibiotics were compared with a susceptible str., and were shown to be different in growth capabilities. These antibiotic resistant strs. were inoculated in ground beef or beef cubes. In experiments simulating precooking contamination, heavily inoculated (10⁶ CFU/g) ground beef meatballs were cooled to 63°C and cooled to either 23 or 4°C within 2-6 h. Increases in the numbers of the surviving pathogens were small (ca. 0.1 log₁₀/g) when the product was cooled to 4°C within 2 h. Surviving *Salmonella* increased greater than tenfold when the meats were cooled over intervals of 6 h. A 4-h chilling interval period for ground beef meatballs sp. held in ground beef at 23°C for 6 h showed less than 1-log₁₀ increase/g. Experiments with S. sp. inoculated onto the surface of beef cubes after cooking also indicated that the 2-h cooling interval prevented substantive proliferation.


Analyses were carried out to study the microbiological quality of fresh, chilled and frozen chicken and beef available in the local market. Results showed that av. initial total viable counts were high, in excess of 10⁸ CFU/g for all 6 types of sample. Although *Salmonella* was not detected in any of the samples examined, the occurrence of *Escherichia coli* and *Staphylococcus aureus* was quite common. Storage at 5°C for 1 week was most suitable for the samples, as the microbial counts increased and sporeform was apparent in most of the samples. On the other hand, none of the samples stored at -18°C was spoiled. The results indicate poor handling of the samples somewhere along the processing line, up to the retail outlet. It is essential that hygiene and sanitation procedures currently observed be upgraded to ensure better quality products.


The effect of various initial chilling treatments on the numbers and types of microorganisms of beef, pork and lamb tongues (n = 60) and livers (n = 60) packaged either in polyethylene (PE) film or in vacuum packages in Texas and transported fresh-chilled via transoceanic shipment to Antwerp, Belgium, was evaluated. Initial chilling treatments included: cooler-tempered (4-6 h at 2°C), cooler-chilled (24 h at 2°C), freezer-tempered (0.5-1 h at -10°C), freezer-chilled (2 h at -20°C), ice-chilled (2 h in ice water) and no chilling (NPC) before packaging and subsequent refrigerated storage at 2°C. After the initial chilling treatments, the microflora was varied with *Micrococcus* spp. or with or without coryneform bacteria being the predominant bacterial types of most samples. After refrigerated storage for 13-15 d, lactic acid bacteria became dominant in most vacuum-packaged samples and in pork and lamb samples stored in PE film. *Brochothrix thermosphaeta* and *Pseudomonas* spp. constituted a greater part of the microflora of beef tongues and livers stored in PE film than that of comparable vacuum-packaged samples. Increases in aerobic plate counts of refrigerated vacuum-packaged samples nearly always were greatest for samples (NPC) that were not pre-chilled before packaging and usually were smallest for samples that were either freezer-chilled, freezer-tempered or ice-chilled.
and delayed microbiological effects of CFU/cm² on pork carcasses. However, the reduction in aerobic colony counts than vacuum-packaging delay of lactic acid. The percentage of samples positive perfrinffns which corresponded with a mean reduction of hot-boned and vacuum-packaged cuts.

A total of 108 shawarma (cooked meat) samples, aspcti­cally collected from various fast-food restaurants in Riyad, were examined for determination of aerobic plate count (APC), and counts of coliforms, Staphylococcus aureus, Clostridium perfringens and the detection of salmonellae. The APC ranged from 10³ to 10⁸ CFU/g. The counts for coliforms, S. aureus and C. perfringens ranged from <10 to 10³, <10 to 10⁶ and <10 to 10⁸ CFU/g, respectively. Twelve percent of samples was positive for Salmonella spp. It is suggested that foodborne pathogens present in shawarmas constitute a potential public health hazard.


Concentrations up to 1.25% (v/v) of lactic acid did not produce unacceptable discoloration inveal carcasses and counts up to 2% (v/v) were not significantly different from controls in terms of flavour. When measured 24 h postmortem, aerobic colony counts (3 d, 30 °C) were reduced by 0.8 and 1.3 log₁₀ CFU/cm² on breast and perineum, respectively, by 1.25% lactic acid sprays. Enterobacteriaceae colony counts (approx. 10⁶ CFU/cm² initially) were reduced below their limit of detection (<1.3 log₁₀ CFU/cm²) as a result of lactic acid treatment.


As a result of 1.25% (v/v) l-tactic acid treatment, aerobic colony counts (3 d at 30 °C and 5 d at 17 °C) were reduced by 0.8 log₁₀ CFU as compared with initial counts of approx. 3.0 log₁₀ CFU/cm² on control carcasses. However, the reduction increased to 1.3 log₁₀ CFU at 14 d postmortem, indicating some delay effect of lactic acid. The percentage of samples positive for Enterobacteriaceae was reduced from 50% to approx. 10% which corresponded with a mean reductio of 0.3 log₁₀ CFU/cm². Vacuum-packaging virtually completely inhibited growth of bacteria, yeasts and moulds on hot-boned cuts, but 1 wk after breaking the counts reached values similar to controls. Wet met to decontaminate carcasses and vacuum-packaging alone. At 14 d postmortem, this was still the case for cuts that had not been subjected to an additional decontamination with 2% (v/v) l-tactic acid immediately before vacuum-packaging. The Enterobacteriaceae colony count of hot-boned vacuum-packaged cuts remained under its limit of detection of 1.3 log₁₀ CFU as a result of lactic acid decontamination. Lactobacillus colony counts were extremely low in all treatment groups.


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ranged from $10^2$ to $>10^7$/g and from $10^2$ to $>10^9$/cm$^2$ on the surface after 3-5 months of storage. Pasteurized hams that had been inoculated with *Clostridium sporogenes* spores before pasteurization followed by a secondary heat treatment at 121°C for 10 min showed a delay in the occurrence of swollen packages when stored at room temperature compared with hams not receiving the secondary heat treatment. However, the secondary heat treatment did not prevent spoilage of hams.

**POULTRY AND EGGS**

*See also abst. 547*


Using a tracer str. of *S. typhimurium* and a direct-counting technique, a procedure was developed for evaluating the effect of immersion treatments on the S. contamination of chicken carcasses. The population of carcasses in 60°C water for 10 min gave 100-fold reduction in S. counts. Addition of 200 p.p.m. chlorine or 2.5% potassium sorbate increased the reductions to 1000-fold with elimination of S. from most carcasses. Other immersion treatments were not as effective. Sensory evaluation indicated the acceptability of treated carcasses on the basis of appearance and flavour.

**DAIRY PRODUCTS**

*See also absts. 397, 399, 684, 697*


A method was found to be suitable for rapid screening of milk products for thermolabile enterotoxins from *Escherichia coli*. ELISA was a more sensitive and accurate method; using GM-ganglioside as antigen receptor it had a min. sensitivity of about 1 ng/ml. The baby mouse test for the thermo­labile *E. coli* ELISA was based on the detection of 7.4% for positive and 7.6% for negative strains and was sensitive to 2 ng toxin. Of 141 *E. coli* strains from milk and milk products, 1 produced thermolabile toxin and 1 produced both toxins. A procedure for isolating and identifying enterotoxin-producing strains of *E. coli* in foods is described.


The International Standards (7251) method was more sensitive than the membrane-agar method for detecting *Escherichia coli* in milk and cheese, lower limits for MPN being 0.3 and 3/g for the former and 1 and 10/g for the latter method. Of 83 samples of liquid milk examined, none contained *E. coli* and 72 contained no enterobacteria or coliforms; the highest count was 110/g. Of 94 infant formulas, 32 and 18%, resp., contained enterobacteria or coliforms, and *E. coli* was found in only 2 samples. However, most samples of semi-hard cheese (75-85%) contained enterobacteria and coliforms, and

40% contained *E. coli*, although only 5% had high counts. Almost all of 103 samples of soft cheese contained enterobacteria and coliforms and 70% contained *E. coli*; 15% had *E. coli* at >1000/g and 30% had >10 000 coliforms/g.


Details are given of a new method for counting viable bacteria in milk and milk products without using the large number of Petri dishes (4-12/sample) needed in the standard plate count technique. In the new method, 6 triangles are marked off on a plate, and 0.05 ml from each dilution, in duplicate, is streaked out over each triangle with a pipette. After incubation, the count/g (x) can be calculated by the formula $x = a 	imes 10^b$, where $a$ is the colony count in the 2 triangles inoculated with a particular dilution and $b$ is the reciprocal of this dilution. Results for 22 samples with the pipette method were in good agreement with results obtained by the conventional standard plate count technique ($r = 0.99$).


A Memorandum of Understanding was negotiated between the New Zealand dairy industry and the Ministry of Health in 1974 to enable certain tests (phosphatase, penicillin and salmonella) to be carried out on products prior to export to the USA. The effects of this Understanding, which came into force in 1976, upon salmonella testing in New Zealand, is discussed. Certified testing for salmonella proved necessary, and a Regional Dairy Laboratory was opened at Wallaceville in 1979 to serve primarily as a national salmonella testing centre. Analytical procedures used at Wallaceville are outlined, and preventative systems established to trace the source of any salmonella, to eliminate salmonella from the country, and where necessary, imports. The number of samples was 6000 milk and 3000 cream, 700 heat treated milk and cream packages.


Pasteurized samples of milk, single cream (SC) and double cream (DC) obtained from shops, and samples of pasteurized milk from 140 dairy industries in and around Ayr, Scotland, were examined bacteriologically. The % samples with initial total counts <10$^4$/ml and psychrotrophic counts <10$^3$/ml resp., were: milk, 58 and 69; SC, 60 and 50; and DC, 44 and 40. Corresponding % for milk samples grouped according to retail outlets were: doorstep deliveries, 68 and 69; small retailer, 49 and 46; and large supermarket, 59 and 81. The % of milk, SC and DC samples with a stated residual shelf-life of >7 days was 3, 38 and 42, resp., whereas the actual % of samples having a shelf-life of >7 days (bacterial count <10$^7$/ml after 7 days at 6°C) was 26, 28 and 32. Further investigations of residual shelf-life indicated that storage temp. in retail outlets were inadequate for inhibiting growth of psychrotrophs. The value of the pre-incubation test for predicting shelf-life of pasteurized products is discussed.


Out of 224 DNAase-positive enterococci recovered from milk products, 21.9% survived pasteurization at 63°C for 30 min. Among the latter, 26 exhibited thermonuclease activity and 14 were haemolytic. Thermonuclease positive loops was exhibited by 13 strains, whereas 14 strains were pathogenic to mice. Resistance to penicillin and streptomycin was shown by 51 and 47 strains, resp. There appears to be a positive
relationship between heat resistance, thermonuclease activity, enterotoxin production by these organisms and their resistance to penicillin and streptomycin. As many as 37 heat-resistant strains were recovered from samples of dried skim milk, infant formulae and condensed milk. The predominant heat-resistant strain was Enterobacter cloacae subsp. faecalis, S. faecium and S. faecalis subsp. zymogenes.


The Limulus test was used to measure lipopolysaccharide (LPS) concentration in raw milk and in cheese of various ages. LPS increased from an av. concn. of 0.3 ng/ml in raw milk and in cheese to 0.8 ng/ml in cheese which was stored at 25-26°C for 10 days. Corresponding decreases in LPS concentration were found in cheese with a low concentration of the LPS in the cheese milk and partly from growth of Gram-negative cells. In cheese, LPS originated partly from contamination of raw milk before preparation of dahi.


Samples of cows' milk khoa, buffaloes' milk khoa and commercial khoa, resp., had the following mean microbial counts/g: standard plate count, 4400, 8000 and 17 000; acid-producing Escherichia coli, 2200, 3200 and 2500; proteolytic organisms, 1500, 2200 and 2500; chromogenic organisms, 1200, 1500 and 2400; lipolytic organisms, 250, 340 and 430; aerobic sporeformers, 71, 77 and 31; and yeasts/moulds, 7.5, 10 and 38.


Milk contaminated with Escherichia coli or Enterobacter aerogenes at 100 organisms/ml was used to prepare dahi by incubation for 18 h at 27-28°C with Streptococcus lactis C10 starter, at 25-26 or 4-5°C for 10 days. During incubation, E. coli counts increased from 10 000 to 980 000/g and acidity from 0.18 to 0.91% lactic acid; Ent. aerogenes counts increased from 10 100 to 6.6 million/g and acidity from 0.17 to 0.38% lactic acid. E. coli counts then decreased during storage at 4°C to 3.100 at 90 days, while acidity increased to 1.0% after storage for 2 days at room temp. and then decreased to 0.96 at 7 days and 0.89 at 10 days. At 4-5°C, acidity increased to 0.94% after 3 days and then decreased to 0.80% after 8-10 days. Corresponding decreases in Ent. aerogenes counts during storage were to 300 and 90 000/g at 7 days and 10 000 at 10 days, with acidity increasing to 1.0 and 0.95% during the first 3 days of storage and then decreasing to 0.85 and 0.81% at 7 days and 0.84 and 0.76% at 10 days.


The survival of Salmonella spp. at concn. ranging from 1100 to 110 000 cells/ml was studied in a series of fresh cheeses made from raw and pasteurized milk [see preceding abstr.]. In raw milk cheeses, at low initial concn. (<3100 cells/ml), survival rates were lower than those related to pH of cheese; at an initial concn. of 1900 cells/ml, Salmonella spp. were only detected in 20 g cheese. At 110 000 cells/ml, the organism was present after 96 h in 0.1 g cheese. Counts of both Salmonella spp. (artificially inoculated) and Staphylococcus aureus (present in original milk) were greater in pasteurized- than in raw-milk cheeses, possibly due to lower counts of lactic acid bacteria. In raw milk cheeses containing Salmonella at a concn. of 10 000 cells/ml, addition of a culture of lactic acid bacteria had no effect on counts of Salmonella at 4 or 5 days after manufacture, but S. aureus was detected at 100 cells/g cheese up to only 2 days, vs. 3 days in control cheeses.


A sample of dahi (a cultured milk product) was obtained from a shop in Karachi and examined for microbial counts, chemical composition, acidity development at 34-43°C and volatile aroma compounds. Dahi was composed of 4.7% fat, 14.0% TS, 3.2% protein and 1.1% lactic acid, with a pH of 3.80; volatile aroma compounds detected were acetaldehyde, acetone, ethanol and diacetyl (found at 12.55, 1.75, 8.32 and 0.17 p.p.m.). Counts of lactic acid bacteria were greater in pasteurized- than in raw-milk cheeses, possibly due to lower counts of lactic acid bacteria. In raw milk cheeses containing Salmonella at a concn. of 10 000 cells/ml, addition of a culture of lactic acid bacteria had no effect on counts of Salmonella at 4 or 5 days after manufacture, but S. aureus was detected at 100 cells/g cheese up to only 2 days, vs. 3 days in control cheeses.


82 milk samples collected from farms in the Po valley during Feb.-April 1984 were analysed for aflatoxin M1 contamination. Between trials and years between 1982/83 and 1983/84, aflatoxin concentrations were generally low.
This is a review-type lecture presented at the Kiel dairying week (Kieler Milchwirtschaftliche Woche) on 22-25 May 1984, based largely on publications of the authors and their collaborators. Particular attention is given to the most recent findings. [See DSA 46, 8366; Archiv für Lebensmittelhygiene (1984) 35, 32.]


Pasteurized milk and cream were purchased from local shops and analysed for initial counts of psychrophilous and mesophilous bacteria. Portions of the samples of milk (10 ml) or cream (10 g) were measured into sterile containers, mixed with 0.1 ml of a sterile solution containing Crystal violet (2 mg/ml), nisin (40 300 U/ml) and penicillin (20 000 U/ml) and incubated at 21 °C for 25 h. The bacterial content of the incubated samples, in which growth of Gram-positive microorganisms was inhibited by the Crystal violet-penicillin-nisin solution, was inversely proportional to the shelf-life of the product at 6 °C. The bacteria may be enumerated by plate count, giving results within 50 h, or by ATB estimation by bacteriocinogenesis, giving results within 26 h of sampling. Impedance measurements on pasteurized milk and cream containing Crystal violet-penicillin-nisin solution at 21 °C, using either modified plate count agar or milk agar, showed that detection times are directly proportional to shelf-life at 6 °C.


An attempt was made to develop a standardized technique for estimating the number of *Closstridium tyrobutyricum* spores in milk, using Reinforced Clostridial Medium with lactate substituted for glucose (RCM lactate). Effects of the following factors on growth of the bacteria were studied: commercial origin of the ingredients used in the culture medium; pH of the medium (4.40-6.50); conditions of pasteurization of the sample; and incubation time (2-14 days). Results demonstrated the importance of always using ingredients of the same commercial origin for the RCM lactate. Best results were obtained at pH 6.2, with pasteurization of the sample for 10 min at 90°C and incubation for 7 days at 37°C.


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Use of the square root model of Ratkowsky (Journal of Bacteriology 1982) [49, 1], the basis of temp. function integration, to monitor the shelf-life of pasteurized homogenized milk, was investigated with particular reference to storage temp. at <15°C at this temp. it follows a curve with a T° (conceptual temp. below which no growth can occur) of about 254°K. A study of the bacterial flora of pasteurized milk indicated that the bacteria responsible for spoilage were psychrotrophic post-pasteurization contaminants, initially present in very low numbers, but which multiplied during refrigerated storage and rapidly outnumbered the thermobacteria that survived pasteurization. It is concluded that temp. function integrators, programmed with growth characteristics of typical psychrotrophic pseudomonads, can be used to predict the shelf-life of milk. [There is no list of references to work cited in this paper.]

590 Mahmoud, S. Z.; Naguib, K.; El-Nokrashy, S.; Tawfeek, N. Use of hydrogen peroxide as a dairy preservative. Egyptian Journal of Dairy Science (1984) 12 (2) 162-172 [En, ar, 26 ref.] Food Sci. Dep., Ain Shams Univ., Cairo, Egypt. Various concns. of H₂O₂ (0.03, 0.05, 0.075 or 0.1%) applied to pure cultures or microorganisms commonly occurring in raw milk in the Cairo area. At 30°C, 0.1% H₂O₂ and a variable inhibitory effect. The most resistant organisms were Bacillus cereus, Streptococcus thermophilus, Clostridium butyricum and C. perfringens in descending order. The most susceptible were the vegetative cells of aerobic and anaerobic sporeformers, Bacillus anthracis, B. sphaericum, B. subtilis, and B. megaterium. Intermediate results were Escherichia coli, Staphylococcus aureus, St. lactis and St. faecalis. Similar results were obtained at 54°C, but the rate of destruction was accelerated. At both temp., increasing the H₂O₂ concn. increased the rates of destruction of the test organisms.

591 Shehata, A. E.; Magdous, M. N. I.; El-Samragy, Y. A.; Hassan, A. A. Utilization of sub-lethal heat-shock, L-alanine, β-alanine and nisin to induce germination of psychrotrophic sporeformers. Egyptian Journal of Dairy Science (1984) 12 (2) 197-207 [En, ar, 16 ref.] Food Sci. Dep., Ain Shams Univ., Cairo, Egypt. Effects of L-alanine, β-alanine, nisin and sub-lethal heat-shock as well as the cumulative effect of various combinations of these on germination of spores of some psychrotrophic sporeforming strains were studied. Evaluation of data suggested that heat-shock at 85°C in combination with L-alanine + nisin, followed by incubation at 37°C for 30 min, achieved the highest germination response of spores of B. cereus, B. pumilus, B. badicus and Pseudomonas aeruginosa. Elimination of nisin from this stimulation system reduced the germination response of these spores by about 7, 2, 12 and 26%, resp. However, elimination of L-alanine from the system reduced spore germination by approx. 9, 2, 6 and 5%, resp. The lowest spore germination response of all bacilli examined occurred after stimulation with L-alanine. The greatest germination response occurred after 30 min incubation at 37°C, and further incubation did not cause appreciable changes in the response of these spores.


593 El-Shibiny, S.; Mehhana, N. M.; Girgis, E. S. Chemical and microbiological quality of ultrafiltered reconstituted milk. Egyptian Journal of Food Science (1984) 12 (1/2) 115-119 [En, ar, 7 ref.] Lab. of Food Tech. & Dairying, National Res. Ctr., Kaf El-Shiekh, Egypt. Imported dried skim milk, reconstituted to 10% TS, was ultrafiltered (conc. factor 4) in a DDS Lab-20 ultrafiltration unit at 45°C. Mean microbial counts in milk, retenate and permeate, resp., were: total count 650 000, 4.7 million and 1.1 million/ml; coliforms, 170, 5000 and <10/ml; sporeformers, 6, 10 and <10/ml; and yeasts/moulds, 31, 37 and <10/ml. Mean chemical composition of retenate and permeate on DM basis was, resp.: 70% to 85% to 95%, 3.0 to 4.0%, and 120 to 200 g/l.

594 Bialke, D. Post-pasteurization contamination case history No. 2. Dairy and Food Sanitation (1985) 5 (4) 142-143 [En] Food & Dairy Quality Management Inc., St. Paul, Minnesota, USA. A case is reported of a dairy producing liquid milk products with flavour defects and short shelf life. Detailed examination of all available data showed that microbial quality of raw milk was adequate, and that products had low initial plate counts and no coliforms present. 7-day plate counts in homogenized milk samples were high, and Gram-negative psychrotrophs were isolated from these plates. The problem was traced to post-pasteurization contamination in a pasteurized milk storage tank; after replacement of this tank, the incidence of high 7-day counts and product spoilage was greatly reduced.

595 Kim, J. W. [Studies on the shelf-life of UHT milk.] Korean Journal of Dairy Science (1982) 4 (3) 175-180 [Ko, en, 35 ref.] Dep. of Dairy Sci., Chungsam Univ., Daejon 300, S. Korea. UHT milk from 6 areas of S. Korea was tested for changes during storage at 5 or 25°C for up to 10 days. At 5°C, 1 sample gave a positive alcohol and cloi-on-boiling test, but titratable acidity increased in this sample after 6 days, and it also developed the highest bacterial count (>10⁹/ml after 10 days). The other samples were all negative for the alcohol and cloi-on-boiling test, but titratable acidity increased in 4 of them from 0.174% to 0.18-0.23% after 264°K. All samples showed zero peroxide at these temp.

596 Hui, C. S.; Kim, H. U. A study on the mesophilic Bacillus species occurring in Korean market milk treated by ultra high temperature. Korean Journal of Dairy Science (1983) 6 (2) 120-125, 161-162 [Ko, en, 10 ref.] Lab. of Dairy Tech. & Microbiol., Seoul National Univ., Suwon 177, S. Korea. Samples of UHT milk from 8 manufacturers in Korea were examined for the presence of mesophilic aerobic sporeformers, Bacillus spp. and Pseudomonas spp. were detected in 14 of the 60 samples. Milk from 5 manufacturers contained B. subtilis and milk from 4 manufacturers contained B. circulans and/or B. megaterium. No Bacillus spp. were detected in 2 brands of UHT milk. Of 67 strains isolated, 54 were identified for growing on blood agar, and B. megaterium, 5 as B. circulans, 4 each as B. licheniformis and B. spharicium, 3 as B. cereus and 1 each of B. larvae and B. coagulans. 17 of these isolates could grow at 7°C (B. subtilis, B. cereus and B. megaterium strains). Vegetative cells of B. subtilis and B. cereus survived heating at 65°C for 40 min. These results indicate that many samples of Korean UHT milk contain thermotolerant psychrotrophic Bacillus spp., which may cause spoilage during storage. Improvements in the UHT milk processing and filling operations are required.

597 Park, H. Y.; Yoon, Y. C. [Changes in the bacterial content of raw milk during storage at low temperature.] Korean Journal of Dairy Science (1984) 6 (2) 120-125 [Ko, en, 10 ref.] Coll. of Anim. Husbandry, Kon Kuk Univ., Seoul 133, S. Korea. Raw milk samples stored at <7°C were analysed for counts of aerobic bacteria, psychrophilic bacteria, Pseudomonas spp. and coliforms, and for contents of pyruvate and lactate. Numbers of aerobic bacteria did not increase significantly during 2 days storage on the farm, but problems were encountered during further storage (at 5°C) at the milk processing plant. Non-acid-forming bacteria increased more rapidly than acid-forming bacteria. Psychrophilic bacteria and Pseudomonas spp. increased during cold storage. Contents of lactate were not high enough to be used for classification of milk stored at low temp. It was not possible to recommend a pyruvate content that could be used as a standard for estimating the quality of raw milk.

Proteinsases from 4 *Pseudomonas* spp. were added to pasteurized milk at 0.18, 1.8 and 18 units of proteinase activity/ml, followed by incubation at 5°C for 18 days. During this period milks were analysed every 2 days for proteolysis. Sensory analysis was done weekly for 18 days. There were no significant differences in proteinase activity when ninhydrin and absorption at 280 nm were used to measure proteolysis; however, only 18 units proteinase activity/ml were detected by the Hull test *Journal of Dairy Science* (1947) 30, 881. Sensory evaluation confirmed the test data. Isolates of *Pseudomonas* spp. were added to milk at 50 or 500 cells/ml, a curvilinear increase in proteolysis was noted by 11 and 13 days, resp., of the 4 *Pseudomonas* spp. as detected by the Hull method. Heat inactivation of the proteasenses at 62.8°C for 30 min was 0.73% and 10-35% as measured by the Hull and agar diffusion methods, resp.


At intervals of 4 to 6 wk, samples of raw milk and silage were examined for moulds and numbers of moulds in 10 different cowsheds. The 100 raw milk samples contained relatively few moulds: 77% contained <100 moulds/ml. In the 129 samples of silage, the moulds were higher, with 20 samples containing >10°g. *Phoma*, *Pusarium*, *Mucor*, *Penicillium roqueforti* and *Aureobasidium* spp. predominated in milk, whereas *P. roqueforti*, *Mucor*, *Absidia*, *Monascus* and *Scopulariopsis* spp. predominated in silage. The yeast *Geotrichum candidum* was present in 46% of raw milk samples at up to 10,000/ml; in silage it was present in 24% of samples at up to 10°c.f.u./g. A comparison was made of moulds occurring in raw milk and silage in individual cowsheds. Use of enrichment cultures showed that heat-resistant moulds (such as *Byssoscholemyces* spp.) were present in both milk and silage.


Studies have shown that the DEFT can assess the bacteriological quality of a sample of milk within about 25 min. The precision (repeatability) of the method can be expressed by a coefficient of variance (CV) of 14.6%. If 3 different persons counted the same membranes filters, a CV of 27.7% was found. Examining raw milk samples (n = 639) a coefficient of correlation of r = 0.77 was calculated between Standard Plate Counts (SPC) and DEFT counts. The regression line showed that at higher bacterial counts, DEFT counts were lower than the SPC. Preservation with a freeze-dried mixture of orthoboric and sorbic acids (0.6 and 0.009% final conc.) resulted in an identical classification of 76.5 and 75.0% of the milk samples after a storage period of 24 and 48 h. Trials with pure cultures in UHT milk at 9°C showed that Gram-negative bacteria gave markedly lower DEFT results compared with SPC and Breed counts. Incubation of the pure cultures at 6°C gave higher DEFT counts than at 9°C. After heating inoculated milk in a water bath, microbiocides (DEFT, Breed) showed higher values than SPC. This limits the use of the DEFT for analysis of heated milk and foodstuffs. For standardization of the DEFT method, formalin-treated raw milk appears suitable. There was no systematic change in DEFT counts during storage of formalin-treated raw milk for 300 days at 6°C.


400 quarter milk samples from 100 buffaloes at private farms in Giza were tested for corynebacteria. 40 contained Corynebacterium pyogenes, 52 *C. ulcerans* and 20 *C. bovis.


Unmean total bacterial counts of 129 samples of raw and pasteurized milks were examined for types and numbers of moulds in 129 samples of raw milk from 7 factories and 20 pasteurized milk samples from the University (22), 159 and 32/ml; from dairies (3), 59, 733 and 18,427/ml; and from cows' milk at home and in local markets (mean *E. coli* count >100/ml) was 30.84 in water samples and 20.52 in swabs. 4 of 23 isolates from raw milk, but none of 2 from pasteurized milk or 4 from swabs, were designated enteropathogenic by the rabbit ileal loop method.
from 1 or 2 samples from each factory were inoculated into 3 mice each, 5 isolates were lethal to 2 of 3 mice within 30 to 40 h, and 7 isolates were lethal to all 3 mice with 24 to 38 h. All 12 pathogenic strains survived heating at 63°C for 30 min. Survival rate of coliforms at room temp. in (i) was 10% after 3 months in 33.3% of samples, but none survived for 6 months. Coliforms were able to grow in reconstituted infant formulas from >10/ml initially to 250,000 and 205,000/ml within 8 h at 45 and 37°C resp., but did not show any increase in numbers for up to 12 h at 7-10°C.

596 SUN, M. Desperately seeking salmonella in Illinois. Science, USA (1985) 228 (4701) 829-830 [En]

Investigations that have been undertaken in an attempt to find the source of Salmonella typhimurium contamination of milk at Hillfarm Dairy, Chicago, USA are discussed. The milk, which has been the cause of an outbreak of food-poisoning affecting >14,000 people, has been found in batches of milk from this dairy processed on 3 isolated occasions since the outbreak was identified, but extensive examination of the plant has not so far failed to reveal the source of the contamination.

597 SUN, M. Illinois traces cause of salmonella outbreak. Science, USA (1985) 228 (4702) 972-973 [En]

Further investigations into the food-poisoning outbreak described above, suggest that the source of Salmonella typhimurium may lie at a small section of the pipeline where 2 air-pressure valves separate raw from pasteurized milk. The possibility of leaking occurring between the 2 milk lines was demonstrated and it was also shown that up to 0.75 US gal of milk could remain for a considerable length of time in this unrefrigerated section of pipe. Other possible sites for bacterial growth were a 2-in section of dead-space in one of the valves that was not flushed out during routine maintenance, and a 1-shaped piece of pipe that was attached to one of the valves and also had a dead space; threaded caps from raw or pasteurized milk lines were freely interchanged to close off one of the 3 ends of the pipe, thus providing another possible source of contamination. Investigators are now trying to locate dairy herds that may be harbouring S. typhimurium.


Under the Milk Quality Ordinance which came into force in the German Federal Republic in 1981, milk from all farms must be tested at least twice/month for bacteriological quality. Some of the new methods are given in the Ordinance and in a booklet from the Kocih method and manual pyruvate determination as reference methods for this purpose, but the regional authorities can allow use of alternative methods more suitable for routine testing. 2 regions, namely Baden-Württemberg and Hessen, have decided to use the Bactoscan method. Initial results with this method are reported and their relationship with the Koch plate count and direct microscopic count is discussed.


Initial results of a study of the quality of milk sold from 2 types of dispenser in the German Federal Republic are discussed. Milk from the dispensers did not differ significantly in organoleptic or bacteriological quality from milk sold in conventional single-trip containers, but tended to have a much shorter storage life because of recombinations. The storage life of up to 5 days claimed by some dairies for dispenser milk appeared unrealistic.


This review examines the causes of and changes associated with flavour deterioration that could occur in good quality pasteurized milk between production and consumption; work on raw, UHT or sterilized milk is included where relevant. Changes considered are those related to the pasteurization process, oxygen-related changes (factors affecting O2 consumption by milk including bacteriological factors, ascorbic acid, sulphuric, Cu-induced lipid and light-induced lipid oxidations, and packaging); vitamin-linked deterioration; oxidation of lipids; light-induced deterioration (and the role of riboflavin); deterioration due to microorganisms and to hydrolytic changes (lipolysis and proteolysis). It was concluded that the greatest danger to keeping quality is pre- and post-pasteurization bacterial contamination.

601 GAJDOSK, S. [Assessment of microbiological quality of milk by a modified resazurin test.] Hodnoecel mikrobiologiska jakosti mléka upraveným resazurínom testem. Životináry Výroba (1985) 30 (2) 105-116 [Cs, ru, en, 14 ref.] Vysoká Škola Zemědělská, Brno, Czechoslovakia.

The resazurin test was carried out directly on samples of raw milk, or after pre-incubation of samples for 1 h at 37°C or for 24 h at 5°C; incubation was for 30, 60, 90, 120 or 240 min at 37°C. Counts of total microorganisms, psychrotrophic bacteria and coliforms were made directly on the samples by stated methods. Catalase and nitrate reduction tests were also carried out from results obtained with 3 datasets. Complete agreement with total bacterial counts was found for any of the test variants, but pre-incubation for 1 h at 37°C followed by test incubation for 90 min at 37°C gave the best approximation. The test has good agreement with counts of psychrotrophic bacteria, but ranges of counts in the different classes were too great for objective evaluation of microbiological quality.


In a 1-yr survey from June 1982, a total of 2493 (2477 raw, 14 pasteurized and 2 unstated) goats' milk samples from various sources were tested; 43% of samples were frozen milk. 61% of samples were from farms, smallholdings or private households and 32% from retail sources such as healthfood shops and supermarkets. There was little difference in bacterial counts between liquid and frozen raw milk samples, 76 and 79%, resp., having total count of <10/ml at 22°C and 80 and 85% at 37°C; 99 and 97% had <10 Phystographic aureus/ml and 90 and 93% had <10 Escherichia coli/ml. No Salmonella spp. were found; Campylobacter jejuni was found in 1 sample, Versinia enterocolitica in 2 and Bacillus cereus in 8 samples. 86.7% of samples passed the methylene blue test, and all samples passed the Brucella ring test although results were sometimes difficult to read due to the difference in fat globule size and distribution between bovine and caprine milk.


The extent to which legislation in the UK, notably the Food Act 1984, Food Hygiene (General) Regulations 1970 and labeling regulations, applies to goats' milk, is discussed. The possibility of various viral, rickettsial and bacterial diseases being transmitted through goats' milk, and the occurrence of mastitis organisms, are considered. Pasteurization of goats' milk is recommended or, where this is not feasible, labeling on containers to recommend boiling of the milk prior to consumption.


Goats' milk is regarded as a food under the Food and Drugs (Scotland) Act 1956, but is not covered by Scottish milk and dairies legislation. The possible role of goats' milk in the direct and indirect transmission of diseases and milk-borne infections is discussed, and the development of a Code of practice is
described. Particular attention is given to the following 5 controversial points that had to be investigated prior to incorporation into the Code of practice: health benefits; heat treatment; antibiotic residues; legislative interpretation of various Acts relating to goats' milk; and bacteriological standards.


70 milk samples from 3 factories in N. Greece were studied. After heating at 80°C for 10 min, mesophilic bacteria were found at 1 to >10^7/ml in all samples, most commonly (63%) at 10^5/ml. Thermophiles were found at 10^3/ml in 64 samples. After heating at 100°C for 30 min, mesophiles were found in 52 samples, mainly at 1-100/ml, and thermophiles were found in 54 samples. 177 strains of spore-forming bacteria were isolated from milk heated at 80°C for 10 min; 101 strains were of Bacillus licheniformis, and 9-16 were of (in descending order) B. cereus, B. polymyxa, B. coagulans, B. pantothenicus and B. macreans. 62 strains were isolated from milk heated at 100°C for 30 min, of which 20 were of B. licheniformis, 12 were of B. pantothenicus and 10 each were of B. cereus and B. coagulans. Other spp. represented in the total number were B. circulans, B. firmus, B. sphaericus, B. alvei and B. megaterium; 9 strains in milk heated at 80°C were not classified.


Of 860 Gram-negative strains isolated from 85 samples of bulk-pooled raw milk, 31.2, 20.2, 14.9, 12.5 and 3.3% were of pseudomonads (Pseudomonas fluorescens, 21.4%), enterobacteria (lactose-negative, 13.9%; Escherichia spp., 3.8%; Klebsiella spp., 6.0%), Aeromonas spp. (A. salmonica, 8.8%; A. punctata, 7.9%), Flavobacterium spp., Actinobacter spp. (Ac. lwoffi, 9.3%) and Moraxella spp. Characteristics of the isolated strains are tabulated.


Pseudomonas fluorescens UQM2490, resistant to rifampicin at 1000 p.p.m., was derived from P. fluorescens JC1, a proteolytic psychrotroph isolated from raw milk. Growth of UQM2490 was studied in 13 raw milk and in 4 samples of UHT milk, using initial inoculum levels of 100, 1000 and 10 000 c.f.u./ml and incubation at 4°C. Growth of the bacteria withdrawn and viable counts made on rifampicin-containing agar. Growth curves obtained showed a slower growth in raw milk than in UHT milk. The higher the inoculum, the longer the final population of P. fluorescens in milk incubated for 7 days at 4°C. Generation times were 9.5-14.1 h in UHT milk and 9.6-33.0 h in raw milk, indicating activity of natural antimicrobial systems and/or competition among microbial species in raw milk. Use of antibiotic-resistant strains may prove useful in understanding the ecology of important spoilage bacteria in raw milk.


Samples of milk from cows, goats and sheep were studied after storage for up to 24 h at 1 or 10°C without or with pre-incubation for 3 h at 25°C. Methylene blue reduction time (without pre-incubation) was about twice as long for goats' and ewes' milk as for cows' milk and ewes' milk (P < 0.001 and 0.05, resp.) but not for goats' milk, and reduction times were lower during storage at 10°C than after the same time at 1°C, and lower after 24 h at 10°C in all samples except pre-incubated goat milk.


860 strains of Gram-negative bacteria were isolated from 85 samples of bulk-pooled raw milk (see Delto Ethnikis Epitropis Galaktos Ellados (1984) 1, 61). Gram-negative bacteria ranged from 1.2 x 10^3 to 9.9 x 10^9/ml. Of the samples, 52, 31, 45, 35, 22 and 41, resp., contained Pseudomonas spp. (P. fluorescens, P. taeniolatus, P. putida and P. putrefaciens), Flavobacterium spp. (F. aquae, F. breve, F. luteus and F. devorans), Aeromonas spp. (A. salmonicida, A. punctata and A. hydrophila), Actinobacter spp. (Ac. lwoffi and Ac. anitratum), Moraxella spp. (M. lacunata and M. bovis) and Enterobacteriaceae (Escherichia spp., Klebsiella spp. and Enterobacter spp.).


Individual milk samples from (i) 50 cows, (ii) 50 ewes and (iii) 50 goats were tested. Data for bacteriological quality included the following for (i), (ii) and (iii), resp.: total viable count on tryptone glucose yeast extract agar, 1.5 x 10^5 to 1.0 x 10^7 /ml; Enterobacteriaceae on deoxycholate agar, 1.5 x 10^5 to 2.8 x 10^6 /ml (on MacConkey agar), 320, 4400 and 3.0/ml; total count on Staphylococcus 10 medium, 4000, 450 000 and 11 000/ml; and Staphylococcus aureus, 80, 100 and 0/ml. 75.3% of S. aureus isolates from (i) and 63.6% of those from (ii) were thermonuclease-positive. Growth of food poisoning organisms at 37°C was tested in (i) and (ii). S. aureus reached a hazardous count (approx. 1 million/ml) after approx. 5 h at 31°C or approx. 18 h at 17°C. Max. growth at 31°C was achieved after 18 h in (i) and 24 h in (ii). S. aureus max. counts were reached after 3 days in all 3 milks. At 31°C, S. aureus disappeared from all milks after 7-8 days. At 17°C, it disappeared from (i) after 17 days, from (ii) after 18 days and from (iii) after 14 days. No salmonellae were detected in any sample. Of 19 suspect isolates, 11 were identified as Proteus spp. and 8 as Citrobacter spp.


Cows' milk (CM) and buffaloes' milk (BM), containing potassium thiocyanate at 25 or 50 p.p.m., were necessary for the presence of H_2O_2 at 240, 260 or 280 p.p.m. (CM) or 160, 180 or 200 p.p.m. (BM). Keeping quality of the milk was assessed by measurement of titratable acidity, pH, total bacterial counts and coform count. Addition of H_2O_2 at 25 p.p.m. improved the keeping quality of both types of milk at room temp. At higher temp. (35°C), higher concn. of H_2O_2 (160-200 p.p.m. for BM and 240-280 p.p.m. for CM) were necessary for improving the keeping quality. These higher concn. are recommended for milk preservation during summer if refrigeration is not available.


The aflatoxin M_1 content of naturally contaminated milk was not affected by storing at 4 or 10°C for 48 h or heating for 4 min at 71°C; however, 5-6% of the aflatoxin M_1 was lost by concentration at medium temp. and up to 30% was lost by concentration at high temp., and a further 15% was lost in the process of spray drying. The aflatoxin M_1 content of milk was decreased by passing it through animal charcoal but not by a commercial adsorbent. Ingestion of aflatoxin B_1 at 9 mg/day
produced changes in the blood and liver parameters of cows within 3 wk. However, 70 mg daily for 4 days affected feed intake and milk yield, decreased the activity of glutamate dehydrogenase to pathological levels, reversed the ratio of granulocytes to lymphocytes in blood and decreased total leucocyte count.


This lecture on problems of hygiene and quality of milk, presented at a meeting of the Polish Society of Veterinary Sciences held in Lublin, Poland on 28 Sept. 1984, deals with residues of pesticides, polychlorinated biphenyls, Hg, As, Pb, Se and Cd, nitrates and nitrites, radionuclides (137Cs, 54Mn, 131I, 134/137Cs) and mycotoxins in milk in Poland. [See: Medycyna Weterynaryjna (1985) 41, 17 for part 1; DAS 48, 821.]


Results are reported of inter-laboratory tests organized by a working group of the Swiss Federal Food Code Commission to find acceptable methods for determining aflatoxin M1 in milk and dried milk. The tests, involving artificially contaminated milk and naturally contaminated dried milk, were made on 3 methods, used at the Cantonal Laboratories in Zurich, Thurgau and Berne, resp. The Thurgau method involved preliminary sample treatment with phosphoric acid and acetone, extraction with dichloromethane, cleanup by silica gel column chromatography, separation by TLC (with 3 developers) and densitometric estimation. All 3 methods gave results in good agreement and are therefore recommended for use as official methods. The Zurich and Berne methods have been described in DAS 45, 7434 and 42, 4728.


The procedures evaluated incorporated the most widely used stages of clean-up including solvent extraction and silica gel chromatographic clean-up, selective solvent extraction of the extracted residue, the use of deproteination prior to hydrophobic column liquid-liquid partition or solvent extraction and the use of pre-packed reversed-phase cartridges for the direct extraction of aflatoxin M1 from the milk. Analysis times for each method, recoveries and relative costs are reported together with fluorescence high-performance liquid chromatograms, obtained under identical conditions to compare the relative cleanliness of the final extracts produced by each method. A pre-packed reversed phase cartridge method was the most satisfactory in terms of speed, cost and cleanliness of final residue.


A method which enables the determination of aflatoxin M1 (AFM1) in reconstituted powdered milk at levels of less than 2 ng/litre is described. The separation of AFM1 from milk and its clean-up are performed using only a Sep-Pak C18 cartridge and modest quantities of solvents.

Butter


The toxicity of 4 Aspergillus spp. isolated from oiled-off butter was tested against Panamecum caudatum, white mice injected intraperitoneally and 9-day-old chick embryos. Mycotoxins from A. versicolor and A. nidulans were highly toxic to all 3 test organisms and their toxicity was positively correlated with the content of sterigmatocystin in a chloroform extract of cultures (3-1064 μg/100 ml). A. flavus and S. sydowi were non-toxic or only slightly toxic and any toxicity was confined to their mycelia. A. flavus did not produce sterigmatocystin, and none of the fungi produced aflatoxins B1, G or M. Spores of A. versicolor and A. nidulans were isolated from the air of butter warehouses.

Cheese

See also abst. 410


In tests on 165 cheese samples, kuranycin/acsutin/azide (KAA) agar gave similar counts of enterococci to enterococció-selective differential (ESD) agar, except in hard cheese when the ESD agar gave higher values (P < 0.01). Membrane-filter enterococció-selective (ME) agar was less sensitive than KAA agar when applied to surface-riveted soft cheese and semi-hard cheese, and gave higher values for hard cheese (P < 0.05). Of 400 bacterial colonies isolated, 88% were identified as enterococci by KAA agar vs. 70% with ME agar. Counts and types of enterococci in 476 cheese samples estimated with KAA agar differed between and within cheese varieties, with counts ranging from 10-1000/g in processed cheese to 10-10/g in Bryndza, and tending to be greater in hard than in semi-hard and soft cheeses. There was also considerable variation between and within factories, with counts of enterococci in white mould cheese from 1 factory ranging from <10 to 105/g. The ratio of enterococci to enterococcus ranged from 1:144 in semi-hard cheese with eyes to 1:106 in other semi-hard cheeses. The tyramine and histamine contents of 103 samples of semi-hard cheeses varied in relation to enterococcus count, but correlation coeff. were only 0.34 and 0.09 for tyramine and histamine, resp.


Applied to 12 samples of 4 types of soft cheese (wine cheese, Camembert, Limburger and Romadur), Violet red bile agar gave counts of enterobacteria 10-times higher than those with Brilliant green bile lactose broth, and VRBG agar gave higher coliform counts than Mossel broth. Of 44 isolates, 10 were identified as Enterobacter aerogenes, 6 as Enteroheacter liquefaciens, 6 as Escherichia coli, 6 as Citrobacter spp., 3 as Hafnia spp. and 2 as Proteus spp.; 10 were unclassified. However, the pattern of isolates varied among cheeses and even among Camembert samples produced on different days at the same factory.

In Violet red bile (VRB) agar, 4 coliforms (Klebsiella oxytoca, Hafnia alvei, Citrobacter freundii and Enterobacter cloacae) and 5 strains of Escherichia coli and Salmonella were isolated at 44.5°C and 30°C. The findings indicated that parallel cultures on VRB agar at 30°C and 44.5°C could not differentiate between Esch. coli and other coliforms. However, in brilliant green bile lactose broth (BGL) all but 1 Esch. coli strain grew at 44.5°C whereas only Enterobacter cloaceae of the other coliforms grew at that temp. Multiplication of mixed cultures containing Esch. coli was 10% lower at 44.5°C than at 30°C. In conclusion, parallel culture in BGL broth at 30°C and 44.5°C could only as a rough guide to the differentiation of Esch. coli from other coliforms. However, examination of 200 Camembert cheese samples showed that 66% of those which gave viable cultures at 44.5°C contained Esch. coli and 66% of the remainder were negative for Esch. coli.

621 SONNENMÖSER, H.; KESSLER, W. [Technical possibilities of controlling coliform microflora.] Technische Möglichkeiten zur Kontrolle der Coliformen-Flora. Deutsche Milchwirtschafts-Zeitschrift, 1984, 31 (38) 125-128 [De, 1 ref.]

Edelweiss-Milchwerke, Kempten, German Federal Republic.

This lecture presented at the Kiel dairy week (Kiefer Milchwirtschaftliche Woche) on 22-25 May 1984 discusses growth of coliforms and possibilities of recontamination at different stages during the manufacture of Camembert cheese. Remedial measures to check the spread of coliforms and Escherichia coli in soft cheese are outlined and their applicability and effectiveness are considered.


The effects of cooking temp., time and temp. of brine-salting, and temp. of ripening on the behaviour of Enterobacteriaceae in Manchego cheese were studied. Cheeses from ewes' raw milk, manufactured and ripened under different conditions, were investigated throughout a 60-day ripening period. Differences in pH due to temp.-dependent whey retention accounted for the effects of temp. on Enterobacteriaceae counts. Temp. of brine-salting had no effect on Enterobacteriaceae counts; a significant effect of salting time on Enterobacteriaceae and faecal coliform counts was detected. Ripening temp. was the manufacturing variable with greatest influence (P < 0.01) on Enterobacteriaceae and coliform counts during the whole ripening period.


Veterinärmedizinische Univ., A-1030 Vienna, Austria.

Tabulated data are presented on the incidence of Lancefield group D streptococci (Streptococcus faecalis subsp. faecalis, S. faecalis subsp. liquefaciens, S. faecalis subsp. zymogenes, S. faecium, S. faecium subsp. durans und unclassified) in cheeses. The best medium for quantitative determination of group D streptococci was kanamycin-aceclohexamide agar. Counts in 476 cheese samples ranged from <10 to 10^5/g, with high counts commonly detected in hard cheeses, mould-ripened cheese and Brinsen. Wide variations were also observed in the types of enterococci in different cheeses. No correlations were found between the numbers of enterococci and coliforms or between enterococci and histamine or tyramine contents. It was concluded that enterococci could not serve as indicators of good cheese manufacturing practices or of health hazards.


Lab. de Microbiol. Laitière, INRA, 78350 Jouy-en-Josas, France.

Samples of Camembert cheese at different stages of ripening were examined throughout 1 yr at 6 different cheese factories. 3 of the factories made cheese from non-refrigerated milk, 2 used a 50-80% mixture of refrigerated milk in non-refrigerated milk, and in 1 factory cheeses were made either from refrigerated milk or non-refrigerated milk. During ripening, the content of lactic acid bacteria in the rind was lower in cheese made from refrigerated milk, while Serratia liquefaciens, a psychrophilic bacterium, varied considerably from one factory to another. The orange-pigmented colonies were mainly coryneform bacteria which were able to utilize methanoline with the production of methanethiol, which is a precursor of compounds giving the characteristic cheese aroma. Characteristics of 652 lactic acid-positive and 343 lactic acid-negative strains of coliforms are summarized and the occurrence of Escherichia coli, Hafnia alvei, Serratia liquefaciens and Citrobacter freundii in rind is discussed. The association between moulds and bacteria on the surface of the cheese was studied using scanning electron microscopy. Growth of coryneform bacteria on the Penicillium mat suggests that these organisms do not play an important role in cheese ripening.

625 KARAOSANNOGLOU, P.; KOPIS, P.; PAPAGEORGIAD, D.; MANTS, S. Survival of Yersinia enterocolitica during the manufacture and storage of Feta cheese. Milchwissenschaft (1985) 40 (4) 204-206 [En, de, 18 ref.]

Dep. of Food Hygiene & Tech., Sch. of Vet. Med., Aristotelian Univ. of Thessaloniki, Thessaloniki, Greece.

The ability of Yersinia enterocolitica 4360 serotype O:9 to grow and survive during the manufacture and storage of Feta cheese (from ewes' milk) was studied. When the acidity of the cheese developed rapidly and the pH of the curd was below 4.5 within 48 h at 22°C, counts of Y. enterocolitica decreased from 3.361-8.1760 log c.f.u./g cheese curd after 4 h at 22°C to 1.6899-5.596 log c.f.u./g after 48 h and to undetectable amounts after 3-5 days. When cheese acidity developed slowly and the pH remained at about 5.3-5.5, Y. enterocolitica was able to multiply in curd during storage for up to 4 days at 22°C and survived in cheese stored for 30 days at 4°C.


Zakład Higieny Zwojenia i Zwyżenia, Katedra Zwyżenia Czołowiaka, ART Olsztyn, Poland.

The literature on the production of the aflatoxins B1 and G1 by Aspergillus flavus and A. parasiticus in cheese is reviewed, with particular regard to the effects of temp., surface moisture, pH, sugars, proteins, NaCl, atmospheric O2 and CO2, fungistatic agents (sorbic acid, pimarin and nisin) and other microorganisms (Streptococcus lactis, Lactobacillus casei, Brevibacterium linens and Penicillium candidum).


Teleme cheese blocks inoculated with Aspergillus parasiticus were placed in plastic screw-cap containers and held at 5, 16 and 25°C. No mould growth appeared at 5°C and no aflatoxins were produced when cheese was kept completely immersed in brine. Aflatoxin B was lower in amount at 16 than at 25°C and also lower in cheese partially immersed in brine than in cheese without brine. Aflatoxins B1 and G1 appeared to increase during all the incubation time of 7 weeks at 16°C, while at 25°C they reached a max. in 3 weeks and decreased thereafter. Aflatoxin B increased to the order of 8 x 10^6, while aflatoxin B increased to the depth of not more than 2 cm and the amount decreased as the distance from the surface increased.
ANIMAL PROTEIN


Four isolates of Bacillus, from museum leather or parchment showing signs of deterioration, were identified as B. subtilis, B. licheniformis, B. megaterium and B. pumilus. The collagenolytic capacity of these 4 isolates was assessed by the evolution of oxyproline, in cultures to which insoluble collagen was added. B. subtilis was the most active, followed by B. megaterium and B. pumilus. B. licheniformis showed no collagenolytic activity. All 4 isolates also split casein.

Leather


From deteriorating specimens of mediaeval manuscripts on parchment, or of ancient leathercraft, a total of 38 bacterial strains were isolated. All but 2 isolates grew in media containing either peptone, or yeast extract, as the sole carbon source. The proteolytic capacity of these isolates was determined by incubating them in the presence of casein. Protease synthesis was demonstrated in 46 of these isolates. It is suggested that the capacity for growth using collagen, linked with the elaboration of extracellular proteases, justify the assumption that such bacteria may be responsible for damage to parchments and leather.

Animal fibres

See also abst. 679

Adhesives

See also abst. 387

PLANT FIBRES

See also absts. 690, 841


C. sublaevis rotted inadequately dried flax straw. The fungus was not highly cellulolytic and xylanase, a constitutive enzyme of the organism, apparently played a major role in degradation of flax fibre.

Lignin

See also absts. 641, 705, 786

Cellulose


Five white-rot fungi were grown on cellulose fibres of cotton and degradation was observed by SEM and TEM. The white-rot fungi produced hyphal sheaths when grown on cellulose fibres, but sheaths were not generally present between the hyphae and fibres. Autolyzing hyphae and diffusion of cytoplasmic material into sheath or fibre was seldom observed. It is suggested that the sheath may not be involved in the degradative process but functions for attachment, nutrition, and protection. The fungi disrupted fibre surfaces. Degradation was not localized around hyphae but occurred at a distance from them. Thinning of fibres did not occur. It was concluded that the white-rot fungi produce a diffusible cellulose-degrading system.

TIMBER

See also absts. 689, 827, 840-841


On 13 Nov. 1972 a windstorm with gale velocities of up to 200 km/h struck NW Germany, particularly Lower Saxony, and flattened more than 100 000 ha of forest. Clearing of 17.6 million m² of timber was necessary. Of this total, 1.39 million m² were placed under wet storage in sprinker yards and in lakes and ponds. Assessments of the logs after several years of storage showed that in order to avoid decay water storage must be restricted to high quality logs with a high moisture content which are free of fungal infection. Water-stored spruce logs showed very dark staining to a darker colour in the outer 5-10 mm of the stem. This creates problems if the wood is used for pulp. Spruce yards had no detrimental effect on surface or groundwater systems, and there were no difficulties in marketing sprinkled logs.


Test crosses, constructed from 12 different softwoods and 9 different hardwoods, were exposed on test fences for 9 to 12 years in both southern Mississippi and Wisconsin. The woods tested in Mississippi were classified into aboveground decay resistance groups. The sapwood of lodgepole pine, balsam poplar (Populus balsamifera), alder (Alnus rubra), and sweetgum and the heartwood of sugar maple, yellow birch (Betula alleghaniensis), and basswood decayed within an av. of 7 years and were classified as nonresistant. Woods estimated to last over 20 years above ground, and thus classified as most resistant, included heartwood of Douglas-fir, redwood (Sequoia sempervirens), western white pine (Pinus monticola), red and white oak, and sapwood of red oak. The remaining woods fell in between these classes. In Wisconsin most woods decayed too slowly for estimates to be made of their longevity. The predominant fungi in the Mississippi test site were: Gloeophyllum sepiarium, the white-rot fungus 'Unknown T," Schizophyllum commune, the brown-rot fungus 'Unknown U," and a Xylaria sp. In Wisconsin, Peniophora clausa, Phanerochaete chrysosporium, Merulius lop. corium, Irpex lacteus, and Peniophora violaceo-livida predominated.

Terrestrial fungal attack

See also absts. 678, 700, 704

A review of major decay-causing agents and types of decay of aspen (Populus tremuloides) and balsam poplar (P. balsamifera), relations between decay and age, site and clone, decay management and the significance of decay and discoloration to utilization. Broadleaved trees (mainly P. tremuloides and P. balsamifera) represent about 40% of Alaska's forest resources, because of a cessation in the repression of cellulases when all the hemicelluloses and lignin was observed in progressively thinner ground portions of weathered power transmission poles of *P. AP-planatum*, Holzforschung (1984) 38 (2) 61-68 [En, de, 12 ref., 2 p. BLL] Dep. Agric. Univ., PO Box 5488, Raleigh, NC 27615, USA.

Western hemlock sawdust degraded by *G. AP-planatum* was examined microscopically. Small electron-dense particles were found in the cell wall at early stages of attack as well as in the hyphae and hyphal sheath. At advanced stages of degradation, large localized areas of deterioration were seen in the cell walls. These areas were in contact with hyphae that released vesicles in the cell walls, thereby degrading the cellulose framework clearly visible under minimum treatment, Aug. 1979 and Feb. 1981. Three treatments had been removed at the time of the examination. Reference is made to the relevance of felling season to resistance to decay.

### 635 Murmanis, L.; Highley, T. L.; Palmer, J. G.


Western hemlock sawdust degraded by *G. applanatum* was examined microscopically. Small electron-dense particles were found in the cell wall at early stages of attack as well as in the hyphae and hyphal sheath. At advanced stages of degradation, large localized areas of deterioration were seen in the cell walls. These areas were in contact with hyphae that released vesicles in the cell walls, thereby degrading the cellulose framework clearly visible under minimum treatment, Aug. 1979 and Feb. 1981. Three treatments had been removed at the time of the examination. Reference is made to the relevance of felling season to resistance to decay.

### 636 Ruel, K.; Barnoud, F.; Ericsson, K. E.


Various changes were observed at different stages of attack. In order to collect diffracted electrons and hence obtain filaments of cellulose fibrils down to elementary fibrils 3-4 nm in width. The crystalline diffraction pattern of cellulose I was seen in areas of slight decay but disappeared when the fungus is grown on wood.

### 637 Braid, G. H.; Line, M. A.


Some blocks of *Picea abies* sawdust were impregnated with glucose, to 2% of their dry wt., and inoculated with wild-type *S. pulverulentum* for 6 wk. Microscopic observations were made by TEM, using a wider diaphragm than that normally used in order to collect diffracted electrons and hence obtain filaments of cellulose fibrils down to elementary fibrils 3-4 nm in width. The crystalline diffraction pattern of cellulose I was seen in areas of slight decay but disappeared when the fungus is grown on wood.

### 638 Rackowski, J.; Krauss, A.; Pacha, R.


Studies were made on the hydration of Portland cement (s) alone, or with the addition (at the rate of about 10% wt./wt.) of particles prepared from pine sawdust (b) with or (c) without brown stain caused by the fungus *Cystospora pini* (syn. *Discula brunnea-tingens*). Hydration rate in (b) was on av. 7.0% greater, and that in (c) 9.8% less, than that in (a). Time to max. hydration temp. in (c) was approx. 40 min less than in (c). Cold- and warm-water soluble and reducing sugar contents were about 1/3 less in stained than in unstained particles. It is concluded that pine wood exhibiting this brown stain can be used without restriction in the manufacture of wood cement.

### 639 Kerner-Gang, W.; Grinda, M.


A culture medium consisting of vermiculite + nutrient liquid + wood sample was evaluated in laboratory tests. Wood samples included solid wood and 5-ply plywood made from birch or beech alone, or in combination with silver fir or Norway spruce. Among various nutrient liquids tested, best results were obtained with a buffer (HCl + KCl)-malt sol. Either glass bottles or plastic test containers were suitable for use with the procedure. Effective decay rates were obtained using plywood attacked by *Trametes versicolor* (*Coriolus versicolor*), *Lentinus cyanthiformis*, *Stereum sp.*, and *Gloeophyllum trabeum*. In the case of *C. versicolor*, considerable mass losses were obtained even when a high concn. of wood preservative was present in the adhesive.

### 640 Ważyń, J.; Krajeski, K. J.


Bore samples were taken from 85-year-old pine trees in Poland over the course of a year (March 1980-March 1981), at 2-wk intervals corresponding to new- and full-moon cycles. The samples were subjected to attack by *Coniophora puteana*, *Streptomyces bursaticus* or *Chaetomium globosum* in pure culture for 12 wk. In general, resistance increased from May to mid-Feb., and decreased from mid-Feb. to March or April. Samples obtained from Nov. to mid-Feb. were most resistant to attack. No influ-

### 641 Shinamoto, M.; Oku, T.; Ishikawa, H.


Three white and two brown pigments were grown on savagurumi (*Pterocarya rhoifolia*) wood chips or in savagurumi dioxane lignin cultures. Fungi were of 2 types: phenoloxidase-lacking, *Phanerochaete chrysosporium* or *Phanerochaete chrysosporium*-rich, e.g. *Coriolus versicolor*. Chemical and physical analyses of the wood chips and dioxane lignin cultures are reported. Lignin degradation was more slowly degraded than dioxane lignin in the cultures. The chemical mechanisms of degradation are discussed. Lignin in the wood was degraded by both types of fungi, whereas dioxane lignin was depolymerized to a lower molecular weight by phenoloxidase-lacking fungi, but predominately polymerized by phenoloxidase-rich fungi. Anomalous changes occurring during decay of the wood chips were examined by SEM. All the fungi secreted extracellular enzymes which reacted with the cell wall. Various mutants caused different accumulation of the chip by attacking the middle lamella. Other fungi caused a progressive thinning of the cell wall starting from the lumen of the cell.
finally a total destruction of cell-wall structure. Decay in latewood was characterized by localized dissolution of the secondary wall, and the formation of "soft-rot" type cavities in the S₃ layer of the secondary walls. Chemical analysis showed little or no difference in proportions of hemicellulose and lignin in earlywood and latewood of sound and beetle-killed wood. Solubility in 1% NaOH, of both earlywood and latewood, was significant. In both sound and beetled-killed wood, similar characters suggest that the primary fungus responsible for decay may be *Peniophora* sp., a fungus commonly found in stored southern pine logs. The results indicate that the beetle-killed wood could be used successfully as a pulpit and reconstituted board products.


Approx. 1320 fungi were isolated and studied from 246 creosote- or PCP-treated southern pine poles in service in the eastern United States. The fungi identified were Basidiomycetes, soft rot fungi, and microfungi. White rot fungi predominated in the 262 Basidiomycetes isolated from 180 poles. The major Basidiomycetes isolated by radial position from poles of varying service ages appeared to develop initially in the outer treated zones and were then associated with checking checks. Some decay origins, however, appeared to be caused by preinvasion and escape of preservative treatment. Five species of soft rot fungi comprised nearly 85% of 211 isolates obtained from 131 poles. They were isolated primarily from creosote-treated poles in the outer groundline zones and were often associated with checking checks. Differentiation of 92 poles indicated 6 developmental decay patterns, and certain fungi were associated commonly with a pattern. The pole mycoflora was relatively uniform in distribution throughout the eastern United States. The soft rot fungi and white rot group of Basidiomycetes appear to be more important components of the treated southern pine pole mycoflora than has been recognized previously.


Six soft rot fungi, commonly isolated from preservative-treated southern pine poles in service, were tested for their capacities to cause wt. loss, anatomical damage, and tensile strength loss in southern pine (*Pinus taeda*) and American beech (*Fagus grandifolia*). The fungi isolated were: *Chondrostereum purpureum*, *Phialocephala dimorphospora*, *Phialocephala aplophora*, *Philosphora hirsuta*, *Alternaria alternata* and *Cladosporium resinae* (a non-decay reference fungus). The fungi caused significant losses in both wood wt. and strength in laboratory tests at 2- and 4-month periods. The wt. losses were greater in beech than in pine and increased with incubation time, although not linearly. Wt. losses caused by some fungi varied substantially with incubation temp. Strength was reduced more rapidly in pine, and losses attained up to 88% of the original wood tensile strength. Strength losses were up to 100% in beech, but appeared to be delayed. Residues for this variation are presented, and the potential of soft rot fungi to affect the service life of treated wood products in ground contact considered.


The study was made on samples of *Cyclocidiscus gabunensis*, *Fagus crysophylla*, *Fruneden a. friderici-dreeri*, and *Kleiniodoxa busergii* obtained from trees grown in Cameroon. In laboratory tests, samples were exposed to attack by *Ceratocystis pustulata*, *Trametes versicolor* (*Coriolus versicolor*), and *T. hirta*. The results of fungicide and fungicide-free treatments in the testblocks for four-month period. Resistance to decay (expressed as mass loss) was analysed in relation to radial position across stem, wood density, and pale-ness of heartwood colour. In *C. gabunensis*, *F. a. friderici-dreeri*, and *K. busergii*, heartwood in a 5-10 cm zone adjacent to sapwood was more resistant than interior heartwood. In decay resistance was found to reflect to density or colour. Considerable within-stem variation in decay resistance was found, indicating potential grading problems in these species.


Small wooden pieces, pruned for different periods in dry humidified soils and fresh soils, were incubated with wooden pieces in inoculated lucerne as a baseline. The same treatment was done using sterile soils. Drying and rehumidification reduced and modified the soil microflora. The lignophagous microflora colonizing wood pruned in dry humidified soils is different from that in wood pruned in the corresponding fresh soils. In all cases the lignophagous microflora limited the colonizing capacity of the basidiomy­cetes. This capacity did not depend on the period of wood pre­incubation in the soil. The results do not confirm the idea that basidiomycetes colonize only after colonization by a microflora with constituents with a more reduced enzyme system.


*M. nagassarium* wood from Kerala, India, was tested for its natural resistance to 7 spp. of decay fungi under accelerated lab. conditions. *Polyporus palustris* was most virulent, followed by, in order of decreasing virulence, *P. [Pycnoporus] sanguineus*, *Lentzius trabea* (*Gloeophyllum trabeum*), *Polyporus* (*Coriolus*) versicolor, *P. [C.] hirsutus* and *P. meliae*. *Irpex flavus* was least virulent. *C. versicolor* caused the greatest wt. loss in the test blocks. *M. nagassarium* wood was classified as highly resistant, although there was considerable variation among the 5 trees tested.


When >400 beech (*Fagus sylvatica*) logs (10-20 cm diam. 30-40 cm long) were cut from freshly felled trees and placed upright 1 m apart with their bases buried up to 10 cm deep in a plot of 600 m² in a mixed deciduous woodland in Gloucestershire many ascomycetes, basidiomycetes and fungi of other phyla were isolated. *Fagus* imperfecti were identified from the vicinity of the aerial cut surface within the first 6 months after cutting. Some such as *Coriolus versicolor* subsequently increased in occurrence, while others, such as *Abies alba* and *Cortinarius purpurascens* declined. Isolation onto malt agar from thin sections of wood revealed some fungi, notably a *Fusarium* sp., not detected by direct means. In addition, homokaryons of *Bjerkandera adusta* (*Polyporus adustus*) and *C. versicolor* were isolated from near the aerial cut surface up to 2 yr after cutting, and lower down, mostly up until 6 months after cutting (thereafter heterokaryons predominated). Moreover, evidence was obtained that small volumes of discoloured, but not strongly decayed wood sometimes contained large numbers of mutually antagonistic individ­uals (up to 30/cm²) of "soft-rot" spp. *P. adustus*, *C. versicolor* and *Stereum hirsutum*. Vertical penetration from the aerial cut surface showed a marked lag phase, such that for the first 6 wk after cutting, overt colonization was restricted to c. 3 mm depth. Thereafter the depth occupied increased rapidly.


The study was made on samples of *Cyclocidiscus gabunensis*, *Fagus crysophylla*, *Fruneden a. friderici-dreeri*, and *Kleiniodoxa busergii* obtained from trees grown in Cameroon. In laboratory tests, samples were exposed to attack by *Ceratocystis pustulata*, *Trametes versicolor* (*Coriolus versicolor*), and *T. hirta*. The results of fungicide and fungicide-free treatments in the testblocks for four-month period. Resistance to decay (expressed as mass loss) was analysed in relation to radial position across stem, wood density, and pale-ness of heartwood colour. In *C. gabunensis*, *F. a. friderici-dreeri*, and *K. busergii*, heartwood in a 5-10 cm zone adjacent to
the ascomycete *Xylaria hypoxylon* and various rhizo-
morph/mycelial cord-forming basidiomycetes, including
*Armillaria bulbosa*, *Phallus impudicus*, *Phanerochaete velu-
tina*, *Stereum hirsutum*, and *Chaetomium globosum*. Apart from *A. bulbosa*,
these generally readily formed mycelial mats on the basal sur-
face, between which somatic incompatibility reactions were
often observed. Most frequent reactions occurred with *X. hypoxylon* of which numerous isolates produced numerous individ-
uals in discrete decay columns. By contrast, lack of somatic
incompatibility reactions between isolates of *A. bulbosa*
indicated that a single extensive mycelial type occurred through­
out the site. The occurrence of somatic incompatibility between
isolates of *X. hypoxylon* from different logs indicated independent
establishment, presumably via basidiospores, and this was con­
sistent with the unusually late arrival of the sp. at the site. In no
cases were numerous somatically incompatible individuals iso-
lated from small volumes of wood lacking demarcation into
discrete columns, as had been observed near the aerial cut sur-
face. Vertical penetration from the base was more rapid than
from the aerial cut surface, with *X. hypoxylon* a major pioneer
species, and there was no pronounced lag phase.

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**Coates, D.**; **Rayner, A. D. M.**. *Fungal population and
community development in cut beech logs. III. Spatial dynamics,
Claverton Down, Bath, BA2 7AY, United Kingdom.

The spatial development, in beech logs exposed to air-borne
and soil-borne inoculum, of mature fungal communities con­
taining mutually or unilaterally exclusive mycelia of decay sp.
was described and related to the ecological strategies and interac­
tion of participant individuals. A competitive hierarchy is recog­
nized between (i) ruderal and/or stress-tolerant individuals
which are spatially dominant early on and then decline, through
(ii) air-borne competitive individuals of decay fungi, such as
*Coriolus versicolor* and *Stereum hirsutum*, which establish
mutually exclusive decay columns expanding away from the
aerial cut surface, and culminating with (iii) highly competitive
individuals of mycelial cord-formers such as *Phallus impudicus* and
*Phanerochaete velutina*, which invade slowly from the base,
ultimately occupying large volumes of wood, causing intense
decay, and replacing many of the pioneers.

Addition of basidiospore suspensions of *Bjerkandera adusta* (*Polyergus adustus*), *C. versicolor*, *Hypholoma fasci­
culare* or *S. hirsutum* to the aerial cut surface appeared to
affect community dynamics and functioning markedly. Devel­
opment of decay columns by air-borne fungi was inhibited and
associated with enhanced vertical penetration by basally colo­
ned fungi. The persistence of non-compatible decay sp. was
**651** 

**Redhead, S. A.**; **Ginns, J. H.**. *A reappraisal of agaric
genera associated with brown rots of wood.* Transactions of the
Mycolological Society of Japan (1985) 26 (3) 349-381 [En, 159
ref., 10 fig.] Biosystematics Res. Inst., Agriculture Canada,
Ontario, Ont., Canada K1A 0C6.

The taxonomic value of rot type in the higher basidiomy­
cetes is discussed. Genera in the Agaricales known to cause
brown rots are reviewed and 3 new genera are proposed.

**652** 

**Hegarty, B. M.**; **Curran, P. M. T.**. *The biodeteriora-
tion of beech by marine and non-marine fungi in response to
temperature, pH, light and dark.* International Biodeteriora-

The wood-rotting ability of marine and non-marine fungi
(*Alternaria sp.*, *Cladosporium herbarum*, *Dendryphilla salina*,
*Monodictys pelagica*, *Stackeberga acre*, *Zalerion nigriunganum* and *Sordaria fibuligera* ) in response to temperature, pH, light and dark was investigated
using vermiculite burial and wood block weight loss estimation
methods. Max. wood decay by the majority of test fungi was recorded in the 15°-25°C range; while additions of Abrams' salts increased the weight losses produced at opt. temp., they
had little effect on decay at the extremes of the temp. range.
Results of the pH studies showed that the fungi produced greatest
weight losses of wood in the 3.0-8.0 pH range. Generally,
comparable weight losses were recorded for test fungi under
light and dark conditions.

**653** 

**Sutherland, J. B.**. *Regulation of cellulolytic activity in
the white-rot fungus *Fuschnodermia resinosum.* Mycologia (1986)
78 (1) 52-55 [En, ref. 1 fig., 2 tab., Inst. Wood Res.

The fungus, which can selectively remove lignin from
wood, was grown on soluble media in stationary submerged
cultures to investigate the effects of various carbohydrates on
activity. The activities of extracellular cellulases (filter paper activity and carboxymethyl cellulase) were higher in cultures grown on carboxymethyl cellulose than in those on
xylan or glucose. Carboxymethyl cellulase was induced in succe­
nate-grown cultures after the addition of cellulbiose or carboxy-
methy1 cellulose; β-glucosidase was induced by cellubiose.
Supplemental xylose, arabinose, fucose, gluconic acid, and
several other carbohydrates were catabolite repressors of cellu­
labse activity.
Impregnation of finished boards was difficult with plywood and drying was slow for both plywood and particleboard. Treating wet veneers with acidic, oxidative or boron containing preservatives weakened the strength of the glue joint. Ammoniacal copper compounds gave good results. Immersion and spraying of dry veneers gave better protection against decay than adding preservative to the adhesive. TBTO applied by immersion or spraying was effective against C. puteana but not against mould. Impregnating veneers with CCA prior to gluing, at a conc. effective against decay, weakened the glue joint. Preservative treatments did not affect the strength of particleboards. Adding preservatives to the adhesive was more effective against termites than against decay. Permethrin applied before gluing gave better protection in plywood than did treating the adhesive. Edge sealing of plywood boards reduced preservative leaching and increased decay resistance in untreated plywood. Edge sealing with acrylic paint and primer with phenolic film prevented leaching and decay in treated and untreated plywood and in PF particleboard.


Small specimens of oven-dry Scots pine sapwood containing PCP preservatives were placed on maltose agar inoculated with a spore suspension of Aspergillus niger. After incubating for 4-5 days at room temp. the effectiveness of treatment was determined from the width of the fungus-free zone on either side of the specimen. In aging tests, specimens treated to saturation with PCP-Na (up to 5% aqueous sol.) retained their antifungal properties much better than specimens treated with the commercial PCP-containing agents Xylamon and Xyladecor. Results suggest that Xylamon treatments on surfaces exposed to weather should be repeated every 1-2 yr.


Trihaloallyl and trihaloacryl derivatives were synthesized for wood preservative evaluation. It was shown in bioassays that the presence of iodine in the compounds is important for antifungal activity. Among the compounds tested, triiodo- and monobromodiodo-allyl esters and -acrylic esters were found to possess marked protective action to wood moulds and wood destroying fungi. 3-Ethoxycarbonylethyl 2,2-dibromodiethylene glycol was chosen as the most promising potential wood preservative as it also showed insecticidal activity.


Matched samples of southern pine lumber and plywood were treated with CCA-preservative and then air-dried. When conditioned at the same RH and temp., the treated lumber and plywood had significantly higher m.c. than untreated samples. The CCA treatment and air-drying did not affect the bending and shear properties of lumber when retention level was 0.6 lb/ft². Compressive strength of treated lumber was reduced by 9% and it was attributed to the higher e.m.c. associated with treated lumber. Bending and glue-line properties of treated plywood were reduced by about 10% because of the increased e.m.c. of treated material.


The thatched roofs of Fiji"'s 'bure' structures, treated with Boliden K33 or Tanalith NCA (2 CCA treatments) by various methods, were observed for several years and the uptake of preservative and a visual assessment of performance were recorded. The thatch materials tested were dry leaves (Metroxylon vitiense), reed stalks/leaves (Miscanthus floridulus), grass stalks (Vetiveria zizanioides), and guinea grass (Panicum maximum). Trials were also set up with test panels of palm leaves treated with one of several preservatives. On the basis of the
results it is recommended that for large undertakings the material should be air-dried and then pressure-treated with a 3% CCA solution by weight, or by means of a 10% CCA solution on 1 lb/GF of wood. For small undertakings the thatch should be dipped or sprayed in CCA or a combination.

667 ALSTON, A. S.; Sapstain and its control. Fiji Timbers and their Uses, Department of Forestry, Fiji (1984) No. 77, 10 pp. [En, 1 pl.]

A brief guide to the prevention of sapstain in 'Fiji' pine (Pinus caribaea), slash pine (Pinus elliottii), kauvala (Endospermum macrophyllum) and mavota (Gonystylus punctatus). Recommendations include prompt exclusion and milling, dipping or spraying with chemicals, and proper seasoning procedure. A table of 10 anti-sapstain chemicals shows formulation, suggested concen., costs, etc.


A report on laboratory tests on some new wood preservation methods: acetylated Scots pine sapwood (20% acetyl content) were completely resistant to attack from fungi (Coniophora puteana and Poria placenta) and wood borers (Hylotrupes bajulus larvae), but not termites. The treatment of wood flour with various organic reagents under mild reaction conditions (60°C) is also discussed. Studies were made on wood treated with high molecular weight synthetic organic oligomers; solutions of a sulphenamide dimer provided effective resistance to fungal attack. Results indicate that surface treatment with certain oligomers can provide long-term protection, with minimal environmental side effects (from atmospheric contamination etc.).


Distribution and retention of Cu, Cr, and As were studied in tests involving two timber species (wood flour prepared from Pinus radiata or Eucalyptus grandis), 3 treatment temperatures (21, 50, 80°C), 3 CCA (Cu-Cr-As) sol. concn. (0.018, 2.5, 8.0%), and 4 sol. pH (1.2, 1.8, 2.4, 5.0). Multivariate equations (21, 50, 80°C), or up to extreme treatment conditions. Sludging reactions are reported. The effect of varying pressures (5.3-8.8 kgfcm 2 ) and time of treatment (1-3 h) were evaluated on penetration and retention of the preservative. Increased pressure and time of treatment together tended to increase preservative retention (range 22.3-122.2 kg/m 2 ) but not penetration (range 0.53-1.7 cm) but results were rather variable. A more severe schedule is recommended to ensure better and more uniform results.


From review in Holz-Zentralblatt 111, 1042.

A collection of relevant DIN standards, including those for wood preservation in building construction.


A study of the effect of varying pressures (7.03-10.54 kgf/cm 2 ) and treatment periods (2-6 h) at 82°C were evaluated on penetration and retention of preservative. Both penetration and retention increased significantly with pressure and time of treatment, with the highest values (152.6-175.4 kg/m 3 and 2.43-2.89 cm) occurring at 10.54 kgf/cm 2 pressure for 6 h. In a further experiment, coefficients of variation of retention and penetration were measured in 8 poles (3 samples/pole) with av. sapwood depths of 3.78-8.1 cm, treated at 8.79 kgf/cm 2 pressure for 3 h; respective values were 2.1-22.6% and 2.1-14.8%. Retention and penetration were directly correlated with sapwood thickness (r=0.87 and 0.77 respectively).


Poles (for power lines) were collected from a 20-year-old plantation in the Chittagong Hill Tracts District. Bark was removed with a scraper and the poles were stacked and air dried in the open to a m.c. of 20-25%. Av. diam. was 11.2 cm, sapwood thickness 1.5 cm and sapwood percent 45. Poles were treated with 40:60 creosote/ash oil diesel fuel by the Bethel full-cell process, starting with a vacuum treatment of 1 h and finishing with one of 15 min. The effects of varying pressures (5.3-8.8 kgf/cm 2 ) and treatment periods (1-3 h) were evaluated on penetration and retention of the preservative. Increased pressure and time of treatment together tended to increase preservative retention (range 22.3-122.2 kg/m 2 ) but not penetration (range 0.53-1.7 cm) but results were rather variable. A more severe schedule is recommended to ensure better and more uniform results.


Southern pine composite (6M-PLY) joists were made with various types of core to determine treatability and drying characteristics. Southern pine veneer was used on the edges. The joists were impregnated commercially with CCA preservative in two charges to a target retention of 0.60 lb/ft 2 . Treatment was followed by kiln-drying in the laboratory using two schedules. Retention of preservative in composite joists compared very favourably with retentions of sawn joists treated in the same charge. Ninety percent of the retentions for the composite joists exceeded 0.54 lb/ft 2 . The composite joists dried rapidly without defects and matched the sawn joists in moisture loss rates. Drying to 10% required 4 to 7 days depending on the severity of the kiln-drying schedule. No delamination of veneer or veneer-core joints occurred. No warp appeared in the composite joists but over 99% of the best of the sawn joists (No. 1 Common) exhibited slight warp. Appearance was uniformly good in the composite lumber. Knots, pitch streaks, and stain, typical of sawn lumber (particularly No. 2 Common and lower grades of southern pine), were absent in composite joists.


A review of the literature on the use of coal tar oils (including creosote and carbolineum) as wood preservatives, under the heads: general aspects (definitions etc.); composition, specifications, and properties; effectiveness; environmental considerations; and use.


Muds produced in the gas refining of aluminium in the USSR contain 15-30% of F compounds. Preliminary tests showed the muds to be as toxic as NaF to Coniophora cerebella (F. puteana). The muds were formulated with various binders as a paste for brush application to wood. The most effective formulations, containing 40-50% of muds with latex and kaolin, were as effective as NaF in graveyard tests. Results suggest that F compounds may not be the only biologically active constituents of the muds.

A third collection of abstracts, obtained by searching the CAB database (1983/84) for records on the preservation and fire-proofing of wood and wood-based materials. Abstracts are arranged under the following main headings: General; Wood preservation from a national point of view; Methods of preservation; Standards; Conferences; Comparative tests; Fire-proofing; Control of termites; Panels, boards, etc.; Marine timbers; Treatability of timbers; Test methods; and Toxicity, pollution problems, etc. An author index is included.


A continuous flow apparatus was designed that maintained a constant fumigant concn in the air surrounding infested wood blocks. The effectiveness of the fumigant (MIT) on P. carbonis in Pseudotsuga menziesii heartwood blocks was determined by comparing estimates of fungal populations before and after treatment. The product of MIT concn and exposure time necessary to kill 90% (CT90) of the fungal propagules ranged from 46 to 179 µg h/cm² air, being lower with higher wood moisture content (40% or 70%, as opposed to 20%) and with longer fungi exposure (32 h, as opposed to 6 h). Although P. carbonis was least susceptible to MIT in blocks at 678 BLOW, was found in quantities capable of preventing decay. The MIT sorbed by the wood during fumigation (32 and 26 mg MIT/g oven dry wood for spring and summer wood respectively) was rapidly lost during aeriation, indicating that MIT was loosely sorbed to the wood structure.


The movement of boron from fused borate rods was studied in both European redwood (Pinus sylvestris) and European whitewood (Picea abies), using 2 techniques. The first method studied the movement of boron from borate rods when water was being taken up via the end grain of test pieces (mass transport). The second examined the diffusion of boron from borate rods in pre-soaked timber pieces (passive diffusion). The distribution of moisture and boron within the pieces was determined. In addition samples were exposed to Coniothyrium puteana to establish if sufficient boron was present to prevent decay at various distances along the length of test pieces. It is concluded that the minimum moisture content required for boron movement to occur is difficult to establish precisely but is probably near 30%. In general where moisture contents were in excess of 30% boron was found in quantities capable of preventing decay.

PAPER
See also abst. 756

TEXTILES


In laboratory studies in the USA, spray applications of a pressurized solvent-based formulation of fenvalerate at 0.25% to woolen cloth gave protection against feeding damage by larvae of Attagenus unicolor, Antherurus flavipes and Tineola bisselliella, both initially and after the treatments had aged for 15 months. Similar treatments that had aged for 6 months were also effective against adults of the 3 species.


Members of the International Biodeterioration Research Group tested standard textile strips for tensile strength on their tensiometers. It was concluded that, in terms of variability shown by standard cloth, tensile strength data are not machine dependent.

HYDROCARBONS, FUELS AND LUBRICANTS


Polymers obtained by graft co-polymerization of acrylic monomers with humic substances, pre-treated with protein (collagen hydrolyzate), possess a high degree of microbial resistance, irrespective of the conditions of their synthesis and of the molecular weight of the protein. This resistance of polymers to the action of microorganisms is due to the fungicidal and bactericidal properties of the compounds studied. These properties of polymers make them highly reliable as additives for drilling liquids. The drilling liquids used are in constant contact with soil which is a natural source of microorganisms.

PLASTIC POLYMERS

Rubbers
See also abst. 813

Plastics


Tensile strength, weight losses, and percent ultimate elongation changes were used to monitor the effects of the growth of 4 fungi (Gliscoladium roseum, Chaetomium globosum, Penicillium citrinum, Aspergillus fumigatus) on polyester and polyether polyurethanes elastomers. A comparison was made between the use of a base pulp and a complete medium for growth of the fungi in the presence of the polyurethanes samples over 28 days. In addition the effects of including competitive substrates in the medium were monitored using urea, a polyester (based upon ethylene glycol, butan-1, 4-diol and adipic acid), and gelatin. The polyester polyurethanes were significantly more susceptible than the polyether polyurethanes. The complete medium delayed the utilisation of the susceptible polyurethanes but after 28 days the recorded changes were similar for both media. The presence of competitive substrates in general decreased the changes in the properties measured with the exception of gelatin which enhanced the activities of P. citrinum and A. fumigatus on the polyester polyurethanes.

PAINT

See also abst. 387
STONE AND CONCRETE

See also absts. 387, 814

CONSTRUCTIONS

See also absts. 387, 756

683 GRANT, C.; BRAVERY, A. F. Laboratory evaluation of algidical biocides for use on constructional material. 3. Use of the vermiculite bed technique to evaluate toxic washes, surface coatings and surface treatments for eradication or prevention of growths of algae on asbestos-cement test panels. Toxic washes containing aqueous formulations of tri-n-butyltin were the most effective of those tested and the algal resistance was conferred on an organo-inn water repellent by incorporation of tri-n-butyltin oxide. Algical emulsion paints for masonry prevented growth of algae but with those studied the protection effect was completely lost following artificial weathering of painted panels.

Packaging

See also abst. 837


Bacteriological rinses were performed on ice cream cups, their lids and wooden or plastics spoons from 5 ice cream factories. Mean bacterial counts for samples from each factory ranged from 73 to 102/cup and from 60 to 120/lid; yeast/mould counts ranged from 35 to 85/cup and from 18 to 45/lid. Plastics spoons from 1 factory had a mean bacterial count of 25/spoon and no yeasts/moulds; at the other 4 factories, using wooden spoons or sticks, mean total bacterial count ranged from 670 to 8540/stick and yeast/mould count from 55 to 1600/stick. Main bacterial contaminants were Escherichia coli, Bacillus subtilis, Clostridium botulinum, and Actinomyetes. Moulds found were Aspergillus, Penicillium and Micor spp.


Reflectivity, smoothness and geometry of several types of food packaging board were studied in relation to the effectiveness of decontamination treatments involving ultraviolet (UV-C, 254 nm) irradiation. Surfaces containing aluminium in the laminate reflected more light in the 325-350 nm range and showed a lower lethal effect when Bacillus subtilis spores were irradiated. Visible light of wavelengths between 325 and 550 nm is known to cause photoactivation of UV damage in vegetative cells and it is suggested that a similar phenomenon might occur in spores on reflective surfaces. Smoothness of the board surface was not an important factor in the extent or the variability of the lethal effect. The geometry of the irradiated surface was shown to be important for aluminium/polyethylene laminate-laminated surfaces only, as more spores were killed on board normal to incident UV-C irradiation than in cartons with reflective angles. Spores on the inner sides of this type of carton may have received more reflected light of photoactivating wavelengths.

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Instruments and Equipment


Microbiological surveys of post-processing can handling equipment were conducted in 3 low-acid food canneries to identify the source and numbers of mesophilic anaerobic sporeformers isolated from post-processing spoilage of cans packed at those canneries. Significant numbers of spores of these organisms were found on various equipment and can tracks. The spores were also isolated from the can cooling waters in 2 of the canneries and in numbers higher than have been reported previously. No correlation was noted between mesophilic anaerobic spore counts and total aerobic counts in samples obtained from the surveys. Clostridium botulinum was not isolated from any of the survey samples. A medium useful in the isolation of mesophilic anaerobic sporeformers is described.

Works of Art, Museum Specimens and Books

See also absts. 628-629


Larvae of Idaea bonifata are reported infesting herbarium specimens in France on 4 occasions in 1968-82. This geometrid is a native of the south-western USA and Mexico and has occasionally been reported from Europe before. The adult, larva and pupa are illustrated and notes on the biology and injuriousness are provided. The larvae appear to develop exclusively on dry plants, attacking the flowers, leaves, roots and rhizomes. Seven kinds of dried plants that were infested in France are listed. It is suggested that various herbarium, aromatic and medicinal plants could be infested. Larval development appears to take about 2 or 3 months. While 3 or 4 generations a year are possible, populations are highest at the end of autumn, in winter and at the beginning of spring, and it is in this period that damage has been reported. It is not known whether I. bonifata can survive out of doors. Possible control measures discussed are burning infested material and the use of insecticides as a vapour action such as dichlorvos.

Structures and Vehicles

See also absts. 666, 708, 824, 827, 839

688 [Control of Hypotrupes bajulus in a stave church.] Hausbockbekämpfung in einer Stabkirche. Holz-Zentralblatt (1985) 111 (67/68) 1020 [De, 1 pl.]

A report on the treatment of (three) approx. 800-year-old churches in Norway in 1984, including a 1100 m³ volume church at Hopperstad. The buildings were (reasonably effectively) sealed with plastic film etc. and fumigated with phosphine (H₃P) at a target concn. of 1 to 2.8 g/m³ (the upper limit chosen to avoid damage to artwork). Atmospheric toxicity was monitored using test pieces containing Anobium punctatum larvae. Complete mortality of Anobium larvae was obtained after 5/7-day fumigation treatment.


About half of the 13 000 bridges in the US Forest Service transport system have wood as a major structural component. Reasons are put forward for using wood in favour of competitive materials. Inspection procedures to detect decay and conditions conducive to decay are discussed together with suitable preservative treatments. A method for determining strength loss due to decay is described. The maintenance of timber bridges is
discussed and examples of rehabilitation (major restoration) of deteriorated and substandard bridges are described.


A simple, cheap and effective method for extending the life of the perishable coconut leaf thatch and making it fireproof is described. Thatched roofs normally need replacement every year but their life span has been extended to 4 years by treating the thatch with chemicals that are neither washed away by rain nor degraded in sunlight. Five types of fungi were isolated from decayed thatched roofs. In the treatment recommended by the Regional Research Laboratory in Trivandrum copper sulphate is used as a fungicide followed by spraying the dried thatch on both sides with a liquid extract from the shell of cashew nuts (CNSL) and mixed with kerosene. CNSL is an excellent water repellent. If spraying is carried out with phosphonylated-CNSL then protection from fire is also conferred.

FOULING

Aquatic surface fouling - marine and freshwater

691 Curtin, M. E. Trying to solve the biofouling problem. Bio/Technology (1985) 3 (1) 38 [En]

Research on non-toxic marine coatings takes 2 forms: making products that organisms cannot stick to (low-energy coatings along the line of Teflon) or using knowledge of bacterial and invertebrate settling mechanisms to make products the organisms will not want to stick to. The first step in fouling is the adhesion of macromolecules. Research is concentrating on the second step (settlement of bacteria and microalgae) and the third step (invertebrates that settle on top of the initial bacterial or algal colonizers).


The attempt by the Department of the Environment to cut the use of organotin antifouling paints on small boats has been postponed to "allow the paint industry and its customers time to adjust". From next January only the worst offenders among the organotin paints, those copolymer paints containing severe constraints imposed on such an apparatus by the requirement to allow TBT to curtail the use of organotin antifouling paints on small boats has been postponed for the study of defined interactions of toxic metals and biofilms. Quantification of the partitioning of lead and trace metal lead and marine bacterium Pseudomonas atlantica into simple media was explored with the mathematical modelling of these interactions was explored with the the thatch with chemicals that are neither washed away by rain nor degraded in sunlight. Five types of fungi were isolated from decayed thatched roofs. In the treatment recommended by the Regional Research Laboratory in Trivandrum copper sulphate is used as a fungicide followed by spraying the dried thatch on both sides with a liquid extract from the shell of cashew nuts (CNSL) and mixed with kerosene. CNSL is an excellent water repellent. If spraying is carried out with phosphonylated-CNSL then protection from fire is also conferred.

BIODETERIORATION ORGANISMS

See also abs. 681, 842

Bacteria

See also abs. 388, 396-398, 403-405, 527, 538, 541-543, 546, 549, 553-556, 558-560, 562, 564-565, 567, 572, 577, 579, 581-583, 586, 588, 590-598, 606-607, 609-610, 618-623, 625, 628-629


This brief review discusses spoilage of refrigerated foods due to psychrotrophic microorganisms and the enzymes (particularly proteinases) produced by them. The foods considered are mainly milk and milk products. The effects of psychrotrophs on the keeping quality of milk and on cheese-making processes are mentioned.


The roles of streptomyces in biodeterioration and as agents of human disease are discussed. Their responses to environmental factors are briefly summarized and the principles and problems of methods for their detection and isolation considered. The current taxonomic status of Streptomyces and related genera is defined. Procedures for the preliminary grouping of isolates and the alternative schemes for their identification are outlined and evaluated.

Terrestrial surface fouling


Thirty commercial, 9 inorganic and 3 organic compounds were screened for effectiveness against laboratory-cultured T. odorata using a rapid laboratory method. A limited number of paint samples containing selective biocides were rapid-tested on glass slides using the same cultured alga. Of those biocides incorporated into paints, Antifoulant C9211M showed promise, but needs extensive field testing to evaluate its effectiveness against the algae outside the laboratory. The urgent need for rapid methods in the search for an effective biocide against T. odorata is discussed.
Fungi


Two extracellular endo-cellulases (of the endo-1,4-β-glucanase type) were highly purified from a brown rotting fungus, *Tyromyces palustris* (*Polyergus palustris*) by column chromatography on molecular sieving and anion exchanger. The two cellulase preparations (II-2-1 and IV-3-1) showed a broad, single protein band on disc gel electrophoresis but it was found that still each contained a trace of xylanase activity with a slightly different isoelectric point on isoelectric focussing. The mol. wt. and the isoelectric point were shown to be respectively. Substrate specificities and some further properties of the two cellulases were investigated.


The rate of mycelial growth of the true dry rot fungus *S. lacrimans* was found to be essentially the same over nutrient and pH 3.35 for IV-3-1, respectively. Substrate specificities and some further properties of the two cellulases were investigated.


Of 9 speeies investigated, only Schizophyllum commune grew at ψ below -4 MPa. Clitocybe aurantiaca (*Hygrocyphus aurantiacus*) and Daecyymyces sp. were especially sensitive to water stress. Max. growth for 7 species occurred at -0.5 MPa or higher.


The enzymes were purified and their properties compared. Both are haem proteins containing protoporphyrin IX. IR and RPR spectroscopy indicated that Fe ions are coordinated with the enzymes' prosthetic groups as high-spin ferritheminate complexes. The roles that the enzymes are believed to play in lignin degradation are discussed.


Eight isolates of *Aspergillus flavus*, 3 of *A. parasiticus*, 1 of *A. ochraceus* and 10 of *Penicillium* spp. were evaluated for their ability to hydrolyze protein, fat and hydrogen peroxyde when the moulds were grown in the presence of different amounts (0-10%) of NaCl. Proteolytic and lipolytic activities of *A. flavus* generally increased with an increase in the amount of NaCl in the medium. This was true for proteolytic and less so for lipolytic activity of *A. parasiticus* and *A. ochraceus*. Of the *P*. spp. tested, 5 isolates exhibited a marked increase and 5 a smaller increase in proteolytic and lipolytic activity at 2, 4 and 8% NaCl, but such activity either remained constant or decreased at 10% NaCl. Peroxidase activity in mycelia of all isolates of *A*. spp. increased with an increase of NaCl in the medium. Most isolates of *P*. spp. exhibited maximum peroxidase activity at 2% NaCl, and some reduction in activity when the amount of NaCl in the medium exceeded 2%.

Algae and Lichens

See also absts. 683, 695

Insects

See also absts. 453-455, 466-471, 473-474, 481, 496-497, 503, 530, 533, 634-660, 679, 687-688, 727-742, 744-750, 752-753, 755, 834-836, 838


Eight isolates of *Aspergillus flavus*, 3 of *A. parasiticus*, 1 of *A. ochraceus* and 10 of *Penicillium* spp. were evaluated for their ability to hydrolyze protein, fat and hydrogen peroxyde when the moulds were grown in the presence of different amounts (0-10%) of NaCl. Proteolytic and lipolytic activities of *A. flavus* generally increased with an increase in the amount of NaCl in the medium. This was true for proteolytic and less so for lipolytic activity of *A. parasiticus* and *A. ochraceus*. Of the *P*. spp. tested, 5 isolates exhibited a marked increase and 5 a smaller increase in proteolytic and lipolytic activity at 2, 4 and 8% NaCl, but such activity either remained constant or decreased at 10% NaCl. Peroxidase activity in mycelia of all isolates of *A*. spp. increased with an increase of NaCl in the medium. Most isolates of *P*. spp. exhibited maximum peroxidase activity at 2% NaCl, and some reduction in activity when the amount of NaCl in the medium exceeded 2%.

When the stored products pest *Plodia interpunctella* was reared in the laboratory in Brazil on an artificial diet at 25 ± 2°C and 70 ± 10% RH, the egg stage averaged 4.51 days and 63% of the eggs hatched. The 5 larval instars together lasted for an average of 24 days.


On 17 June, 1982, aggregations of adult males of *Collops hirtellus* were reported from northern Idaho, one each in Lewis- ton and Moscow. The beetles were swarming around houses in such numbers that they were considered a nuisance, In June 1983, beetles again aggregated at Moscow. In all instances, sun decks or other surfaces had been treated with a wood preservative. When the attractiveness of the wood preservative (containing 0.5% bis(tributyltin) oxide, 0.5% N-(trichloromethyl)phthalamide and 99% inert material) to adult males of *C. hirtellus* and *Formica* spp. was determined in trap tests near Moscow in 1982-83, both species exhibited
attraction. Further research was needed to identify the attractant.

709 SUZUKI, T. Presence of another aggregation substance(s) in the frass of the red flour beetles, Tribolium castaneum (Coleoptera: Tenebrionidae). 

Males of the stored-product pest Tribolium castaneum are known to produce an aggregation pheromone, (4R,5R) - 4, 8-dimethyldecane. Laboratory studies in Japan revealed the presence of another aggregation substance in this species. The new substance (or substances), which was successfully extracted with hexane and was clearly different from the pheromone produced by males, was produced with the frass of adults of both sexes.


When adults of Ctenoborus maculatus in 19 successive generations were selected by exposing them for 24 h to cowpea [Vigna unguiculata] seeds treated with carbaryl, malathion and lindane at concentrations causing 50-60% mortality in laboratory studies in Egypt, the development of resistance to carbaryl was marked and rapid, whereas that to the other 2 compounds was weak and slow. The LCS50s of adults in the F1, F2, F3 and F4 generations were 34.2, 113, 282 and 655 ppm, respectively, for carbaryl, 1.2, 2.2, 2.8 and 2.8 ppm for malathion and 1.6, 3.8, 3.7 and 3.8 ppm for lindane. Overall, there were 38.5, 2.5- and 1.02-fold increases in resistance to carbaryl, malathion and lindane, respectively.


The effects of relative humidity, temperature and food on the development of the stored-product insect Trogoderma granarium, a quarantine pest in Poland, was studied in the laboratory. The pest was highly resistant to low air humidity. At 28°C, its development was most rapid. Complete development took place at temperatures between 25 and 30°C. Keeping mature larvae at -15°C for 10 days caused 96% mortality, while 91% of larvae kept at -5°C for 5 months survived. Of 36 kinds of food tested, wheat germ and oat flakes were the most suitable. Larvae survived for 816 days without food at 15°C, and when transferred to wheat grain at 28°C their diapause was interrupted and some of them completed their development.


Studies carried out in Poland showed that feeding by Tyrophagus putrescentiae caused a decrease in weight of stored sugar beet seeds that was dependent on the duration of the infestation and the degree of mechanical damage to seeds during processing. The greatest decrease in seed weight occurred over about 70%. The occurrence of mites in sugar beet seed had little effect on germination of polygerm seeds, while infested monogerm seeds showed some decrease in germination capacity. Incrustation of seeds appeared to be a good method of protecting them against mite infestation.


The greatest decrease in seed weight due to irradiation and malathion-resistant (R) strains of Tribolium castaneum exposed to gamma radiation at 5, 10, 20 or 30 krad, followed by a 5-h exposure to malathion at the LD99.9 for the susceptible strain 1, 8, 15 or 22 days after irradiation, was assessed for up to 5 weeks after irradiation. Exposure of S strain adults to malathion, 1, 8 or 8 days after irradiation at all doses hastened the onset of death. Irradiation at 10, 20 and 30 krad was the dominant cause of death in the R strain. There was some evidence that exposure of the R strain to malathion 1 or 8 days after irradiation at 10 krad accelerated mortality. 2 and 3 weeks after irradiation, no progeny were produced by either the S or R strains irradiated at any dose. Irradiation of the S and R strains did not influence their response to malathion.


In surveys of migration by adults of the stored-product pests Tribolium castaneum, Rhysopertha dominica, Sitophilus oryzae and Cryptolestes spp. in a grain farming area of southern Queensland in 1979-82, T. castaneum and R. dominica were the predominant migrants, being active in the field from October to May. Traps in farm buildings and central storages caught many more beetles than did traps in the field. There was no clear relationship of catch with nearby infestations, except over short distances. A truck trap demonstrated beetle flight in the field between a wheat area 1600 and 1600 or deeper away. Only two traps were placed at 20°C above 26°C. The average beetle density at these times was estimated to be about 52/Mm². These insects represented a serious potential for reinfestation of stored grain.


Capture and analysis by gas-liquid chromatography of volatiles from adults of Cryptolestes ferrugineus infesting rolled oats disclosed that the naturally-produced ratio of the aggregation pheromones (5E,8E)-4,8-dimethyl-4,8-dodecadien-10-olide [(5E,8E)-3,9-dimethylhexacycloundeca-5,9-dien-2-one] (I) and (3Z,11S)-3-dodecen-11-one [(3Z,12S)-12-methylundecylcyclododec-4-en-2-one] (II) is 1:6.1:10. Approximately 990 and 640 pg/male beetle/h of the 2 pheromones, respectively, were produced in a culture with 76 beetles of mixed age and sex per gramme of oats. A newly designed trap, which confines beetles responding to attractive odours within an enclosed chamber, was very effective in recapturing released adults of C. ferrugineus when baited with a 13:16 mixture of I and (±)-11 released at 1.25µg/24 h, and also in recapturing released adults of Tribolium castaneum, S. oryzae and S. granarius. Effect of the aggregation pheromone, 4,8-dimethyldecane, released at 0.08µg/24 h. However, the capture of each species to traps baited with both species pheromones was no different than to traps baited with their pheromone alone. Thus the pheromones of both species can be used together in the same trap in semiochemical-based, pest monitoring systems.

716 UNGSUNANTWAT, A.; MILLS, R. B. Influence of rearing medium on size and weight of adults of four Sitophilus populations and larval weight of host kernels (Coleoptera: Curculionidae). Journal of Stored Products Research (1985) 21 (2) 89-93 [En, 10 ref.] Department of Entomology, Kansas State University, Manhattan, KS 66506, USA.

Four populations of Sitophilus (S. zeamais from Mexico, S. zeamais from Arkansas, S. oryzae and S. granarius) were
studied. Hard red winter wheat (‘Cloud’ variety), brown commercial sorghum and yellow and white ‘dent’ maize (mixed varieties) were used to determine the effect of parent- and progeny-rearing media on the adult elytron length in each population. Kernel weight loss caused by individuals of each population and weevil weights were determined. Elytron length of S. oryzae progeny were significantly (P<0.05) but only slightly longer, when parents were reared in wheat than sorghum or maize; otherwise parent-rearing medium had no effect. Progeny-rearing medium, not parent size, was most responsible for differences in elytron lengths. S. granarius progeny from wheat were significantly larger than those from maize or sorghum; progeny from all other populations were significantly larger from maize than from wheat or sorghum. S. oryzae caused less weight loss in kernels of wheat and sorghum, and were significantly lighter (P<0.05) than other insects. The Arkansas strain of S. zeamais was significantly heavier than the Mexican one in wheat, otherwise their weights were similar. Weight losses (actual and percentages) of kernels of all grains were similar for the Arkansas and Mexican strains of S. zeamais. S. granarius was heavier and produced greater weight loss in wheat kernels than other species did.


Studies on the inheritance of resistance to malathion in a field-collected malathion-resistant strain of Tribolium castaneum by making genetic crosses between the resistant strain and a susceptible strain revealed that resistance in this strain is controlled by an autosexually inherited single major gene which is incompletely dominant.

718 MONDAL, K. A. M. S. H. Response of Tribolium castaneum larva to aggregation pheromone and quinones produced by adult conspecifics. International Pest Control (1985) 27 (3) 64-66 [En, 38 ref.] Department of Agricultural Biology, The University, Nalbari, Assam, India.

The responses of larvae of Tribolium castaneum to flour conditioned by adults of the same species were studied in the laboratory. Larvae were attracted to flour conditioned for 3-6 days by males alone or in combination with females (1:1) but showed no preference for flour conditioned by females alone for 3-6 days. Larvae were repelled by flour conditioned for 30 days by either sex alone or both sexes together. This suggests the involvement of a male-produced aggregation pheromone in the attraction of the larvae and the involvement of quinones produced by both sexes in the repulsion of the larvae. An olfactory basis for these larval responses was demonstrated.


Phosphate-resistant strains of the stored-grain pests Rhizopertha dominica and Tribolium castaneum from New South Wales were tested for cross-resistance to 6 insecticides. Two strains of R. dominica and one of T. castaneum showed no cross-resistance. One strain of R. dominica was 7.2 times as resistant as a susceptible strain to malathion and 4.8 times as resistant as the susceptible strain to fenitrothion. One strain of T. castaneum was resistant to lindane (by a factor of 5.2) and malathion (by a factor of 77.9). Although these strains showed some multiple resistance to pesticides, there was no obvious correlation between the response to phosphate and resistance to DDT, lindane, organophosphates, carbaryl and bioresmethrin in the strains tested.

720 JAY, E. Imperfections in our current knowledge of insect biology as related to their response to controlled atmospheres. In Controlled atmosphere and fumigation in grain storages. Proceedings of an international symposium ‘Practical aspects of controlled atmosphere and fumigation in grain storages’ held from 11 to 22 April 1983 in Perth, Western Australia [edited by Ripp, B.E.]. Amsterdam, Netherlands; Elsevier (1984) 493-508 [En, 10 ref., 1 fig.] Stored-Product Insects Research & Development Laboratory, ARS, USDA, Savannah, Georgia 31410, USA.

Recent (mainly unpublished) research in the USA on the relation between laboratory studies on controlled atmospheres for controlling insect pests of stored products (especially Tribolium castaneum, Sagothrips oryzae, Rhyzopertha dominica, Sitophilus oryzae, S. zeamais, Trogoderma glabrum, T. variabile and Ephestia cautella) and their application under field conditions are reviewed. The responses within and between species and life-cycle stages and their relationship to transfer stimuli in controlled-atmosphere composition are examined. Shorter exposure times are generally needed to control stored-product pests in field situations than would be expected from laboratory results. Attempts are made to explain this phenomenon, how it relates to the overall information transfer of eggs is thus one to field application, and how the available laboratory data can be applied in the field.

721 MESSINA, F. J.; RENNICK, J. A. A. Ability of ovipositing seed beetles to discriminate between seeds with different loads. Ecological Entomology (1985) 10 (2) 225-230 [En, 18 ref.] Boyce Thompson Institute for Plant Research, Ithaca, New York 14853, USA.

Previous work has shown that ovipositing females of Callosobruchus maculatus avoid seeds already bearing eggs and thereby reduce competition among their larval progeny within seeds. The authors demonstrate that females also detect small differences in egg density and preferred to oviposit on seeds (Phassulus vulgaris) with a lower-than-average number of eggs. A nearly uniform distribution of females was observed even after all seeds bear several eggs. In addition, variation in egg load influenced the oviposition rate. Transfer of females from seeds with few eggs to seeds with many eggs inhibited oviposition; the reverse transferred stimulated oviposition. The appearance of the egg chorion or egg ‘cover’ remained intact on the seed surface after the larva entered the seed and continued to deter egg-laying for at least as long as the period required for larval development. Fourteen-day-old egg covers provided as much deterrence as freshly laid eggs.


Observations made in India on some unrecorded aspects of the biology of Trogoderma granarium on stored cereals indicated that no feeding took place during the rather short adult lifespan. Adults normally flew only when briefly disturbed, and persistent disturbance resulted in hastened oviposition. Oviposition began 1-2 h after mating, the eggs being laid singly among the grains. The males passed through 5 and the females through 6 larval instars. Characteristics of the moulding and mating processes are described.


Laboratory-reared adults of Callosobruchus chinensis in India were exposed 3 days after emergence to sublethal concentrations of six insecticides (representing the L50, L30 and L40 of diazinon, fenitrothion, fenthion, Metasystox [demeton-S-methyl] or dimefoxetho, by contact for 1 h with residual films from these compounds. Treated males and females were placed in pairs in petri dishes containing fresh gram seeds [Cicer arietinum] for mating and oviposition. Treated adults of both sexes had a shorter lifespan than did untreated ones, but females survived longer than males and laid more eggs per day than did untreated females; this increased fecundity, of which the rate differed with
different insecticides, was less marked in females treated with the equivalent of the LC20 and LC30 than with the LC40. Most of the eggs laid by treated females hatched. Despite the shorter lifespan, the eventual result of sublethal treatment was a population increase in comparison with untreated insects.

**Mites**

See also absts. 400, 468, 735, 838


The biology, ecology, occurrence and harmfulness of Tyrophagus longior were studied in Poland. The duration of development of the mite averaged 15.8 days at 20°C and 13.8 days at 25°C and 85% RH. The optimum conditions for development of the pest were 20–30°C and 85–95% RH, and the most suitable rearing substrates included wheat germ, bakers' yeast and oleaginous seeds. Under these conditions, each female laid an average of 327.6 eggs during its lifespan. The lifespan increased with decreased temperature, reaching an average of 166.8 days (female) at 10–15°C. Marked inhibition of mite development and oviposition occurred at temperatures below 15°C or above 35°C and at low humidities (65% RH). T. longior was shown to be a polyphagous pest of stored food products, seeds and greenhouse cucumber. Heavy infestation could cause damage to even total destruction of food products, seeds and plants. In a station of storage seeds by T. longior, especially those of oleaginous plants (hemp, poppy and sunflower) reduced their germination capacity and energy. The mite also occurred in beehives and birds' nests.


The mortality of newly emerged adults of the stored-product pest Acrasius soro was assayed by exposing them to 2, 4, 6, 10 or 21% oxygen in nitrogen, or to 10, 20, 30 or 40% carbon dioxide in 21% oxygen and with the balance of the atmosphere made up of nitrogen. The tests were carried out at 15 or 26°C and 75% RH in a specially designed apparatus. The mean duration of mite survival in air was 11 and 7 days at 15 and 26°C, respectively. Exposure to 2% oxygen was required to obtain 100% mortality in 72 h at 15°C, the same result was obtained within 120 h at 20°C by exposure to 10% oxygen. At 15°C, 30% carbon dioxide was required for 96 h to achieve 100% mortality; at 26°C, 20% carbon dioxide or more produced the same result within 72 h.

**Rodents**

See also abst. 832

**TECHNIQUES**

See also absts. 400, 639, 715


A simple and accurate technique for the determination of the heat resistance of spores is described. The technique combines a modified capillary tube method with a solid heating block. The come-up time of spore suspensions was found to be short and stable and an accurate technique is suggested for the correction of the come-up times. Experimental results are presented for the destruction of spores of Bacillus stearothermophilus at 120°C which indicates the accuracy and reproducibility of the new method.

**CONTROL**


727 Rabindra, R. J.; Balasubramanian, M.; Jayaraj, S. Effect of heat and gamma irradiation on the infectivity of Fari­ nocestis tribolii to Tribolium castaneum. Journal of Invertebrate Pathology (1985) 45 (3) 365-366 [En, 3 ref.] Department of Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

The schizogregarine Farnocestis tribolii is highly infective to larvae of the stored-products pest Tribolium castaneum in all instars. Laboratory studies were carried out in India to determine the effect of dry heat, moist heat and gamma radiation on the infectivity of F. tribolii to the beetles. The spores of F. tribolii were more sensitive to moist heat than to dry heat; the IT50 (the temperature to which the spores should be exposed for 30 min so that the mortality in inoculated larvae is inhibited by 50%) was lower in moist heat (45.64°C) than in dry heat (55.71°C). Spores exposed to 60°C moist heat or 62°C dry heat lost their virulence completely; the relevance of this finding to the safety of pathogen-treated wheat flour is discussed. Spores exposed to gamma-irradiation at a dose of 35 krad were completely inactivated; the IT50 (the quantum of gamma-radiation to which the spores should be exposed so that mortality in inoculated larvae is inhibited by 50%) was 13.4 krad. This indicated that F. tribolii and gamma-irradiation should not be used together in the control of T. castaneum.


The combined effect of gamma-radiation and 4 commonly used fumigants (ethylene dibromide, a mixture of ethylene dibromide and carbon tetrachloride (EDCT), carbon disulfide and methyl bromide) on larvae and adults of Tribolium castaneum was studied in the laboratory in India, the irradiation preceding or following fumigation. Larvae and adults exposed to 5 and 10 krad required higher doses of fumigants if fumigation occurred within 6 h of irradiation than if it was performed 7 days after irradiation. Irradiation preceding fumigation affected the susceptibility of the insects to subsequent fumigation, but fumigation did not affect the susceptibility of the survivors to subsequent irradiation.


In Ephesia cautella, Corcyra cephalonica and Tribolium castaneum, multiple and viable supernumerary larval moults were brought about by treatment of the last-instar larvae with the juvenoids Ro 10-3108 [epoxonene] and ZR-619 [triprene]. The normal last larval instar responded to such treatment throughout its life, but the number of extra moults was age- and dose-related. Supernumerary instars showed progressive increase in weight, size and head width. Day's law of growth was not strictly applicable. Until a certain stage, a single treatment or removal from a medium containing juvenoid was followed by 2 or 3 further moults and the insects then metabolized normally. Administration of juvenoids in the food was more effective than topical treatment.


The effects of 14 sesquiterpene lactones on feeding by Corcyra cephalonica and Tribolium confusum on wafer discs were studied in the laboratory in Poland. All the tested compounds considerably reduced feeding.
by these pests; the most effective were helenalin, eupatoriopicrin and linifolin A.


The feeding deterrent effects of extracts and lactone fractions of various plants of the Compositae against *Tribolium confusum*, *Trogoderma granarium* and *Sitophilus granarius* fed on wafers were studied in the laboratory in Poland. The greatest deterrent effects on adults of *S. granarius* were caused by lactone fractions of *Teckelia speciosa* and Asteriscus mari­tieus. Larvae of *Trogoderma granarium* consumed the greatest amount of wafers treated with chloroform and petroleum benzene extracts of *Pezesia multiflora* and *Venidium hirsutum*. The same extracts of *V. hirsutum* had the strongest deterrent effect on adults of *Tribolium confusum*.


As part of a study of the suitability of ultrasound as a means of controlling stored-product pests such as *Sitophilus granarius*, various levels of ultrasonic waves on the germination and growth of wheat were studied in the laboratory in Poland. Exposure to ultrasonic sound for 1-5 min accelerated germination and growth, while treatment for 10 min caused slowing of development and sometimes killed plants.

7.3 PRADZYNSKA, A. [The suitability of ultrasound for controlling stored-product pests. II. The effectiveness of treatment with ultrasonic waves on all stages of the granary weevil (*Sitophilus granarius*).] Przydatność ultradźwięków w zwalczaniu larw i nóg *Sitophi/us granarius*. II. Skuteczność działania fal ultradźwiękowych na wszystkie stadia rozwojowe woli zbożowego (*Sitophilus granarius*). *Prace Naukowe Instytutu Ochrony Rolni* (1982, recd. 1985) 24 (1) 77-80 [Pl, en, ru, 33 ref.]

A laboratory study was carried out in Poland on the effectiveness of ultrasonic sound against all developmental stages of the stored-grain pest *Sitophilus granarius*. The negative effects on the pest were manifested by reduced adult emergence and by mortality. In order to kill all the stages of *S. granarius* in wheat grains, treatment for 5 min at 14.5 W/cm² was necessary. At this intensity, adults were killed within 2 min when treated outside the grains and within 4 min in the kernels. During treatment at about 5.4 W/cm², 100% mortality of adults was obtained in 5 min when treated outside grains and after 5 min when treated inside grains.

7.3 NAWROT, J.; BŁOSZYK, E.; GRABARZYK, H.; DRODZÓ, B.; DANIENSKI, W. M.; HOLUB, M. Further evaluation of feeding deterrency of sesquiterpene lactones and growth of wheat were studied in the laboratory in Poland. The feeding deterrent effects of extracts and lactone fractions of various plants of the Compositae against *Tribolium confusum*, *Trogoderma granarium* and *Sitophilus granarius* fed on wafers were studied in the laboratory in Poland. The greatest deterrent effects on adults of *S. granarius* were caused by lactone fractions of *Teckelia speciosa* and Asteriscus mari­tieus. Larvae of *Trogoderma granarium* consumed the greatest amount of wafers treated with chloroform and petroleum benzene extracts of *Pezesia multiflora* and *Venidium hirsutum*. The same extracts of *V. hirsutum* had the strongest deterrent effect on adults of *Tribolium confusum*.


The deterrent effect of 11 sesquiterpene lactones on adults of *Sitophilus granarius*, adults and larvae of *Tribolium confusum* and larvae of *Trogoderma granarium* was studied in the laboratory in Poland. The activity of the test compounds was compared with that of helenalin. Deterrent activity equal to or greater than that of helenalin was caused by artdolte, usni­oide, A, lactarurufin A and B and erivarin against adults of *Tribolium confusum*, and by artdolte, desacetylarcolde and lactarufin A against larvae of *T. confusum* and *Trogoderma granarium*.


Long-term studies were carried out in Poland on the structure of the sense organs of the stored-grain pest *Sitophilus granarius* and on the behaviour of the pest in the presence of feeding attractants, repellents and antifeedants. On the basis of the reaction of the pest to substances isolated from wheat grain, it was found that those acting as phagostimulants were triglycerides containing residues of palmitic, linolic, oleic and linolenic acids; these compounds showed similar activity against other stored-product pests. More than 50 small-scale experiments, 24 aldehydes, 10 alcohols and 54 extracts from 28 plant species were tested for their antifeedant activity, and the most effective compounds were the lactones helenalin, linifolin A, eupatoriopicrin, yaritin, bakkenolide and bisabolangelone. The most effective repellent was caraway oil, the main component of which is carvone.


The susceptibility to methyl chloroform of *Cryptostyes farrugineus*, *Ephesia kuehniella*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitotroga cerealella*, *Sitophilus granarius*, *S. oryzae* and *Tribolium confusum* in all developmental stages, diapausing larvae of *E. elutella*, *E. cautella*, *Platax interpunctella* and *Trogoderma granarium* and adults of *Tribolium castaneum* was studied. Tests were conducted at 15, 20 and 25°C, 55-75% RH with concentrations ranging from 25 to 200 mg/litre and exposure times from 8 to 16 days. The toxicity of methyl chloroform was in general similar to that of carbofuran and the best results were obtained with *Trogoderma cerealella* and *Sitophilus spp.* and diapausing larvae of *Trogoderma granarium*, showed the highest levels of tolerance. Methyl chloroform was more effective at the higher temperatures but a concentration of 50 mg/litre was below the threshold level for full toxicity against all 5 species at 25°C. Depending on the species concerned, the concentration × time (CT) product required for control varied from 3700 mg.l/h/litre at 25°C to 60000 mg.l/h/litre or more at 20°C. At 15°Cfewer species were tested, but 5 of 9 survived CT products in the region of 30 000 mg.l/litre. There was no appreciable difference between the susceptibility of the immature stages of malathion-resistant and susceptibles strains of *O. surinamensis*. Neither were any consistent differences observed between adults of normal susceptibility and those resistant to methyl bromide or phosphine.

7.37 EVANS, N. J. The effectiveness of various insecticides on some resistant beetle pests of stored products from Uganda. *Journal of Stored Products Research (1985)* 21 (2) 105-109 [En, 8 ref.] Tropical Development & Research Institute, Storge Department, Slough, Berk, SL3 7HL, United Kingdom.

Laboratory tests were carried out in Uganda to compare the toxicity of *Tribolium castaneum*, *T. confusum*, *Sitophilus zeamais*, Callosobruchus maculatus and Zabrotes subfasciatus from Uganda, firstly to determine whether they were resistant to malathion and/or lindane and secondly to measure the effectiveness and stability of pyrimethanil, fenithrothion, etrimos, permethrin and deltamethrin dilute dusts in protecting cereals and pulses from these pests. All populations tested were resistant to malathion and lindane and some were resistant to both. Of the insecticides tested, deltamethrin at 1 p.p.m. was generally the most effective. The organophosphorus compounds were only effective against the *Tribolium* spp. and *S. zeamais*. Permethrin was the least effective, only controlling *C. maculatus*.

7.38 LAMBERT, J. D. H.; GALE, J.; ARNASON, J. T.; PHILLOGÈNE, B. J. R. Bruchid control with traditionally used insecticidal plants *Hypis spicigera* and *Cassia nigricans*. Insect Science and its Application (1985) 6 (2) 167-170 [En, fr, 9 ref., 6 fg.] Department of Biology, Carleton University, Ottawa, K1S 5B6, Canada.

The effectiveness of the plants *Hypis spicigera* and *Cassia nigricans* (from Upper Volta) used by farmers to control insect infestation in stored cowpeas *Vigna unguiculata* was studied in the laboratory. The oviposition and hatching of *Acanthoscelides obtectus* and *Platypus recessus vulgaris* were reduced when extracts of the plants (1 g plant material to 1 ml ethanol) were applied at low rates; the EC50 varied from 0.3 to 14 µl extract/g bean.
The importance of formulating insect growth regulators with surfactants and their blends for the control of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *Insect Science and its Application* (1985) 6 (2) 183-186 [En, 15 ref.]. Department of Entomology & Economic Zoology, Cook College, New Jersey Agricultural Experimental Station, Rutgers University, New Brunswick, NJ 08903, USA.

The use of surfactants and their blends to improve the penetration of hydrophrene and R-2045B ([E]-3-[4-(ethylphenoxyl)-3-methyl-3-pentenyl]-2,2-dimethyloxirane) into wheat kernels for the control of *Sitophilus oryzae* was investigated in the laboratory. The formulation of these insect growth regulators with the surfactant Tween-85 (polyoxyethylene trioleate) increased mortality of the weevil. The formulation of hydrophrene with a blend of Span-80 (sorbitan monooleate) and Tween-80 (polyoxyethylene-polyoxypropylene) produced results similar to those with hydrophrene plus Tween-85. However, formulations of the insect growth regulators with surfactants with high or low hydrophilic-lipophilic balance (HLB) failed to provide suitable control of the weevil, probably due to the type of emulsions achieved. The same results were obtained with hydrophrene formulations with blends of Spanes and Tweenes, even though their HLBs were close to that of Tween-85. The residual half-life of hydrophrene was about 3 months; formulations with Tween-85 or a blend of Span-80 did not enhance this residual activity.

Commercial potential of methyl bromide and carbon dioxide mixtures for disinfecting grain in controlled atmosphere systems or grain storages. Proceedings of an international symposium 'Practical aspects of controlled atmosphere and fumigation in grain storages' held from 11 to 22 April 1983 in Perth, Western Australia [edited by Ripp, B.E.]. Amsterdam, Netherland; Elsevier (1984) 327-341 [En, 16 ref., 4 fig.] CSIRO Division of Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.

Using examples from recent full-scale field trials in Australia, defects in the retention, distribution and application of fumigant that can lead to build-up of phosphine (methyl and ethyl) and the survival of insect pests are illustrated. The ratio of minimum to maximum concentration is used as an indicator of distribution in unsealed systems with uniform admixture of phosphine-releasing agents and sealed systems with surface application. A set of criteria is proposed by which the success of a treatment can be judged. In increasing order of stringency the criteria are (a) the grain be found free of insects by inspection after treatment, (b) the average maximum concentration of phosphine be over half that expected theoretically, (c) the concentration at the end of the exposure period be greater than the minimum effective concentration and (d) the ratio of the minimum to maximum concentration exceed 0.25 after not more than 25% of the exposure period.

Ethyl formate has been used successfully for the fumigation of individual packages for many years, and in tests reported from India it has shown promise as a safe general fumigant for stored foods in large quantities. Dosages of 300-600 g/nm² in an exposure period of 48-72 h controlled all stages of insects (including *Tribolium castaneum* and *Sitotroga cerealella*) in stored grains and their products. Large-scale desiccation is carried out on various agricultural commodities, including cereals, pulses, spices, dry fruits, nuts and dried tubers. Fumigation methods are discussed. The residues of ethyl formate or formic acid are much below the permissible limit of 250 p.p.m. Simple methods of detecting the above have been developed.

Tests with mixtures of methyl bromide and carbon dioxide for the control of insect pests (including Tribolium castaneum, Cryptolestes sp., Sitophilus granarius, S. zeamais, Rhizopertha dominica, and Oryzaephilus surinamensis) were conducted in silo bins containing stored grain at 3 sites in South Africa. Very high methyl bromide concentrations and complete kill of insects were obtained when the carbon dioxide was applied as a gas. Where the carbon dioxide was applied as snow or dry ice, the methyl bromide concentrations were much lower and there were distinct areas where very low concentrations and some insect survival occurred. It was concluded that air was trapped under the heavy gases and that there might be a need for recirculation to disperse methyl bromide more uniformly.


Fumigation (against Sitophilus oryzae, Rhizopertha dominica, Tribolium castaneum, Latheticus oryzae and Cryptolestes sp.) of grain stored in bolted-iron farm silos was investigated at 3 places in New South Wales in 1976-82. Fumigant leakage from unsealed bins reduced the effectiveness of carbon disulfide applied at dosages that were effective for 24-h fumigation in bins enveloped in plastic sheeting. Fumigation with 2, 6 or 12 g phosphine/l, applied as tablets containing aluminium phosphide probed into the grain, was effective both in unsealed bins and in bins enveloped in plastic sheeting for 7-10 days. Another tablet formulation containing aluminium phosphide, urea and ammonium bicarbonate, tested in conjunction with the aluminium phosphide-ammonium carbonate formulation used previously in these trials, gave similar results at 6 g phosphine/l after 7-8 days in unsealed bins.


The topics discussed in this review of fumigation as part of an integrated pest management programme in stored grain include hazards and safety precautions; health standards for fumigants; detection and analysis of fumigants; effectiveness of fumigants in controlling insects; properties of fumigants and fumigation and pest management programmes.


This account of the development of grain protectants for use in Australia includes information on test procedures (including the use of resistant strains of insect pests and field testing), combinations of grain protectants, protectants to control major pests (including multi-resistant strains of Rhizopertha dominica), synergism of synthetic pyrethroids, comparison between grains, summaries of application rates that have been shown to be effective for up to 9 months' storage, and residues.


Australia is recognized as a world leader in pesticide studies for the protection of grains. All South-East Asian countries have requested collaborative studies on pesticides. Little basic information exists on how pesticides behave when exposed to the high ambient humidities and commodity moisture contents prevailing in these countries. The project here briefly reported will study the chemical processes causing loss of insecticides during the storage and processing of tropical grains, nuts and legumes. The basic studies of pesticide decay will be carried out in Australia and field trials will be carried out in South-East Asia.

The results are presented of laboratory investigations and a field trial in Manitoba on the cold tolerance of insect pests of stored grain. Most of the species tested were susceptible to freezing temperatures, and it is suggested that this is the main reason why Cryptolestes ferrugineus, Sitophilus granarius, S. oryzae and S. zeamais are rarely or never found in stored grain in the Prairie Provinces of Canada. Cryptolestes ferrugineus is a common pest of stored grain there because it can acclimate to low temperatures and survive for several weeks at temperatures as low as −5°C. Tribolium castaneum and O. surinamensis cannot survive at temperatures below freezing for more than 2 weeks. The reason they have become established as pests of stored grain must be because they are able to find heated habitats in which to survive the winter.


With special reference to conditions in Canada, a review is presented of research on contact insecticides used as structural treatments to control insect pests of stored products, especially grain. A historical review of the main pests and their control in Canada is followed by discussions on the classes of insecticides, the residual activity of insecticides (including the effects of surfaces, formulations and application), insecticide uptake into stored products from treated surfaces and resistance to malathion in Tribolium spp. in Canada.


This evaluative report examines English, German, Dutch, French and Norwegian language literature published mainly between 1981 and 1984. It briefly considers surveys of the current situation in Germany, the Federal Republic of Germany, the United Kingdom, the Netherlands and Iceland and reports on the occurrence of pests in the German Federal Republic, Netherlands, Cape Verde and India (Orissa), before covering research on the biology and ecology of the following groups of pests: Acarina, Psocoptera, Coleoptera and Lepidoptera. The section on storage protection ecology considers the creation of conditions for normal storage (drying and cooling of harvested cereals), storage under modified atmosphere, refrigerated storage as a protection against pest infestation, residual and contact pesticides to these ecological measures (e.g., diapause, tolerance to CO2 and adaptation to unfavourable temp. and moisture conditions), use of resistant cultivars of rice, maize, Phaseolus vulgaris and Vigna unguiculata, and use of biotechnological methods for detection and control of pests (sexual and aggregation pheromones and light traps).


Disks (400 mg) of open-cell polyurethane foams containing 2.5% organotin fungicides, (tributyltin acetate and tributyltin chloride) prevented all growth on paper inoculated with fungi (Aspergillus flavus, A. niger, A. ochraceus, Paecilomyces variotii, Fusarium sp., Penicillium janthinellum, P. citrinum, Syncophlastrum racemosum) in 50 litre vessels for 6 months and inhibited some species after 12 months. The potential of this type of system to protect stored goods, optical equipment and documents against fungi in humid conditions is discussed.


Glutaraldehyde 4%, sodium/dichloroisocyanurate dihydrate (2400 mg/l active chlorine) and peracetic acid 0.25% demonstrated after 30 min of exposure at 20°C in the presence of 4% horse serum a clear activity against spores of Bacillus cereus. Under the same conditions formaldehyde 4% and glutaraldehyde 2% were also sporidical, but only after a longer time of exposure. Spores of B. anthracis and B. cereus appeared to be comparatively resistant against these disinfectants, whereas conidiospores of Aspergillus fumigatus and A. niger were less resistant. Of the microorganisms tested Candida albicans were the least resistant, and spores of B. subtilis the most resistant. It is concluded that B. cereus spores and A. fumigatus conidiospores appear to be suitable test organisms.

BIODEGRADATION - GENERAL


In harnessing the beneficial activities of microbes for national development, a number of factors, some social, others cultural, are encountered in either the acceptance or rejection of such technology by different peoples. Specific examples are cited, particularly with the development of technologies for the developing world in the food and energy sectors. Attention is also given to the role of women and to the use of resources that are acceptable to some communities and unacceptable to others due to prevailing sociocultural traits. Recognition of the different aids and barriers in development involves an understanding of the existing social and intercultural factors and their implications.


Written to appeal to a wide audience, the various aspects of this area of science and technology are clearly described. The author also endeavours to present the conflicting interests, problems and challenges which are raised by biotechnologies in their industrial development, their transfer to less privileged countries and their adaptation to various economic, social and cultural situations.

Planning and Plant Design
See also abst. 771

Hygiene and Pathogens


Composted sewage sludge may be used to improve soil quality, but there remains some doubt concerning the microbiological safety of the product. Sewage sludge composts from 30 municipalities were sampled and 4 samples (12%) contained Salmonella spp. All 30 composts were inoculated with salmonella. The populations decreased at a specific death rate of about 0.15h−1 over 24h at 36°C. In irradiation-sterilized composts inoculated with salmonellae, the salmonellae grew at a rate of 0.65 doublings per h over 24h. Growth and death rates were found to be moisture and flora associated. The growth or death rates for antibiotic-resistant strains were not different from those of non-resistant str. It was concluded
that the active indigenous flora of compost establishes a homeo-
static barrier to colonization by salmonellae, and in the absence
of competing flora, re inoculated salmonellae may grow to poten-
tially hazardous densities. The active microflora of moist com-
posts eliminated contaminating salmonellae (10^7/g) after 6
weeks.

761 WALTER, M. V.; VENNES, J. W. Occurrence of multi-
ple-antibiotic-resistant enteric bacteria in domestic sewage and
oxidation lagoons. Applied and Environmental Microbiology
Forks, ND 58202, USA.

The coliform bacterial population in the Grand Forks,
North Dakota sewage system was examined for multiple-antibiotic-
resistant organisms over a 1-year period. Multiple-antibiotic-
resistant coliforms were found to be common in the sewage,
and their numbers remained fairly constant relative to the total
coliform population throughout the year. Resistance to
kanamycin, tetracycline and ampicillin was found to be trans-
ferrable at variable rates. Transfer rates were found to be tem-
perature sensitive and were optimal at 35°C. Although 75% of
the multiple-antibiotic-resistant coliforms were capable of
transferring resistance at some level, only 25% were capable of
transferring resistance at rates greater than 10^-7 transconju-
gants per initial donor.

ENVIRONMENTAL POLLUTION


*Pseudomonas* str. capable of mineralizing 2,4-
dichlorophenol (DCP) and *p*-nitrophenol (PNP) in culture
media were isolated from soil. Growth of *Pseudomonas* and its
ability to mineralize DCP or PNP in lake water (sterile and non-
sterile), sewage (sterile and non-sterile) and soil (sterile and
non-sterile) was studied. It was suggested that microorganisms
able to degrade organic pollutants in culture sometimes may fail
to function when inoculated into natural environments because
the concentration in nature may be too low to support growth or
because the organisms may be susceptible to toxins or predators
in the environment, may use other organic compounds in prefer-
cence to the pollutant, or may be unable to move through soil to
sites containing the chemical.

BIOLOGICAL WASTE TREATMENT

Composting

See also abst. 760, 777-778, 803, 828

763 MCKINLEY, V. L.; VESTAL, J. R. Effects of different temperature regimes on microbial activity and biomass in com-
pposting municipal sewage sludge. Canadian Journal of Microbi-
Biol. Sci., Univ. Cincinnati, Cincinnati, OH 45221, USA.

Municipal sewage sludge was composted under 2 different
temp. regimes. The temp. within the composting piles were regu-
lated using a temp. feedback system controlling aeration
fans which blew air up through the piles. Each composting run
lasted c. 2.5 weeks. In one part of the composting pile, the mean
temp. were kept below c. 59°C, while in the other part the mean
temp. reached 84°C. Two experimental composting runs of this
type were performed. In both cases the major treatment effect
was the highly significant differences in microbial activity
(1^14C]acetate incorporation into lipids) per g of compost or per
unit of microbial biomass, with the low-temp. part of the piles
having the greatest activity. Microbial biomass (measured as
lipid phosphate) was greater in the low temp. part of the pile by
day 10 of the 2nd composting run. The pH of the low-temp. piles
to also increased more rapidly than that of the high temp.
piles. These results confirm other work in which increased rates
of CO2 production were detected in materials composting at
lower temp. It is suggested that the efficiency of batch compost-
ning processes is greatest at moderate temp. (not exceeding c.
55°C), since higher temp. greatly inhibit the growth and metabo-
lisum of the microbiota.


The pH, EC and water-extractable phenolics of fresh bark
composts increased rapidly at the initial stage of composting,
decreased with the process of decaying and reached almost
constant. The C/N ratio decreased in proportion to the decom-
position of carbohydrate during the process of composting. Most
of the organic nitrogen was rapidly transformed into the
organic nitrogen at the initial stage of the composting. From
the results, the indices for estimating the degree of maturity of
bark composts was considered to be as follows: pH of 6.5-7.5, EC of
below 3 mmho/cm, water-extractable phenolics of below 2 X
10^-3 M, C/N ratio of below 25 (hardwood bark) or below 35
(softwood bark), a ratio of carbon in reducing sugar to total
nitrogen of below 6 (hardwood bark) or below 10 (softwood
bark), and a ratio of carbon in reducing sugar to total carbon
of below 20% (hardwood bark) or below 30% (softwood bark).

Ponds, Lagoons, Oxidation ditches

See also abst. 761

Anaerobic digestion

fontein 9300, South Africa.

Cheese whey and deproteinized whey preparations with different
C/N ratios ranging from 7.5 to 73 were digested anaerobically
in a downflow stationary fixed-bed reactor at 35°C with a hydraulic retention time of 5 days. Effluent and biogas param-
ters indicated that no adverse effect on digestion and stability
occurred at the highest C/N ratio. Chemical oxygen demand (COD) removal averaged 88%, while volatile fatty acids
were maintained at a low level (<300 mg/l). Biogas yield averaged 0.423 m^3/kg COD and the methane content of the biogas varied
between 57 and 63%. Ammonia toxicity occurred at the C/N ratio of 7.5 in the substrate feed. Virtually no ammonia N was
detected in the effluent when whey substrates with C/N ratios of 50 or 73 were fed. Titrination curves showed that buffer intensities
in the effluent were not affected by a decrease in ammonia level.
A decrease in the biomass content of the effluent which occurred as a result of the increase in C/N ratio of the substrate did not
cause any rate limiting effect on biogas production. The removal
of protein from the whey caused a reduction in the ratio of
COD/total carbon in the whey. On average 59% of the C in the
substrate was converted to biogas.
These studies indicate that a collection of bacterial antibodies can be used to identify and map bacteria in situ in mixed samples.


The activity of methanogenic bacteria in anaerobic reactors can be studied using analysis of coenzyme F₄₂₀ and its derivatives.

WASTE DISPOSAL (BIOLOGICAL ASPECTS)


The study of a desulphurizing, denitrifying reactor has shown that the dominating autotrophs were present and at least 30% of the population is mixotrophs. The dominant heterotroph is a str. of Klebsiella. Among the dominant mixotrophs is a new species, Thiosphaera pantotropha, which can grow autotrophically, heterotrophically and mixotrophically under both aerobic and anaerobic conditions.

Land treatment and Irrigation


The biodegradation of domestic waste separates in soil at various dosage rates was followed over 18 months by assessing changes in ethanol/water solubles, organic carbon and nitrogen and the formation of humus substances. Biodegradation followed an exponential pattern, with rates decreasing at the highest dosage level (65 tonnes/hectare). The effect of the separates on certain soil parameters such as pH and water capacity was also followed together with an investigation into the release of zinc, lead, copper, cadmium and boron from the biodegrading refuse separates into the receiving soil.

Landfill

770 GILL, T. Landfill gas — a fuel from waste for industry. Industrial Biotechnology (1985) 5 (3) 5 pp. [En, 5 figs.] NCB (Coal Products) Ltd., P.O. Box 16, Wingerworth, Chesterfield S42 6JT, United Kingdom.

The production of gas from a landfill site in Essex is described. Generation of the gas (consisting of 60% methane and 40% carbon dioxide) can be controlled and even enhanced by new biological processes introduced to the landfill. Extraction techniques enable the gas to be collected, compressed and utilised profitably.


The production of landfill gas is an inevitable consequence of the deposition and subsequent degradation of organically-based waste materials in landfill sites. However both the rates of gas production, and also the quality of the product, are unpredictable. This article seeks to provide landfill site operators/owners with a range of options for managing their sites to ensure that maximum production and recovery of good quality (methane-rich) landfill gas is achieved.


In a model landfill kept under aerobic conditions, refuse from medical consulting rooms showed a low rate of mineralization. A mixture of municipal and medical refuse showed a similar mineralization rate to that of municipal refuse alone. Almost no mineralization occurred in all types of refuse disposed of in model landfills under nearly anaerobic conditions.

Feedstuffs

773 AFRIKAN, É. K. [Some aspects of the microbiological production of food and feedstuffs]. Biologicheskii Zhurnal Armenii (1984) 37 (10) 825-836 [Ru, en, 10 ref., 2 tab.] Institut Mikrobiologii AN Armianskot SSR, USSR.

The present state of the microbiological production of feedstuffs in various countries (including the USSR) is reviewed. The microbial production of feedstuffs on a large scale in Armenia is considered with regard to local resources and conditions.

Biological upgrading to feedstuffs

See also absts. 805, 811


Whey protein concentrate (WPC) had the following composition before and after incubation, resp., of a 200 g/l solution with calcium alginate-immobilized Kluyveromyces fragilis for 4 h at 28°C and pH 4: protein, 33.8% and 72%; fat, 2.8% and 5.5%; lactose, 54.2% and 0%; and ash, 9.1% and 22.4%. Demineralization could result in a 93% protein product.

Biological upgrading - other

See also absts. 783, 812


An improved fermented whey having greater mycotoxic activity is produced from the fermentation of whey with Pseudomonas acidilactici NRR NL 3568. This species gives a higher content of propionic acid, the mycostatic agent, than did P. Shermanii. The dried fermented whey can be used as a mycotoxic agent in bread and other bakery goods, particularly in those claimed to be natural.


When 4 organisms (Bacillus subtilis, Lactobacillus hilgardii and Klebsiella pneumoniae) were grown in a medium containing 50 g lactose, 5 g yeast extract and 2 g K₂HPO₄/L, only K. pneumoniae NCIB 8017 produced reasonable amounts of 2,3-butanediol, max. yield being 4.5 g/l (0.24 g/g lactose utilized) after 72 h at 30°C. When K. pneumoniae was grown in rennet whey permeate, a max. 2.3-butanediol concn. of 7.5 g/l (0.46 g/g lactose utilized) was obtained after 96 h at 30°C; ethanol and acetoin concn. were 0.3 and 1.1 g/l, resp. Corresponding yields when K. pneumoniae was grown in hydrolysed lactic acid casein whey permeate were 13.7 g 2,3-butanediol (0.39 g/g sugar utilized), 0.2 g ethanol and 1.1 g acetoin/l. It is suggested that lactose utilization may be a limiting factor in the fermentation process.
MUNICIPAL WASTES

See also absts. 760, 769

Solid wastes
See also abst. 772


Continuous thermophilic composting was examined with a 4.5 litre reactor placed in an incubator maintained at representative temperatures. Feed was a mixture of dried table scraps and shredded newspaper wetted to 55% moisture. One run at 49°C (run A) employed a 1:4 feed-to-compost ratio, while the other was used at 55°C. Both reactors were incubated at 50, 55, 60, and 65°C. Due to self-heating, internal temperatures of the composting mass were 0 to 7°C hotter than the incubator. Two full-scale composting plants (in USA and England) were also examined. Fifteen taxa were isolated, including 10 of genus Bacillus, which dominated all samples except that from run A. Species diversity decreased markedly in laboratory composting at 60°C and above, but was similar for the three runs incubated at 49, 50, and 55°C. The maximum desirable composting temperature based on species diversity was 50°C, the same as that previously recommended based on measures of the rate of decomposition.


The thermophilic microflora of solid-waste composting, with major emphasis on Bacillus spp., was examined with Trypsicase soy broth with 2% agar as the initial plating medium. Five 4.5 litre laboratory units at 49 to 69°C were fed a mixture of dried table scraps and shredded newspaper. Two composting plants (treating refuse in the USA and England) were also sampled. Of 652 randomly picked colonies, 87% were identified as Bacillus spp. About 15% of the Bacillus isolates could be assigned to species only by allowing for greater variability in one or more characteristics than has been reported by other authors for their strains. In particular, growth at higher temperatures than previously reported was found for strains of several species. A small number of Bacillus isolates (less than 2%) could not be assigned to any recognized species.

Liquid wastes
See also abst. 763

AGRICULTURAL WASTES

See also abst. 785

Cattle waste


The role of (hemi)cellulolytic bacteria in the degradation of plant cell walls during the methanogenic fermentation of cattle manure was investigated. Bacteria were enriched and isolated on a basal medium containing filter paper or xylan as sole carbon and energy source. A variety of anaerobic, Gram-negative, thermophilic aciditrophic bacteria were isolated on a basal medium containing filter paper or xylan as sole carbon and energy source. A variety of anaerobic, Gram-negative, thermophilic aciditrophic bacteria were isolated. Most of the isolates were able to grow on cellulose or xylan. Some of the isolates could use fibres from manure as growth substrate, degrading about 20% of the untreated fibre material.

SPECIFIC WASTE MATERIALS


The use of 2-chlorobenzoic acid (2-CBA) as a nutrient for P. cerasi was investigated. The bacteria grew better in the presence of riboflavin, but was inhibited by nicotinic acid. Possible pathways for the breakdown of 2-CBA, first to cis, cis-muconic acid, then to 3-ketoadipic acid are discussed.

781 KARASEVICH, YU. N.; ZAITSEV, G. M. [Utilization of 4-chlorobenzoic and 2,4-dichlorobenzoic acids by a mixed culture of microorganisms.] Mikrobiologiya (1984) 53 (3) 374-380 [Ru, en, 19 ref., 4 fig., 2 tab.] Inst. Mikrobiologii, AN SSSR, Moscow, USSR.

From an enrichment culture from soil samples, pure cultures of Pseudomonas putida and Corynebacterium sepedonicum were obtained. These 2 bacteria in association utilized 4-chlorobenzoic acid and 2,4-dichlorobenzoic acid as sole source of hydrocarbons and energy, and liberated 100% of the theoretically possible chlorine in the process. However, P. putida on its own was unable to metabolize either acid, while C. sepedonicum on its own metabolized them incompletely, accumulating 4-hydroxybenzoic acid and protocatechuic acid in the medium. These were utilized by P. putida. The two bacteria were synergistic in the further sense, that C. sepedonicum was unable to grow on its own without added vitamins, unless it was in association with P. putida.

Cellulosic wastes
See also absts. 815-819


The enzyme, purified from culture filtrates of S. rolfsii, is homogeneous as determined by disc gel electrophoresis, with a molecular weight of 60,000. The enzyme was not inhibited by acetate, which is a major metabolite of the organism. The enzyme was not inhibited by dithiothreitol, which is a major metabolite of the organism. The enzyme was not inhibited by dithiothreitol, which is a major metabolite of the organism. The enzyme was not inhibited by dithiothreitol, which is a major metabolite of the organism. The enzyme was not inhibited by dithiothreitol, which is a major metabolite of the organism. The enzyme was not inhibited by dithiothreitol, which is a major metabolite of the organism.

The two bacteria were synergistic in the further sense, that C. sepedonicum was unable to grow on its own without added vitamins, unless it was in association with P. putida.


A mixed culture of facultatively anaerobic, obligatorily thermophilic spore-forming bacilli, including Clostridium thermocellum and C. thermohydrosulphiicum, isolated from Buryat hot springs, was grown in a simple medium, containing 2% filter paper as a source of cellulose. Up to 0.85-1% of ethanol was evolved in the culture fluid, following anaerobic incubation under a water seal. Formic, acetic, propionic and butyric acids, and CO₂ and H₂ were also evolved. Ethanol was likewise produced when other sources of cellulose (malt sprouts, beet pulp or newsprint) were used.


Cellulase production by A. terreus can be affected by the "inductor", i.e. the type of cellulose present in the growth.
medium. Accordingly, _A. terreus_ p17 was grown in media containing malt sprouts, conifer sawdust (mechanically produced, with or without subsequent phosphoric acid treatment) or microcrystalline cellulose. The cellulase activity in the supernatant was assessed by the production of reducing sugars from filter paper. The protease and xylosence activity were also measured, and mycelia grown were examined microscopically. The mycelia of _A. terreus_ were found to produce malt sprouts were short with much branching, while those grown in microcrystalline cellulose were slender, longer and more uniform. The endoglucanase and cellobiase activity, and the digestion of filter paper, were much the strongest in cultures grown with microcrystalline cellulose.


The topics studied included straw disposal and the carry-over of crop pests and diseases at Long Ashton; rapid counting of cellulose-decomposing microbes at the Rowett Res. Inst.; a microbial kit to degrade straw at the Glasshouse Crops Res. Inst., and the ultrastructure and digestion of barley-straw cell walls at the Hannah Res. Inst.


A critical review of English, French and some German language literature, it mainly from 1983 onwards. It examines experimental evidence on the behaviour as bacteria to biodegradation of lignins and phenolic polymers such as tannins and suberins. The different molecular mechanisms of lignolysis by fungi (mainly), actinomycetes and bacteria are examined. A new biochemical approach to the physiological mechanisms of regulation of lignolytic activities is suggested based on the discoveries of lignolytic enzymes: effects of nitrogen, oxygen and substrate are discussed. It is concluded that a better knowledge of the structure and reactivity of phenolic barriers is needed in order to control the process of lignolysis.


The incidence of such bacteria was studied in 4 Karelian lakes in mid-July. The capacity of the bacterial isolates to decompose filter paper was estimated, using Winkler's reaction to measure the oxygen used, and also using C14 to estimate the CO2 evolved. The greatest cellulose-decomposing activity was found in lake water near wood-processing plants, at points with a temperature gradient and on the surface of water plants.


The bacterium produced a carboxymethyl cellulase (CMCase). The production of enzyme was strongly inhibited by the addition of glucose. After purification the enzyme gave a single band in AGE. The enzyme hydrolyzed carboxymethylcelullose with an optimum at pH 9.0 and a Km of 0.48 mg/ml; no activity was observed at pH 6.0. The enzyme had a molecular weight (SDS-PAGE) of 92000 and an isoelectric point of 3.1. The maximum degree of hydrolysis of carboxymethylcelullose was about 30% and trans-glucosidase activity was also observed.


Microorganisms metabolizing xylan as a carbon source, and the enzyme systems they employ for its breakdown, may become important tools in elaborating economically and ecologically beneficial processes for using the second most abundant renewable polysaccharide. Critical factors in converting xylan into useful products with xylanolytic enzyme systems are considered.


Bioconversion of cellulose and hemicellulose substrates to 2,3-butanediol by a sequential coculture approach was investigated with the cellulolytic fungus _T. harzianum_ and the fermentative bacterium _K. pneumoniae_. Vogel medium optimal for the production of the cellulolytic and xylanolytic enzymes of the fungus was found to be inhibitory to butanediol fermentation. This inhibition appeared to be due to a synergistic effect of various ingredients, particularly the salts, present in the fungal medium. The removal or replacement of such ingredients from Vogel medium led to the relief of fermentation inhibition, but the treatment also resulted in a significant decrease in fungal enzyme production. Resting cells of _K. pneumoniae_ could be used for butanediol production in the fungal medium, indicating that the inhibitory effect on solvent production under such conditions was due to the indirect result of growth inhibition of the bacterial cells.


Thermophilic (55°C) anaerobic enrichment cultures were incubated with [14C]-labelled lignin, cellulose, and hemicellulose. Significant but low percentages (2 to 4%) of synthetic and natural pure lignin were recovered as gaseous end products. The results overall indicated that elevated temperatures can greatly enhance rates of anaerobic degradation of lignin and lignified substrates to methane and low molecular weight aromatic compounds.

792 GRBIC-GALIC, D. Fermentative and oxidative transformation of ferulate by a facultatively anaerobic bacterium isolated from sewage sludge. _Applied and Environmental Microbiology_ (1983) 50 (4) 1052-1057 [En, 26 ref., 4 fig.]. Dep. Civil Engineering, Stanford Univ., Stanford, CA 94305, USA.

The bacterium was isolated from methanogenic consortia digesting 3-methoxy-4-hydroxyxinnonate (ferulate). (Methoxylate aromatic compounds are released during the aerobic catabolism of lignin). The isolated bacterium transformed ferulate under anaerobic and aerobic conditions. The pure culture has been tentatively assigned to the genus _Enterobacter_ with the type strain DG-6. Tentative pathways for both fermentative and oxidative degradation of ferulate are now proposed.


Of 20 strains of _C_. _spp._ screened, 17 hydrolysed larch wood xylan. Two strains of _C. acetobutylicum_ hydrolysed xylan but failed to grow on solid media with larch xylan as the sole carbon source; however one of these was subsequently found to grow on xylan under specified conditions in chemostat. These two strains possessed cellulytic activity and were selected for further studies. The xylanolytic enzymes of _C. acetobutylicum_ were capable of degrading in vitro, 12% of the larch xylan in a 24-h hydrolysis period. The resistance of xylan to degradation by the enzymes may be attributable to end product inhibition or the structure of xylan itself or both rather than to the instability of the enzymes, since the addition of fresh enzymes near the end of hydrolysis did not increase the extent of degradation significantly.
Study of plasmids controlling naphthalene biodegradation by catechol cleavage, while O controlled the leaching of uranium from ores. \[Review\]. Moscow, USSR; UNEP (1985) 69 pp. [En, 225 ref., 13 tab.]

The role of microorganisms in the oxidation of sulphide minerals and leaching of metals from ores and concentrates, and the factors underlying the kinetics of the above processes, as well as technical, technological, economic and environmental aspects of the biogeotechnology of metals are reviewed.


Long axes of the filamentous cyano bacterium LPP O.1.3 were incubated with samples of uranium-bearing coal from several sources. The influence of leaching parameters such as coal concentration (pulp density), initial biomass, particle size, temperature and composition of the growth medium on the leaching of uranium from the ore by the cyano bacterial strain were studied. It was found that the variations in leaching parameters were different from reactions of acidic leaching organisms.

Hydrocarbons, Petroleum

Kochetkov, V. V.; Boronin, A. M. \[A comparative study of plasmids controlling naphthalene biodegradation by Pseudomonas putida and Pseudomonas \[Microbiology (1985) 53 (4) 639-644\] \[Ru, en, 15 ref., 4 tab.\] Inst. Biokhimii i Fiziologii Mikroorganizm AN SSR, g. Pushchino, RSFSR.

From soil samples, 25 strs. of \[Pseudomonas \[12 P. putida, 7 P. fluorescens, and the remaining 6 unidentified, though allied to P. putida,\] were grown with naphthalene as the only source of carbon. Of these 14, were shown to transfer the ability to catabolise naphthalene to one or both of two resistant strs., through conjugative plasmids. These plasmids were of high copy number. The capacity of \[ClB to grow on, and oxidize, a range of primary and secondary alcohols\] suggested that the constitutive alkan-2-ol dehydrogenase activity was specific for \[D-isomers.\] Growth on secondary alcohols induced synthesis of \[3-alkan-2-ol dehydrogenase\] and also greatly increased the capacity of extracts to oxidize racemic alkan-2-ols in the range C1-C12. An enzyme with high activity towards symmetrical alcohols was also induced by growth on secondary (especially symmetrical) alcohols. Collectively, the various alcohol dehydrogenase activities detected would enable \[P. C12B oxidize all the alcohols liberated from mixed alkyl sulphate detergents by the organism's complement of 2 primary and 3 secondary alky sulphates.\]

PHENOLS

See also abst. 762


The biofilm did not significantly affect the maximal suspended cell concentration in the effluent but it increased the maximal phenol reduction rate. The increase in phenol reduction rate was linear up to the surface area/volume ratio of 1.4 cm²/ml. The continuous cultures with biofilms could tolerate a higher phenol concentration of the medium (3.0 g/litre) than the nonbiofilm system (2.5 g/litre). At higher dilution rates an intermediate product, 2-hydroxyxynoic semialdehyde, accumulated in the culture. When the biomass of the effluent started to decrease, the concentration of 2-hydroxyxynoic semialdehyde reached a peak value. It is concluded that biofilms in continuous culture have the potential to enhance the aerobic degradation of aromatic compounds.

ALCOHOLS

Krauzova, V. I.; Komarova, G. N.; Il'chenko, A. P.; Gulevskaya, S. A. \[Oxidation of alcohols by Candida guilliermondii grown on hexadecanol. \[Microbiology (1984) 53 (4) 621-627\] \[Ru, en, 17 ref., 3 fig. (1 electron micrograph), 1 tab.\] Inst. Biokhimii i Fiziologii Mikroorganizm AN SSR, g. Pushchino, RSFSR.

C. guilliermondii grown in the presence of hexadecanol showed by electron microscopy many "dark cells" in the cell walls, which appeared to communicate between the cell surface and the plasmamembrane. These cells also showed well-developed mitochondrial apparatus. They, and their mitochondrial and
LEAD

See also abstr. 802

SPECIFIC INDUSTRIES

Food industry


It has been estimated that 2-4% of black tea produced is waste which cannot be processed for human consumption. Under Indian conditions this may amount to 10 000 t/year. The various by-products reviewed are as follows: caffeine; polyphenols and pigments; phenolic materials as extenders in polyomers; organic fertilizer; animal feedstuffs; foamng agents; the plant growth regulator triacontanol; carbon hydrates for fermentation products; seed oil and saponins; and pentosan for furfural production.


The possible utilization of wheat bran as a cheap substrate rich in carbohydrates for the production of Saccharomyces cerevisiae was investigated. Complete hydrolysis of the bran was obtained through heating with 0.2% sulphuric acid, S:R ratio 1:20 at 121°C (15 p.s.i.) for 60 min. This opt. level revealed 84.3% conversion value. The best yeast yield was obtained under the following conditions: (1) hydrolysis rate can be determined to contain 20 g/litre reducing sugar (as glucose); (2) pH adjusted to 5.0; (3) propagation carried out at 30° for 48 h; and (4) N source added as ammonium phosphate at 3 g/litre.


A liquid rich in sugars and nitrogen is derived from residuals after coffee pulp is pressed and composted for use as an organic fertilizer. The liquid from coffee pulp was used as a culture medium for C. utilis (characteristics: 5 g/l of carbohydrates; pH 5-4.5). Nutrients were provided by the addition of ammonium sulphate as a nitrogen source, and phosphoric acid as a phosphorus source. These cultures were performed under constant conditions of 30°C, l p.p.m. aeration; agitation at 250 r.p.m. and inoculation at 15%.

Fermentation industry


The productivity of the S. maxima cultures that received the analysed gas was 0.74 g. h-1 higher than the control cultures, these showed 0.56 g. h-1. The protein content of the biomass was higher and more homogeneous than the control (respectively 68.1-58.6% and 67.9-43.9%). There was an increase in the carbohydrates content in control cultures, but the ash and lipids did not change significantly during 700 hours of growth.
810 BODIE, E. A.; SCHWARTZ, R. D.; ANDERSON, T. M. (USA) (STAUFFER CHEMICAL CO.) Improved fermentation processes - European Patent Application (1985) EP 0 141 642 A1 [En] An improved process for manufacture of fermented whey having a high concentration of propionic acid is disclosed in which the whey medium, containing preferably 6-12% dried whey and 0.5-1% yeast extract, is sterilized at 121°C for 15 min or by direct steam injection for 4-20 s. The sterilized whey medium is then inoculated with mixed culture of a propionic acid bacteria (e.g. Propionibacterium shermanii ATCC 39393) and a lactic acid bacteria (e.g. Lactobacillus casei ATCC 39392) and incubated for long enough to maximize the fermentation, with yields of up to 30% propionibacteria. The lactobacilli overcome the inhibition of the propionibacteria in sterilized whey. The high propionic acid content is useful as a mycostatic agent when the fermented whey is used in bakery goods.

Sugar refineries
See also abst. 818


Supplementation of bagasse with peptone and malt extract, followed by fermentation by S. pulverulentum wild type, increased the in vitro digestibility of bagasse from 27 to 36 and 39%, respectively. Yeast extract and simple nitrogen sources, e.g. NH₄NO₃, NH₄Cl and asparagine, reduced the in vitro digestibility of bagasse below the untreated control value. With all the above nitrogen sources, the mutants 44-2 and 65-2 of S. pulverulentum also reduced the digestibility of bagasse below the control value.


A. globiformis isolated from garden soil was cultured on phosphate salt medium containing phytosterols extracted from subphitation pressmud, a sugar industry waste, as sole source of carbon. The bacterium grew well on starch and transformed them into precursors of steroidal drugs and hormones. Addition of α-α-dipropyl and sodium arsinite as metabolic inhibitors in the culture medium enhanced the accumulation of a 17-ketosteroid which acts as precursor of steroidal drugs. The opt. temp. and pH conditions for its max. accumulation were found to be 32 ±0.5°C and 7.2. Spectroscopic analyses of the 17-ketosteroid confirmed it to be androsta-1,4-diene-3,17-dione (ADD). The bioconversion of sugar-cane sterols into 17-ketosteroid in varying culture condition is discussed.

Rubber and Plastics industry


An actinomycetes Nocardia sp. grows well on unvulcanized rubber and synthetic isoprene rubber, but not on other types of synthetic rubber. Not only unvulcanized but also various kinds of vulcanized natural rubber products were more or less utilized by the organism as the sole source of carbon and energy. The thin film from a latex glove was rapidly degraded, and the weight loss reached 75% after a 2-week cultivation period. Oligomers with molecular weights from 10⁴ to 10⁶ were accumu­lated during microbial growth on the latex glove. The partially purified oligomers were examined by infrared and H nuclear magnetic resonance and 13C nuclear magnetic resonance spectroscopy and the spectra were those expected of cis-1,4-polyisoprene with the structure, OHC-CH₂-[CH₂-CH₄-CH₃]=CH-CH₃, n-CH₃-C(=0)-CH₃, with the average values of n of about 11 and 19 for the two oligomers.

Mining industry
See also abst. 796


The capacity of Thiothrix the oxidation. T. thioparus and Bacillus mucilaginosus to cleave the Si-O-Si bonds in quartz was investigated, in crushed samples in Ashby's medium. The silicon liberated into the medium was measured. T. thioparus slightly acidified the medium, in a similar way, the cleavage of siloxane bonds was reduced; but in the absence of bacteria, these bonds were only broken at very alkaline pH. Thus this process occurred at a much wider pH range in the presence of T. thioparus, in association with the increase of bacterial biomass. A similar phenomenon occurred with B. mucilaginosus, grown in the presence of saccharoses. Again the release of Si was associated with an increase of bacterial biomass, and was quantitatively greatest when the medium was chemically made alkaline.

BIODEGRADATION ORGANISMS

Microorganisms
See also abst. 773

Bacteria
See also absts. 760, 766, 775, 779-781, 783, 787, 793-794, 797-799, 803-804, 810

Fungi
See also absts. 774, 776, 782, 784, 801, 809, 811


Spawn was prepared on sterilized, chopped rice straw or sorghum grain with different amounts of added chalk powder (0 to 10%) or gypsum (0 to 5%). The growing medium was dry rice straw soaked in water for 16 h and pasteurized by dipping in hot water (80-85°C) for 10 minutes. On cooling, the straw was tied into 1 kg bundles and used to make 5, 10, 15 or 20 kg beds. The beds were spawned at 12% of the fresh straw weight and
covered with transparent 200 gauge polyethylene sheets. The sheets were removed when fruiting began. Production of spawn on rice straw was best with 4-5% added guaymi, while for sorghum grain 4-6% chalk powder was best. The 20 kg beds produced the highest number of fruiting bodies (202) and yield (4686 g = 23.43% of bed weight) followed by 15 kg beds with 65 fruiting bodies weighing 1696 g (11.32% of bed weight). The bed temperatures increased with increasing bed weight, from 23-30° with 3 kg beds to 31-38° with 20 kg beds.


The opt. biosynthesis of cellulolytic enzymes (cellulase and glucosidase) was obtained after 3 days incubation, at 30-35°C, with a 13% wheat straw concn for Trichoderma viride and its M_2514 mutant, and at 30-34°C, with 10% wheat straw concn for M_2515. Pretreatment of the medium with water or NaOH solution had an adverse effect on enzyme biosynthesis.


A mutant strain, 52, was produced from G. candidum 3, using N-nitrosomethylacetate. The enzyme system of the filtrate from G. candidum 52 was studied after fractionation, the activity of the 4 fractions upon Na-CMC, cellulose and cotton fibres being tabulated. Fraction 1, identified chromatographically with endo-1,4-β-glucanase (C_x), was able to catalyse cotton fibres freely, only when fraction 4 (cellulohydrolase, C_y) was added. Fraction 2 (a different form of endo-1,4-β-glucanase, C_y) was unable to hydrolyse cotton fibres even when C_x was added, but was active against Na-CMC. Fraction 3 also hydrolysed cotton fibres if C_y was added. It is suggested that C_y is not involved in the hydrolysis of native cellulose, as C_y, but might cleave cellodextrins and natural glycans containing 1,4-β-glucoside bonds.


M. albomyces was isolated from soil and compost by incubating solid glucose/sorbite/ammonium liquor medium, followed by enrichment culture in medium containing sugar cane bagasse as carbon source. The culture filtrate protein of the fungus grown in the presence of bagasse or xylose hydrolysed xylan and some other polysaccharides but cellulose was not hydrolysed. A high extracellular xylanase activity was produced by cultures grown on xylose or hemicellulose materials. The fungus is one of the most active producers of xylanase discovered so far.


A method of fractionation of microme and a variety of vesicular bodies from T. resesi Rut-C30 mycelium is described. The microme (i.e. light- to medium-density fraction) contained a significant proportion of the cell-bound CMCase activity; the heavy-vesicle fraction, which contained amorphous or crystalline electron dense material, showed low CMCase activity. However, anti-CMCase antibody binding, determined by direct radioimmunoassay, was high in the heavy-vesicle fraction. In contrast to the cytochrome c reductase, the majority of the ATPase was concentrated in the organelle-free cytosol. However the residual particle-bound ATPase was concentrated in the heavy-vesicle fraction.

Algae and Lichens


Studies have indicated that many cyanobacteria co-metabolize naphthalene, biphenyl, anilines and their derivatives, and toxic substances present in the water-soluble fraction of crude oil. These are commercial sources of foreign chemicals released from chemical plants and dye works. It is suggested that mutant cyanobacteria, once isolated, could be exploited for breaking down the chemicals in the waste-water stabilization ponds that receive industrial and urban effluents. Modern recombinant DNA technologies should make possible the development of novel strains of cyanobacteria capable of degrading a variety of chemicals, by cloning and expression of bacterial chemical-degrading plasmids from aerobic and amnobiobacteria that are otherwise environmentally less successful.


Cyanobacteria (blue-green algae) have a great deal of potential as a source of fine chemicals, as a biofuelizer and as a source of renewable fuel. This potential is being realized as data from research in the areas of the physiology and chemistry of these organisms are gathered and as the knowledge of cyanobacterial genetics and genetic engineering increases. The present (and possible future) uses of cyanobacteria and the state of the art with respect to the genetic manipulation of cyanobacteria are assessed.

REPORTS


The Tropical Products Institute and the Centre for Overseas Pest Research (in the United Kingdom) merged in 1983 to form the Tropical Development & Research Institute (of the Overseas Development Administration). This is the final report of the Tropical Products Institute, and includes reviews of the work of departments for storage (including pest biology and control, and storage engineering); industrial development; animal products and feed; plant food commodities; non-food commodities; and marketing and industrial economics.


Reviews are presented of about 20 research projects in various countries on the taxonomy, identification, incidence and control of grasshoppers and locusts; the physiology, biotaxyonomy, biology and control of Nilaparvata lugens on rice; the biogeography, forecasting and control of Spodoptera exempta in East Africa; the integrated control of cotton pests (including Pectinophora gossypiella, Spodoptera littoralis, Heliothis armigera and Earias insulana) in Egypt using pheromones and viruses; the taxonomy, identification, biology and control of termites (as pests of crops and timber); interactions between termites and other work on insect pests and rodents.

CONFERENCES

824 DOOD, G. D. (EDITOR) (UNITED KINGDOM, SOCIETY OF FOOD HYGIENE TECHNOLOGY) Aspects of pest control in the food industry. The paper presented at the developments seminar held on the 27 September 1984 at the Novotel, Long Eaton, Nottinghamshire. Potters Bar, United Kingdom; Society of Food Hygiene Technology (1985) 65 pp. [En, many ref., 305×216 mm] PO Box 186, Potters Bar, Herts, EN6 1QU, United Kingdom.
A review of pest problems facing the food industry (by P.L.G. Bateman) is followed by 6 papers including pest control on aircraft (by M.J. Kelly), an introduction to booklice (Psocidae, especially Liposcelis granulata [L. bostrychophilus], Lepinus patruellus and Trogium pulsatorium), the Society of Food Hygiene Technology survey of the problem in the United Kingdom (by G.D. Dodde), the results of this questionnaire survey (by S.F. Downing) and a report (by D.B. Penny) on recent research on the identification and classification of the 2 main pest species (L. patruellus and Liposcelis bostrychophilus), life-history and damage, detection and control (with fumigants and insecticides).


The proceedings contains contributions on triazine-resistant canola, weed control, stored products, management of biting flies and plant protection against diseases and insects. Relevant items are abstracted separately and can be traced via the subject index under Conferences, Canadian Pest Management Society.


Eighteen papers and 16 poster abstracts from this international conference. Papers were presented under 4 main themes; conservation of old timber; biological control of preservatives; and test methods. Many of the papers were also published individually in Holz-Zentralblatt; these are noticed separately elsewhere.


Twenty papers and 7 posters were presented, dealing with the technology of composting, including modelling and microbiological processes, compost analysis, odour control, mechanisation, heat recovery and utilisation, the use of compost as crop (including mushrooms) substrates, vermiculture and pathogen control. Relevant papers have been abstracted and may be traced in the index under Conferences, Composting of agricultural and other wastes.


This compiles the Proceedings of the Mycotoxic Symposium held during the Third International Mycological Congress, Tokyo, 1983. There were 5 symposia, viz. Ecology of mycotoxin-producing fungi; Taxonomy of mycotoxin-producing fungi; Food and feed mycology in relation to mycotoxins and food hygiene; Detection and analytical methods of mycotoxins — risk to human health. Individual papers are abstracted elsewhere.


This proceedings of the seminar held at the Cont. Advanced Studies in Agric. Microbiol., Coimbatore comprises abstracts of papers presented at sessions on microbial ecology of plants (64); microbial ecology of soils (19); microbial ecology of animals, insects and aquatic systems (27); microbiology of food (13); and microbial transformation of agro-industrial residues (24).


Individual papers are abstracted elsewhere.


Papers published here were presented at the symposium as a contribution towards the formulation of a comprehensive long-term rodent-control policy in Kuwait.

BOOKS


This book contains a comprehensive account of pîne bark beetles and their distinctive effects on forests and forest products in the USA and Canada. Since insecticides are no longer relied upon to control such pests, new and improved technologies have been developed. A summation and overview are given of the current status of knowledge and experience in managing the 3 main species, Dendroctonus brevicomis, D. ponderosae and D. frontalis, and associated destructive agents, in their respective host forest ecosystems. Special emphasis is placed on current information and guidelines from recent National Science Foundation/Environmental Protection Agency programmes and related USDA Forest Service programmes. There is a total of 9 papers, contributed by different authors, on various aspects of the subject, and these are noticed individually elsewhere. There is a subject index.


Notes are provided on over 1300 species of insects of agricultural importance in Cameroon, about half of them having been recorded by the author on one of the food-plants. The major part of the text is devoted to an illustrated systematic account of species arranged alphabetically under families in orders, and includes information on their common names, appearance, injuriousness (to relevant food-plants or commodities) or beneficial action [for example as natural enemies of pests] and distribution (and in some cases natural enemies) in Cameroon, together with notes on these species elsewhere in Africa. Pests are dealt with first, followed by parasites and predators. Host-plants are also alphabetically listed, with lists of relevant pests being provided [under the headings cultivated or useful plants, forest trees, ornamental plants and wild herbaceous plants]. Fifty-one species of pests of stored products are
listed. Indexes of fungus and other diseases of plants; pests, parasites and predators and host-plants are provided, as are lists of plant families and genera and common names of insects and plants.


The authors describe chiefly pests of field crops in Britain and the book is intended primarily for students in agricultural colleges and universities, but some pests of gardens and orchards are mentioned. In this 3rd edition, new nomenclature has been introduced for many pests, the sections on pesticides and pest management have been updated, the 1st chapter (on the origin and nature of pests) has been shortened and some of the references to earlier works have been omitted. Sufficient morphology and classification are included for preliminary identifications to be made. The major part of the text is devoted to accounts of the insects of minor economic importance (Colembola, Orthoptera, Dermaptera and Thysanoptera) and major importance (Hemiptera, Lepidoptera, Coleoptera, Hymenoptera and Diptera). Vertebrates, molluscs, nematodes and arthropod pests other than insects are also dealt with. Sections on the different animal groups include accounts of their general structure, classification, biology, injuriousness and control. Most economic importance are dealt with individually. The final chapters are on pests of stored grain, types of crops and their pests, pest management (including natural control, biological control, control by disrupting reproduction, hormones, pheromones, indirect control measures, resistant varieties, direct methods, selectivity of control measures, physiological control, integrated pest control, pest monitoring and simulation modelling and legislative measures) and pesticides (including resistance to pesticides).


This handbook on the prevention of insect attack on pasta in Italy is concerned mainly with localities in the factory and the site where infestation may originate or remain hidden. It is divided into sections on primary materials (not only the pasta or its ingredients but also the containers and vehicles in which they are transported and the packaging materials which were used), pastas (whether of flour or those which had been wrapped); the outside environment (previous factories through which the cereals have passed and nearby storehouses); the pasta factory and its processing machinery; places of storage of the finished products; conclusions regarding measures for cleaning all the localities, as so as to reduce the infestation. The book describes in detail the insect breeding sites and different means of chemical control (mainly contact insecticides, toxic gases and fumigants) and the regulations relating to them. A list of specific insecticides is appended, together with a list of the 23 species of insects most commonly found in stored pasta. Attention is drawn to the vulnerability to insects of all types of packaging used to wrap pasta products.


This manual was compiled by entomologists of the Storage Department of the Tropical Development & Research Institute to assist in training courses held there. It brings together, for the first time, identification keys and information on the basic biology of all the pests and arthropods of stored products, as well as many others of lesser importance. The Coleoptera, Lepidoptera and Acari are dealt with in turn, and a section on the ecology of stored-product arthropods follows. In this, insect population growth, the strategy of storage pests and the effects of physical and biotic factors (including predators and pathogens of stored-product pests) are dealt with. General entomological techniques, the use of identification keys and the laboratory culture of stored-product insects are dealt with in appendices, and an index to scientific names is provided. In addition to its role as a training aid, this manual should be useful as a reference book for researchers and those concerned with the management of pests in tropical and other stored products.


A book dealing with the main types of structure and their agricultural applications, and also with methods of protection against moisture, fire and biodeterioration.


A comprehensive reference book, copiously illustrated with photographs, covering both common and rarer decay fungi and insect pests.


A textbook containing 22 chapters by various authors covering the structure of wood, the localization of polysaccharides and lignins in wood cell walls, metabolism and synthetic function of cambial tissue, cell organelles and their function in the biosynthesis of cell wall components, biosynthesis of plant cell wall polysaccharides, lignin, cutin, suberin and associated waxes, phenolic acids and monolignols, quinones, flavonoids, tannins, stilbenes and terpenoid wood extractives, the occurrence of extractives, the metabolism of phenolic acids, wood degradation by micro-organisms and fungi, and biodegradation of cellulose, hemicelluloses, lignin, and aromatic extractives of wood. An index is included.


This contains the following:

- Stoloff, L. General introduction (xxv-xxvii, 51 ref., 1 fig., 2 tab.) [lists the fungal metabolites reported to produce cellular aberrations, their chemical structure and producing mould(s)].
- Castegnaro, M. Hazards in handling mycotoxins (xv-xlix, 7 ref.).
- Stoloff, L. Analytical methods for mycotoxins — an overview (33-62, 77 ref., 4 tab.).
- Preparation and testing of mycotoxin standard reference materials (63-84, 4 ref.).
Friesen, M. Quality assurance for mycotoxin analysis. Mycotoxin check sample programmes (85-106, 17 ref., 10 fig., 2 tab.).

Schuller, P.L. (et al.). Limits and regulations (107-116, 2 tab.).

Methods of analysis of aflatoxins:
This section details the detection of total aflatoxins and of various aflatoxins in groundnuts and groundnut products, cottonseed and cottonseed products, milk and cheese and animal tissues (all adapted from the official methods of analysis of the Association of Official Analytical Chemists) and in feeds (adapted from the Council of the European Community Methods).

Toxicity, occurrence and methods of analysis of other mycotoxins:
Egmond, H.P. Van (et al.). Sterigmatocystin (283-310).
Scott, P.M. Patulin (311-327).
Thorpe, C.W. Penicillic acid (329-348).
Wilson, D.M. Citrinin (349-364).
Mirocha, C.J. Trichotheccenes (365-383).
Ueno, Y. Luteoskyrin and other Penicillium islandicum toxins (399-417).


This book provides a thorough introductory account of mycotoxins, for use by undergraduates and postgraduate research workers in various fields. The multidisciplinary nature of research on mycotoxins and the diseases they cause is emphasized. Chapters include: Introduction; Toxigenic fungi; Structure and formation of mycotoxins; Implications of mycotoxins in animal diseases; Human mycotoxicoses; Natural occurrence of mycotoxins; Mycotoxin analysis; Control of mycotoxins. Each chapter ends with a list of references for further reading.


This up-to-date mycology textbook contains 23 chapters, the first 5 of which are devoted to classification. Other topics include yeasts, lichens, spore dispersal, fungal physiology, genetics and ecology, plant pathology in agriculture and forestry, fungicides, fungi as agents of biological control, fungi exploiting microscopic animals, fungal symbioses with animals, mycorrhizas — mutualistic plant-fungus symbioses, fungi as food, fungi in food processing, food spoilage by fungi, mycotoxins in food and feed, poisonous and hallucinogenic mushrooms, and medical mycology. There is a glossary and an index.
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CAB Identification Services

In any serious attempt to control the diseases and pests that menace the crops and livestock of world agriculture, the first step must be the accurate identification of the organism concerned.

CABI through its Institutes of Entomology, Mycology and Parasitology, offers efficient and reliable identification services to agricultural and biological scientists throughout the world.

The service covers the identification of:

- insects and mites
- microfungi and bacteria
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compiled by G ERIC TIDBURY

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C·A·B INTERNATIONAL

Advancing Agricultural Production in Africa

Proceedings of CAB's First Scientific Conference
Arusha, Tanzania, 12–18 February 1984
edited by D L HAWKSWORTH

The Proceedings of C·A·B International's (formerly Commonwealth Agricultural Bureaux) First Scientific Conference held at Arusha, Tanzania, 12–18 February 1984, in collaboration with the Government of Tanzania was published August 1984. This valuable book summarises all the papers given and discussions which followed.

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