Field Control of Aflatoxins in Cottonseed

Tom E. Russell, Plant Pathologist

Damage to immature cotton bolls by the pink bollworm, (PBW) through exit holes created by larvae cut-out, or feeding of Heliothus spp. have been reported as portals for entry by the fungus Aspergillus flavus. These reports are the result of work conducted in small tightly controlled tests. Until now the degree to which this "worm" damage contributes to aflatoxins contamination of cottonseed under commercial field conditions has not been established.

In 1977 and 1978, we established sampling sites in commercial fields within the "aflatoxin belt" which includes most of the major irrigated acreage in Arizona (below 1800' elevation) and southern California. In 1977, eleven fields were selected and 16-13 row foot (1/1000 Ac) sampling sites were established in each field. In 1978, four to eight 13 row foot replications (1/1000 Ac) were established in each of 28 fields in these same areas.

Two major studies were conducted in each field during both years. The initial study was a seasonal sampling program. All the plants in each replication were stripped of all mature (open) bolls on a bi-weekly basis commencing at initial boll opening and culminating when no mature bolls were present at the end of the normal production season. All bolls were examined for "worm" damage and a percent infestation calculated. The intent of this study was to determine if worm damage was more critical to the contamination of seed by aflatoxins during one portion of the season than another.

The results can be seen in Table 1. The correlation (r) of aflatoxins with percent pbw or worm damage was low. The r values for the 1977 and 1978 early August sampling dates were r = .51 and .56, respectively. These values are low and suggest only a slight relationship. The r values decrease for later sampling dates to the point that worm damage is apparently not a factor at all in the contamination of aflatoxins in bolls maturing after the middle of September. There was no relationship between worm damage and aflatoxins in "stub" or "ratooned" (perennial) cotton. The degree of correlation coefficient significance (asterisks) after each r value indicates the probability that you would obtain the same value if the tests were repeated in the same manner.

The secondary study involved the year-end sampling of all plants in a sister replication across the furrow from each seasonal sampling replication. All mature bolls that were deemed harvestable were removed from plants at the end of the season. Each boll was examined and categorized as to whether they, (1) were fully fluffed (FF) and apparently undamaged by insects or A. flavus, (2) had one or more unfluffed (tight) locules apparently resulting from microbial activity and not insect damage, and (3) were damaged by the pink bollworm (PBW) or Heliothus spp. (collectively called "worm" damage). We had expected that seeds from "worm" damage (WD) bolls would contain most of the aflatoxins but this was not the case. In Table 2 you can see that these bolls in 1977 and 1978 accounted for only 33% and 40%, respectively, of the aflatoxins detected. Removal of the WD bolls from the test would not drastically change the overall mean value of aflatoxins.

In Table 3 you can see the distribution of aflatoxins in 1977 fully-fluffed (FF) and WD bolls. There is little difference between FF and WD bolls in the proportion of bolls showing non-detectable levels of aflatoxins. The same observations were made in 1978 (Table 4). The only basic difference lies in the proportion of bolls with levels of aflatoxins in excess of 5-10,000 ppb. Very few FF bolls in 1977 contained levels of this magnitude and none were found in 1978, while a much higher proportion of WD bolls contained levels in excess of this figure.

It is because of this apparent distribution of aflatoxins and the poor correlations in our seasonal sampling date that we conclude:

"Seeds from bolls with worm damage (PBW or Heliothus damage) are no more likely to be contaminated with aflatoxin than bolls with damage."

or

"Seeds from bolls with no worm damage are as likely to be contaminated with aflatoxin as bolls with worm damage."

and

When aflatoxins are present worm damage appears to cause the levels to be higher than detected in undamaged bolls.

The previous worm damage data suggests there are other major factors in the epidemiology of *A. flavus* and aflatoxin formation. The presence of worm damage in cotton bolls which apparently produces an ideal portal for the fungus *A. flavus*, an extremely aggressive saprophyte, but no aflatoxin suggests the absence of *A. flavus*. The lack of ideal environmental conditions for inoculum development or an insect carrier of *A. flavus* might also account for this situation.

With this in mind, we surveyed a number of fields in 1980 for the presence and seasonal fluctuation of *A. flavus* propagules. Utilizing Andersen 2000 spore traps, we were surprised to find that it is difficult to detect *A. flavus* in fields until the middle of July (Table 5). This is about two weeks prior to initial boll opening. After this period, populations increased rapidly in some fields but increased only slightly in others. The key to the problem may be with the effect of sub-canopy microclimate on developing *A. flavus* populations or possibly the emergence of antagonistic or competitive organisms.

There is some evidence from past work to indicate that irrigation plays a role in the contamination of cottonseed by aflatoxins. During the 1980 season, we studied the effect of the timing of the final irrigation on aflatoxin levels in seed from several varieties grown in replicated plots under commercial conditions. The results from the three locations can be seen in Tables 6, 8 and 9. In all cases, the additional irrigation was responsible for a significant increase in aflatoxins. The mechanism of this cause and effect relationship is unknown.

The irrigation termination tests were harvested by two different machines. One was the standard spindle picker and the other a brush roll stripper. The spindle harvester was designed for fluffed lint and is inefficient in the collecting of lint and seed from tight loculed, worm damaged, or rotted bolls. The stripper harvester collects everything in much the same manner as a ground gleaner except that ground recovery is not made. Comparison of the amount of aflatoxins detected in seed harvested by each method reveals a substantially higher level in stripper harvested seed (Tables 7, 8 and 9). It is quite obvious that these data should be considered before cotton production areas with detectable levels of aflatoxin make a major switch to stripper harvesting.

Segregation of seed cotton or fuzzy cottonseed into "high" and "low" lots is limited to a direct analysis procedure at this time. Only a limited amount of separation is being attempted on the basis of spindle versus ground recovered seed and nothing is being done commercially with fluorescence.

Interpretation of data in Table 10 suggests that the screening of trailers in a gin yard on the basis of bright greenish-yellow fluorescence (BGY) is a viable way to segregate for various degrees of aflatoxin contamination. At least under the dry harvest conditions which prevailed in 1979, approximately 82% of trailers with zero to two spots contained seed with levels below 10 ppb and 90% contained 20 ppb or less. More research in this area is in order.

Table 1. Seasonal formation of aflatoxins ($B_1 + B_2$) as related to the percentage of bolls exhibiting one or more pink bollworm (pbw) exit holes in 1977 and 1978 cottonseed.

	Late July		Early Aug.		Late Aug.		Early Sept.		Late Sept. to harvest	
	PBW %	Aflatoxins ppb	PBW %	Aflatoxins ppb	PBW %	Aflatoxins ppb	PBW %	Aflatoxins ppb	PBW %	Aflatoxins ppb
1977 (Planted)	--	---	5	185	4	732	10	336	15	136
r =			0.51***		0.35***		0.20**		0.10 NS	
1978 (Planted)	--	---	8	368	7	198	8	107	4	26
r =			0.56***		0.40***		0.47***		0.10 NS	
1978 (Stub)	12	68	6	270	9	30	8	14	--	---
r =	0.01 NS[a]		0.10 NS		0.17 NS		0.05 NS		--	---

[a]NS = not significant ($p > 0.05$). Triple (***), double (**) or single (*) asterisks indicate correlation coefficients significant at $p = 0.001$, 0.01 or 0.05, respectively. Correlation coefficients calculated by using natural log transforms of total aflatoxin data ($\ln [x + 1]$).

Table 2. Distribution of aflatoxins ($B_1 + B_2$) and meal weight among fully fluffed, tight loculed and "worm" damaged cotton bolls (1977 and 1978).

	1977			1978		
	Aflatoxin		Meal wt	Aflatoxin		Meal wt
	$\bar{x}$ ppb	%	%	$\bar{x}$ ppb	%	%
Fully fluffed	268	30	80	59	43	92
Tight loculed	898	37	14	495	17	4
"Worm" damaged	4036	33	6	3310	40	4

Table 3.

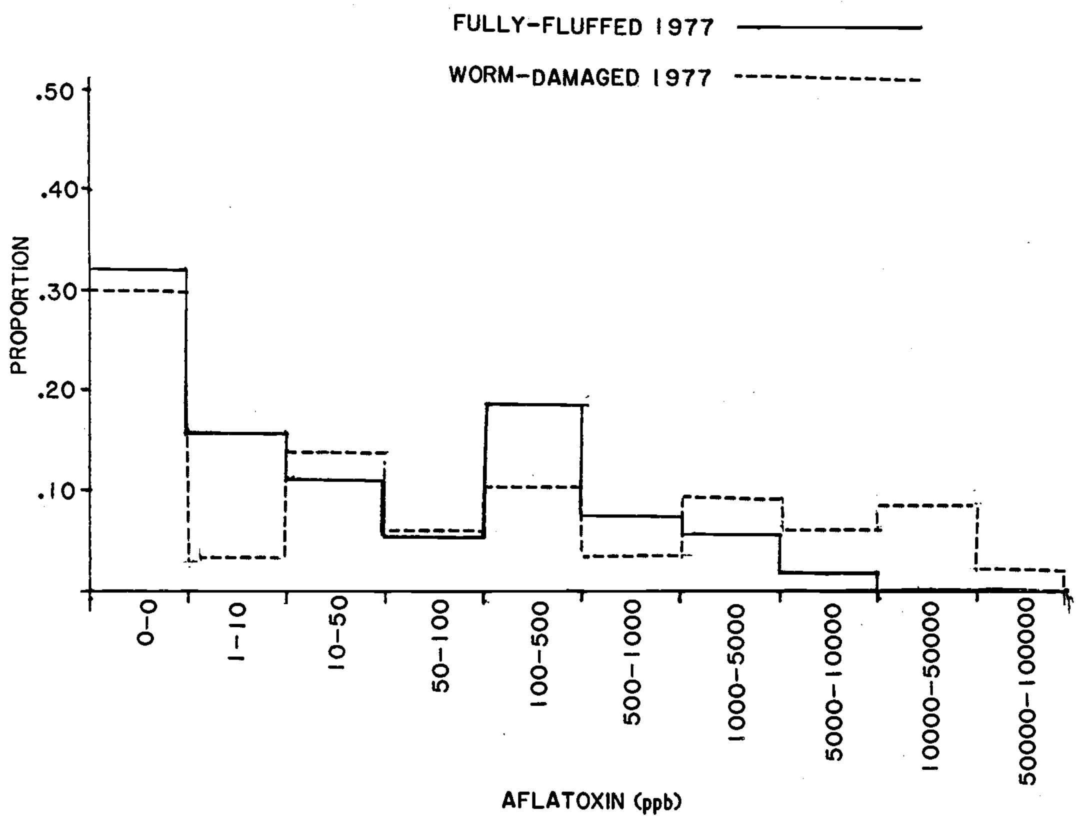


Table 4.

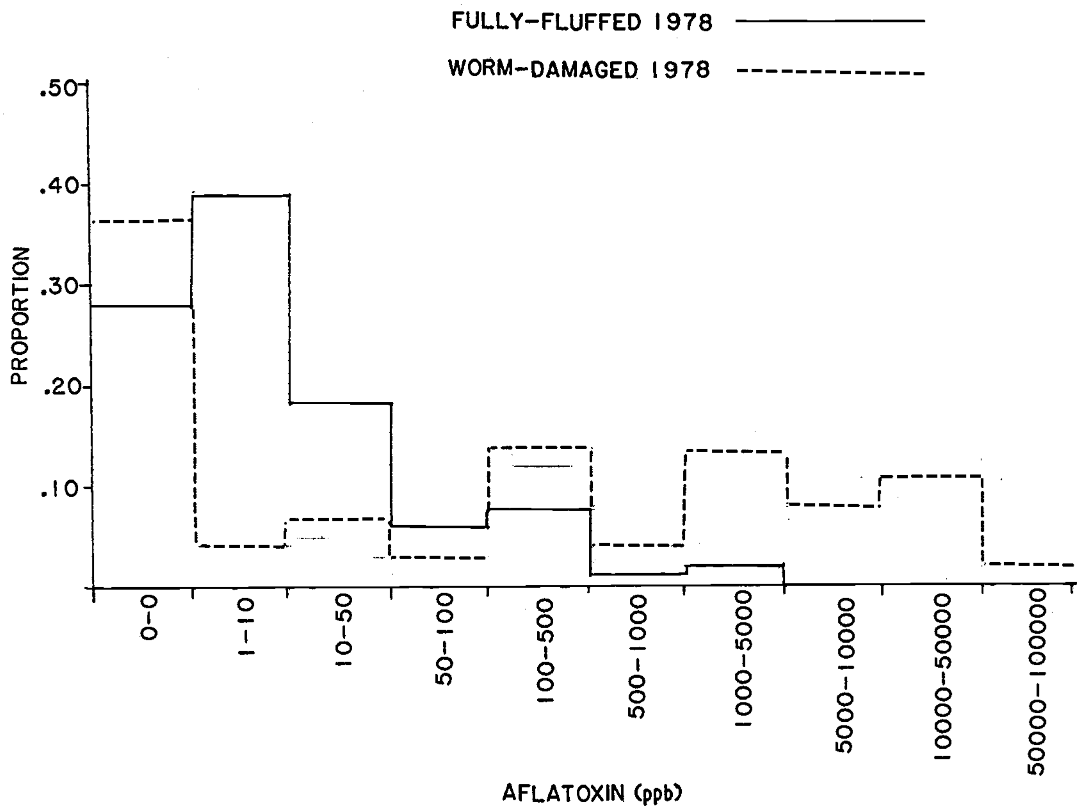
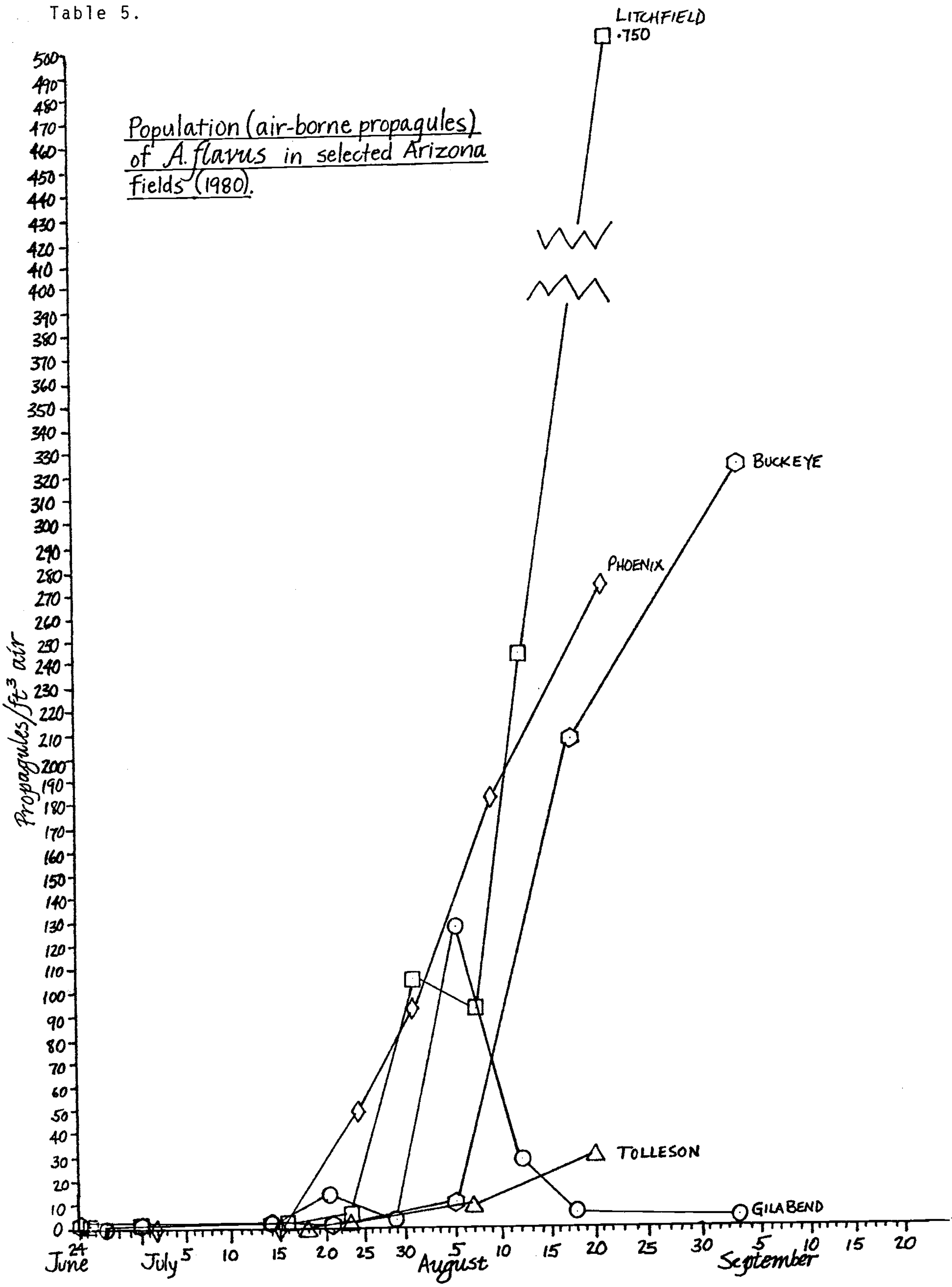

FULLY-FLUFFED 1978
WORM-DAMAGED 1978
.50
.40
.30
.20
.10
PROPORTION
0-0
1-10
10-50
50-100
100-500
500-1000
1000-5000
5000-10000
10000-50000
50000-100000
AFLATOXIN (ppb)

Table 5.



Population (air-borne propagules) of A. flavus in selected Arizona fields (1980).
Propagules/ft³ air
LITCHFIELD
·750
BUCKEYE
PHOENIX
TOLLESON
GILABEND
June
July
August
September

Table 6. Relationship of variety and irrigation termination date on formation of aflatoxins (B_1 + B_2) in cottonseed produced and spindle harvested in Gila Bend, AZ (1980)

Variety	Aflatoxins B_1 + B_2 (ppb) $\bar{x}$[a] Aug. 25[b]	Sept. 9[b]	Sept. 16[b]
Deltapine 70	ND[c]	ND	46
Deltapine 41	ND	3	48
Stoneville 825	1	ND	18
Fisher 7203	ND	ND	14
Fisher 7209	ND	ND	10

[a]Mean ($\bar{x}$) value based on analysis of 16 cottonseed samples (4 field reps. x 4 rep. sub-samples).
[b]Date of final irrigation.
[c]Aflatoxins non-detectable.

Table 7. Comparison of aflatoxin levels detected in stripper and first pick spindle harvested cottonseed (paired plots)[a]

Variety	Stripper $\bar{x}$ Aflatoxin (ppb)	Spindle $\bar{x}$ Aflatoxin (ppb)
DP-70	8	ND[a]
DP-729	40	ND
ST-825	48	ND
7203-14	57	ND
7209-10	137	T
7209-30	51	ND

[a]Non-detectable.

Table 8. Relationship of variety, irrigation termination dates and harvest method on formation of aflatoxins B_1 and B_2 in cottonseed produced in Coolidge, AZ. (1980)

	Aflatoxins B_1 + B_2 (ppb) $\bar{x}$[a]					
	August 22[b]		September 5[b]		September 19[b]	
Variety	Spindle	Stripper	Spindle	Stripper	Spindle	Stripper
DP-70	ND[c]	1	2	8	<1	28
DP-41	ND	6	3	7	1	31
SV-825	ND	1	1	9	<1	30
7203	7	8	20	12	4	39
7209	3	37	65	10	1	43
$\bar{x}$	2	11	18	9	1	34

[a]Mean ($\bar{x}$) value based on analysis of 16 cottonseed samples (4 field reps x 4 rep sub-samples/rep).
[b]Date of final irrigation.
[c]Aflatoxins non-detectable.

Table 9. Relationship of variety, irrigation termination dates and harvest method on formation of aflatoxins B_1 + B_2 in cottonseed produced in Phoenix, AZ (1980)

	Aflatoxins (B_1 + B_2) $\bar{x}$[a] ppb							
	August 1		August 12		August 28		$\bar{x}$	
Variety	Spindle	Stripper	Spindle	Stripper	Spindle	Stripper	Spindle	Stripper
DP-70	5	31	15	27	4	138	8	60
DP-712	4	14	3	54	3	164	3	77
SV-825	5	26	11	44	>1	104	6	58
SV-506	4	96	3	59	12	159	6	105
7203	10	45	5	44	22	178	12	89
7209	26	--	6	--	12	387	15	--
$\bar{x}$	9	42	7	46	9	186		

[a]Mean value from four field replications.

Table 10. The relationship between the number of bright greenish-yellow (BGY) spots observed when cotton trailers contains seed cotton are irradiated with UV (blacklight) light and aflatoxins detected in trailer sub-samples. (1979)

BGY spots	cotton trailers	Range of Aflatoxins ($B_1 + B_2$) in ppb				X^{2a}
		ND-10	11-20	21-50	50+	
#	#	#	#	#	#	
0	63	50	7	4	2	--
1	26	24	1	0	1	3.46 NS
2	16	12	0	3	1	6.21 NS
3	16	10	1	3	2	9.19*
4-10	13	7	2	2	2	8.93*
11-38	20	2	0	5	13	264.16***
Total	154					

[a]NS = not significant (p >0.05). Triple (***), double (**) or single (*) asterisk indicate significant difference from expected value by Chi-square (x^2) evaluation at p = 0.001, 0.01 or 0.05, respectively.

1981 Arizona Cottonseed Treatment Study

Tom E. Russell, Plant Pathologist

Cottonseed treatment plots were established on the Yuma, Phoenix (CRC) and Marana Experiment Stations in order to test the efficacy of fungicides and combinations thereof for the control of Rhizoctonia soreshin seedling disease of cotton.

All seed treatments were provided and applied by the distributors to a common lot of Deltapine 61 acid delinted seed. The only exceptions were the Terracoat L-21 and Terraflo 21 treatments which were applied by us.

The Rhizoctonia for soil infestation was prepared by growing cultures on steam sterilized wheat seed at 24C. After two weeks of incubation the infested seed was dried, ground to a granular size and packaged in 10 g lots.

Planting was by cone planters and the addition of Rhizoctonia or Super X granules was made into the hopper just prior to incorporation into the soil. The seedling rate was 100 seed/28 feet (46,643 plants/acre).

Stand counts were made at 10 and 30 day post-planting intervals. Only the data for the 30 day counts in Yuma (Tables 1 and 4) and Phoenix (Tables 2 and 5), and the 10 day Marana (Tables 3 and 6) are presented.

Conclusions

The best and most consistent commercially available treatment for the control of Rhizoctonia in these tests was Super X granules at 10 lbs/Ac. It was quite evident that the Terracoat L-21 and Terraflo 21 seed treatments played little or no role in achieving the stands observed when combined with Super X.

The next best treatment, and at times it was comparable to Super X, was Chloroneb (Demosan) at the 2.66 oz and 4.23 oz rates. The lower rates of Chloroneb and higher rates of Carboxin (Vitavax) (2.4 oz and 3.00 oz) were the third best treatment with the lower rates of Carboxin including the commercially applied 1.75 oz rate, Terracoat L-21 and Terraflo 21 being the least effective of all materials and concentrations tested.

A new material BAS 389-01F (Gustafson's "Campogran") which is not registered as a cottonseed treatment performed better than any of the other "seed treatments" for the control of Rhizoctonia.