ROLE OF PHYTOALEXINS IN RESISTANCE OF TOXIGENIC ASPERGILLI IN PEANUTS

R. J. COLE, J. W. DORNER, P. D. BLANKENSHIP and T. H. SANDERS

USDA, ARS, National Peanut Research Laboratory, 1011 Forrester Drive, S. E.
Dawson, Georgia 31742 (USA)

The concept of plants being able to produce defensive substances, called phytoalexins, in response to infection was first proposed by Muller and Borger (ref. 1). Phytoalexins are presently defined as low molecular weight antimicrobial compounds that are synthesized by and accumulate in plants after their exposure to microorganisms.

The first report of phytoalexin production in peanuts appeared in 1972 when Vidhyasekaran et al. (ref. 2) reported that they detected phytoalexin production in response to invasion by storage fungi. Keen and Ingham (ref. 3) reported that two phytoalexins from A. hypogaea were cis- and trans-isomers of 3,5,4'-trihydroxy-4-isopentenylstilbene or Arachidin II. Simultaneously, Ingham (ref. 4) isolated two related compounds from the fungal-infected hypocotyls of an African-grown cultivar of A. hypogaea. These were identified as cis- and trans-3,5,4'-trihydroxystilbene or resveratrol. Aguamah et al. (ref. 5) isolated 3 antifungal compounds from A. hypogaea that were identified as 4-(3-methyl-but-1-enyl)-3,5,3',4'-tetrahydroxystilbene (Arachidin I), 3,5,4'-trihydroxy-4-isopentenylstilbene (Arachidin II), and 4-(3-methyl-but-1-enyl)-3,5,4'-tri-hydroxystilbene (Arachidin III).

Recent studies by Wotton and Strange (ref. 6) provided circumstantial evidence for phytoalexin involvement in the resistance of peanuts to Aspergillus flavus. Their results indicated that resistance of peanut kernels to invasion by A. flavus was correlated with their capacity to synthesize phytoalexins as an early response to wounding. Also, conditions that promoted invasion of peanuts by A. flavus inhibited phytoalexin production. Thus, kernels of drought stressed plants, which are more susceptible to A. flavus than kernels of nondrought stressed plants, produced less phytoalexin in response to wounding by slicing than kernels from non-stressed plants.

This review summarizes a recent study on the role of stilbene-based phytoalexin resistance in peanuts (ref. 7).

This study showed that peanut kernels of all maturity stages from an irrigated control plot maintained a kernel a_w of 1.0 throughout the experiment. These peanuts consistently produced relatively high quantities of stilbene phytoalexins and no samples had significant aflatoxin contamination (>5 ppb) during the experimental period (ref. 7). As the study progressed, the a_w and

moisture of drought-stressed peanuts decreased. The rate of moisture loss was faster in the 29°C treatment than in the 25°C treatment, particularly in the more immature stages (ref. 7). However, the moisture loss was not uniform within a given maturity stage in a given treatment. For example, the moisture content of kernels in the yellow 2 maturity stage (next to most immature stage) from the 29°C treatment ranged from 40.3% to 17.0% (a_w range of 1.00 to 0.92). The yellow 1 (most immature stage) and orange stage (intermediate maturity) had similar ranges, but the moisture range in the mature stages (brown, black) was much more uniform. This phenomenon was observed in both the 29° and 25°C treatments, but as expected, the number of drier kernels within a maturity stage was greater in the 29°C treatment. Kernels in the yellow 1, yellow 2, and orange maturity stages were further segregated based on visual assessment of kernel moisture. All kernels were separated into groups of high, medium, and low moisture before determinations of moisture, a_w , phytoalexin-producing potential, and aflatoxin contamination were made. These segregations were essential to testing the hypothesis that kernel a_w was the primary factor that directly or indirectly led to aflatoxin contamination. Since a_w and moisture of peanuts in the brown and black maturity stages were generally quite uniform, it was not necessary to further segregate kernels from those categories.

Comparisons of moisture content of particular maturity stages from the 29° and 25°C treatments were difficult, since there were up to four moisture contents for each maturity stage. For example, in the yellow 2 group from the 29°C treatment samples at 148 days after planting (DAP)(45 treatment days [TD]), the moisture content of the different segregations within the group were: very low moisture, 11.7%; low moisture, 15.0%; medium moisture, 29.3%; high moisture, 37.3%. Each sampling from both the 29° and 25°C treatments yielded kernels of similar moistures within a maturity stage. However, the 29°C treatment consistently had more kernels at lower moistures than the 25°C treatment. Therefore, to arrive at a single moisture content for a segregated maturity stage, the amount of kernels at a given moisture was taken into account and a "weighted moisture" was used for each maturity stage (ref. 7).

As expected, the more immature peanuts lost moisture sooner in the 29°C soil than in the 25°C soil. Generally, it took about a week longer for kernels in the cooler treatment to reach moistures attained in the warmer treatment. The data clearly showed a direct relationship between soil temperature and the rate of water loss from peanut kernels under late-season drought stress.

Relationship between kernel a_w and phytoalexin production. As kernel a_w decreased during drought stress, the capacity of kernels to produce phytoalexins also decreased and eventually was lost. This trend was

independent of maturity and soil temperature. When a_w was high (0.97-1.00), kernels of all maturities and from both temperature treatments produced abundant phytoalexins. No significant phytoalexin production was observed when a_w was below 0.95. This was consistent throughout all maturities and in both treatments and illustrated the correlation between lower a_w and reduced phytoalexin production irrespective of maturity and soil temperature.

Relationship between phytoalexin production and aflatoxin contamination.

Many samples that had lost the capacity for phytoalexin production had no detectable aflatoxin while in other similar samples aflatoxin levels ranged as high as 2400 ppb. Significantly, however, no sample of peanuts that had moderate to high phytoalexin-producing capacity contained >5 ppb aflatoxin. Therefore, it can be stated that as long as peanut kernels had the capacity for phytoalexin production (high kernel moisture), significant amounts of aflatoxin was not formed. However, the loss of phytoalexin-producing capacity resulted in aflatoxin formation in a portion of these susceptible kernels (ref. 7).

Relationship between kernel a_w and aflatoxin contamination. In order to evaluate the relationship between a_w and aflatoxin contamination, it was useful to determine the percentage of samples contaminated with aflatoxin both above and below a_w of 0.95 (the a_w below which the capacity to produce phytoalexins was lost). In the 29°C treatment, when a_w was >0.95, only 5% of all samples contained greater than 5 ppb aflatoxin. However, 50% of all samples with a_w <0.95 had greater than 5 ppb aflatoxin. In the 25°C treatment 7% of samples greater than 0.95 a_w had more than 5 ppb aflatoxin compared to 26% of samples when a_w <0.95. These data are for all maturities combined.

When samples from all maturities and both treatments are combined, 94% had <5 ppb toxin when a_w was >0.95. This is compared to 65% having <5 ppb when a_w was >0.95. When 20 ppb is used as the cut-off, the percentages were 98% for a_w >0.95 and 73% for a_w <0.95. The difference in contamination between samples above and below a_w of 0.95 was greater in the more immature peanuts. For example, when treatments are combined, only 13% of the yellow 1 samples had >5 ppb aflatoxin when a_w was >0.95, but 67% of yellow 1's were >5 ppb when a_w was <0.95, but when a_w was >0.95, only 17% of the samples from each maturity were >5 ppb. Proceeding from yellow 1 (most immature) to black (most mature) when a_w was <0.95 the percentage of samples >5 ppb decreased accordingly - 67%, 50%, 24%, 19%, 17%.

A higher percentage of samples from the 29°C treatment had contamination than from the 25°C treatment. The percentages of samples from each maturity (yellow 1-black) containing >5 ppb aflatoxin when a_w was <0.95 were 80%, 50%, 50%, 50%, and 20%, respectively, in the 29°C treatment. Corresponding values

from the 25°C were 57%, 50%, 8%, 0%, and 14%, respectively.

Therefore, when a_w falls below 0.95 where phytoalexin-producing capacity was lost, immature peanuts were more likely to become contaminated than mature peanuts, particularly in high temperature soil. However, in mature peanuts, low a_w and the loss of the capacity to produce phytoalexins does not result in the same degree of contamination (both in numbers and levels) as it does in the immature peanuts. This indicates that after protection by phytoalexin production is lost ($<0.95 a_w$) other as yet unknown factors are providing a high degree of protection from preharvest aflatoxin contamination to mature peanuts.

Conclusions

Phytoalexins appear to be at least partly involved in resistance to drought-induced preharvest aflatoxin contamination of peanuts. Phytoalexin-based resistance appears to be more important in immature peanuts than in mature peanuts. Therefore, mature peanuts are more resistant to preharvest aflatoxin contamination than are immature peanuts. The exact mechanism of this resistance is not known.

REFERENCES

1. Muller, K. O. and H. Borger. Experimentelle Untersuchungen über die Phytophthora - Resistenz der Kartoffel, Arb. Biol. Aust. Reichsanst. (Berl.), 23 (1940) 189-231.
2. Vidhyasekaran, P., D. Lalithakumari and C. V. Govindaswamy, Production of a phytoalexin in groundnut due to storage fungi, Indian Phytopathology, 25 (1972) 240-245.
3. Keen, N. T. and J. L. Ingham, New stilbene phytoalexins from American cultivars of Arachis hypogaea, Phytochemistry, 15 (1976) 1794-1795.
4. Ingham, J. L., 3,5,4'-trihydroxystilbene as a phytoalexin from groundnuts (Arachis hypogaea), Phytochemistry, 15 (1976) 1791-1793.
5. Aguamah, G. E., P. Langcake, D. P. Leworthy, J. A. Page, R. J. Pryce and R. N. Strange, Two novel stilbene phytoalexins from Arachis hypogaea, Phytochemistry, 20 (1981) 1381-1383.
6. Wotton, H. R. and R. N. Strange, Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to Aspergillus flavus, J. Gen. Microbiol., 131 (1985) 487-404.
7. Dorner, J. W., R. J. Cole, T. H. Sanders and P. D. Blankenship, Interrelationship of kernel water activity, soil temperature, maturity and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts, Mycopathologia, in press.