Aflatoxins remain the most important mycotoxin problem in the world, and peanuts are a major source of these toxins.

Control of aflatoxin levels in peanuts on a commercial scale is possible by colour sorting and aflatoxin assays on shelled peanuts, and this is widely practised in developed countries. However, this procedure is expensive.

Prevention is a much more attractive proposition than control, so a great deal of effort has been devoted to developing methods for reducing aflatoxin formation, mostly by traditional approaches using plant breeding. This work has been largely unsuccessful, principally because the species which produce aflatoxins, Aspergillus flavus and A. parasiticus, are not pathogens in peanuts, but are endophytes or commensals. These species grow in peanut plants and developing peanuts without causing the host-pathogen interactions essential in cultivar selection techniques.

A much more promising approach is biocontrol or, more correctly, competitive exclusion – the addition of non-toxigenic strains of A. flavus and/or A. parasiticus to peanut soils to compete with the naturally occurring toxin forming strains for sites on the developing peanuts.

The non-toxigenic strains to be used must be chosen carefully. Ideally, strains should be isolated from the same region where they will be used ultimately. They must be incapable of producing toxins, and be competitive under field conditions.

We studied more than 200 isolates, looking for those which did not make aflatoxins. We tested first for absence of toxin production when isolates were grown on coconut cream agar, then after growth on moistened cracked maize, where sensitive chemical assays, including HPLC, were used.

Isolates which showed no aflatoxin production were then screened in young peanut plants for competitive ability against toxigenic strains of A. flavus. Soil in horticultural pots was inoculated with equal numbers of spores of selected non-toxigenic and toxigenic strains, then peanut seeds were planted, and allowed to grow in a glasshouse for three weeks.

Plants were then cut into short (50 mm) sections, surface disinfected and plated on A. flavus and parasiticus agar, and the relative numbers of infections assessed. This relatively simple technique permitted results to be obtained in about four weeks, and provided a useful indication of the performance of strains under natural conditions.

The most competitive non-toxigenic strains from these small pot tests were then studied in full scale glasshouse experiments, with peanuts grown to maturity in large, automatically watered garbage bins, under controlled environmental conditions. Each experiment took four months.

Peanut stems plated on a differential medium, Aspergillus flavus and parasiticus agar, showing colonies of A. flavus (bright orange reverse colour) growing out of cut ends of surface disinfected stems.

Spreading biocontrol inoculum on young peanut plants in field trials near Katherine, NT.
The most successful non-toxigenic strains competed effectively with the toxigenic strains, on a 1:1 basis, i.e. no competitive advantage or disadvantage. These experiments clearly established that aflatoxin production plays no role in infection of peanut plants or peanuts by A. flavus or A. parasiticus, and that these two toxigenic species compete equally in peanuts, which is not the case with maize, colonised only by A. flavus.

The most competitive naturally occurring isolates were also studied genetically by collaborators at the University of Sydney, to select ones with the least potential to revert to toxigenicity. This work was very successful with A. parasiticus, allowing selection of an isolate incapable of reversion to aflatoxin production, but less successful in the case of A. flavus. This work is continuing.

Because A. flavus is a known human pathogen, the introduction of non-toxigenic spores on the large scale requires care. It is likely that distribution over plants or soil by spraying or dusting will never be permitted. Therefore we chose to spread the spores directly into soil on a carrier, by means of standard farm machinery such as fertiliser spreaders.

After looking at a variety of alternatives, we chose cracked barley as substrate. Spores of the selected non-toxigenic isolates are coated onto the barley particles in molasses, which provides an even inoculation, reduces dust and acts as an additional nutrient source.

The inoculated cracked barley has been broadcast over peanut fields at the rate of 0.5 to 1 tonne per hectare. Some trials have given very good results. Experiments have been successful when the non-toxigenic strains have grown well, i.e. spore numbers have reached $10^4$ to $10^5$ per gram of soil, and the ratio of non-toxigenic to total spores present has exceeded 20:1 (proportion of total exceeds 95%).

Under those conditions, aflatoxin formation can also be reduced by 95% or more (Table 1), to well below the statutory Australian limit for peanuts of 15 µg/kg. In some successful trials, the inoculum contained both A. flavus and A. parasiticus, while in others, either species was effective by itself.

The work we have carried out, in the glasshouse and then in the field, has shown conclusively that biocontrol of aflatoxin in peanuts is a numbers game, involving raising the ratio of non-toxigenic to toxigenic spores to 50:1 or more. Successful commercial application of this technology depends on obtaining consistent growth of the fungus after inoculum has been applied to the fields. Soil moisture and temperature, i.e. the right climatic conditions, appear to hold the key to this final obstacle to success. It is expected that commercial use of this technology will commence soon.

Acknowledgements

Our thanks to the Grains Research and Development Corporation, Peanut Company of Australia and Queensland Dept of Primary Industries for financial support and/or cooperation in this work.

References


Table 1. Some results of semicommercial application of inoculum (1 tonne/hectare) to soils in which peanuts were grown, and resulting reduction in aflatoxin formation, harvest years 2000 and 2001.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Total count of A. flavus and A. parasiticus at harvest (cfu/g soil)</th>
<th>Non-toxigenic strains in soil at harvest (% of total)</th>
<th>Aflatoxin (µg/kg) in peanut samples from control plots after drying</th>
<th>Aflatoxin (µg/kg) in samples from treated plots after drying</th>
<th>Reduction in aflatoxin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>960</td>
<td>99</td>
<td>24</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>H</td>
<td>28,000</td>
<td>97</td>
<td>38</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>J</td>
<td>280</td>
<td>88</td>
<td>6</td>
<td>19</td>
<td>Nil</td>
</tr>
<tr>
<td>M</td>
<td>16,000</td>
<td>97</td>
<td>240</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>R</td>
<td>19,000</td>
<td>96</td>
<td>196</td>
<td>11</td>
<td>94</td>
</tr>
<tr>
<td>P</td>
<td>8,000</td>
<td>88</td>
<td>56</td>
<td>139</td>
<td>Nil</td>
</tr>
<tr>
<td>R</td>
<td>12,600</td>
<td>94</td>
<td>56</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>W</td>
<td>120,000</td>
<td>99</td>
<td>242</td>
<td>5</td>
<td>98</td>
</tr>
</tbody>
</table>