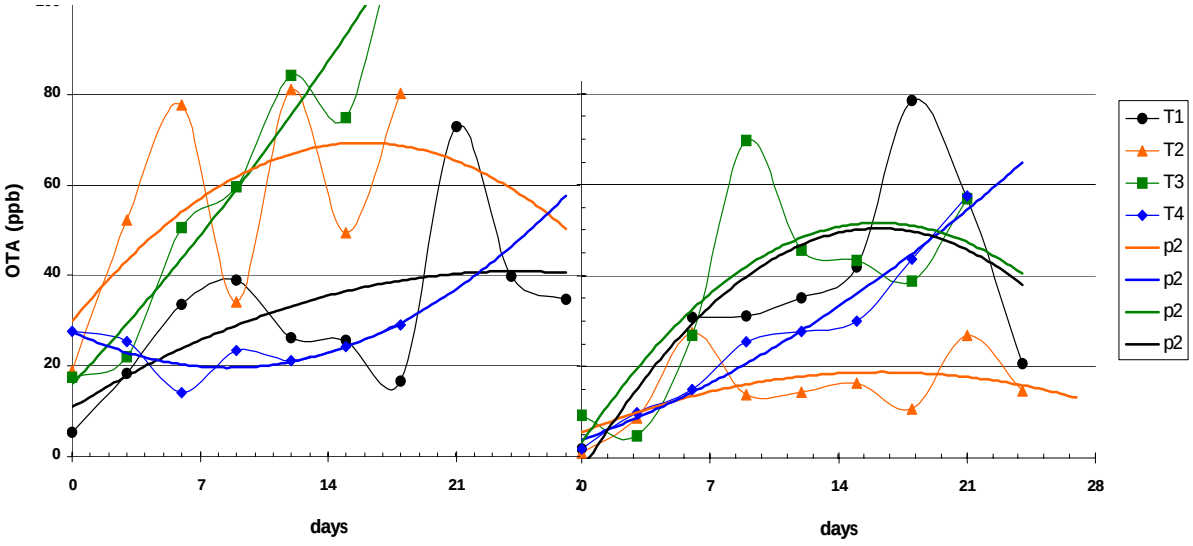
Annex C.7

Rate of OTA Production in Two Field Trials

Figure A reports two trials where periodic sampling, followed by oven drying and OTA analysis, were conducted during drying. Unfortunately, due to changes in personnel at the collaborating institution, full details of the four treatments are not known, but they involve different drying management parameters. The data provided are from replicate procedures where the replicates were not run simultaneously. There is a lot of noise, as always, in the OTA data but since adjacent points support each other, trends form something equivalent to a mean, thus the trend is more significant than the points comprising it.

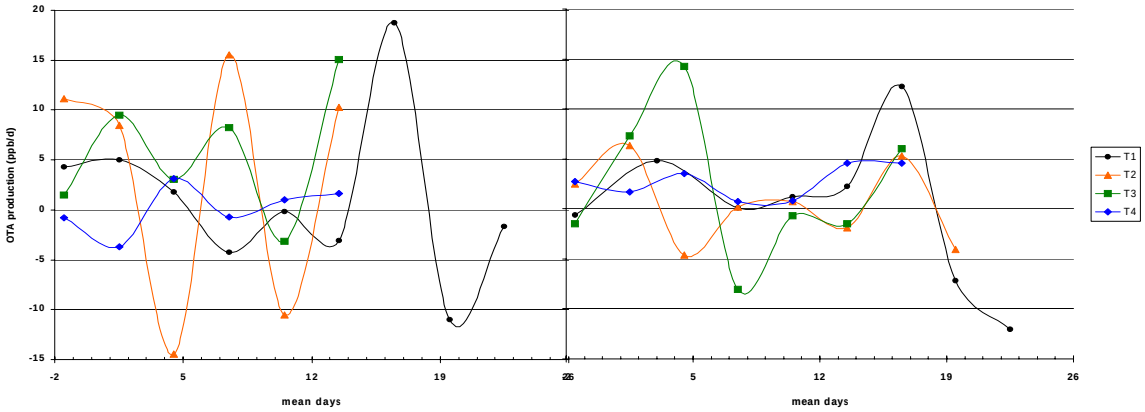
Figure A: OTA content of samples taken at three-day intervals over a drying time-course applied to four treatments. Second order polynomials have been fitted to each set of data, the points of which are linked by lines for ease of viewing.



In both instances an upward trend is clear in six of the eight treatments though of different magnitudes. The relative magnitudes of increase of the different treatments are not consistent demonstrating a variation in performance. As the trials were discontinued at full dryness any increases in OTA after about day 14 must be suspect since the A_w at this time should be approaching the minimum requirement for OTA production of abut 0.80.

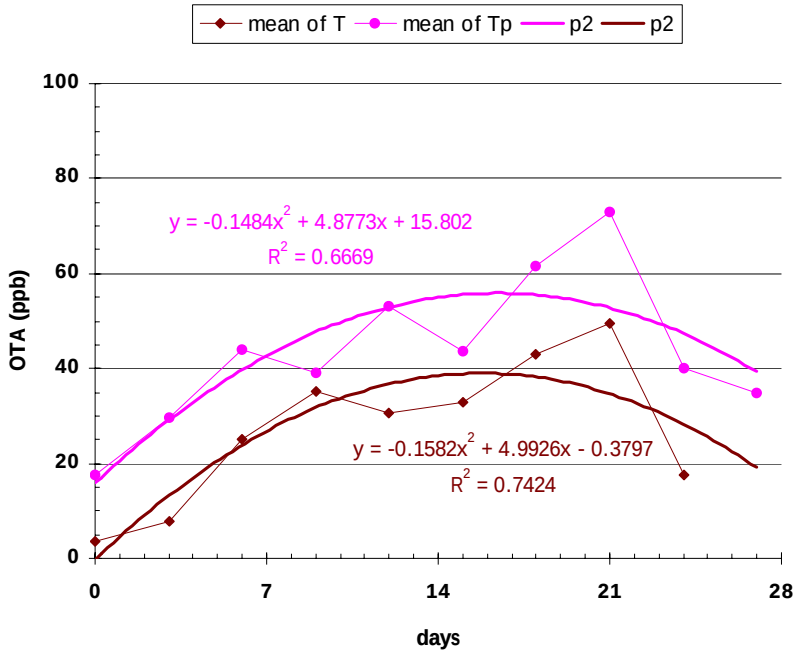
If the data is recalculated to show rates of net OTA production, it is clear that there appears to be periods of loss and these tend to alternate with the periods of net positive production. Of course we know that total methodological error is very high in OTA analysis but the data could mean that OTA accumulation is much more dynamic than normally assumed with high production rates and active mechanisms of destruction.

Figure B: The net rate of OTA production during drying of four treatments. 'Mean day' is the interpolation between two values used in calculating each change.



Given that the variation is very high and the treatments manifestly do not determine the outcome, it could be justified to consider all the treatments as replicates. This is explored below in Figure C.

Figure C: Grand means of all the data in each of the two replicate trials. The second order polynomial of best fit and the r2 values are recorded.



If the variations in Figures A and B were caused by an overweening factor, taking the mean would accentuate the swings since the swings would be synchronised in time and polarity by the factor. Instead we see a reduction in variation when calculated in this way. This could be because the variation is largely noise as discussed above and as such will not synchronise, rather tend to cancel out peaks as random coincidence of peaks with troughs occurs. Another explanation could be that the treatments are producing opposite effects which cancel out the differences when a mean is taken. Of course this is the problem of taking means but in this case no consistent trend, a

necessary corollary of a treatment effect, can be identified to lend credence to this possibility.

If we consider Figure C at face value, the similarity between the two sets of data is striking. If the points that fall away at the end of the drying periods are removed to enable a line to be fitted to the remaining data (not shown), the slope indicates a production rate of 2.1µg/kg/day net increase (Ta = 2.0 and Tb = 2.2) with r^2 values of 0.90 and 0.87 for the two data sets.

The initial OTA concentration would be expected to be roughly proportional to the biomass though not precisely so since mycotoxins are not concomitants of growth (such as is organic acids, for example). Nevertheless, from the data one would hypothesize that biomass is not rate limiting in this range of biomass since the slopes in the two cases, which apparently differ in biomass, are the same. It could be that assimilation or biosynthesis limits OTA accumulation independently of growth and respiration and this is certainly a known phenomenon in mycotoxin expression.

Table A: OTA analysis of husk from the first and last samples of the set derived from the trials described above. All figures are in ppb.

Treatment	Day 0	Last day
T1	66	755
T2	81	614
T3	163	744
T4	59	647
Tp1	253	699
Tp2	677	652
Tp3	866	890
Tp4	661	890

Table A presents results of the analysis of the husk from the cherry in these experiments. There is 10 to 50 times the amount of OTA in the husk of these cherry samples than in the bean. This pattern is reversed in some other studies that were carried out in the course of the project. As the husk/bean weight is close to parity, wet or dry, this means there is a massive concentration gradient from husk to bean. Before drying has progressed to the point where the bean shrinks away from the husk at about 25% m.c., the 2 µg/kg/day net OTA accumulation rate could reflect the diffusion rate limit of OTA. There is also the possibility of cross contamination of bean by husk causing errors in the OTA determinations of the beans since the husk seems to be universally contaminated to a high degree.

For completeness, two other experiments are reported. OTA analysis data of samples from drying trials are presented in Table B. Details of the drying treatments are not known due to a change in personnel at the experimental station. From the drying time course data it seems that the treatments were able to affect the required drying period, but accumulation of OTA is not closely related to the drying period. The

grand average of all data with respect to OTA accumulation rate is 3.8 µg/kg/day, somewhat higher than seen in the similar data set discussed at length above.

Table B: More OTA analysis of samples with uncertain provenance. The grand mean of net OTA accumulation rate is 3.8 ug/kg/day.

	Protocol 1					Protocol 2			
Treatment	T1	T2	T3	T4	T5	T1	T2	T3	T4
Initial	2.7	6.5	7.5	1.2	1.8^{4}	0.8	7.0	3.5	52.3
Intermediate	17.9^{10}	16.6^{9}	19.2^{4}	19.6^{10}	7.0^{10}	5.9^{6}	81.5^{6}	82.2^{8}	32.5^{5}
Final	46.5^{13}	17.9^{12}	22.8^{9}	38.5^{13}	8.0^{13}	4.5^{11}	126.2^{10}	81.3^{8}	73.9^{8}