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The Needs and Problems of the Food Industry

Chairman: Mr A. JONES
Session Organiser: Dr G. D. DODD

FOOD INDUSTRY STANDARDS - THEIR TRENDS AND SIGNIFICENCE IN STORED PRODUCT PEST CONTROL.

G. BARNES, F.I.E.H., F.R.S.H.

Technical Manager, Marks & Spencer plc, England.

INTRODUCTION

The purpose of this paper is to review briefly and broadly the development in the Food Industry to-day and to see how these developments might influence the standards we seek for the control of pests of stored products. The paper will reflect the changes that have taken place to the required standards together with their applications and benefits during the period of the last 20 years. The paper does not attempt to deal with all aspects of food handling but concentrates on food manufacturing together with the associated considerations of raw material production and also the storage and distribution of food products.

CURRENT DEVELOPMENT

Perhaps the first observation to be made is the ever widening divergence in the systems, processes and standards seen to-day - high technology has its representation in the Food Industry. For example, the application of ultrafiltration and reverse osmosis techniques to manufacture dairy products and the aseptic processes and packaging system used to allow perishable products to be stored at ambient temperatures. And there are many other examples of science and technology being applied to the production of foods. At the same time there are parts of the Industry which claim, either intentionally or through complacency, standards and processes which have not changed in decades. This adds to difficulty in discussing general standards.

If we then look at the pressures which influence the manager involved in food production what do we find:-

1. The varying standards previously mentioned will apply to raw material sources. This difference in approach will apply to quality control systems and may result in the manufacturer dealing with a raw material supply point in which he can have every confidence, after agreeing a buying specification, but at the other end of the scale is the producer of raw materials whose standards are suspect and where the manufacturer has to spend a disproportionate amount of time and energy in monitoring and generally propping-up the raw material producers technical team - if there is one!! It is not always appropriate to dismiss the substandard supplier to be replaced by another - for a number of reasons monopoly or near monopoly situations exist and have to be accommodated.

2. New raw materials and their sources serve to give the manufacturer further considerations - not necessarily problems as they may act to his benefit - but the consideration of knowing for example whether developing countries which previously produced base raw material are now involved in further processing. If this is the case then are they operating in a competent manner that will save time, money and effort by producing the further processed raw material in a consistently safe manner to the prescribed quality.

An increasing development is that of tailor-made raw materials in which several components and processes are undertaken to produce a product which

can be incorporated at a more advanced stage in the manufacturing sequence towards the finished product. The same considerations will have to be applied as with further processed raw materials from Third World Countries in deciding on the level of confidence that can be invested in multi-component raw materials.

3. Distribution

Means and techniques of distributing and applying intermediate storage of raw materials have changed and paradoxically some of these changes designed to furnish improvement can also bring problems. The bulking of deliveries to replace units of sacks, boxes etc. clearly offers the advantage of efficient handling but the scale of the bulking-up is such that any adverse analysis will see the rejection of considerably larger volumes than may be the case with smaller unit systems where repeat sampling may allow partial and selective rejection.

Temperature controlled distribution systems offer clear advantages for perishable commodities and those vunerable to insect infestation. However, their use must be coupled with compatible storage systems at the manufacturing site, otherwise, what may appear to be a "clean" parcel of raw material on arrival at the factory may turn out to be badly infested after it has been held at ambient temperatures for two or three weeks.

The complexity of distribution systems, especially those which are not dedicated to the Food Industry, can contribute problems of product contamination by means of transfer from previous loads. Chemical tainting has frequently occurred this way, but more appropriately, as far as the subject matter is concerned, so has the transfer of pest infestation. Damaged insulation systems have provided pest harbourage and the well-known occurrence of certain insect pests on wooden pallets still presents real problems.

An interesting situation experienced recently was the extensive rodent damage to hazelnuts which had been transported in sealed freight containers. The nuts had been fumigated in both the country of origin and at the receiving factory. Investigation showed that following the latter fumigation the nuts had been stored in the freight containers and during this period of storage mice had gained entry through drainage holes, approximately ¼ inch diameter situated at the corners of the container floor. The proofing of these drainage points was a minor detail but the consequence of not anticipating this particular problem was the loss of many thousands of pounds of raw material.

4. Factory developments

The last few years have seen the acceleration in capital investment in both new factory buildings and elaborate manufacturing equipment. Much of this development has centred on that part of the manufacturing Industry involved with perishable products of the convenience food type - the so-called "High Risk" products. With the design of these factories, there has been far more attention given to hygienic layout and sanitary maintenance. Whilst there is room for further improvement there has been a genuine move on the part of some architects and civil engineers to "build-out" the future problems. This is a distinct and welcome change from the attitude of a few years ago when the building was put up, and then details of fittings, finishes and equipment location were accommodated as best as possible.

A very clear trend in building systems for food factories is the use of pre-fabricated systems, which not only provide the advantage of speed of erection but, if well conceived, can make easier the subsequent alterations

to the factory. The latter will inevitably occur when marketing departments demand new products which in turn may result in the need for new product specific equipment housed in modified buildings.

A well established design feature now accepted as the norm is that of service areas which carry pipework, ducting and other ancillary service equipment. There are many advantages in terms of efficient maintenance - but, in terms of pest control there remain major potential problems which will be realised if insufficient thought is given to access for inspection and means of cleaning - especially if such areas are subject to leakage from pipes conveying powdered materials such as flour, milk powder etc.

Finishes to walls have been aided considerably by the development and application of "sandwich panels" which are the sealed units incorporating good insulation properties with an easily cleanable finish. Apart from their ease of installation they can naturally be replaced easily should damage occur. For existing factories which are in need of modernising, the use of plastic sheet wall cladding systems is a major advantage if properly applied i.e. so as not to create cavities and allow easy access for pests to such cavities. It is necessary to consider the balance between the reasonable cost of transforming sub-standard fabric by using this method with care taken in its execution to avoid future problems.

Another interesting example of problems associated with wall cladding systems has been with the growth of moulds on the rear surface of the panelling. This was due to a combination of the nature of the surface and the lack of ventilation behind the panels. The moulds attracted plaster beetle and although the latter are not true pests of food commodities they did cause a degree of contamination to foodstuffs stored adjacent to the affected walls.

The layout of the factories in terms of allocation of space continues to find problems for the adequate storage of raw materials. Whilst it is understandable that production space will always command first priority, the consequence of cramped space is well known to most people who are involved with pest control. The congestion that leads to poor access for inspection and cleaning and to damage of containers and, therefore, spillage is the prime ingredient for ideal infestation developments. In recognition of this problem more bulk silo systems are being installed. However, it is easy to replace one problem with another and, therefore, the design of silos and their associated transfer mechanisms and dust control services must be well thought through in respect of the design and construction for self-cleaning, access and general maintenance.

The external location of silos has caused problems of fungal growth on internal surfaces due to condensation, and mite problems have followed. Attempts to insulate the silos have overcome the condensation problem but have resulted in the insulating material providing harbourage for insects. However, there is little doubt that more and bigger bulk systems will be employed and that satisfactory solutions will have to be found for some of the problems previously mentioned.

A further point in the allocation of space for specific functions is that of how and where to mechanically screen raw materials for the presence of foreign matter including infestation. There is now an increasing move to screen all powdered raw materials both in bulk and unit systems. Clearly this operation is best achieved in a separate area located conveniently for the arrival of raw materials. Unfortunately, this is not always possible, and it is, therefore, likely that the main small goods dispensary will be used. It is at this point that cross-infestation can

easily occur - especially if space allocation is tight. The future demand for raw materials of known integrity which do not need subsequent monitoring for insect pests is over-whelming.

5. Food manufacturing machinery

It has been stated previously that Architects are beginning to appreciate that hygiene maintenance has to be taken into account at the earliest stage in the design and development of a factory - similarly, the designers of manufacturing equipment are giving more thought to the cleaning and maintenance of the equipment that they produce. Of the construction materials used wood now finds little acceptance, except in some very specific pieces of equipment used for chocolate and sugar confectionary manufacturing. Other materials considerations in equipment for handling powdered materials show gaskets and their mountings still causing difficulties in providing the location for harbouring the larval stages of some stored products insects.

It is lack of access to non-self cleaning surfaces which cause most problems of infestation in equipment. Whilst major improvements have been made, a thorough inspection of a modern bread bakery will reveal many areas that are still difficult to clean to the required standard. There has to be more activity in transferring to bakery equipment and plant used for handling infestable materials, the thinking that has gone into the cleaning of dairy and brewery equipment, much of which has been automatic. Naturally, the emphasis would need to be on pneumatic and vibratory systems rather than water based systems for obvious reasons of keeping moisture levels to the minimum.

6. Manufacturing processes

A continuing trend which can have severe repercussions in terms of poor pest control is that of continuous processing during which many tons of raw materials will be used during a full shift. From the point of view of production efficiency there is little argument - but if one of the component raw materials were to be infested there would be no chance of selective rejection as is the possibility with batch production. This is a point of major importance to both the supplier and user of the commodities as well as the maintenance staff responsible for the hygienic upkeep of the equipment.

An interesting technique which is being harnessed increasingly by the food industry is that of modifying the environment in which the raw material or finished product is stored - the so-called system of modified atmosphere packaging. The technique has been used for many years for the bulk handling of fruit to control the rate of ripening. However, the last few years have seen its application to retail packs - mainly to retard biochemical changes, and bacteriological deterioration. Clearly the use of atmospheres which are modified to exclude oxygen or to reduce it significantly will have an additional benefit in suppressing insect activity.

This could be an interesting avenue for the future development in the handling of certain pest vulnerable products - especially where vacuum packing may not give the appearance required for the retail shelf.

Another interesting development referred to earlier is the increasing development of chilled, high risk products. It is reckoned that this market is increasing at approximately 20% per annum. It has developed to meet a public that is not only requiring convenience foods but which is increasingly discerning in its demand for high quality - in all respects. From the manufacturers point of view providing products which are of a

"high risk" nature and, yet also have a manageable life, has been further complicated by the publicity to minimise the use of chemical additives.

This clearly means that the already high standards of hygienic manufacturing have to become even tighter to allow for the reduction or removal of preservatives. The cost of maintaining such standards is high and added to this must be the energy costs in running manufacturing environments at low temperatures. It goes without saying that such manufacturers will be demanding cleaner raw materials - cleaner, not only in forms of freedom from pest problems - but also in terms of pesticide residues.

In the drive for accelerated product development we are seeing manufacturers crossing borders of known expertise and discipline. For example, raw material producers are further processing as mentioned before, and we are also seeing manufacturers based on one main raw material now using a wider range of materials. I believe that when the manufacturer is one used to handling highly perishable materials such as meat or milk, he will want and demand to apply similarly high standards to other materials he brings in - he may not take the view that insect pests, for example, are an acceptable, albeit regrettable, consequence of handling nuts or flour. He will also reason that if he cannot use chemical preservatives systems to retard bacterial decomposition he will certainly not accept pesticide residues.

7. Legislation

Of the many pressures which afflict the food producer, grappling with ever-increasing amounts of legislation is not one of the least onerous. In the U.K. I believe we are fortunate in taking general and balanced views towards food standards. In drawing-up these standards the legislators fairly listen to the views of the manufacturer. It is within the field of international legislation and its interpretation that problems are to be found. As food industries saturate their own home markets they will turn increasingly to the export fields, and in order to avoid the defences put up to protect home markets the exporters will need to demonstrate the highest standards. As exports in food stuffs increase I believe that the results of filth tests for the presence of pest activity will form an ever increasing part of the acceptance/rejection pattern.

8. Retailers

Throughout this paper there have been references to the scale of operations seen to-day. Nowhere is this truer than in some of the distribution and storage systems, especially those developed by some of the retailers. The value of products in a distribution depot can be worth many millions of pounds at any one time and, therefore, the demands on the pest-free quality of the foods stored becomes greater.

A very high proportion of the retail food business is in the hands of a few major companies. Their influence is increasingly extending towards manufacturing standards and as this happens they become more knowledgeable and exercise common standards. These standards will be applied not only to manufacturers but also to key raw materials and there will be an expectation that manufacturers will be expected to take a greater interest in raw materials - which will also include packaging - an area often ignored but which has been associated with infestation problems.

CONCLUSION

These then are some of the changes taking place in the world of food manufacturing, where the demand for excellence is increasing at a great rate and where the scale of operation will increasingly demand that there

cannot be a weak link in the chain.

In this general review of trends I must very briefly reflect on what changes have occurred within pest control standards within the food industry. I believe the situation can be summarised by four periods:-

1. The period of 10-15 years following World War II when allowances were made to tolerate the major shortages in raw materials, and, where in the face of food rationing only products that were dangerous would be rejected.

2. The early sixties saw the food industry wrestling with major mouse problems as warfarin resistance developed. It was during this period that the basic hygiene controls to combat rodent problems were applied. It was also a period of major inner-city redevelopment which saw demolition playing its part in transferring rodents from building to building, many of which were food premises.

3. The early seventies saw the food industry start to take the problems of stored products insects seriously. This activity went back to the mills and in the latter regular cleaning and treatment took over from the annual fumigation.

4. Today the food industry is looking somewhat nervously at the future and anticipating that the regular use of residual pesticides inside food factories will be severely limited. This must accelerate the demands on the pest control industry that it becomes more knowledgeable of food industry practices and is prepared to work much more closely in an anticipatory fashion rather than a reactionary mode.

PEST CONTROL IN CEREAL PROCESSING : A HEALTH FOOD INDUSTRY VIEW

P G REDGATE

Booker Health Foods Ltd., West Byfleet, Surrey KT14 6NE

ABSTRACT

There is a well established consumer demand for foods which are free from 'contamination' by preservatives, other additives and residues of chemicals such as those used to control insect infestations.

The paper describes how such considerations are taken into account during the milling of wholemeal flour by the traditional method of stonegrinding. Quality Assurance of raw materials and milled product is discussed, and precautions taken to avoid infestations are described with brief reference to the relevant stored product pests.

The same standards are applied to the manufacture of other cereal products, particularly those which are grown to 'organic' standards.

The special problems associated with 'organic' products are described, together with a brief assessment of likely future trends for cereal processing in the health food industry.

INTRODUCTION

The health food industry is undeniably one of the great 'boom' industries of recent years, maintained firstly by the publication of successive reports (Anon.1984, Anon.1985) expounding the virtues of a high-fibre, low-fat diet and secondly by doubts concerning the safety of certain commonly-used additives.

The type of products which Booker Health Foods (BHF) markets leaves us particularly vulnerable to infestation. These products are of vegetable origin and undergo a minimum of processing. Therefore they do not rely for their keeping qualities on the addition of preservatives, antioxidants, stabilisers and other additives. They include those derived from cereals, e.g. bran, wholemeal flour and wheatgerm, but also nuts, beans, dried fruit and juices.

A conflict of interest can already be seen to have arisen. Paradoxically people buy these foods because they want them free from additives and other 'contaminants'. Insects count as contaminants but so do the chemicals used to control them! Our typical consumer is no more sympathetic towards an insect in her bran than she is to the presence of tartrazine (E102) in an ingredients list.

Furthermore we, like other manufacturers, are subject to the demands of the Food Hygiene Regulations (1970). These require that we continue our business 'at a premises where there is no risk

of contamination of the food' (Regulation 6) and of which the state of repair is such that the risk of infestation by rats, mice and insects is avoided (Regulation 25). Further, under Section 2 of the Food Act (1984) we must supply food which is of the 'nature, substance and quality' demanded by the purchaser.

So for our industry there are no easy options and it is the purpose of this paper to show how we cope with these conflicting demands.

Although BHF markets an extensive product range, many cereal-based, under the Allinson and Prewett's brands, it is proposed to concentrate upon the problems encountered in the milling of stoneground flour, briefly considering other cereal products and the future of the industry.

SETTING THE SCENE : THE OLD MILL ON THE RIVER

One of the primary requirements in ensuring a pest-free premises is building design. Easy when starting from scratch with a generous budget; far more difficult when updating a building such as our Mill at Castleford which has been operational for more than 200 years. Pest problems were presumably a way of life in past times, with little or no incentive being given to overcome them.

The Mill, while unquestionably ideal for receiving barge-loads of wheat transported up the River Aire, was certainly not conducive to good pest control. The Aire has always been a polluted river with an industrial hinterland, providing in its banks an ideal home for Brown Rats (*Rattus norvegicus*). When the river rises after heavy rain they may be driven out, occasionally searching for weaknesses in the building structure which would give them access to food, shelter and possible breeding sites inside.

The original design of the Mill and its equipment showed little consideration for good hygiene and pest control practice. There was a proliferation of wooden structures, limited access for cleaning and a profusion of 'dead spaces' where, without major structural work, such access was virtually impossible.

However, our investment in the Mill accelerated to cope with increased demand for stoneground wholemeal flour and the requirement for a top quality product has largely dictated the measures taken to improve hygiene and pest control. These include:

(a) Improvement to the building structure, e.g. the damp coursing, rendering and painting of all internal walls to prevent the mould growth attractive to certain stored product insects (SPI), the filling of cracks between floorboards, the repair/replacement of doors and windows.

(b) The replacement of the original infestation-prone wood fittings, e.g. conveyors, machinery and wheat storage bins, where possible by stainless steel.

(c) The replacement of mechanical methods of moving wheat and flour by aspiration wherever possible, with the effect of removing many of

the aforementioned 'dead spaces' previously to be found at the end of worm conveyors and at the bottom of bucket elevators. Here, access for cleaning is frequently restricted with a consequently increased risk of insect infestation.

(d) Improvements to the existing preventive pest control programme to increase its effectiveness.

(e) The establishment of a comprehensive cleaning schedule to ensure that every area receives the required attention.

MAINTAINING AN INFESTATION-FREE PRODUCT

Wheat arriving at the Mill comes from many different sources. We therefore rely greatly on the integrity of our supplying merchants to provide high quality, infestation-free wheat. Precautions taken to ensure that these criteria are met include:

(a) Wheat is purchased by our Mill Manager 'by sample'. Thus he provides a specification detailing required variety, protein content, moisture, Hagberg, and the merchant returns a small sample of a proposed wheat for examination. This is thoroughly tested in the Mill laboratory and, if suitable, the remainder of the consignment is purchased.

(b) On arrival at the Mill and before the wheat is tipped it undergoes:

(i) Thorough visual examination for general quality and evidence of insect or mite activity.

(ii) Random sampling and microscopic examination in the laboratory for evidence of infestation, particularly by flour mites (*Acarus siro*), or bored out grains which could indicate the presence of Grain Weevil (*Sitophilus granarius*).

(iii) Laboratory tests to ensure that the consignment matches the sample.

It is obviously of vital importance that any infestation is detected at this stage as, although our modern wheat cleaning plant will eliminate it, the possibility of introducing infestation to the premises must be avoided.

(c) When our technicians are satisfied that the wheat meets our specification the load can be tipped into the wheat intake, whence it is conveyed to the appropriate silo. There it remains until required in the Mill.

(d) The vast complexity of modern flour mills is due largely to the range and intricacy of the different machines designed to thoroughly clean the wheat and, in our particular case, to ensure that a perfectly balanced grist is presented to the grinding stones.

To achieve this the following machines are used:

(i) A preliminary separator consisting of a set of sieves of decreasing coarseness to remove the largest impurities, e.g. sticks, stones, mud-balls etc.

(ii) A finer separator which removes smaller objects such as large foreign seeds (e.g.maize), straw, small stones, broken kernels and insects. The 'clean' wheat is then aspirated to remove the lightest particles including dust and insect parts.

(iii) A concentrator, also strongly aspirated, which allows wheat to be separated into light and heavy grains with impurities again being drawn off.

(iv) A scourer, consisting of a mesh 'jacket' against which the grain is thrown at high velocity, cleaning and polishing it with husks, insects and mites being removed from its surface.

(v) An entoleter, which is specifically designed to eliminate any remaining insect infestation. It is, in effect, a centrifuge consisting of two opposing metal plates with pin-like projections on the faces. These rotate at approximately 3000 rev/min, battering the grains and killing insects and eggs even when inside the grain.

(vi) A dampener to ensure that the required moisture content of the grain is achieved before it is blended with other wheats to produce the required grist. This is then introduced between pairs of slowly rotating millstones which grind the grain to produce wholemeal flour.

(vii) Sifters and a further entoleter before the flour is blown under positive air pressure into holding bins from which it is packed. To summarise, our flour-milling process takes full account of the risk of infested grain passing through it and is specifically designed to remove from it any foreign matter whether of animal, vegetable or mineral origin.

MAINTAINING AN INFESTATION-FREE MILL

The precautions taken to keep our premises free from infestation are collectively known as preventive pest control measures.

They depend for their success on the maintenance of a high standard of hygiene and housekeeping and are of particular importance in the context of the manufacture of health foods. This is due to the highly infestation-prone nature of the products and the ethical requirement to keep the use of chemical treatments to an absolute minimum.

We consider an effective preventive pest control programme to encompass the following basic procedures:

Housekeeping

Poor housekeeping must be the single most important reason for the occurrence and maintenance of an infestation, whether inside or outside the building. Therefore:

(a) All waste materials, flour spillages etc. are kept in covered skips which are waterproof and not allowed to overflow.

(b) The exterior of the Mill is kept as clear as possible of vegetation which could harbour rodents. Unfortunately, the river bank itself is outside our control and does at times sustain brown rats, which we must ensure cannot enter the Mill.

(c) All spillage is removed immediately it occurs. We operate a 'clean as you go' system rather than having cleaners as such: this discourages operatives from creating spillage in the knowledge that someone else will clean up behind them.

(d) Cleaning equipment must be suitable for its purpose. Thus sweeping is fine for most floors but vacuuming is required for removing flour dust from less accessible areas, particularly inside machines.

(e) Cleaning of each area must be ensured by the use of a cleaning schedule which is rigidly adhered to and ensures that more 'difficult' areas are not neglected.

(f) An inspection and reporting procedure has been established to ensure that cleaning is being carried out to the required standard.

(g) Specialised cleaning, for instance of damp flour which inevitably accumulates on the sides of holding bins, is normally carried out by a contractor.

Storage conditions

Good housekeeping is only possible if good storage conditions are maintained. This entails:

(a) Proper storage of raw material and packed product in suitable containers: thus wheat in concrete silos rather than wood; bulk flour in stainless steel bins; packed flour on pallets rather than the floor; pallets stacked so that access is available all around them.

(b) Quick rotation of raw materials and packed product which in practice is easy because our capacity for wheat storage is very limited. Less than a week's supply can be kept on site. A similar lack of warehouse space ensures that packed flour is loaded directly onto waiting lorries for transport away from the Mill, often direct to bakeries.

(c) The maintenance of dry conditions to prevent mould growth which attracts certain insects (e.g. booklice (Psocids)) and could result in tainting of the flour.

Proofing

These are the measures taken to deny entry to the premises by rodents, birds and insects. In practice it is extremely difficult to achieve completely because of the small size of hole through which a mouse or particularly a flying insect can pass. However, many sensible precautions are taken including the elimination of any gaps in the building structure which could be used by rodents, e.g. around doors, pipework, electric cables, dust extraction vents, window frames, roof eaves etc. Rodent proof brushes have been fitted along the bottom of doors, which are in addition protected by bird-proof plastic curtains. Fly screens cover all opening windows.

THE ROLE OF THE PEST CONTROL CONTRACTOR

A vital part of the preventive pest control system is to employ the specialist knowledge of a reputable pest control company through frequent inspections of the premises by trained operatives.

Our requirements for a pest control contractor are that he must:

(a) Be capable of advising on the exclusion and control of all pests likely to be encountered in the Mill. These include SPI, rodents and birds.

(b) Provide for frequent routine inspections of all areas of the site, internal and external, and at the appropriate frequency.

(c) Establish a proper reporting procedure by contact with a manager on site and written visit reports.

(d) Be capable of providing a prompt emergency service if necessary.

THE HEALTH FOOD CONSIDERATIONS TO PEST CONTROL

The nature of our business with the emphasis on pure, unadulterated foods means that we may use our pest control contractor rather differently from other food manufacturers. Thus we discourage the use of precautionary pesticide treatments for which we compensate by:

(a) The maintenance of the highest standards of housekeeping.

(b) An increased frequency of inspections by the contractor including 'technical' inspections which are supplemented by internal hygiene audits.

(c) Maintaining a very rapid stock turnover.

(d) Increasing the frequency and depth of cleaning of flour bins, wheat pits, the remaining worm conveyors, stone covers and other particularly vulnerable areas to obviate the necessity for insecticidal treatments.

These procedures have enabled us to:

(i) Greatly reduce the number of rodent baits on the premises and hence the risk of contamination of the product. Much of the Mill plant, e.g. the grinding stones, is at floor level so the potential risk of contamination by spilled bait would be high.

(ii) Virtually dispense with the routine use of surface spray residual insecticides such as fenitrothion.

(iii) Carry out precautionary insecticidal treatments on a strictly controlled basis. Only non-residual insecticides of low mammalian toxicity are used as space sprays.

(iv) Dispense with the annual mill fumigation, one of the great milling traditions and always considered to be the ultimate in mill pest control. However it is expensive, entailing several days' lost production, and labour intensive in completely sealing the premises to make it gas tight. Further, the ethics of treating the premises with a gas such as methyl bromide which can leave a residue on grain, flour and surfaces with which it comes into contact have led us to dispense with it. We are now in our third year without a fumigation and, by means of the described precautions, infestation problems have so far been avoided.

THE PESTS

It is now appropriate to briefly survey the pest species to which we consider our products and premises to be vulnerable should our preventive pest control prove inadequate. The following table lists some of the species attracted to cereals and the premises where they are processed and stored. It also details their favoured habitat and the 'risk' they present to our operation.

Group	Species	Favoured Environment	Hazards
Rodents	Brown Rat (*Rattus norvegicus*) House Mouse (*Mus musculus*)	Both are ubiquitous, found anywhere that food harbourages and breeding sites are provided.	Damage to building structure and product.Fouling of product.Carry diseases (e.g.leptospirosis)
Insects	Saw-toothed Grain Beetle (*Oryzaephilus surinamensis*)	May be present in arriving consignments of wheat.	Infestation of premises and product.
	Grain Weevil (*Sitophilus granarius*)	Arriving wheat, inside grains themselves.	Infestation if not detected before acceptance.
	Spider beetles (e.g.*Ptinus tectus*)	Cracks and crevices in building structure (e.g.between floorboards).	Infestation of premises, rarely found in products.

	Flour Beetles (eg. *Tribolium confusum*)	'Dead spaces' in milling machinery (e.g.worm conveyors, stone covers).	Infestation of packed flour.
	Mealworm Beetle (*Tenebrio molitor*)	Damp, dark, inaccessible areas where wheat accumulates.	Can cause spoilage of grain, but rarely found in products.
	Booklice (Psocids) (e.g. *Lepinotus patruelis*)	Damp areas, especially where moulds occur.	*Lepinotus* is a pest of premises, virtually never found in packed products.
	Mill Moth (*Ephestia kuhniella*)	Very mobile, so anywhere that access is possible to wheat or flour.	Infestation of packed flour, larval webbing fouls machinery.
Mites	Flour Mite (*Acarus siro*)	Any stored cereals where moisture exceeds c.16%	Soiling and tainting of cereals. Infestation of premises.
Birds	Sparrows (*Passer domesticus*)	Ubiquitous. Found anywhere that food, shelter and nesting sites are provided.	Fouling of product/packaging. Damage to product. Introduction of insects and mites to premises.

WAREHOUSING AND DISTRIBUTION

For flour warehousing the following procedures ensure that the product remains infestation-free:

(a) Proofing to guard against the entry of rodents and birds.

(b) A comprehensive pest control contract.

(c) Maintenance of good, dry storage conditions with ease of access for the cleaner and pest control operator.

(d) Prompt removal of spillages.

(e) Strict stock rotation and control. Our major bakery customers require that the flour will be less than two weeks old on receipt, and all bags are coded with the date of production to ensure that this is achieved.

The production of infestation-free stoneground flour which complies with our own demanding specification sees the fulfilment of most of our responsibilities as a food manufacturer.

But it is also our responsibility to ensure that it arrives at our customers' premises in this same perfect condition. Distribution is often direct to the bakeries and we endeavour to maintain the highest standards of hygiene during transport in the way described above.

This means our trailers are:

(i) Suitable for the task, being curtain sided so that they can be opened up completely for steam cleaning.

(ii) Constructed to eliminate any 'dead spaces' where flour which is sometimes spilt during handling can accumulate undisturbed; for instance, no false floors.

(iii) Scheduled to allow time for weekly steam cleaning which will kill any residual infestation by penetrating deep into the inevitable cracks and crevices between floor panels.

OTHER CEREAL PROCESSING

Although stoneground flour is the cornerstone of Booker Health Foods' business, a range of other cereal based products is marketed under the Allinson and Prewett's labels. These include mueslis, other breakfast cereals, biscuits, cakes and vegetarian main meals.

Some of these products are manufactured under contract by specialist packers who, in order to pack for us must satisfy many quality-related criteria, including:

(a) An understanding of the inherent problems of SPI infestation associated with cereals.

(b) The ability to screen raw materials arriving at the factory and during packing, and to recognise signs of insect and rodent infestation.

(c) A firm commitment to the maintenance of the highest standards of hygiene.

(d) The ability to manage a comprehensive preventive pest control programme in conjunction with a reputable contractor.

(e) An understanding of the concept of a 'health food' and the consequent requirement for the responsible use of insecticides, particularly materials with residual activity and fumigants (e.g. methyl bromide), which are not generally permitted.

In the majority of our cereal products we specify the use of 'micronised' cereals which have been cleaned, rolled and toasted on an infra-red grill to 'stabilise' them. The moisture content is reduced, thereby prolonging the shelf life and reducing the attraction to SPI, as well as killing any residual infestation remaining after the cleaning process.

It is virtually unknown for any of our contract manufacturers to supply us with infested product. Yet consumer complaints relating to insect infestation are occasionally received. Cereals are vulnerable

to infestation even when sealed in prepacks and distributed to the retailer and the consumer.

Much of our sales volume is achieved through the independent retail trade, where shops are particularly prone to such insect infestation. Often, being small, they have to carry far more stock than is desirable so housekeeping suffers, with increased risk of cross-infestation. Our own products are stored and displayed in close proximity to those of other manufacturers who may not be as diligent in their pest control as we are. The risk increases further because, in the absence of air conditioning many shops suffer from the 'green-house' effect with very high temperatures occasionally being experienced. Further, certain current merchandising/marketing trends are incompatible with good pest control practice. Thus the increased use of false wall and shelf panels creates 'dead spaces' behind them which are accessible to insects and debris but inaccessible for cleaning.

One insect which is particularly adept at exploiting the retail environment is the Indian Meal Moth (*Plodia interpunctella*), which can maintain itself in this environment from year to year, causing severe infestations in a great range of products in the summer and autumn. The enforced removal of wall and shelf panels can then reveal a mass of larvae and pupal cases hitherto undisturbed and unnoticed.

The domestic premises can also be the site of infestation; biscuit beetles (*Stegobium paniceum*) are common inhabitants of the domestic larder and booklice (Psocids) are a cause of consumer complaints of infested flour. Yet the species involved, (*Liposcelis bostrychophilus*) has not been found in our mill, factories or warehouses, and very rarely in our retail premises.

Consumer complaints concerning flour infested with *P.interpunctella* and Psocids account for the majority of our total infestation complaints, illustrating how, although we take every practical precaution to keep our own premises pest-free, we are still vulnerable to problems occurring subsequent to distribution and in conditions of storage over which we have no effective control.

ORGANICALLY GROWN CEREALS

The consumer lobby for a healthy, balanced diet, which in the last few years has seen a greatly increased demand for additive-free foods, has now identified another source of unnecessary 'contamination' of cereal based products; the use of chemical treatments such as insecticides, herbicides and fungicides, which can leave residues in the end product.

The Food and Environment Protection Act (1985) has drawn further public attention to the question of pesticide residues by seeking under the Control of Pesticides Regulations (1986) to regulate the sale, supply, storage and use of pesticides. Further statutory controls are likely on the levels of pesticides permitted in products for human consumption.

What effect is this demand for pesticide-free cereals having on our milling and cereal processing?

As regards milling, little as yet. We do not claim to mill flour which is grown to organic standards and it would be impractical for us to do so. The main reason for this is that the grist we use to produce flour of sufficiently high quality for supplying plant bakeries must contain at least 70% Canadian wheat, which is not available in 'organic' form in the quantities required.

However, we could easily produce a softer general purpose all-English flour from organic wheat as our Mill Manager is confident that he could obtain sufficient supplies from the large cooperatives. This would be suitable for the housewife but not for the modern bakeries due to its insufficient protein content.

The milling of organic wheat would affect our operation in the following ways:

(a) All wheat received would have to be certified as grown to the standards of the Soil Association, Organic Farmers and Growers or an equivalent body. These require that crops are grown in soil totally free from chemical residues, that growing crops can only be fertilised with organic matter and that only natural pesticides, herbicides and fungicides are used.

(b) This certification would have to be supplemented by independent analyses of wheat received at the Mill.

(c) Arriving wheat would have to be inspected particularly thoroughly for overall quality and especially the presence of infestation, bearing in mind the restriction on the use of insecticides.

(d) Organic wheat would have to be stored in a separate silo area from 'mainstream' wheat to reduce to an absolute minimum the risk of the wrong wheat being used.

(e) The use of insecticides in this storage area would have to be avoided totally. For ethical reasons we would be reluctant to use even the permitted natural pyrethrins.

(d) Organic and 'mainstream' wheat would have to be kept separate at every stage of milling and flour would be bagged directly off the mill rather than stored in holding bins.

If an SPI infestation was to be experienced in a silo holding organic wheat then obviously it would be treated urgently using whatever means necessary. But the flour produced therefrom would no longer be organic and the silos could not be used for the storage of organic wheat until all residues from the treatment had disappeared. Residual insecticides would be used only as a last resort.

We are currently launching two other organic cereal products; organic oats and organic muesli. These are both being packed for us under contract and my earlier comments regarding the requirement for a

great degree of competence on the part of the packer could not be more relevant than in the case of organic products. Our requirements are strictly specified to ensure that the product and its ingredients are grown to appropriate organic standards, that they are certificated as such, that sufficient independent checks are carried out, that suitable conditions of storage and packing are maintained and that very accurate batch records allowing each ingredient to be traced, are kept.

The Muesli presents more complex problems. As well as the home-grown organic cereal component there are also dried fruits, nuts and seeds from abroad. Obviously these ingredients are more difficult to control, thereby emphasising the importance of independent analyses for pesticide residues. A more practical difficulty is that supplies are frequently spasmodic and unreliable; however Food Labelling Regulations forbid the substititution of ingredients so the muesli may be a 'seasonal' product if any ingredient becomes temporarily unavailable.

THE FUTURE

The health food/organic food lobby is here to stay. But it is becoming more competitive with the result that standards of product and operating practice will continue to rise as they have done rapidly over the last ten years.

We will continue to utilise whatever new advances in Pest Control we can in order to ensure that our premises and products remain infestation-free.

Of recent developments, one that has proved of particular use is the pheromone trap, which operates by attracting male moths with a lure that mimics the sex-attractive scent of the female. Obviously if moths are caught in the trap, the presence of an infestation in the premises is indicated and appropriate disinfestation steps can then be taken.

However, irradiation will not be available to us as the health food lobby is totally opposed to the concept. This is mainly on the grounds that it can change the vitamin and mineral content of treated foods, that it can cause the formation of radiolytic products whose effects on health have been inadequately investigated; that it affects the taste and texture of foods and that inconclusive studies have suggested that animals fed irradiated foods show lower birth rates, higher mortality, lower growth rates, more tumours and cataracts.

It is not for me to comment on the pros and cons of irradiation as a treatment for cereals, but it shows once again the difficulties we face in satisfying the apparently conflicting demands of the health food consumer.

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LIMITING THE DAMAGE FROM STORAGE PESTS AND FROM THEIR CONTROL – A GOVERNMENT VIEW

P J BUNYAN

Research and Development Service, ADAS, Great Westminster House, Horseferry Road, London SW1P 2AE

ABSTRACT

The background to the introduction of the Food and Environment Protection Act, the probable actions and possible results flowing from it and their likely effects in the area of stored product pest control are briefly reviewed. Particular problems resulting from the use of chemical pest control methods in the storage of cereals, nuts and pulses, including residues and pesticide resistance are considered. The future need for research and development in the area are suggested and the potential of alternative control methods explored. The role of Government, the Pest Control Industry and the Food Industry in these developments are examined.

INTRODUCTION

Man has sought to store food successfully between harvests since he ceased to be a nomadic hunter-gatherer and began to husband crops and animals. From the earliest times certain groups of foods were found to be amenable to long-term storage in their natural dry state (eg cereals, nuts and pulses), others had relatively short storage lives (eg certain fruits and vegetables), whilst a further group (e.g. meat, fish and green vegetables) could not be stored for any reasonable period without resort to some form of preservation such as salting, fermentation, pickling or drying. The modern agri-food industry still has need to store as well as to transport food in order to satisfy an increasingly complex and demanding market. Modern technology in the form of freezing, chilling, heat treatment and gas packaging has both greatly extended the storage life and improved the final quality of many traditional high value perishable foods. It has had less impact on the storage of cereals, nuts and pulses, because of the bulk involved in storage and because of their relatively low value. Improvements in the storage of these commodities have been confined mainly to the use of various pesticides to limit damage and deterioration in the long term. Recently, even these materials, fumigants, insecticides and rodenticides, have been subject to difficulties, either because increasingly the public with its media-led views of healthy eating is questioning the use of chemicals on food, or because of the failure to control the target pests. There remains nevertheless the need to control damage by rodents, insects, mites and fungi and improved methods more acceptable to the trade and the consumer must continue to be sought. They must also comply with an increasingly complex framework of law.

THE FOOD AND ENVIRONMENT PROTECTION ACT

Despite a long and impressive record of safety under the non-statutory but nevertheless formally negotiated arrangement of the Pesticides Safety Precautions Scheme (PSPS), criticism of the control over the use of pesticide in the United Kingdom grew steadily in the early 1980s, both from the general public and from a number of interested organisations at home and abroad. Doubts were expressed on a number of aspects including the use of chemicals on stored products with particular concerns about the effects of pesticides on health and on the environment. The former were particularly for applicators, members of the public who might be exposed at the time of application and consumers exposed to residues of pesticides on food. Various trade organisations pointed to and questioned the use of unauthorised imports, whilst others raised doubts about whether agreements aimed to support PSPS in the distributive sector violated European Community competition rules.

The Food and Environment Protection Act (FEPA) was promulgated in 1985 in order to deal with a number of problems caused by chemicals and Part III provided for specific controls over the use of pesticides in the United Kingdom, including the food storage sector. The Control of Pesticides Regulations (1986) and the consents given under Regulation 6 implement most (but not all) of the provisions of Part III and those responsible for the storage, supply and use of pesticides, including those in the storage sector, must comply with them from 6 October 1986 or at a later specified date. Most users of pesticides in the food storage sector will have little difficulty complying with the new regulations if they followed the previous guidance issued under PSPS and most storage pesticides approved previously received consents initially. A few users may need to re-examine their methods of application which may not have been cleared for use and some will need to reconsider how they can comply with recommended application intervals since these now become mandatory. The majority are unlikely to attract the attention of the Health and Safety Inspectors or Local Authority Officers for contravening the regulations and consents. In the longer term there may however be problems for the use of pesticides in the storage sector.

Maximum residue levels

Pesticide residues in stored foods have always presented particular problems to regulators, firstly because unlike residues on growing crops there is far less opportunity for weathering to cause them to decline to very low levels and secondly, because the methods of application often lead to uneven distribution of the pesticide causing some samples to have relatively high residue levels. Moreover the use of pesticides in storage has immediate impact on the consumer. Until now the presence of pesticide residues in food exposed for sale has been controlled under the general provisions of the Food Act (1984), or its predecessor the Food and Drugs Act (1955) and the Imported Food Regulations (1984). These make it an offence to add anything to food so as to render it injurious to the health of the purchaser and specify that food must be of the nature, substance and quality demanded by the purchaser. More specifically the Preservatives in Food Regulations (1979) made under the Food Act make it an offence to sell food for human consumption containing any "added preservative" which is not expressly permitted in that food and in amounts expressed in the regulations.

Whilst many storage pesticides meet the definition of preservatives, only thiabendazole on bananas and citrus fruit is expressly permitted by the regulations, so that the presence of any other residue could constitute an offence. For this reason the regulations contain a defence clause (Section 10) which allows the presence of a non-permitted preservative provided it was used "as an acaricide, fungicide, insecticide or rodenticide for the protection in each case of food whilst in storage". Storage is defined by the regulations for this purpose.

The Food Advisory Committee which advises Ministers on food regulations still consider there is no practical alternative to a defence of this kind but have advised that they would prefer to see the substances and their levels identified more specifically. They have also made it clear, as has the Minister, that they wish to see the use of pesticides regulated to keep residues in food at the point of sale to a minimum. Finally, they have recorded their view that chemicals used in food storage should not be allowed on foods where their policy would be not to recommend the use of added preservatives. When preparing the FEPA the Government took the opportunity to include provisions enabling it to set legal limits on pesticide residues in food, crops and feedingstuffs and it has now issued a consultative document with a view to implementing these powers by regulation. Such a move will be in line with the advice of the Food Advisory Committee and make the framework of law far more specific and enforceable than hitherto. FEPA also contains a defence clause:

> "for the person charged to prove that he took all reasonable precautions and exercised all due diligence to avoid the commission of an offence".

The likely effect for stored food is to lead to a commercial requirement for the keeping of more accurate records of treatments and for the availability of authenticated records for packages of produce moving in trade.

Monitoring of home-grown and imported foodstuffs has been undertaken by the Working Party on Pesticide Residues since 1976. Use has been made of a combination of Total Diet Study, regular monitoring of basic foodstuffs and surveys targetted at specific problem areas to maintain an overall assessment of residues to which the consumer may be exposed. This approach supplements the clearance procedure which examines data supplied by the notifier on the residues likely to arise from a treatment and evaluates their impact on the consumer. The Working Party has issued two reports (1982, 1986) which indicated that whilst there are a few areas where steps were necessary to reduce residue levels, in most foods residues are very low or non-existent. This work will continue under the FEPA but in the future industrial contributions will finance that part of the survey concerned with home-grown food. Future surveys are also likely to provide data for the structured review of consents.

Consents

During the parliamentary passage of the FEPA the Government undertook to review all existing PSPS clearances within a reasonable period. This could lead to the curtailment or withdrawal of certain consents unless new data meeting modern toxicological criteria are forthcoming. Since many storage pesticides are 'commodity chemicals', this could be difficult to obtain. The review process has already been occurring in a piecemeal fashion,

particularly in the storage sector where, for example, the use of ethylene dibromide and recently diazinon have been banned or curtailed by PSPS and the use of carbon tetrachloride and ethylene dichloride only retained pending the substitution of suitable alternatives, but these reviews are now likely to be positively initiated and structured. These moves are being reinforced by action taken or contemplated in the European Community. The use of ethylene oxide as a fumigant must be banned in national law by July 1987 under Directive 79/117. Although derogations had been granted until 1989 for most major UK food uses they may be difficult to extend if necessary and they do not include the use on nuts and cocoa beans. The future use of methyl bromide is also being discussed by the Scientific Committee for Pesticides which provides the European Commission with technical advice on pesticides. The United Kingdom Government is questioning this piecemeal approach, since methyl bromide is the only alternative for many of the uses of ethylene oxide and has argued strongly that attention should be given to the relative merits of various chemicals in the review process.

RESISTANCE TO PESTICIDES

The use of chemicals to control invertebrate and vertebrate pests has led to many recorded cases of the development of resistance to their lethal action. In this context resistance is defined as the ability of the target organism to survive a discriminating dose of toxicant designed to kill all normal or susceptible organisms.

The current position

For storage pest insects, whilst the presence of resistant strains as defined does not automatically indicate probable control failure, there are important combinations of species and chemical where it will do so. It is therefore important to be aware of the current position in order to know where the real threats to successful control lie. Since the major storage pests are spread worldwide by trade, resistant strains are also easily spread and information on resistance should ideally be collected on this basis, although the resources required for this are considerable. The only comprehensive survey of this type was carried out by FAO in 1973 (Champ and Dyte, 1976) and it demonstrated resistance in many storage insect species, although predominantly in certain geographical areas. The position in the United Kingdom at that time appeared to be acceptable. Subsequent more limited surveys have continued to show a deteriorating position, with more species and more chemicals of a wider variety giving positive results (eg Champ, 1986).

Insecticide resistance

In the United Kingdom, the most serious pest of stored grain, the saw-toothed grain beetle (*Oryzaephilus surinamensis*) provides a good indicator of the changing situation. At the time of the FAO survey all tested field strains of UK origin were susceptible to malathion. This position in which inland premises were free of resistant strains was maintained until 1979 by a rigorous inspection scheme on imported animal feedstuffs with complete eradication by fumigation if resistance was indicated. However the increasing containerisation of imports, and the diversion of scarce manpower resources to barley export inspections which help to deal with surpluses combined to curtail import inspection leading to the establishment of malathion resistant strains on farms by 1980.

Partly due to the development of resistance and partly due to the end of patent protection, commercial pressures during the 1980s have dictated a move away from control by the use of malathion, as well as by gamma-HCH and fenitrothion in favour of control by the more persistent organophosphorus compounds, pirimiphos-methyl and chlorpyriphos-methyl. Indeed the former is now by far the most widely used compound in storage, with the use of the latter increasing. Testing carried out by the Ministry in 1984-5 where farmers sought advice following a control failure, showed that a substantial proportion (31%) of strains tested (74) were resistant to malathion and (39%) to pirimiphos-methyl (28 strains tested), whilst a very high proportion (93%) were resistant to chlorpyriphos-methyl (27 strains tested). This investigation was followed up by an examination of six of the farms involved a year later and all were found to still have organophosphorus resistant O. surinamensis present. On one of these farms a carefully controlled and monitored treatment by skilled ADAS personnel at the recommended dose rate failed to eradicate the resistant population and after the treatment the proportion of resistant individuals in the population was found to have increased. Limited evidence such as this, taken together with the rising incidence of insecticide-resistant O. surinamensis on farms suggests an increasing risk of control failures with the accompanying damage. There is also the possibility of larger residues as operators seek either to exceed the recommended dose rate or to apply multiple pesticide treatments.

The demonstration of resistance to a particular organophosphorus insecticide does not necessarily mean that resistance to others will also be exhibited. Strains with specific resistance to malathion and chlorpyriphos-methyl are well known and specific resistance to pirimiphos-methyl is postulated but not yet proven. However strains with multiple resistance to all three are also known. Strains have also been detected where a single mechanism appears to confer cross-resistance to a wide range of different insecticides. For instance, one such strain from a UK farm was found to have cross-resistance to malathion, fenitrothion, pirimiphos-methyl, bromophos, bioresmethrin, dioxacarb, methomyl, gamma-HCH and DDT as well as separate specific resistance to chlorpyrifos-methyl.

Fumigant resistance

The development of resistance to fumigants which are small molecules of simple structure has been considered far less likely than the development of resistance to large biocidal molecules with known specific toxic actions. For insects to develop an appreciable degree of resistance it was argued that a relatively large number of biochemical changes would be required. Hydrogen cyanide was found to be an early exception since it acts as an irreversible inhibitor of the major respiratory enzyme cytochrome-c oxidase and an alternative enzyme is thought to occur in resistant strains (Price, 1980). As the refinement of testing techniques allowed small changes of tolerance to fumigants to be demonstrated it became apparent that fumigant resistance is present in a number of strains and the FAO survey (Champ and Dyte, 1976) showed that resistance to methyl bromide and phosphine was present. Whilst resistance to methyl bromide and the halogenated hydrocarbons has proved to be of little consequence economically, resistance to phosphine with consequential control failures is now well documented. Work at the ADAS Slough Laboratory has shown that phosphine, like hydrogen cyanide, has a specific mode of action in the respiratory chain, causing a reduction in oxygen uptake, inhibition of mitochondrial oxidation and also physical and chemical effects on cytochrome-c oxidase. Resistance

can be conferred by changes in any of these areas. So far, high levels of resistance have only been recorded among strains from Bangladesh and the Punjab where it seems likely that misuse of phosphine is the cause. Resistant populations will be imported into the United Kingdom unless measures are taken at source. With the development of insecticide resistance and the increasing curtailment of use of the halogenated hydrocarbon fumigants, phosphine is one of the few chemicals still available for dealing with serious breakdowns in control. Although resistant populations can still be controlled by phosphine by increasing the length of exposure under gas-tight conditions this is economically undesirable and the position could deteriorate so that the development of resistance must be viewed with disquiet.

Rodenticide resistance

Specific resistance and some cross resistance have also developed in certain rat and mouse populations to the variety of anticoagulant rodenticides now available. These compounds were developed to replace the older less satisfactory acute poisons and are undoubtedly much safer to use for rodent control in food stores. The development of resistance was mainly monitored in field populations but in recent years, during a period when a second generation of anticoagulant rodenticides have become established, resources have not allowed for systematic monitoring. Thus as with insect resistance, the current position on resistance is not known with precision. Cases of resistance in mouse populations have been reported in Government stores, but a policy of rigorous inspection of control operations and the use of calciferol appears to have contained the problem. Our knowledge of the position in other stores is inadequate to make any judgement. However careful monitoring would now be of great benefit, since if some of the newer, more toxic rodenticides are used in proscribed ways to combat resistance to the older chemicals, the situation could deteriorate, because if cross resistance develops the option of using these chemicals against resistant populations will not be available.

MYCOTOXINS

Cereals, nuts and pulses are also subject to spoilage by moulds. Some spoilage originates in the field (although fungal species occurring in crops in the field generally do not survive long in store). Fungal spoilage more usually arises from poor storage conditions and can be exacerbated by insect damage. Apart from simple biodeterioration, mould damage may under certain circumstances which are not well understood give rise to toxic compounds known as mycotoxins. These vary greatly in their chemical composition and the type and severity of their toxic effects and it is often difficult to establish any link between illness in man or animals and the presence of a particular mycotoxin in food or feed.

There is substantial evidence for animal and human illness caused by aflatoxins. These are mycotoxins arising from Aspergilus flavus, and these have been detected widely in nuts, particularly groundnuts. The Government already controls the contamination of animal feed under the Feeding Stuffs Regulations (1986) which set maximum permitted levels for aflatoxin B_1 of between 10 and 50 µg/kg depending on the type of feedstuff and the animal for which the feedstuff is intended. Although the general provisions of the Food Act (1984) control the presence of mycotoxins in food for human consumption, the Government is now proposing a specific maximum level of 10 µg/kg for aflatoxin B_1 in nuts and nut products in a regulation under

the Act. The Government is currently supporting work to develop methods for degrading aflatoxin in groundnuts, but although some progress has been made with chemical degradation it is still not yet clear whether the degradation products are themselves toxicologically safe. Other mycotoxins, most notably vomitoxin arising pre-harvest from Fusarium spp. have been found mainly in imported cereals. This compound has been implicated in refusal of feed by pigs.

The Ministry continues to undertake work to define the extent of contamination of susceptible commodities by mycotoxins. However prevention of mould infestation by good storage practice, in order to avoid both primary and secondary infestation is the best way to ensure that further problems do not arise. The use of fungicides is not a practical answer. Indeed the use of the fungicide tridemorph has been shown to enhance the production of T-2 toxin whilst inhibiting the growth of the mould responsible for its production, whilst the under-application of propionic acid to stored grain can promote aflatoxin production in the subsequent animal feed.

FUTURE RESEARCH AND DEVELOPMENT NEEDS

The strategies required to deal with the problems posed by the presence of residues following treatments to control infestations in store and by the development of resistant strains of storage pests tend fortuitously to be the same. Development work in ADAS is pursuing a number of lines which appear to be the most promising of those currently suggested, concentrating particularly on the more common pests and on control or prevention at the farm level. Work on problems of particular or more special interest to the food processor or retailer, such as infestations by less common insects, for instance psocids, favoured by changes in storage practice, is not currently supported by public money and the industry will need to consider how best to meet these requirements.

Although the Ministry does not seek to find alternative pesticidal chemicals it is very active in developing strategies for the use of new chemicals to reduce infestation in storage. Work on methyl chloroform either alone or in admixture with methyl bromide to improve both the penetration and the uniformity of application is at an advanced stage, although the lack of a commercial sponsor for these materials may be a barrier to full commercial use. The highly specific insect growth regulators and their analogues which appear to be virtually non-toxic to mammals have already found uses in a number of areas of pest control and their use to control storage infestations is being explored. Alternative strategies are also being developed using currently approved chemicals in combination with the earlier detection of infestations using insect attractants and treatments by more selective and less extensive use of pesticides. Such strategies would in themselves ease the pressure of pesticide use and the selection for resistance.

Greater emphasis is also being placed on the development of non-chemical methods of control. Storage under oxygen depleted gas mixtures is a particularly promising field but the potential of various mixtures to control both a range of pests and their various life stages requires careful evaluation as does the more practical aspect of the generation and retention of gaseous atmospheres under commercial conditions. This work will if successful, require support both financially and in the provision of test

facilities from the industry in the final stages. Such support would be in line with current Government policy in the Link Scheme as announced recently by the Prime Minister to Parliament.

Irradiation is a potential disinfestation process for cereals, nuts and pulses. Most insects are killed by radiation doses of less than 0.25 kGray, although mites and certain moths require higher doses. In practice doses need not exceed 1 kGray which is one tenth of the overall level considered safe by the Advisory Committee on Novel and Irradiated Foods (ACNIF). However unlike insecticide treatment irradiation provides no protection against reinfestation so that if used in practice the technique would have to be combined with another such as controlled atmosphere storage. No work is currently in hand in this area, partly because of the adverse publicity which could stop the development of a successful process despite the view of both the FAO/WHO and the ACNIF which show it to be safe, and partly because of potential costs on a bulky low value crop. However, such a development could in due course prove to be of real value for the most valuable packages of cereals, although both the economics and the practical difficulties suggest that it is unlikely to be of use for nuts and pulses.

Finally, the current situation on resistance must be properly delineated. The range of discriminating dose tests is being extended to cover all insecticides approved for use on stored cereals, with priority given to O. surinamensis. Other species will follow as resources allow. Studies on the mechanisms of resistance and its inheritance will also continue as an aid to the diagnosis of problems and to provide possible new strategies for control of resistant populations of both insects and rodents.

CONCLUSION

In all these areas, the existence of surplus cereals is both a promoter and a deterrent to additional work. Export of cereals is highly dependent on quality which in turn is affected by infestation. Intervention standards also require freedom from infestation. But once in store the spur to maintain surplus cereals free of infestation is smaller and is an additional expense to the Intervention Board. The Government is therefore of the view that outside of the farm gate and the export ships hold, work on infestation in store is of benefit primarily to the feed and food industry. Where there are clear benefits to the industry from R and D in Government establishments, the Government is making a major effort to develop a partnership in which the industry carries a larger share of the responsibility for funding and is closely involved in the determination of priorities. There are many possible forms for such funding including the Link Scheme referred to earlier.

Although funding can be channelled through individual contracts between relevant research institutes and individual commercial enterprises, the industries would do well to consider some form of collaborative funding for pre-competitive development work such as that now being raised by the HGCA in the form of a levy. In the area of cereal storage in bulk, the food processing and retailing sectors may consider that such work would be beneficial and it is to be hoped that they will consider making a financial contribution to ensure that it is undertaken.

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Fungal Problems and their Control

Chairman: Professor J. E. SMITH
Session Organiser: Dr J. LACEY

THE OCCURRENCE AND SIGNIFICANCE OF MOULDS AND MYCOTOXINS IN CEREALS AND ANIMAL FEEDSTUFFS IN THE UNITED KINGDOM

K.A. SCUDAMORE, A.E. BUCKLE

Ministry of Agriculture, Fisheries and Food, Slough Laboratory, London Road, Slough, Berkshire SL3 7HJ.

ABSTRACT

Mould spoilage of cereals can occur in the field or during storage. Invasion of cereals in the field by Fusarium spp. may result in contamination with mycotoxins which can produce disease in man and livestock. Surveys of cereals grown in the UK have revealed the wide-spread occurrence of Fusarium spp. and the occasional presence of certain Fusarium mycotoxins. In the UK, mould spoilage of cereals in store sometimes results in the production of mycotoxins and other metabolites mostly by Penicillium spp. Few of these substances have yet been chemically identified and to achieve this, development of appropriate analytical methods is essential. Consumption of mouldy grain may cause harmful effects in livestock. Those attributed to mycotoxins cannot always be adequately explained by the amounts detected which suggests that other toxic substances may be present. However, many of the problems caused by moulds and mycotoxins can be avoided by ensuring that grain is properly stored.

INTRODUCTION

Cereals naturally acquire a mould flora in the field and in store after harvest which is normally of little significance. However, when climatic or environmental conditions strongly favour fungal growth problems can arise. Mould spoilage can devalue the grain by reducing its nutritional content and produce mycotoxins which are potentially hazardous to man and livestock. Mycotoxins may be toxic even when present at very low concentrations when detection may prove a difficult analytical problem. Because of this, sensitive methods are available for few of the many secondary metabolites that may be present in mouldy grain. Lack of information about the variety of mycotoxins, the amounts that may be present, their toxicity and the frequency of natural occurrence, makes any assessment of their overall significance particularly difficult. This paper discusses the occurrence of some of the mycotoxins and current views on their significance, particularly to livestock.

FUNGAL SPOILAGE OF CEREALS IN THE FIELD

The environmental differences between field and store favour different moulds. Mycologists conveniently distinguish between epiphytic "field" species, e.g. Alternaria, Cladosporium, Fusarium and Helminthosporium spp. and "storage" species, e.g. Aspergillus and Penicillium. It is rare for species to flourish both in field and store. One notable exception is Aspergillus flavus which can invade maize in the field as reported in the USA (Shotwell et al. 1983) and can continue to grow in store. In the UK the "field" and "storage" species occurring on cereals are quite distinct. In most seasons the "field" species do not constitute a hazard to man or livestock. However, if the harvest is delayed by wet weather toxic species may

be favoured. Fusarium species thrive on crops which have been lodged by rain and cold and these conditions favour the production of Fusarium mycotoxins. In 1980 the wheat harvest in Ontario, Canada was delayed by wet weather (Trendholm et al. 1981). Many of the grain kernels turned pink indicative of Fusarium spoilage and the Fusarium mycotoxin, vomitoxin (deoxynivalenol), was detected in 44 out of 45 samples at levels ranging between 0.01 and 4.3 mg/kg (Scott 1984).

Fusarium on home-grown cereals

The possible occurrence of Fusarium mycotoxins in cereals grown in the UK has been investigated in three surveys. The 1980 harvest of barley used as animal feed and for malting was examined for vomitoxin. In 90% of the samples it occurred at less than 0.02 mg/kg and exceeded 0.1 mg/kg in only 2.3% of samples (Gilbert et al. 1983). A further survey was undertaken on wheat from the three harvests 1980-1982. Analysis was carried out for seven Fusarium toxins, nivalenol, vomitoxin, fusarenone-X, neosolaniol, diacetoxyscirpenol, HT-2 toxin and T-2 toxin. Only vomitoxin was detected at levels between 0.02 and 0.40 mg/kg in 32 out of 199 samples (Osborne and Willis 1984). In another survey in 1984 utilising a new and improved analytical procedure, nivalenol, vomitoxin and zearalenone were detected in 17 (55%), 20 (65%) and 4 (13%) respectively out of 31 samples (Tanaka et al. 1986).

In a survey of 214 samples of UK-grown wheat harvested in 1982 the incidence of Fusarium species and their potential to produce mycotoxins under laboratory conditions was studied (Niles et al. 1984). F. poae, F. tricinctum, F. culmorum and F. avenaceum were found to occur most frequently, occurring on, respectively, 42, 18, 17 and 13% of samples examined (Table 1). When cultured on Vogel's medium at 20°C for 3 weeks some F. poae isolates produced diacetoxyscirpenol, T-2 toxin and neosolaniol and some F. culmorum isolates produced vomitoxin and/or zearalenone (Table 2).

TABLE 1

Incidence of Fusarium species in 1982 wheat

Species	% samples on which a species is predominant	% of grains infected (100 grains tested)
F. avenaceum	13	13
F. culmorum	17	18
F. graminearum	1	2
F. nivale	9	10
F. poae	42	34
F. tricinctum	18	18
Others	0	<1

To determine possible relationships between degree of Fusarium contamination and the presence of Fusarium mycotoxins, those samples with the highest levels of Fusarium and representative control samples were examined for vomitoxin, nivalenol, fusarenone-X, diacetoxyscirpenol and T-2 toxin.

Vomitoxin was detected in 10 out of 62 samples at levels ranging between 0.03 and 0.75 mg/kg, fusarenone-X was detected in three samples at 0.14-0.57 mg/kg together with vomitoxin, and alone in one sample at 0.03 mg/kg (HMSO 1987). Analysis for zearalenone was carried out on 10 samples contaminated with the greatest amounts of F. culmorum and F. poae using the

TABLE 2

Fusarium toxins found in Fusarium isolate cultures

Fusarium spp.	No. of isolates tested	No. of positive results				
		Vom	Zea	Das	T-2	Neo
F. avenaceum	29	0	0	0	0	0
F. culmorum	39	8	25	0	0	0
F. graminearum	12	2	3	1	0	0
F. poae	34	0	0	17	8	4
F. tricinctum	33	0	0	2	0	0
Others	2	0	0	0	0	0

Vom = Vomitoxin
Zea = Zearalenone
Das = Diacetoxyscirpenol
T-2 = T-2 toxin
Neo = Neosolaniol

multi-mycotoxin method (Patterson and Roberts 1979) with a detection limit of 0.050 mg/kg, but none was found. There was no significant relationship between Fusarium contamination and the occurrence of vomitoxin and fusarenone-X. However, for those samples containing vomitoxin and fusarenone-X, there appeared to be some correlation between the levels of mycotoxins detected and the percentage of grains contaminated with Fusarium.

Fusarium mycotoxins in animal feedingstuffs

In contrast to the low incidence of Fusarium mycotoxins detected in UK cereals they have been found in much larger concentrations in compound feedingstuffs. This results from inclusion of maize and maize by-products grown in North America where zearalenone (Shotwell et al. 1971) and vomitoxin (Vesonder et al. 1978) can occasionally occur in these products at high enough levels to have adverse effects on livestock. Breeding sows are particularly susceptible to the oestrogenic effects of zearalenone (Long et al. 1982) while vomitoxin and other related compounds can cause feed refusal or vomiting in pigs (Vesonder et al. 1981). Compound feeds found contaminated with vomitoxin have been associated with feed refusal by pigs in England in recent years but home grown cereals have never been implicated in these problems.

FUNGAL SPOILAGE IN STORED CEREALS

While spoilage of home grown cereals by field species generally gives no cause for concern, problems can arise in store when grain has not been dried to 15% moisture content or less. The extent and nature of this spoilage depends on the temperature and moisture content of the grain. The temperature ranges and minimum water requirements for growth of some important storage fungi are shown in Table 3.

TABLE 3

The minimum moisture content and temperature range required for growth and mycotoxins produced by important storage fungi on grain.

Storage fungus	Min. a_w*	Equivalent % moisture content at 25°C	Temp. range, °C	Mycotoxins produced
Aspergillus restrictus	0.75	15.4	2-44	?
A. versicolor	0.75	15.4	9-40	Sterigmatocystin
A. candidus	0.75	15.4	10-45	Citrinin
A. flavus	0.78	16.0		Aflatoxins
Penicillium spp.	0.80	16.4	-4-48	Citrinin Ochratoxin A Viomellein Vioxanthin Xanthomegnin
A. fumigatus	0.82	17.0	12-55	Fumitremorgen

* water activity (a_w) is the ratio of the vapour pressure of water in a product to that of pure water at the same temperature.

Aspergillus and Penicillium species are invariably present on grain which has moulded in store on farms in the UK. They can produce many mycotoxins given the right conditions and examples are given in Table 3. Apart from mycotoxins, mould spoilage has other harmful consequences for man and livestock. Spores produced by the fungi make the grain dusty and impart a musty smell decreasing its market value. Some species are pathogenic, e.g. Aspergillus fumigatus can cause respiratory infection (Aspergillosis) in man and poultry when its spores are inhaled. This mould as well as other species found on grain, e.g. Absidia and Mucor species, can also cause mycotic abortion in cattle (Ainsworth and Austwick 1973). Mould spoilage will often lead to grain heating thereby providing optimum conditions for the growth of thermophilic actinomycetes. The two most important species encountered in such grain are Thermoactinomyces vulgaris and Micropolyspora faeni which cause the respiratory allergy known as Farmers' Lung in individuals who have become sensitised to the antigens on the spores. Sensitisation occurs after repeated exposure caused by inhaling the dust released into the air when mouldy grain or hay are handled. However an 'organic dust toxic syndrome' can also occur from a heavy single exposure without prior sensitisation (do Pico 1986). Cattle, too, can also exhibit similar symptoms when challenged by these microorganisms from dusty fodder or bedding.

An aspect of mould spoilage that has received relatively little attention is its effect on the nutritional quality of feeds. Microbial respiration utilises carbohydrates thereby lowering the energy value. Grain with an initial moisture content of 19% held over 16 weeks at 25°C lost 15% of its starch content through microbial activity (D Thorne, MAFF, personal communication). Bartov et al. 1982 reported that spoilage by Aspergillus and Penicillium species in ground maize and sorghum stored at 15% moisture content decreased the metabolisable energy value but did not produce aflatoxin B_1, ochratoxin A, patulin or sterigmatocystin.

Mould spoilage and mycotoxin formation may render grain less palatable

and can result in a reduced intake or feed refusal. Young turkeys consumed less feed when it was spiked with pure ochratoxin A (Burditt et al. 1984). Feed containing 16 mg/kg of ochratoxin A decreased consumption to about half of the control value. The natural occurrence of ochratoxin A and its effects in poultry have been reviewed by Burns and Dwivedi (1986).

Ochratoxin A and citrinin both formed by Penicillium spp. are the two mycotoxins most commonly found in home grown cereals examined by ADAS. In 1985, ochratoxin A and citrinin were detected in respectively 39 and 35 out of 140 samples examined during advisory work. It must be emphasised that these analyses were undertaken because the grain was of doubtful quality and would be atypical of grain used in animal feedingstuffs in the UK. Nevertheless, such findings indicate that conditions can be favourable for mycotoxin production in stored grain and suggest that other mycotoxins might also be identified subject to the availability of suitable analytical methods.

ANALYTICAL METHODS FOR DETERMINATION OF MYCOTOXINS

To date the multi-mycotoxin technique using thin layer chromatography (TLC) (Patterson & Roberts 1979) has been the only screening method used in the regional laboratories of the Agricultural Development and Advisory Service of MAFF. However the chemical and physical properties of such a heterogeneous group of natural products as the mycotoxins differ greatly from toxin to toxin, and any acceptable multi-toxin method must, of necessity, make a compromise between the conditions optimal for each individual toxin. The Patterson/Roberts screen has provided invaluable data on the occurrence of the 11 or 12 mycotoxins for which it was developed. It may even become possible to include further toxins in the screen, but for some compounds specific methods are necessary. For instance, vomitoxin requires a different solvent and clean-up procedure, followed by TLC or the use of a sophisticated gas chromatographic separation with detection based on electron capture or mass spectrometry. Even after extensive clean-up the chromatograms obtained may be very complex and require reliable confirmation. Fortunately these methods can also be used to screen for other closely related trichothecenes, produced by various Fusarium species.

OCCURRENCE OF MYCOTOXINS IN ANIMAL FEEDSTUFFS

The principle toxins found in cereals and animal feedstuffs in the United Kingdom have been summarised by Buckle(1983, 1986). Using data collected by the ADAS microbiology laboratories and by the Central Veterinary Laboratory (CVL) on mycotoxins found in animal feedstuffs in the UK between 1971 and 1980, the frequency of occurrence of the individual mycotoxins on an empirical basis can be plotted against detection limits obtained when using the Patterson/Roberts screen, Fig. 1. Data obtained for vomitoxin using an alternative TLC method and for citrinin using a HPLC method is also included. Hence aflatoxin B_1, ochratoxin A and citrinin with detection limits using TLC of 10 µg/kg or better, have been recorded most frequently and sterigmatocystin and zearalenone with limits of 25 to 100 µg/kg are observed less frequently. T-2 and diacetoxyscirpenol, which can only be detected by the multi-method above 200 µg/kg, have never been found.

The use of alternative, more sensitive analytical methods enables the relationship between detection limit and frequency of detection to be tested. For surveys of trichothenes in cereals, sensitive gas chromatographic and mass spectrometric (GC/MS) methods were used. Still T-2 and

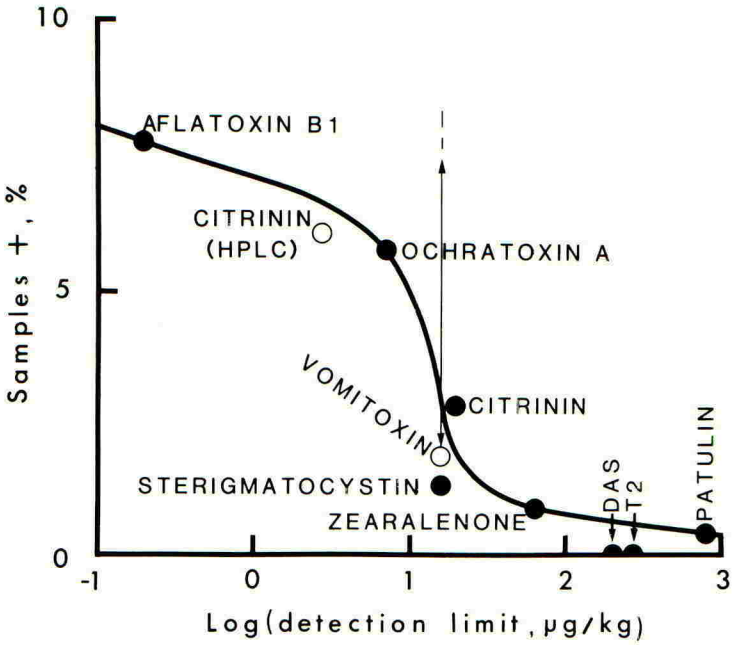

Fig. 1. Relationship between the frequency at which a mycotoxin has been found in animal feed samples examined and the sensitivity of the analytical method used for that mycotoxin.
-●- Patterson/Roberts TLC, -o- Other methods.

diacetoxyscirpenol could not be found but vomitoxin was found in more samples by GC/MS than by TLC. The detection limits by TLC are about 20 times greater than by GC/MS but both methods gave results close to the curve shown in Fig. 1. Similarly, sensitive HPLC methods for citrinin allowed its detection in more samples than with the multi-mycotoxin method and results for both are close to the curve in Fig. 1. Thus for many mycotoxins the relationship between detection limit and frequency of detection shown in Fig. 1 is valid although for others found only in laboratory studies so far, this does not hold. It is therefore better to refer to frequency of detection of a mycotoxin rather than frequency of occurrence. Fortunately, both aflatoxin B, and ochratoxin A can be detected at low concentrations by their fluorescence under UV light as they are of particular consequence.

When examined by TLC, extracts prepared from mouldy samples of feedstuffs reveal many coloured spots in addition to those of known mycotoxins or natural constituents of the feedstuff itself. In general, unmoulded samples give relatively few spots suggesting that most of these additional spots represent fungal metabolites of unknown identity and toxicity. Normally, the worse the condition of a sample, the greater the number of such spots. However mycotoxins are also sometimes found in samples which, at least superficially, appear of good quality.

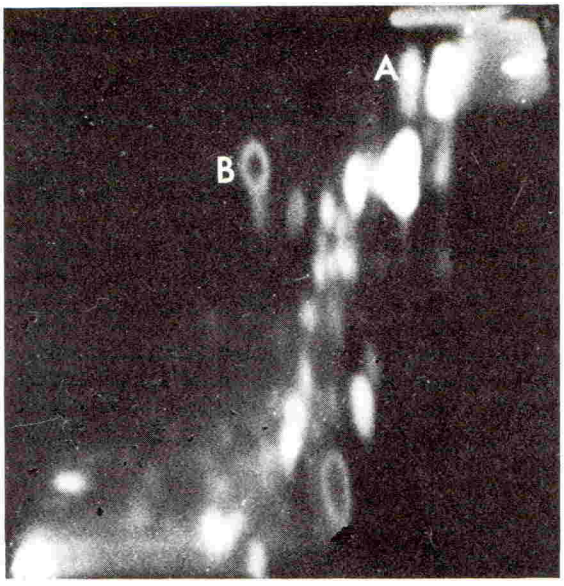

Fig. 2. Metabolites produced in a culture of a species of Penicillium. A = vioxanthin, B = xanthomegnin.

The moulds occurring in animal feeds and cereals can be isolated and cultured in suitable media to give a guide to the range of fungal metabolites that can be produced and may be present in the original contaminated animal feed. Many metabolites may be produced by one isolate as shown in black and white in Fig. 2. This Penicillium isolate came from a sample of wheat and produced xanthomegnin, viomellein and vioxanthin which were also found in the original wheat sample. However most TLC spots remain unidentified. As a mouldy sample may contain several different fungi, the complexity of the metabolic profiles obtained is not surprising.

Significance of mycotoxins in animal feeds

The conclusive implication of mycotoxins in livestock disorders is extremely rare often because the feed held responsible has been exhausted or disposed of before symptoms develop and mycotoxicosis suspected. More often problems arise in livestock which can be tentatively linked to the ingestion of mouldy feed in which one or more mycotoxins are subsequently detected. Even when these toxins are known to cause the observed effects, the amounts found in the feed are often too small to have been responsible. However addition of a pure toxin to feed has frequently failed to produce the same effect or to the same degree as in the affected animals when fed the same amount of toxin in a naturally contaminated sample. The toxicity observed is probably due to the contribution of many other metabolites present but has rarely or never been tested.

At Slough, mycotoxin positive samples are often received from ADAS regional laboratories for further examination. One particularly interesting recent case concerned three fattening bulls which died due to kidney failure. Post mortem examination revealed that the kidneys showed lesions resembling those associated with ochratoxicosis in pigs. Examination of a sample of the barley on which the bulls fed revealed the

presence of ochratoxin A and citrinin at 380 µg/kg and 1550 µg/kg respectively. In addition sterigmatocystin was found at 50 µg/kg and a possible trace of xanthomegnin. Many other TLC spots were also present on the plates but these could not be identified. Lloyd (1980) and others have reported similar disease in cattle caused by cereals containing ochratoxin A and citrinin.

An insidious aspect of the problem is that many mycotoxins can exert subtle effects on the immune system leading to secondary infection by bacteria, viruses or parasites which may then be diagnosed as the primary cause (eg. Pier et al. 1980, Campbell et al. 1983). Additionally, some mycotoxins can produce adverse effects at very low levels which current analytical methods cannot readily detect.

Assessing the significance of mycotoxins is thus extremely difficult. Proven instances of mycotoxicosis are uncommon. More frequently cases of illness, feed refusal, loss of productivity, reproductive problems and other diseases can only be linked tentatively to the presence of moulds and mycotoxins.

Much more needs to be known about the fungal metabolites that occur in cereals and how they interact. With this in mind, work at Slough is aimed at improving methodology for detection of mycotoxins, at developing methods for some metabolites not currently studied and at determining the incidence of mycotoxins in home grown cereals. An example of this is the method published recently (Scudamore et al. 1986a, 1986b) for the detection of a group of naphthoquinones including viomellein and xanthomegnin and determination of their incidence simultaneously with ochratoxin, citrinin or sterigmatocystin.

CONTROL OF MOULDS AND MYCOTOXINS

Because so much remains unknown about the significance of moulds and mycotoxins, it is important to concentrate attention on their prevention and control although this may be difficult with imported feed components which may already contain mycotoxins. Cereals stored properly usually contain few moulds and are free of mycotoxins. Thus, correct drying after harvest and good storage conditions will considerably reduce the risk of problems from moulding. Application of fungicides prior to harvest or protectants, such as propionic acid, during storage should also decrease fungal related problems. Other techniques employed to protect grain from insect damage, such as use of insecticides, fumigation or modified atmospheres rich in nitrogen or carbon dioxide may also inhibit mould growth and mycotoxin production (eg. Richard-Molard et al. 1984, Serafini et al. 1980). Elimination of insects reduces the risk of hot spots and the consequent development of damp areas with subsequent mould growth. While it is difficult to assess fully the significance of moulds and mycotoxins in cereals and animal feedstuffs, many of the potential problems will be avoided by careful attention to proper storage and protection of commodities.

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THE INFLUENCE OF WATER AND TEMPERATURE ON THE GROWTH OF FUNGI CAUSING SPOILAGE OF STORED PRODUCTS

N. MAGAN

Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedford MK43 OAL

J. LACEY

Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ.

ABSTRACT

Grain entering store carries a varied microflora of "field" and "storage" fungi. Field fungi are common on grain before harvest but seldom in store as for growth they require water to be readily available (> 0.90 a_w). By contrast storage fungi, especially Aspergillus spp., develop only after harvest since they can grow at low a_w (0.70-0.75 a_w), enabling them to initiate spoilage of stored grain. Most storage fungi are mesophilic with optimum temperatures for growth between 20 and 35°C but a few are psychrotolerant (able to grow below 0°C) and others are thermotolerant (able to grow up to 60°C). Water availability in the grain, temperature and intergranular gas composition together determine which species are able to grow and initiate spoilage. Bad storage allows a succession of fungi to colonize and utilize the grain, often resulting in spontaneous heating and loss of quality.

INTRODUCTION

Micro-organisms contaminating grain interact with one another, with the substrates that they colonize and with the prevailing environmental conditions. An acceptable storage system should keep the crop dry and not allow significant fungal growth. Fungal activity can cause rapid deterioration of grain, with losses in both dry matter and quality. The latter may be evident in loss of nutrient value, biochemical changes such as increased fatty acid content or contamination with mycotoxins and poor germination. Fungal spores may also present a health risk to farm workers through infection or allergy (Lacey 1975).

Grain is first colonised before harvest by "field" fungi, such as Alternaria, Cladosporium and Fusarium species which seldom develop in store. By contrast, storage fungi, such as Aspergillus and Penicillium species are few before harvest but may contaminate grain during harvesting (Flannigan 1978) or subsequent handlingand then develop rapidly in store when conditions are favourable for growth.

Availability of water in the grain substrate, temperature and the intergranular gas composition are the primary determinants of microbial activity (Sinha 1973, Ayerst 1986). Water availability may also interact with other environmental factors to have a profound influence on the germination, growth and sporulation potential of storage fungi. Knowledge of how these factors affect fungal development may allow manipulation of the environment to minimise their effects on grain

quality.

GRAIN WATER CONTENT AND WATER AVAILABILITY

In the grain industry water content (w.c.) expressed as a percentage of fresh weight (wet weight basis) is the measure most commonly used to indicate the storability of grain. However w.c. gives little indication of the availability of water for microbial growth. Water availability of hygroscopic materials like cereal grain is best measured by water activity (a_w) or water potential (Ψ). Water activity is a concept originated by Scott (1957) and is the ratio of the vapour pressure of water over the substrate to that over pure water at the same temperature and pressure. Water potential is the sum of the osmotic, matric and turgor pressures. a_w is expressed as a decimal fraction of one and water potential is measured in Pascals (Pa). The relationship between water potential and a_w is given by:

$$\Psi = (RT/V) \log_e a_w$$

where R is the ideal gas constant, T is the absolute temperature and V the volume of one mole of water (Papendick & Mulla 1986). An important difference between them is that a_w is temperature dependent while water potential is not. a_w is used predominantly in the agricultural and food industries while water potential is used in other areas of soil microbiology. There is pressure for all disciplines to use the water potential concept (Ayres & Boddy 1986). However, in this paper a_w will be used as it is still more generally understood.

The relationship between w.c. and a_w at a particular temperature is described by a water sorption isotherm. The isotherm, which is sigmoid in shape, will differ with grain type and variety, and with temperature. Moisture sorption isotherms will also differ considerably depending on whether water is being absorbed or desorbed due to hysteresis. A water content equivalent to about 0.65 to 0.60 a_w (=13.0 to 13.5% w.c. in wheat at 25°C) is normally required for safe long term storage of grain. However, for every 10°C change in temperature the water availability may change by as much as 0.03 a_w and could result in favourable conditions for microbial activity.

TEMPERATURE AND GROWTH OF STORAGE FUNGI

Temperature can control the rate of germination and growth of fungi in stored grain and individual isolates, species and genera differ in their responses to temperature (Mislivec & Tuite 1970, Lacey 1980). Most species of *Aspergillus* and *Penicillium* grow between 10-40°C with optima between 20-35°C and are mostly classified as mesophiles. Others, such as *P.aurantiogriseum*, *P.brevicompactum* and *P.viridicatum* can grow below 0°C (Mislivec & Tuite 1970, Lacey 1980). Stored grain which heats spontaneously may reach temperatures of 50 to 60°C and is characterised by thermophilic fungi and actinomycetes particularly *Humicola* (*Thermomyces*) *lanuginosa* and *Faenia rectivirgula* which grow at up to 60°C.

WATER AVAILABILITY AND GROWTH

Stored grain fungi differ considerably in their tolerance of low

a_w. Field species such as *Alternaria* and *Cladosporium* spp. seldom grow with < 0.90 a_w (Magan & Lacey 1984a), but storage spp. are tolerant of much lower a_w and are therefore the main causes of grain spoilage. *Penicillium* spp. can usually germinate down to 0.85-0.80 a_w and *Aspergillus* spp. to 0.75-0.70 a_w. Sporulation and growth of most species occurs over a rather narrower range of available water than that required for germination (Table 1) so that germination of spores can occur at low a_w but not be followed subsequently by growth (Ayerst 1969, Magan & Lacey 1984a).

TABLE 1

Minimum a_w permitting germination, growth and sporulation of some storage species.

Species	Germination	Growth	Sporulation
Aspergillus candidus	0.78	0.80	0.83
A.versicolor	0.76	0.78	0.80
Emericella nidulans	0.83	0.80	0.80
Eurotium repens	0.72	0.75	0.78
Penicillium aurantiogriseum	0.80	0.75	0.85
P.brevicompactum	0.80	0.82	0.85
P.hordei	0.80	0.83	0.86
P.piceum	0.79	0.85	0.89
P.roquefortii	0.83	0.83	0.83

INTERACTION OF WATER AVAILABILITY AND TEMPERATURE

Interactions between substrate a_w and temperature often determine the range of conditions over which individual species may be active and therefore determine their role in spoilage of stored grain. Such interactions affect all stages of the fungal life-cycle from germination (lag time, limiting a_w and germ tube extension), through to sporulation.

Germination

The effect of lowering a_w and changing temperature on the number of days prior to germination of spores of some *Aspergillus* and *Pencillium* spp. on wheat extract agar is shown in Table 2.

The minimum a_w for germination usually occurs at the optimum temperature and changes if temperature is altered. For example, the minimum for germination of spores of *P.aurantiogriseum* is 0.80 a_w at 20 to 25 °C but only 0.90 a_w at 5 °C while for *Eurotium* (*Aspergillus*) *repens* it is 0.72 a_w at 20-30 °C but only 0.85 a_w at 40 °C (Magan and Lacey 1984a) (Table 3).

TABLE 2.

Effect of temperature on the lag time, in days, for germination of spores of grain fungi at 0.85 a_w (N.G., no germination).

Species	Temperature (°C)		
	10	20	30
Aspergillus candidus	14	2	2
A.versicolor	6	3	1
Eurotium repens	4	2	1
Penicillium brevicompactum	6	5	7
P.hordei	9	3	6
P.piceum	N.G.	6	3

TABLE 3.
Effect of temperature on the minimum a_w permitting germination of storage fungi (N.G., no germination).

Species	Temperature (°C)					
	5	10	20	25	30	40
Aspergillus candidus	N.G.	0.85	0.76	0.78	0.79	N.G.
A.versicolor	N.G.	0.82	0.77	0.76	0.79	0.89
Emericella nidulans	N.G.	N.G.	0.82	0.83	0.82	0.84
Eurotium repens	N.G.	0.76	0.72	0.72	0.72	0.85
Penicillium aurantiogriseum	0.90	0.82	0.80	0.80	0.85	N.G.
P.brevicompactum	0.90	0.83	0.80	0.80	0.88	N.G.
P.hordei	0.95	0.87	0.84	0.82	0.87	N.G.
P.piceum	N.G.	0.97	0.84	0.79	0.79	0.85
P.roqueforii	0.93	0.83	0.83	0.83	0.85	N.G.

Germ tube extension is similarly affected by interactions between temperature and a_w. Changing either a_w, temperature or both results in a marked change in the germ tube length (Table 4). Germ tube growth is rapid at high a_w, but slows as a_w is decreased (Snow 1949).

Growth

Growth of most grain fungi increases with increasing water availability but there are exceptions. Some Penicillium spp. grow optimally at a_w between 0.99 and 0.98 (Hocking and Pitt 1979) while Eurotium spp. (Aspergillus glaucus group), A.niger and A.versicolor grow best between 0.95 and 0.90 a_w at 25-35 °C (Ayerst 1969, Magan and Lacey 1984a). The effect of a_w and temperature on growth and also on germination

time can be summarized in two dimensional diagrams with a_w and temperature as axes (Fig. 1). These patterns are modified by changes in available nutrient or gas composition.

TABLE 4.

Effect of interaction between temperature and a_w on the mean germ tube length (μm) of spores of storage fungi after 7 days incubation on wheat extract agar (N.G., no germination).

	Water activity	Temperature (°C) 10	20	30
Aspergillus candidus	0.90	41	> 350	137
	0.85	N.G.	91	46
	0.80	N.G.	48	35
Eurotium repens	0.90	64	282	> 300
	0.85	65	237	> 250
	0.80	N.G.	130	120
Penicillium hordei	0.90	182	200	255
	0.85	10	150	57
	0.80	N.G.	57	N.G.

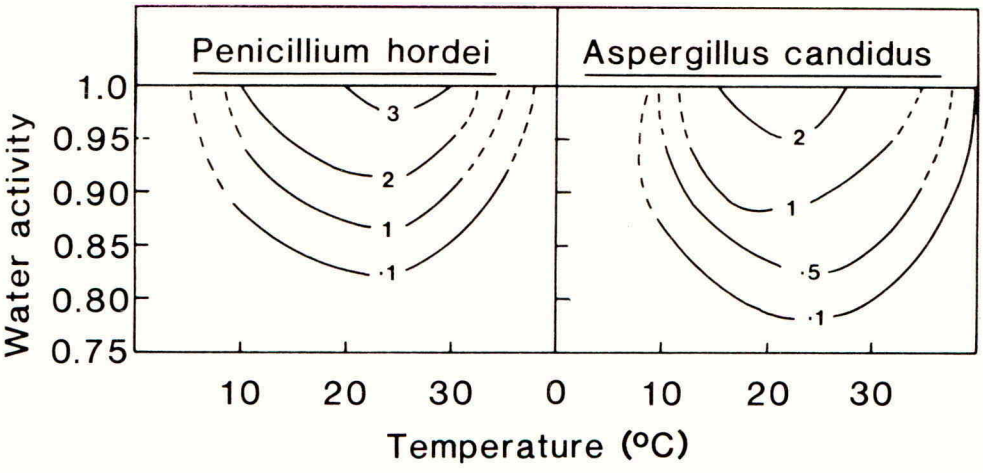

Fig. 1. The effect of temperature and a_w on the growth of two storage fungi. The isopleths represent growth rates in mm/day.

The colonizing fungi on stored grain interact during growth. Both competitive and antagonistic interactions occur which can result in different groups of fungi becoming dominant under different temperature, a_w and nutrient conditions. However, the interactions seen in laboratory studies and on autoclaved grain do not always accurately simulate activity on naturally colonized grain (Magan & Lacey 1984b, 1985).

Sporulation

Interaction of a_w and temperature with sporulation has been little studied although effects on the time and minimum a_w for both asexual and sexual spore formation of some Aspergillus and Penicillium spp. has been described (Snow 1949, Mislivec & Tuite 1970, Magan & Lacey 1984a). The ability to sporulate at low a_w may be important in enabling fungi to survive and to enable spread by insects and mites. In general, for asexual conidia, sporulation occurs more rapidly than production of cleistothecia and development of ascospores (sexual spores) (Snow 1949). A.chevalieri and Eur.repens required 56 days for formation of cleistothecia at 0.90 and 0.93 a_w, respectively (Snow 1949). However, little quantitative information is available on spore production on grain held under different conditions.

Ventilation of grain with ambient or warmed air can remove heat and CO_2 and introduce O_2. This can change a_w of grain bulks and result in differences in a_w of the grain at the grain surface allowing growth of fungi without visible signs of sporulation.

INTERACTION OF GAS COMPOSITION WITH WATER ACTIVITY AND TEMPERATURE

Concentrations of O_2 and CO_2 in the intergranular spaces of grain bulks are important in modifying the effects of a_w and temperature on fungal colonization. Fungi are usually considered obligate aerobes, but the concentrations of O_2 required for growth of spoilage species are often overestimated.

Aspergillus and Penicillium spp. were found to be relatively tolerant of low O_2 and the lag time before growth occurred appeared to be negatively correlated with decreasing O_2 (21% to 0.14%) and a_w (0.98 to 0.85) at both 14 and 23°C (Magan & Lacey 1984c). Below 1% O_2 concentration and 0.90 to 0.85 a_w the lag time prior to growth was 10-20 days for some Aspergillus and Penicillium spp. while in normal air and high a_w (0.98-0.95) this period was only a few days. For example, at 23°C and 0.85 a_w A.candidus required 1 day for growth in 21% O_2 but 13 days in 1.0% O_2. For the same conditions P.roquefortii required 1 and 19 days respectively. Therefore, decreasing O_2, together with a_w and temperature usually increases the period during which germination is inhibited and before fungal colonisation of grain occurs. The tolerance of storage fungi to O_2 and how this changes with a_w and temperature is shown by the the concentrations required to halve (LD 50) colony diameter in laboratory studies (Table 5; Magan & Lacey 1984c).

Usually, large concentrations of CO_2 are necessary before the germination of grain fungi is inhibited. Germination of spores of P.aurantiogriseum (P.martensii) were inhibited by 95% only when CO_2 concentration was increased to 60%, provided O_2 was adequate. The

temperature range promoting germination was also decreased as CO_2 concentration was increased. a_w interacts with gas composition especially to increase the lag phase prior to growth. For instance, the lag time for growth initiation of some Aspergillus and Penicillium spp. was increased to 16-18 days by 15% CO_2 and 0.90 a_w compared to 4 days with 21% O_2 and 0.03 CO_2 at 14 °C. The lag times were shorter at 23 °C (Magan and Lacey 1984c).

TABLE 5

The concentrations of O_2 (%) required to halve growth of some storage fungi at 14 and 23°C on wheat extract agar.

	Temperature (°C)					
	23			14		
Water activity (a_w)	0.95	0.90	0.85	0.95	0.90	0.85
Aspergillus candidus	1.00	0.45	5.00	< 0.17	9.40	N.G.
Eurotium repens	3.00	5.00	10.20	0.90	4.00	N.G.
Penicillium aurantiogriseum	5.30	2.40	13.00	< 0.17	10.20	N.G.
P.brevicompactum	0.60	0.40	1.00	< 0.17	< 0.17	N.G.
P.hordei	< 0.14	1.30	12.50	0.80	1.60	N.G.

COLONISATION OF STORED GRAIN

Harvested grain often has 16-18% w.c. (0.75-0.85 a_w) which is conducive to fungal spoilage and must therefore be dried to about 0.65 a_w (13-14% w.c.) for safe storage. However, grain bulks with, on average a low a_w may contain some grains with higher a_w. In the best conditions the w.c. of individual kernels can differ by as much as 2% (Oxley 1948) which could mean that some were 0.75 a_w or more. Also, when damp and dry grain are mixed, individual grains differ in the time taken to equilibrate to a safe a_w. Such seed can form a focus for fungal growth, with perhaps heating and the rapid loss of dry matter. In some seasons, green, immature grains may be common and also form foci for fungal growth. Moist corn in the U.S.A. with 14-15% w.c. (0.75-0.80 a_w) could have considerable fungal invasion and aflatoxin contamination when only about 0.5% dry matter loss had occurred (Seitz et al. 1982). The initial inoculum of Aspergillus flavus on the corn was a determining factor in the extent of fungal invasion.

At 14-15% w.c. (0.70-0.75 a_w) changes of only 0.5% in barley and wheat grain have a large effect on the rate of development of storage fungi, particularly Eurotium spp. in stored wheat and barley grain (Christensen 1963, Lacey 1971). Between 0.70 and 0.75 a_w Aspergillus restrictus and Eurotium spp. are able to grow slowly, without affecting grain temperature. However, dry matter losses may still

occur. If a_w is higher, fungal growth is more rapid and the rate of moulding increases rapidly. Since grain is a good insulator and cannot conduct the metabolic heat away as rapidly as it is produced the temperature also rises, enhancing fungal growth. Metabolism of the grains also produces water which increases the a_w further. In this way rapid heating can occur, although the initial a_w of the grain determines the maximum temperature reached (Lacey 1980). During the heating process, successive groups of micro-organisms dominate the deteriorating grain. It is important to note that the optimum conditions for activity of storage fungi in laboratory studies are not always the same environmental conditions at which these species occur most abundantly in stored grain. Thus, P.aurantiogriseum and P.viridicatum are most abundant at 0°C and 1.00 a_w and Eurotium spp. at 30°C and 0.70 a_w in stored grain although in in vitro laboratory studies they show optimal growth at 25°C and 1.00 a_w and at 30°C and 0.90 a_w, respectively. Interaction can also occur between fungi and insect pests. Insect activity, either by utilization of grain substrate or sometimes of fungi can create a 'hot spot' and also be responsible for transportation of spores of storage fungi within a grain bulk (Mills 1983).

At marginal temperatures and a_w levels (0.65-0.75 a_w) loss of quality through fungal growth is slow, with little evidence of visible moulding in the first few months. At higher temperatures and a_w, moulding is more rapid. Caked, moulded grain commonly represents dry matter losses, in the range of 10-30% (Burrell 1982). Burrell (1966) found that at 3.8°C barley grain with 26-29% w.c was safe for 150-200 days before moulding, especially with Penicillium spp., was visible while that containing 19% w.c. remained relatively free from spoilage for 400 days with little visible colonization. Periods of safe storage for barley of different w.c. held at 0-25°C are shown in Table 6.

TABLE 6

The effect of water content and storage temperature on the time, in weeks, before visible moulding of barley grain (extrapolated from Burrell 1982).

	Water content		
Temperature (°C)	25%	20%	18%
25	< 1	1-2	4-6
20	< 1	2	6
10	1-2	8	40-50
5	1-2	8-10	50-100
0	5-6	50	> 100

Visible moulding has been an important criterion used to assess the deterioration of stored grain. However, much less attention has been given to fungal activity when levels of water availability is 0.70 to 0.80 a_w (16-19% w.c.) and where fungal growth may not be obvious. More detailed information is still required on the effect such fungal growth has on quality of grain and also on the potential for mycotoxin production.

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EFFECTS OF MODIFIED ATMOSPHERES ON MOULDING AND MYCOTOXIN DEVELOPMENT

N. PASTER

Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

ABSTRACT

Mould growth and mycotoxin production can be affected by modified atmospheres. The relationship between CO_2 and O_2 tensions and the growth of fungi varies between species and genera. Usually, inhibition is due to the presence of CO_2 rather than the absence of O_2. High concentrations of CO_2 can result also in decreased production of mycotoxins. The degree of inhibition by high CO_2 concentrations is dependent on other environmental factors, of which relative humidity and temperature are probably most important. Most fungi are not killed by high CO_2 concentrations and the biosynthetic pathways for mycotoxin production are only blocked but not damaged. When restored to air, growth is resumed and mycotoxins are produced to the usual extent.

INTRODUCTION

Fungi are one of the principal causes of reduction in the quality grade and price of stored grains. The major types of losses due to fungi are discolouration, heating, mustiness, various biochemical changes and the production of mycotoxins which, if consumed, may be injurious to man and domestic animals. Postharvest mould spoilage can be controlled by chemical and/or physical means. The former include the use of fungistatic agents, while the latter involve modified atmospheres (MA), gamma irradiation, drying, cooling, and aeration of the grain bulk.

The use of modified atmospheres has been found effective in controlling storage pests. These techniques are particularly acceptable in grain storage, as they either minimise the quantity of fumigants employed or eliminate their use completely. While considering the use of modified atmospheres for fungal control, the different stages of the life cycle should be taken into consideration. These include sporulation, germination and mycelial growth; in addition, some secondary metabolites can be produced during fungal growth. These processes may be affected differently by changes in the intergranular gas composition. For each species the reaction is also closely related to other environmental, chemical and physical conditions. This paper will present a broad outline of the effect of modified atmospheres on grain moulding and mycotoxin production.

GAS REQUIREMENTS OF MOULDS

Moulds are usually considered to be aerobic, although several reports indicate that certain species will continue to grow, although at a greatly reduced rate, under low O_2 concentrations. Peterson *et al*. (1956), studying the effects of O_2 and CO_2 on mould growth *in vivo* using sound hard red spring wheat, found that there was significant mould growth even in samples aspirated with N_2 (0.2% O_2). *Aspergillus glaucus* was the predominant mould isolated under these conditions, with *Penicillium* spp. and *Aspergillus flavus* present in smaller numbers. When large concentrations of CO_2 were added to atmospheres containing 21% oxygen, mould growth was almost completely inhibited by >50% CO_2. *A. glaucus* was the species most resistant to high CO_2 levels, followed by *A. flavus*, *Aspergillus candidus* and *Penicillium* spp.

However, the CO_2/O_2 ratio is not the only factor that governs fungal growth and some other factors related to the gases' character should also be taken into account. For instance, Brancato and Golding (1953) who worked, *inter alia*, with *Aspergillus niger*, *A. flavus*, *Penicillium notatum* and *Penicillium expansum*, suggested that these fungi could use O_2 for spore germination and growth only in the dissolved state, by absorption through the submerged hyphae. The dissolved O_2 may be the most important source of O_2 in liquid media. Similarly, it was noted that the inhibitory effect of decreased O_2 concentrations is in proportion to its solubility and not directly in proportion to the composition of the gas above the medium (Miller & Golding 1949).

EFFECT OF MA ON *ASPERGILLUS FLAVUS* GROWTH AND AFLATOXIN FORMATION

The ability of *A. flavus* to grow and produce aflatoxin in modified atmospheres has been studied extensively using synthetic media, corn, groundnuts, soya beans and wheat. *A. flavus* grew well and produced aflatoxins B_1 and G_1 in liquid medium at room temperature in an atmosphere composed of 10% CO_2, 1.8% O_2 and 88.2% N_2 (=MA; Table 1) (Epstein *et al.* 1970). However, at 15.6°C fungal growth and toxin production were substan-

TABLE 1

Mycelium and aflatoxin production by *Aspergillus flavus* after 21 days of incubation in liquid medium under various temperatures (Epstein *et al.* 1970)

Temperature (°C)	Atmosphere	Mycelium net weight (g)	Toxin concentration (µg/ml) B1	G1
Room temperature	Air	18.8	48	9
	MA*	16.6	10	3
15.6	Air	18.7	22	18
	MA	0.6	0.06	0.05
12.4	Air	0.1	None	None
	MA	None	None	None
1.1	Air	None	None	None
	MA	None	None	None

*MA = 10% CO_2, 1.8% O_2 and 88.2% N_2

tial in air but minimal in MA. At 12.4°C and below, toxin production was inhibited even in air (Table 1). Studies of the effects of modified atmospheres on *A. flavus* in groundnuts utilized sound mature kernels that had been surface disinfected with 1% sodium hypochlorite (Diener & Davis 1972). *A. flavus* did not grow in concentrations of 40, 60 or 70% CO_2 given in combination with 5% O_2, while with 20% O_2 growth was inhibited when CO_2 exceeded 60%. In another study, Landers *et al.* (1967) found that *A. flavus* occurred on groundnut kernels stored at 1% O_2/99% N_2 and 1% O_2/20% CO_2 but was completely inhibited by 1% O_2/80% CO_2. It is quite evident from these data that, even though the fungus can tolerate quite large concentrations of CO_2, growth inhibition is caused by presence of CO_2 rather than absence of O_2.

By contrast, no inhibition of A. flavus growth by high CO_2 levels was found by Jackson and Press (1967). They found that a high concentration of CO_2 or N_2 (both above 70%) had little significant effect on fluctuations in the numbers of fungi estimated on unshelled and shelled groundnuts and suggested that changes in population of fungi could be related to storage temperatures rather than to storage gases. More studies are needed on this subject to elucidate the effect of all the environmental factors.

Amounts of aflatoxin produced in groundnuts usually decrease with increasing concentration of CO_2 (Table 2; Diener & Davis 1972). Similarly, Clevström et al. (1983) found that CO_2 enrichment hindered aflatoxin formation on defined medium even in the presence of vitamin B which otherwise promotes substantial aflatoxin production in cultures with a limited supply of O_2. Relationships between temperature, relative humidity r.h.

TABLE 2

Effect of various combinations of atmospheric gases on aflatoxin production in groundnut kernels stored at 30°C and 99% r.h. (Diener & Davis 1972)

Concentrations of gases (%)			Total aflatoxins
CO_2	O_2	N_2	(% of control*)
0	15	85	100
	10	90	61.8
	5	95	30.1
	1	99	1.1
20	20	60	24.7
40	20	40	10.7
60	20	20	6.3
80	20	0	0.03

*Control = air

and aflatoxin production under modified atmospheres have been described by Diener and Davis (1972) and Sanders et al. (1968). Their data indicate that in any given combination of gases, aflatoxin formation decreased with a reduction in r.h. and/or temperature (Table 3). Thus the effects of all these environmental factors should be considered when establishing the ideal commercial storage conditions. As high CO_2 or N_2 concentrations, given with very low O_2, both gave good results in controlling insect growth in silos of stored grain, the effect of high N_2 concentration on aflatoxin production in wheat seeds was studied by Fabbri et al. (1980). Production of aflatoxins B_1 and B2 was markedly inhibited in the N_2-enriched atmospheres, with only trace amounts being produced by A. flavus-inoculated grains after 21 days incubation (Table 4). The depressant effect of N_2 on aflatoxin production in groundnuts was noted also by Jackson and Press (1967).

TABLE 3

Effect of relative humidity (r.h), temperature (Temp.) and controlled atmospheres on aflatoxin production in groundnut kernels (Diener & Davis 1972).

Temp. (°C)	r.h. (%)	Concentrations of gases (%)			Total aflatoxins (µg/g)
		O_2	CO_2	N_2	
25	99	Air			206.33
		20	40	40	3.82
		20	60	20	0.24
	86	Air			72.08
		20	40	40	0
		20	60	20	0
17	99	Air			57.05
		20	20	60	0.17
	86	Air			0.21
		20	20	60	0.01

TABLE 4

Aflatoxin content in wheat seeds stored in air and N_2 controlled atmospheres (Fabbri et al. 1980)

Incubation time (days)	Aflatoxin content (µg/kg)			
	B_1		B_2	
	Air	N_2	Air	N_2
7	0.37	-	0.35	-
14	7.04	-	0.75	-
21	118.04	0.45	5.00	0.28

The possibility of using modified atmospheres to inhibit fungal deterioration in freshly harvested high-moisture corn (29.4% moisture content, m.c.) and corn remoistened to 19.6% m.c. was tested by Wilson et al. (1975) using three modified atmospheres: 99.7% N_2/0.3% CO_2; 61.7% CO_2/8.7% O_2; and 13.5% CO_2/0.5% O_2. The modified atmosphere treatments delayed deterioration by A. flavus and Fusarium moniliforme, but did not stop their growth completely (Fig. 1). In another trial, corn at 18.8% m.c.was purged with 14.3% CO_2, 0.5% O_2 and 85.2% N_2, and sealed for 36 and 109 days at 25°C. Aflatoxin production was almost inhibited under these conditions, although A. flavus survived (Wilson et al. 1977).

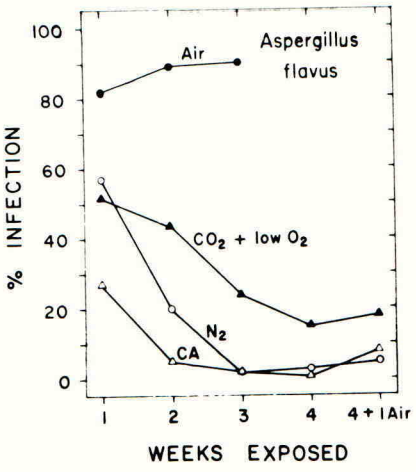

Fig. 1. Percent infection of kernels by Aspergillus flavus in freshly harvested, inoculated, high-moisture corn stored in modified atmospheres. Air = 0.03% CO_2, 21% O_2, and 78% N_2; CO_2 + low O_2 = 61.7% CO_2, 8.7% O_2, and 29.6% N_2; N_2 = 99.7% N_2 and 0.3% O_2; CA = 13.5% CO_2, 0.5% O_2 and 84.8% N_2. Values are averages of two replications, 100 kernels per replication (Wilson et al. 1975).

INHIBITORY EFFECT OF MODIFIED ATMOSPHERES ON MYCOTOXINS OTHER THAN AFLATOXIN

The effect of modified atmospheres on ochratoxin production was studied using Aspergillus ochraceus grown on a synthetic medium. In atmospheres enriched with 10% or 20% CO_2, ochratoxin production was decreased when O_2 concentrations were below 20% and enhanced when they were 40% or 60% (Fig. 2) (Paster et al. 1983).

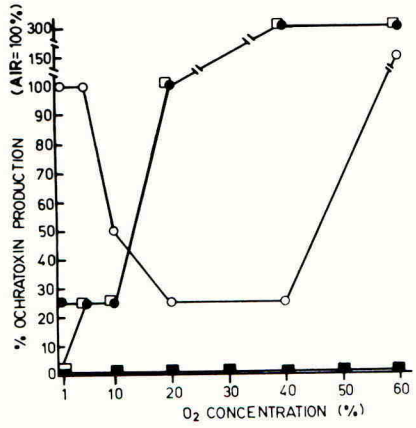

Fig. 2. Ochratoxin A production by Aspergillus ochraceus grown in modified atmospheres. (The production in air atmospheric conditions was 2.0 µg/g). CO_2 concentrations were 0% (o), 10% (●), 20% (□) and 30% (■) (Paster et al. 1983).

Ochratoxin production was completely inhibited by 30% or more CO_2, regardless of the O_2 level. Colony growth was partially inhibited at 60% CO_2 while no growth occurred with 80% or more CO_2. When colonies inhibited by 60% or more CO_2 were subsequently exposed to air, radial growth, dry weight of mycelium and the amount of ochratoxin produced were the same as in the control colonies (Table 5) (Paster et al. 1983).

TABLE 5

Effect of modified atmospheres on ochratoxin production, growth, and sclerotium formation by Aspergillus ochraceus[a] (Paster et al. 1983)

CO_2,O_2,N_2 (%)	Expt I			Expt II			
	Dry wt (mg)	Colony diam (mm)	Sclerotia formation	Dry wt (mg)	Colony diam (mm)	Ochra-toxin (µg/g)	No. of sclerotia
60,20,20	120	35	-	200	55	5.5	1.720
80,20,0	NG			190	55	1.5	1.468
100,0,0	NG			190	55	1.5	1.814
Control[b]	200	55	+	210	55	1.5	1.878
Control[c]				210	55	1.5	1.751

[a]Expt I, After 14 days of growth under modified atmospheres; Expt II, After disconnection from the modified atmospheres and 14 days of exposure to normal air. Ochratoxin determinations, means of two replicates; no. of sclerotia, means of three replicates; other readings, means of six replicates. NG, no growth; -, no sclerotia; +, sclerotia formed.

[b]Control (atmospheric conditions) from the start of the experiments.

[c]Control (atmospheric conditions) started only after CO_2-treated mycelia were disconnected from the controlled atmosphere.

Penicillium patulum was used to study the effect of modified atmospheres on patulin production. The amount of the toxin produced by colonies grown for 7 days under 1% or 5% O_2, but no CO_2, was less than that produced by the control. Less mycelium was also produced (Table 6) (Paster & Lisker 1985). When 10% O_2 was given without CO_2, patulin production and mycelial dry weight were both similar to the controls. Further increases to 60% or 70% O_2 given without CO_2 decreased patulin production, but not mycelial dry weight. Toxin production was also inhibited when CO_2 concentration was raised above 10%, while maintaining the O_2 level at 20% (Table 6).

When spores were kept at 100% CO_2 or N_2 for 7 days, no colonies developed. However, when test chambers were removed from CO_2 or N_2 atmospheres and subsequently exposed to air, colonies appeared after 3 days and the amount of patulin detected 7 days later was the same as in control flasks (inoculated when the test cultures were removed from the CO_2 or N_2 atmospheres). No decrease in mycelial dry weight was recorded.

Penicillic acid (PA) production by Penicillium martensii was studied in mould-inoculated corn over a temperature range of 5 to 20°C in air and in atmospheres containing 20%, 40% or 60% CO_2, with 20% O_2. Production of PA decreased with increasing CO_2 concentrations. Although production was greatest in air at 5°C toxin production was completely blocked by 20% CO_2 at 5°C and by 40% CO_2 at 10°C over a four-week period (Fig. 3) (Lillehoj et al. 1972).

TABLE 6

Effects of selected combinations of atmospheric gases on patulin production and growth of Penicillium patulum in Czapek agar maintained at 26°C (Paster & Lisker 1985)

Controlled atmospheres CO_2/O_2 (%)	Number of colonies (as % of control)	Patulin (mg/40 ml)	Mycelium dry weight (g/40 ml)
Control	100	45.0	0.26
0/1	74.5	1.0	0.10
0/5	81	14.0	0.15
0/10	80	45.0	0.20
0/60	73	20.0	0.24
0/70	80	1.3	0.24
1/1	83	1.0	0.06
1/60	95	45.0	0.24
10/20	100	42.0	0.30
20/20	76	10.0	0.28
60/20	72	2.5	0.12

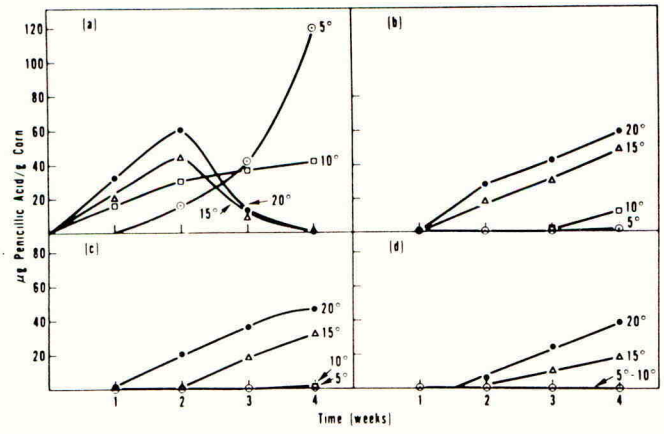

Fig. 3. Effect of temperature (°C) and gaseous environment on production of penicillic acid (PA) by Penicillium martensii on corn. The gases employed were: (a) air; (b) 20% CO_2, 20% O_2, 60% N_2; (c) 40% CO_2, 20% O_2, 40% N_2; (d) 60% CO_2, 20% O_2, 20% N_2. Culture vessels were flushed with the appropriate gas at the rate of 100 cc/min during the trial. PA was extracted from mouldy corn with chloroform-methanol (90:10, v/v) and subsequently analysed by quantitative thin-layer chromatographic methods (Lillehoj et al. 1972).

Working with Fusarium tricinctum that produced T-2, it was found that toxin production was decreased by 80% in atmospheres composed of 50% CO_2 and 20% O_2 (Table 7) (Paster et al. 1986). However, fungal growth was not decreased significantly until the CO_2 concentration was increased to 60%.

TABLE 7

T-2 production and growth of Fusarium tricinctum in controlled atmospheres (Paster et al. 1986)

Controlled atmospheres CO_2/O_2 (%)	Mycelium dry weight (g/15 ml)	T-2 (µg/45 ml)
Control (air)	0.135	21.17±2.33[a]
5/2.5	0.110	20.06±2.75
5/5	0.114	14.98±1.98
5/60	0.113	28.90±3.25
10/20	0.125	19.46±2.87
10/60	0.119	19.87±3.15
20/20	0.120	20.44±2.88
40/20	0.125	19.68±2.97
50/20	0.109	4.01±0.81
60/20	0.07	3.96±0.90
80/20	0.03	1.10±0.04

[a]Means and standard deviation.

CONCLUSIONS

It seems likely that mycotoxin formation could be controlled by enriching atmospheres with CO_2 or by decreasing O_2. However, modified atmospheres, different substrates, a.h. and temperature may interact differently for each mycotoxin. Furthermore, the inhibitory concentrations are not lethal for the mycotoxigenic fungi and after removing the colonies from these atmospheres - mycotoxins can be produced. These findings should be considered when applying modified atmospheres in storage. When studying the effects of modified atmospheres, the following seem to be of major importance: what other metabolites, if any, are produced by the toxigenic moulds grown under modified atmospheres storage; how does modified atmosphere affect toxin production by fungi grown in a competitive environment; and how can modified atmospheres be integrated with chemical control, thus permitting a decrease in the quantity of chemicals used.

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THE INFLUENCE OF IRRADIATION OR AUTOCLAVING OF MAIZE SEEDS ON GROWTH AND AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS

J.E. SMITH

Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, Scotland.

R.G. CUERO

USDA-ARS-Southern Regional Research Center,New Orleans, USA.

J. LACEY

Rothamsted Experimental Station, Harpenden, Herts.

ABSTRACT

Maize seeds were sterilised by autoclaving or by gamma irradiation (cobalt 60) at 1200 krad. When these treated seeds were inoculated with Aspergillus flavus and incubated at 25°C and 0.95 a_w for 14 days, aflatoxin production was considerably less in irradiated seeds than in autoclaved seeds. Combinations of irradiation/autoclaving and autoclaving/irradiation each resulted in little aflatoxin production. In all treatments, visible growth of A. flavus was similar at the end of the experiment.

INTRODUCTION

Long term storage of grain can lead to qualitative and quantitative changes in the grain due to the activities of microorganisms and insects. Traditionally, control of these organisms is achieved by physical and chemical methods. For successful control of microbial growth it is essential to store at low water activities (a_w) < 0.7 a_w and to maintain this throughout storage. However, control of microorganisms colonising stored moist cereal grains, particularly fungi, can be achieved by using chemicals, such as propionic acid in various formulations. Insects may also be controlled chemically using fumigants or other insecticides.

Gamma irradiation (Co^{60}) has been proposed as an acceptable method for food preservation (Anon 1986) killing both microorganisms and insects with apparently no adverse effects on the substrate. The susceptibility of most microorganisms to irradiation has long been recognised (Bridges 1956, Yen et al. 1956, Kiss & Farkas 1977, IAEA-FAO 1970, 1978). Gamma irradiation of grain can decrease fungal growth without either greatly affecting respiration and chemical composition of the grain or producing unacceptable odours (Metta & Connor 1959, Kiss & Farkas 1977). Species of Fusarium, Rhizopus, Penicillium and Aspergillus are more resistant than other moulds to gamma irradiation (Webb et al. 1959, Ito et al. 1969, Mohyuddin & Skoropad 1969) but dry conditions may increase radiosensitivity of Aspergillus and Penicillium species (Ito et al. 1973).

The development of fungi on cereals before and after harvesting is well documented (see Smith & Moss 1985, Lacey 1986). In stored grain, fungi common on the grain before harvest are largely replaced by those typical of storage, especially Aspergillus and Penicillium spp. Some of these are well known as producers of toxic metabolites known as mycotoxins. Residual traces of insect toxins can also create problems when grain is later consumed by mammals (man or animals). The effects of irradiation on fungal growth and mycotoxin production have been studied mostly on synthetic media or natural substrates after autoclaving with most emphasis on Aspergillus flavus and aflatoxin production. Among the changes observed following irradiation were increased aflatoxin production (Jemmali & Guilbot 1969, Applegate & Chipley 1974a,b, Priyadshini & Tulpule 1976, Niles 1978); no effect (Miyaki et al. 1967, Kellebrew et al. 1968, Paster et al. 1985); and decreased aflatoxin formation (Chang & Markakis 1982). Only Harwig & Chen (1974) found ochratoxin A and citrinin production by Penicillium viridicatum on viable, sterile barley and wheat seeds to be less than on non-sterile seeds.

We have recently shown that gamma irradiated (1200 krad) maize seeds are free of viable microorganisms but are able to germinate (Cuero et al. 1986). The study described here compared how seed sterilisation by gamma irradiation or autoclaving affected growth and aflatoxin production by Aspergillus flavus.

MATERIALS AND METHODS

Source of organism and cultivation methods

Aspergillus flavus CMI 102566 was obtained from the Commonwealth Mycological Institute, Kew, and was grown at 25°C for 7-10 days on malt extract agar. Spores were harvested in sterile distilled water, filtered through sterile cottonwool and washed once in distilled water by centrifugation. Spore suspensions were freshly prepared or stored at 4°C for up to 2 days.

Source and treatment of maize

North American hybrid maize seed (Pioneer, 3369a), highly susceptible to Aspergillus flavus, was obtained from the Southern Regional Research Center, USDA, New Orleans, USA.

Maize kernels were cracked in an electronic pilot plant machine (Universal Dryer, Richard Simon & Sons, Nottingham, UK) with variable speed drive, to simulate mechanical damage or insect attack and to further aid invasion by A. flavus. The initial a_w of cracked maize was determined at 25°C using an electronic dew point meter (Protimeter DP 680) and then adjusted to 0.95 a_w by adding sterile water (Pixton & Warburton 1971). Flasks containing cracked maize to which water had been added were sealed with rubber stoppers and stored at 5°C for 1-2 weeks for the a_w to reach equilibrium throughout.

The maize was then sterilised by gamma irradiation at 25°C in a Co^{60} source at the Scottish Reactor Centre, National Engineering Laboratory, East Kilbride, Glasgow (Cuero et al. 1986). A dose of 1200 krad, measured with a perspex dosemeter (IAEA 1977) was used. Samples of maize from the same batch were sterilised by autoclaving at

121°C for 30 min. For irradiation under anaerobic conditions, samples were irradiated within a stainless steel anaerobic jar (Don Whitlet MK II) in which anaerobiosis was created over 24 h. A lead indicator was used to determine anaerobiosis.

Fungal growth and toxin production in maize

After irradiation or autoclaving or combinations of both treatments, 50g of treated maize were placed in a 500ml beaker and inoculated with 1 ml of a suspension containing 10^6 A. flavus spores/ml. After careful aseptic mixing the inoculated maize seeds were transferred to microporous film bags (Cuero et al. 1985), which were sealed and then incubated in a Fison Environmental Cabinet adjusted to 25°C and 95% relative humidity. The bags were carefully shaken daily to prevent mycelial compaction. Fungal growth was visually assessed each day for 12 days using a semiquantitative scale, viz. 1, very little growth; 2, 25% of the grain covered; 3, 50% of the grain covered; 4, 75% of the grain covered; and 5, all of the grain covered.

After 12 days incubation, total aflatoxins were extracted from each bag using standard methods (Gorst-Allman & Steyn 1979, AOAC 1980). Aflatoxins B_1 + B_2, G_1 + G_2 and total aflatoxins were determined quantitatively by HPLC using a Pye Unicam Model LC-XPD. All experiments were repeated at least three times each with three replicates.

RESULTS

Growth of Aspergillus flavus on sterilised maize

A. flavus grew most rapidly on autoclaved maize. Visible growth was first apparent after 48 h whereas visible growth occurred on the irradiated maize only after 60 h (Table 1). Growth continued more rapidly on autoclaved maize than on the irradiated and it was totally colonised by the 8th day. By contrast growth was slower on irradiated maize and full colonisation was barely achieved in 12 days. Combinations of autoclaving and irradiation allowed similar colonisation to that found in maize seed that was only irradiated.

Aflatoxin production by Aspergillus flavus on sterilised maize

Most aflatoxin (10,603 ng/g maize) was produced in autoclaved maize, and much less (1,832 ng/g) on irradiated maize (Table 2). However, when maize was autoclaved then irradiated or irradiated then autoclaved, somewhat less aflatoxin was produced than that in maize seed that was only irradiated.

Irradiation of maize under anaerobic conditions, before treatment in exactly the same way as aerobically irradiated or autoclaved grain, caused almost total inhibition of aflatoxin production (415 ng/g) (Table 2).

TABLE 1

Growth of Aspergillus flavus on maize exposed to various sterilisation treatments

Treatment	Extent of fungal colonisation Time (days)			
	2	5	8	12
Autoclaved	2*	3	5	5
Irradiated	1-2	2	4	4-5
Autoclaved/Irradiated	1-2	2	3-4	4-5
Irradiated/Autoclaved	1-2	2-3	3-4	4-5

* Semiquantitative scale 1-5.

Temperature 25°; 95% r.h. Irradiation under aerobic conditions.

TABLE 2

Aflatoxin production by Aspergillus flavus on maize exposed to various sterilisation treatments.

Treatment	Aflatoxin production (ng/g cereal)			
	$B_1 + B_2$	$G_1 + G_2$	Total	S.E.
Autoclaved	3235	7368	10603	250.8
Irradiated	914	918	918	45.6
Autoclaved/Irradiated	184	1064	1245	70.0
Irradiated/Autoclaved	531	995	1532	30.0
Irradiated anaerobically	239	176	415	

Experimental conditions 25°C, 98% r.h. and 12 days incubation. Irradiation was performed in aerobic conditions except where stated.
S.E. - standard error

DISCUSSION

Previous studies of the effects of environmental conditions, such as temperature, substrate and water activity, on mycotoxin production have mostly used either liquid cultures or autoclaved natural substrates (Diener & Davis 1970, Northolt et al. 1979a,b, Magan & Lacey 1984a,b). In nature, mycotoxin production usually occurs when fungi are growing in a solid matrix, e.g. a cereal or oil seed and often in competition with other species. The seed is living and possesses all the physicochemical features of living systems, in particular, compartmentalisation of component systems. When a seed is autoclaved, it is killed and drastically changed physicochemically, altering considerably the nature and availability of nutrients.

We have found that A. flavus can rapidly colonise autoclaved maize seeds and form large amounts of aflatoxin (Table 2). We have also shown (Cuero et al. 1986) that all microorganisms on maize seeds can be killed by irradiation while leaving the seed still able to germinate, although subsequent colonisation by toxigenic fungi can occur and production of toxins (Cuero et al. 1987). However, the present studies have shown that irradiated maize is colonised by A. flavus more slowly than autoclaved maize and that aflatoxin formation is much less on the irradiated maize. From these observations it perhaps could be deduced, erroneously, that the differences between the two systems result purely from the irradiated maize being physically intact and living while the autoclaved seeds are physically changed and dead. The tissues of the autoclaved maize are softer and more easily colonised by A. flavus while the nutrients are more easily available perhaps allowing more aflatoxin synthesis.

If softening of the tissues and increased nutrient availability were the only reason for the changed pattern of toxin production, it might be thought that grain receiving both autoclaving and irradiation would have allowed much aflatoxin production. However, the reverse was true, so that irradiation appears to have created a chemical impediment to aflatoxin synthesis within the maize seed that is effective whether the seed is living or dead.

Rapid aflatoxin production on autoclaved substrates has been reported on several occasions (Hesseltine et al. 1966, Applegate & Chipley 1974a,b, Bennett & Christensen 1983). Also, production of ochratoxin A in autoclaved barley, rye and wheat kernels is 2-3 times higher than on viable, intact kernels (Chelkowski et al. 1981). The autoclaved kernels were more quickly attacked by the fungus than viable seeds and it was suggested that Zn^{++} ions were released during autoclaving which stimulated fungal growth.

The results reported here generally agree with previous findings and conclusions. However, combining the sterilising treatments prior to inoculation with A. flavus, regardless of whether irradiation was before or after autoclaving, strongly suggests that irradiation produces within maize seed one or more compounds which are inhibitory to aflatoxin biosynthesis or in some way alters essential precursors to aflatoxin formation. The change is not reversed by heat and is most marked when grain is irradiated under anaerobic conditions.

Accumulation of radiolytic products such as $H^+OH^-e^-$ has been reported (IAEA 1970). Recently, a unique chemical, o-tyrosine , has been isolated following irradiation of food (Simic, National Bureau of Standards, Maryland, Times Newspaper Report). Further studies are now required to isolate and identify such possible compounds in irradiated maize.

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DETECTION OF FUNGI IN STORED GRAIN

A. KASPERSSON

The National Veterinary Institute, Box 7073, S-750 07 Uppsala, Sweden

ABSTRACT

Fungal growth and activity in stored grain may be monitored by measuring respiration rates and adenosine triphosphate (ATP), chitin and ergosterol concentrations, and by enumeration of colony forming units by dilution plating and by direct plating of kernels. The ATP assay is fast, convenient and relatively inexpensive and correlates well with other methods. It is thus a valuable alternative to more complicated and time-consuming methods for measuring ongoing fungal activity. Ergosterol assay can detect whether there has been any fungal growth in the grain. It has a high specificity for fungi and correlates well with other methods of measuring fungal growth. All the above methods require representative sampling of the grain for accurate determination. Sampling headspace gases to assay fungal volatiles can be used to monitor fungal activity without the need of sampling the grain.

INTRODUCTION

Fungal growth is a major factor restricting the shelf-life of intermediate moisture foods and feeds (0.6-0.9 a_w), such as cereal grains. In the temperate climatic zone, deterioration of cereal grains and cereal products by mites, insects and rodents is usually of minor importance compared with that caused by fungi (Sinha 1979). Fungal growth in grain causes a decrease in nutritional value and rapidly leads to a loss of palatability. It may also result in health hazards, associated both with mycotoxin production and with abundant sporulation, especially by storage fungi and, in severe deterioration, by actinomycetes.

In order to protect grain from fungal invasion during storage, good methods of assessing fungal activity are essential. Only by monitoring fungal activity is it possible to exercise full control over this activity in grain.

The choice of an assessment method for a specific application must be guided by the answers to such questions as: Is there a need for measuring ongoing activity or is it more essential to demonstrate whether or not there has been any fungal activity? Is representative sampling possible or must activity be monitored without sampling the grain?

The usfulness of various methods for detecting fungal activity in stored grain will be surveyed in this paper, with special emphasis on the use of adenosine triphosphate and ergosterol measurements.

DETECTION AND QUANTIFICATION OF FUNGAL ACTIVITY

Enumeration of fungi

Determination of colony forming units (CFU) by dilution plating and direct plating of whole kernels is probably the most widely used method in grain mycology. However, when counting CFU, it is not possible to ascertain whether the colonies originated from mycelial fragments, aggregated or single conidia or from other spores (Rose & Bradley 1980, Jarvis et al.

1983). Since colonies usually arise only from spores (Christensen & Meronuck 1976), heavily sporulating species will be overestimated and less abundantly sporulating species underestimated. Great caution is thus needed in interpreting results from CFU counts.

Direct plating of whole grains, after surface-sterilization, allows estimation of the number of kernels invaded by important fungal colonizers. This method is also useful for assessment of the fungal flora in stored grain. Sodium hypochlorite is a powerful surface-sterilizing agent and is, perhaps, the most widely used. A quick rinse of the kernels in ethanol prior to hypochlorite treatment ensures the best possible effect. Otherwise, the surface tension of the sodium hypochlorite solution may leave part of the surface of individual kernels unsterilized (Booth 1971, Sauer & Burroughs 1986). Reviews of methods for enumerating fungi in grain may be found in Lacey et al. (1980) and Jarvis et al. (1983).

Respiration

The activity of fungi growing in grain may be studied by measuring the evolution of carbon dioxide and/or the consumption of oxygen. From such measurements, respiration rates as well as estimates of dry matter losses may be derived. These measurements generally require sampling and incubation of samples in laboratory systems. Production of CO_2 may be measured by absorption in NaOH and KOH solutions, or in soda lime (Hlödversson & Kaspersson 1986). Analyses of both CO_2 and O_2 may also be performed by gas chromatography, while continuous measurement of CO_2 production in moulding grain may be made with an infra-red CO_2 monitor. The last method enables particularly accurate estimates of the respiration rate (Kaspersson 1986b).

Fungal volatiles

Where large quantities of grain are stored and sampling of the grain is difficult, indirect measurements of fungal activities are attractive. It may be possible to use the intragranular CO_2 as an indicator (White et al. 1982). It may also be possible to use the volatile substances (odours) produced by growing fungi to detect their activity (Abramson et al. 1983, Hyde et al. 1983, Kaminski et al. 1985).

Adenosine triphosphate

Adenosine triphosphate (ATP) has a central role in energy transfer between catabolism and biosynthesis in all living cells. This compound is found only in living cells and may therefore, be used as an indicator of life (Huennekens & Whiteley 1960). Thus, it has been used in many applications for detection of viable microorganisms and as a measure of microbial biomass in environmental and ecological studies (Karl 1980).

Assessment of ATP

ATP is measured by a bioluminescence assay, using the luciferin-luciferase enzyme system of the firefly (Photinus pyralis). In a very simplified form, the reaction with ATP may be summarized as follows:

$$\text{Luciferin (red.)} + \text{ATP} + O_2 \xrightarrow{\text{luciferase}} \text{oxyluciferin} + \text{AMP} + CO_2 + PP_i + \text{light.}$$

The critical step in any application of the ATP-bioluminescence method is the extraction of ATP from what is often a highly diverse microflora contaminating different kinds of materials. A good extraction method must ensure a quantitative release of ATP from the microorganisms and, at the

same time, totally inactivate all enzyme activity. The extracted ATP must also be stable in the solution.

To extract ATP from grain and feeds in which fungi are important contaminators, a 2.5 % trichloroacetic acid (TCA) solution containing 4 mM EDTA was chosen after testing a number of different types of chemical substances (Kaspersson 1986b). One hundred grams of grain was treated with 100 ml of the TCA solution in 300-ml flasks on a wrist shaker for 5 minutes. The solutions were then filtered and analysed directly or collected in plastic tubes (10 ml) and frozen (-25°C) immediately. The ATP content was analysed on a Lumac Biocounter (M 2010), using Lumit Bioluminescence reagent (Lumac B.V., The Netherlands). The filtrates were diluted at least 100-fold with, and measured in, a 20 mM Tris-H_2SO_4 buffer (pH 7.75) containing 2 mM EDTA. The 100-fold dilution ensured that the TCA did not interfere with the test enzymes. Adenosine 5'-triphosphate disodium salt in 1 mM glycine buffer (pH 10.0), kept on an ice-bath, was used as internal standard.

It might be argued that the TCA also extracts ATP from the stored seeds. However, in order to maintain the germinating capacity over a long period, the metabolic activity must be very low. Moreover, under the conditions used for extraction, TCA penetrates only the outmost parts of a kernel in which the fungal mycelium is situated.

Correlation of ATP with other measurements

Kaspersson et al. (1986) studied the microbial dynamics in airtight-stored barley of two moisture content levels. Samples were taken periodically throughout the storage seasons 1980-82 and 1982-83 from 18 airtight pilot silos. CFU of microorganisms and the ATP content were determined in a total of 267 samples. A multiple regression analysis was performed with ng ATP as the dependent variable, and CFU of bacteria, yeasts and moulds as the independent variables. This resulted in the following regression equation, with a coefficient of multiple determination, $R^2 = 0.80$:

$$\log (ATP) = -16.3 + 2.6 \log (\text{yeasts}) + 2.5 \log (\text{moulds}), \quad n = 267$$

CFU of bacteria were omitted, since a stepwise regression procedure showed that this factor did not improve the fitness of the equation and had a nonsignificant F-value.

In another study (Kaspersson 1986b), the respiration rate and the ATP content were determined in two experiments where two storage fungi colonized barley grain. Simple linear regression analyses, performed on the results obtained from these pure culture experiments, showed that good correlations exist between ATP and respiration rate for *Penicillium verrucosum* var. *cyclopium* (r=0.97 and 0.93 for experiment I and II, respectively). A lower coefficient of correlation (r=0.76) was found for the same regression in the case of *Aspergillus amstelodami*. Good correlation between ATP content and respiration (r=0.91) has also been shown for growth of a *Cladosporium* sp. in liquid culture (Eiland 1985). For the storage fungi mentioned above, good correlations were also found to exist between log ATP and log CFU, r=0.96 for *A. amstelodami* and r=0.88 and 0.92 for *P. cyclopium* in experiment I and II, respectively.

Chitin

Chitin is a linear polymer of α-1,4-linked N-acetylglucosamine. It is found in cell walls of a majority of fungi but not in green plants.

Although chitin is present in all insects and glucosamine is a component of bacterial cell walls, chitin can be used for fungal biomass estimations in applications where insects and large numbers of bacteria are not to be found. The assessment of chitin requires hydrolysis to glucosamine and colorimetric measurements, generally with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) as reagent. Chitin assay has been used to measure fungal growth in cereal grains and cereal products (Donald and Mirocha 1977, Nandi 1978, Seitz et al. 1979, Ghosh and Häggblom 1985).

Ergosterol

Ergosterol (5,7,22-ergostatrien-3-ol) is the predominant sterol of most fungi belonging to the classes Ascomycetes and Deuteromycetes (Weete 1980). It is within these two classes that most of the important deteriorators of dry and medium-moisture foods and feeds are found. Ergosterol is not found to any signficant extent in green plants. (The small amounts found can probably be attributed to contamination with fungal tissue.) This makes the quantification of ergosterol extremely suitable as a method for assessing fungal biomass in stored grain.

Assessment of ergosterol

The use of ergosterol as a sensitive indicator of fungal invasion in grain was first described by Seitz et al. (1977), using high-pressure liquid chromatography (HPLC) for quantification. Ultraviolet spectroscopy of sterols obtained from thin-layer chromatography was used to confirm the presence of ergosterol in the grain.

In our study (Kaspersson 1986b), apart from ATP assay, the ergosterol content was monitored during mycelial growth of two storage fungi. The method used for extraction, purification and analysis of ergosterol was essentially that of Seitz et al. (1979). Dried, ground barley (10 g) was extracted in 40 ml methanol and 10 ml petroleum ether (b.p. 60-70 °C). After saponification of 30 ml of the extract, the triglycerides were removed with water. The hexane residue containing the sterols was evaporated to dryness under N_2. Chromatography equipment (HPLC) consisted of a Millipore Waters Wisp autoinjector, a Waters 501 solvent delivery system, a 5x100 mm NOVA-PAK C_{18} column and a Waters 441 fixed-wave-length detector (280 nm). The mobile phase was methanol-water (95:5, v/v) at 1.5 ml $minute^{-1}$. Peak areas were measured by a computer integrator (Waters 740). Ergosterol (U.S. Biochemical Corporation) dissolved in methanol was used as external standard.

Another method employing a gas chromatographic analysis of all sterols present in grains was described by Kaminski et al. (1985). This procedure permitted the increase in ergosterol content to be monitored when fungi developed on wheat kernels.

Correlation of ergosterol with other methods

Following the report of Seitz et al. (1977) that the ergosterol content showed a close relation to the invasion of field fungi in wheat kernels, the ergosterol assay has been compared with other methods for assessing fungal growth. Cahagnier et al. (1983) stored rice and corn under different conditions of relative humidity and temperature. They found good correlations (r=0.93-0.99) between ergosterol content and the CFU of moulds isolated when grain was stored at a relative humidity exceeding 80 %.

Seitz et al. (1982) measured fungal growth and dry matter loss during bin storage of high moisture corn by using CO_2 evolution, ergosterol content and number of invaded kernels to monitor fungal activity. They found fairly good overall relationships between calculated dry matter loss and ergosterol content.

In work on the colonization of barley kernels by pure cultures of fungi (Kaspersson 1986b), a very close relationship was found to exist between ergosterol content and dry matter loss. Simple, linear regressions gave coefficients of correlation, $r=0.96$, for colonization by A. amstelodami, and $r=0.98$, for colonization by P. cyclopium.

GENERAL DISCUSSION

The ATP-bioluminescence method with a simple extraction technique is a fast, convenient and relatively inexpensive method. It has been shown to correlate well with other methods used to monitor microbial activity and may thus be an alternative to more complicated and time-consuming methods for measuring ongoing activity. The good correlations found between CFU of fungi and the ATP content are, perhaps, somewhat puzzling. As pointed out previously, CFU of storage fungi are, in most cases, entirely of spore origin. Accordingly, the ATP content, reflecting the amount of active cytoplasm, would be expected to show a poor correlation with CFU of fungi. The very good correlations above must be considered typical only of fungi sporulating as abundantly as the storage fungi tested. Good correlations between ATP content and CFU are, however, likely to be found in any application where the predominating microorganisms consist of separate cells. For instance, very good correlations between ATP and bacterial counts have been found in other applications (Gutekunst et al. 1977, Lee and Crispen 1977). However, it is important to remember that the ATP content is dependent on the growth rate of microorganisms present in a sample at the time of extraction. It thus seems more logical to seek relationships with other measures of fungal activity than CFU, e.g., respiration.

The ergosterol assay seems a highly suitable method for measuring both ongoing and past fungal activity on solid substrates, such as grain. It might be argued that chitin assay is as good a method as ergosterol assay. However, ergosterol assay is more fungus-specific than chitin assay. Chitin (N-acetyl-glucosamine) is found also in the cell walls of bacteria and in large concentrations in the cuticles of all insects. Unfortunately, insect parts are common in stored grain. Chitin assay also requires a time-consuming and rather difficult hydrolysis (Seitz et al. 1979).

The long-term stability of ergosterol under different conditions is still not fully known. However, in one experiment (Kaspersson 1986b) the ergosterol content increased steadily through 77 days of storage. Seitz et al. (1982) also found continuous increases in ergosterol content through 65 days of bin storage of high-moisture corn. Cahagnier et al. (1983) reported that various physical treatments of grain, such as high-temperature drying, gamma irradiation or the addition of such chemicals as propionic acid, which greatly reduced the viable microflora, only had a slight effect on the pre-existing ergosterol content. Kaspersson and Hlödversson (1986b) found a general correlation between increase in ergosterol and dry-matter loss in deteriorating hay, despite the fact that the ergosterol analysis was performed on ground hay samples that had been stored for five years at room temperature. Together, these observations support the long-term stability of ergosterol.

Both the ATP-bioluminescence method and ergosterol assay necessitate the sampling of stored grain. A development of a headspace technique capable of monitoring "fingerprints" based on fungal volatiles would be a significant step forward in attempts to control fungal activity in stored grain. The problem of representative sampling could be overcome in this way.

It is important to remember that the quantitative analyses discussed above must be combined with an analysis of the composition of the fungal flora when health hazards are to be evaluated.

Far from all of the methods used to assess fungal activity have been reviewed here. For diagnosis of specific fungi, immunological techniques - such as the use of fluorescent antibodies, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) - and probably analyses of mycotoxins can be used. When, however, the aim is to evaluate fungal activities in a broader sense, the use of respiration, ATP or ergosterol assays may be very valuable.

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A NEW APPROACH TO ROUTINE AFLATOXIN TESTING

D. G. THOMSON

May and Baker Diagnostics Limited, Glasgow, Scotland.

ABSTRACT

Present methods of analysis for aflatoxin in grain and feed stuffs involve lengthy extraction procedures and often expensive capital equipment. In addition a high degree of technical expertise is required to carry out the analysis. The complicated nature of the assay has meant that aflatoxin screening has been confined to central laboratories which are often removed from the intake point. As a result many consumers of grain products are reluctant to analyse the suspect commodity because of the high cost of analysis and the delay involved in obtaining a result. This paper describes a new approach to the problem of aflatoxin testing and illustrates how the use of monoclonal antibodies can allow routine screening in areas where it would not previously have been considered. The use of these highly specific "biological keys" enables a rapid, low cost test to be produced and at the same time decreases the expertise required to carry out the assay. The paper describes two approaches to aflatoxin screening as well as the potential for routine screening of other mycotoxins associated with temperate climates.

INTRODUCTION

The presence of aflatoxin in grain and feedstuffs has been a major problem for exporter and importer alike, often resulting in particular commodities being restricted or even banned from use in animal feed. The result is that the choice available to the compounder has been reduced and often other less attractive alternatives have to be utilised. This will be further enforced by impending EEC legislation on six major commodities which are likely to exhibit toxin contamination (see Table 1). Although material containing mycotoxin will not be completely banned, it will mean that careful control will be required to ensure that commodities meet the required quality criteria for that particular mill.

TABLE 1

Commodities liable for aflatoxin control under EEC legislation.

Commodities	Maximum Permitted
Maize	0.2 mg/kg
Groundnut	" "
Palm Kernel	" "
Babussa	" "
Cotton Seed	" "
Copra	" "

Present methods of analysis prohibit either rapid or regular checking on the incoming material because of the lengthy procedures involved. However, the development of monoclonal antibodies (Kohler and Milstein 1975) has had a dramatic impact on the clinical diagnostic field in improving selectivity of clinical analysis. These biological keys can be applied in the same way to chemical analysis and as a result reduce the complexity of the method and improve the speed. By utilising the power of monoclonal antibody methods, it has been possible to develop two diagnostic kits which have considerable advantages over existing methods of analysis.

PRESENT METHODS OF ANALYSIS

Present methods of analysis fall into two major categories:

1. Thin Layer Chromatography (TLC).
2. High Performance Liquid Chromatography (HPLC).

TLC

In most laboratories this is the standard technique but it involves lengthy extraction procedures using organic solvents (see Table 2). The results can take up to one day to obtain. Usually the technique will enable identification of the toxin but the degree of accuracy is highly dependent on an individual's assessment when the sample is viewed under UV Light. Consequently, analysis has been confined to those laboratories having the expertise to handle the technique. This can often result in delays in reporting the final result.

HPLC

High Performance Liquid Chromotography has become, in recent years, one of the most powerful instrumental techniques in analytical chemistry. The technique is particularly useful where the separation of various organic analytes is required. The application of this technique to mycotoxin analysis is an obvious one since the concentration of each toxin is very low and the sample matrix is extremely complicated. HPLC is now used extensively in laboratories where a profile of the different toxins present is required and there is a spare capacity in terms of the instrumentation available. This technique is used worldwide. HPLC tends to be more accurate than TLC because it is instrumental and fewer extraction steps are required, it does, however, suffer from two major disadvantages, particularly in the less well-equipped laboratory. These are:

1. high capital cost.
2. a high level of instrumental expertise.
3. decreased flexibility because the equipment has to be dedicated to mycotoxin analysis.

These disadvantages preclude use of the technique within most areas which require a rapid, one-off analysis.

NEW TECHNIQUES OF ANALYSIS

Until recently, the use of monoclonal antibodies has been extensively exploited only within the area of clinical diagnostics. Only now have researchers looked outside this area and towards applications in food and agriculture. Because analysis for mycotoxins has been difficult there has been considerable interest in developing antibodies to these compounds (Chu 1984, Candlish et al. 1985, Smith et al. 1986). Having established a suitable antibody to the toxin the next stage is the development of an

analytical technique. May and Baker Diagnostics Ltd., in collaboration with a number of academic institutes,[1] have developed two kits using these antibodies which meet the differing requirements of both the agriculture and food markets. The two kits operate on quite different principals and hence fulfil quite different functions.

TABLE 2

Basic steps in chemical analyses of mycotoxins (after Romer 1976)

Step	Description	Purpose
1. Sampling	Probe of automatic sampler	Representative sample
2. Sample preparation	Grinding, mixing, subsampling	Representative sample
3. Extraction	Shaker or blender	Separate the toxin from compounds insoluble in the extraction solution
4. Clean-up	Liquid-liquid partitioning (separatory funnel) Column chromatography Divalent metal clean-up (Pb^{2+}, Fe^{2+}, Cu^{2+})	Separate the toxin from groups of compounds in the sample extract
5. Final separation	Thin layer chromatography (TLC) Gas liquid chromatography (GLC) Liquid-liquid chromatography (LC) Minicolumn chromatography	Separate the toxin from remaining compounds in the sample that might interfere with the toxin
6. Detection and quantitation	Fluorescence on TLC plate Fluorescence in solution, UV adsorption in solution, GLC-flame detector	Detection and measurement of response
7. Confirmation	TLC separation and detection of derivative of mycotoxin Biological test Mass spectrometry	Identification of chemical compound

ELISA KITS

The technique of ELISA (Enzyme Labelled Immuno Sorbent Assay) is a common technique in clinical chemistry which utilises the selective power of monoclonal antibodies. In this case the antibody is highly specific for aflatoxin B1 and hence binds preferentially to this toxin.

PRINCIPLE OF THE ASSAY

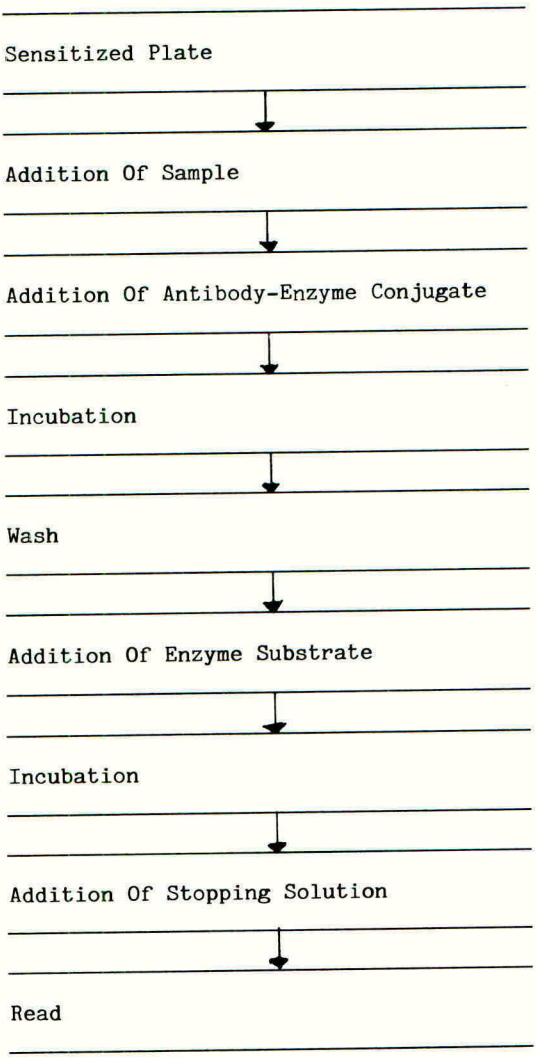
An outline of the test procedure is shown in Table 3. The toxin, in this case aflatoxin B1, has been coated onto the wells of a microtitre plate.

[1] The University of Strathclyde, Boston University and Massachusetts Institute of Technology.

The test sample is added to the respective wells followed by the addition of the antibody-enzyme conjugate. If no toxin is present in the sample, the conjugate will bind specifically to the toxin-sensitised plate. If toxin is present, the conjugate will bind with the free toxin thus competing with the toxin sites on the sensitised plates. During the subsequent washing process, conjugate combined with the sample toxin will be washed from the well, but conjugate combined with immobilised toxin that is bound to the well will be retained. On addition of the colour reagent, a colour will develop, the intensity of which corresponds to the amount of conjugate which is bound to the wells, ie. the colour intensity decreases with increasing toxin concentration. The resultant plate is then read on a microtitre reader.

TABLE 3

Outline Of Test Procedure For ELISA



ADVANTAGES OF THE TECHNIQUE

The particular advantage of this technique is that quality during production of the sensitised plate and enzyme conjugate can be closely controlled during production so that a standard curve can be provided which eliminates the need for toxic standards. This version of the kit is most suited to laboratories already involved in mycotoxin analysis or where there is a need to process samples in batches. For example, about 40 samples can be analysed in duplicate in one hour, enabling a large sample work-load to be handled on one plate. In addition, the plate can be designed in strip format to allow even greater flexibility in the choice of sample work load.

The principle of ELISA can be extended further to allow assay of multiple toxins on one plate, such as Ochratoxin, T-2 and vomitoxin, thus enabling the user to obtain a profile of their occurence in a sample.

This enables a range of agricultural commodities to be analysed rapidly in a central laboratory at low cost per test and, hence, to provide a more efficient service to the interested party.

AFFINITY COLUMN KIT

The principle of this kit is again to utilise the power of monoclonal antibodies to separate the aflatoxin from an extraction mixture. The heart of the system is an affinity column containing antibody (Groopman et al. 1985). The steps involved are shown in Table 4.

Using this method the sample is extracted in 60% methanol for 1 minute and then diluted with water. A sample aliquot is applied to the top of the column containing the antibody using the syringe provided. The sample is then slowly eluted through the column and the eluate is passed to waste. The monoclonal antibody preferentially binds to aflatoxin in the sample and is retained in the column. After washing with distilled water to remove extraneous material, the column is washed with pure methanol which denatures the antibody and releases the bound aflatoxin. The resultant methanolic solution of aflatoxin can be assayed for aflatoxin in two ways:

1. The column is eluted into a plastic tip containing florisil. Fluorescence under UV light is then compared with standard florisil tips calibrated in the range 0, 10, 25 and 50 µg/kg using a UV light box.

2. A more quantitative result may be obtained by collecting the methanolic solution in a test-tube. A fluorimeter calibrated in µg aflatoxin /kg sample is then used to give a direct reading of the aflatoxin content.

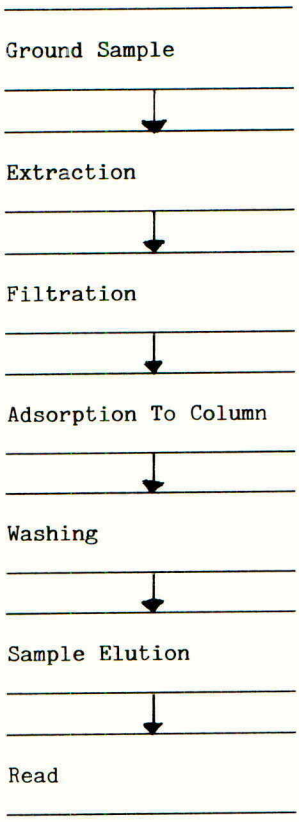
ADVANTAGES OF THE TECHNIQUE

The use of this simple method of analysis allows the possibility of routine aflatoxin screening within the agricultural market. In about 10 - 15 minutes, an operator using basic laboratory apparatus can determine the concentration of an incoming material. At no time during the analysis is there any contact with toxic standards nor the need for extensive fume cupboards. These features allow the feed mill or grain silo manager to accept or reject a suspicious commodity without referring to outside services thus reducing the time delay. Confidence that a commodity is free or safe from aflatoxin may open up the possibilities of utilising those

that were previously regarded as unsafe.

TABLE 4

Outline Of Test
Procedure For
Affinity Column



SUMMARY

The introduction of this aspect of biotechnology to the food and agriculture market can only serve to improve the quality standards of the various cereals and crops. The use of monoclonal bodies as an analytical technique in this area is still in its infancy and it is not unlikely that many more kits will soon become available to enable assay for a variety of contaminants such as pesticides and herbicides.

The advantages of using this novel approach to mycotoxin assay are:

1. Lower cost per test.
2. Reduction in the routine technical expertise required.
3. Faster sample turn-around.
4. Improved quality control.

This can only be to the benefit of both the consumer and supplier.

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