

- 1) Mr. T. Nibe : "Field Management to Control Mycotoxin"
- 2) Mr. M. Kobayashi: "Corn Sheller and Moisture Meter"
- 3) Dr. Mitri Naewbanij: "Batch Dryer and Continuous Flow Dryer"
- 4) Mrs. Prisnar Siriacha: "Bright Greenish Yellow Fluorescence Test (BGYF)"
- 5) Mr. Prawat Tanboon-ek: "Aflatoxin Control in Thai Maize"

Laboratory work on "Sample Preparation and BGYF Test" was demonstrated by Miss Arunsri on August 8, 1989. Participants in the above course visited the MQIRC on August 7, 1989. Dr. T. Yoshiyama gave a presentation "On the History of the MQIRC and the Project Activities".

Mr. K. Arai and Miss Arunsri Wongurai instructed on the use of the experimental equipment and facilities.

3. The second field trip and spot discussion of the Four Sections were held at Phra Phutthabat Field Crops Experiment Station on November 1st, 1989.

4. The 100th meeting of "the Agricultural Seminar" organized by TARC was held on December 1st, 1989 at the meeting room of the Japan External Trade Organization (JETRO). Dr. T. Yoshiyama, Mr. T. Seino, Mr. T. Nibe, Mr. K. Arai, Mr. T. Goto and 20 other members concerned attended. Dr. T. Yoshiyama gave a presentation on the organization system of the MQIRC and research activities.

5. Research Activities

A series of experiments has been carried out under the Tentative Implementation Program, which were confirmed at the Joint Committee Meeting on March 16, 1989. The main subjects implemented in three sections are as follows ;

1) Agronomy Section

Total eleven research subjects were proposed in agronomy section. Ten subjects were already completed. Field experiments were carried out from April 14 to beginning of March. One subject "Relation between Kernel Type and Resistance to Fungus Infection after Inoculation" was delayed due to postponed seed multiplication by field arrangement, that will be carried out end of April to early May. General conditions for maize growing was less rainfall than 1988 and its distribution was also not preferable especially in early growth period. Over one thousand four hundred samples including soil, maize and air were taken for analysis of aflatoxin, A. flavus and soil chemical properties. Level of aflatoxin in general was not high as 1988.

2) Post-Harvest Section

Main activities of this year were divided into four categories. For corn sheller improvement, seven types of cylinders were tested to get data for the prototype of improved corn sheller. For moisture meter, standardization of the oven method and calibration test of moisture meters were under investigation and moisture meter for ear maize was newly developed and tested. For chemical treatment, ammonia and sulfur dioxide

treatment during storage were examined and trickle drying process using ammonia or sulfur dioxide (TAP, TSDP) was investigated. And for storage and drying, increase of damaged kernel during handling, effect of delayed drying in the post-harvest process and internal environment of farmers' storage were analysed, and simple drying method was investigated. Last year one hundred and twelve samples were sent to Microbe Section for aflatoxin analysis.

3) Microbe Section

This field has close relations with the Agronomy and Post-Harvest research. In 1989, crop season, 526 of maize samples for aflatoxin analysis, 235 of maize samples and 288 of soil samples for microbiological studies were received from the Agronomy Section. The Post-Harvest Section sent 112 of maize samples to the Microbe Section for aflatoxin analysis, both shelling and drying experiments.

The Microbe Section carried out their three main subjects, viz. physiological and ecological study on *A. flavus*, analytical method of aflatoxin and physical properties of maize kernels. About 500 of maize samples in field, trade and storing were analyzed on aflatoxin, and more than 400 of maize and soil samples were used for microbiological studies. Both the microbiological techniques and the analytical method of aflatoxin by means of Immunoassay were transferred to the junior scientists in the section.

Experts	1st Year Dec. 1986 ~ Dec. 1987	2nd Year Dec. 1987 ~ Dec. 1988	3rd Year Dec. 1988 ~ Dec. 1989	4th Year Dec. 1989 ~ Dec. 1990	5th Year Dec. 1990 ~ Dec. 1991
<u>Team leader</u> Dr. Taketoshi YOSHIYAMA Mr. Sei-ichi UEDA				(Jul. 21. '87 - Dec. 20. '89)	
		(Dec. 8. '89 - Dec. 14. '91)			
<u>Long-term experts</u> Mr. T. SEINO (Coordinator) Mr. N. KOBAYASHI (Post-harvest) Mr. M. HARADA (Post-harvest) Mr. T. NIDE (Agronomy) Mr. K. ARAI (Microbe)				(May 20. '87 - May 19. '90)	
		(Dec. 8. '87 - Dec. 14. '91)		(May 20. '87 - Nov. 19. '89)	
				(Jun. 30. '87 - Jun. 29. '90)	
				(Jul. 8. '88 - Jul. 7. '90)	
<u>Short-term experts</u> 1987 Japanese fiscal year					
Mr. Y. IDEGUCHI (Engineer)		(1)	(Dec. 15 - 26, '87)		
Mr. H. SHIMADA (Microbe)		(2)	(Mar. 10 - Jun. 17, '88)		
Mr. Y. TOMIOKA (Engineer)			(Mar. 10 - Apr. 8, '88)		
1988 Japanese fiscal year					
Mr. N. ISHITANI (Moist. tester)			(Aug. 1 - Aug. 19, '88)		
Mr. Y. AZUMA (Corn sheller)			(Jul. 20 - Sep. 19, '88)		
Mr. M. KAMO (Ammonia treatment)			(Jul. 20 - Sep. 19, '88)		
Dr. O. TSURUTA (Microbe)			(Aug. 19 - Oct. 27, '88)		
1989 Japanese fiscal year					
Mr. N. ISHITANI (Moist. tester)				(Aug. 1 - Sep. 9, '89)	
Mr. M. KAMO (Ammonia treatment)				(Jul. 6 - Aug. 24, '89)	
Mr. Y. AZUMA (Corn sheller)				(Jul. 6 - Nov. 11, '89)	
Mr. K. INOUE (Drying method)				(Jul. 25 - Sep. 24, '89)	
Dr. O. TSURUTA (Microbe)				(Jun. 16 - Aug. 15, '89)	
Mr. T. GOTO (Analyze)				(Sep. 14 - Dec. 12, '89)	
Mr. M. TSUIKI (Simulation)				(Sep. 27 - Nov. 26, '89)	

Remark : Assignment of experts in 1990 Japanese Fiscal Year will be informed by the Technical Guidance Team's summary report.

Training in Japan	1st Year Dec. 1986 ~ Dec. 1987	2nd Year Dec. 1987 ~ Dec. 1988	3rd Year Dec. 1988 ~ Dec. 1989	4th Year Dec. 1989 ~ Dec. 1990	5th Year Dec. 1990 ~ Dec. 1991
1987 Japanese Fiscal Year Mr. Narongsak Semarong (Field Crops Research Institute) Mrs. Sriwai Singhagajen (Division of Agri. Engineering)		■ (Sep. 28 - Oct. 17) ■ (Sep. 28 - Oct. 17)			
1988 Japanese Fiscal Year Ms. Arunsri Kongurai (Division of Plant Pathology and Microbiology) Mr. Sukapong Vayupari (Field Crops Research Institute) Mr. Naitri Naebanij (Division of Agri. Engineering) Dr. Vijai Nopamornhodi (Administration)		■■■■ (May 16 - Sep. 15) ■■■■ (Jun. 20 - Oct. 21) ■ (Oct. 2 - Nov. 30)	■ (Mar. 6 - Mar. 21)		
1989 Japanese Fiscal Year Mr. Suparat Kositchareonkul (Division of Plant Pathology and Microbiology) Mr. Prason Uepayasuvan (Field Crops Research Institute) Mr. Chaival Paosantadpanich (Division of Agri. Engineering) Mrs. Siripoin Simhasake (Administration)			■ (Jan. 14 - Apr. 17) ■■ (Jul. 10 - Sep. 2) ■■■■ (Oct. 30 - Jan. 28)	■ (Mar. 5 - Mar. 28)	
1990 Japanese Fiscal Year (Division of Plant Pathology and Microbiology) (Field Crops Research Institute) (Division of Agri. Engineering) (Administration)				□□□ (3 months) □ (1.5 months) (4 months) □□□□ (3 weeks) □	

■ = attended □ = scheduled

Equipment (budget)	1st Year Dec.1986 ~ Dec. 1987	2nd Year Dec.1987 ~ Dec. 1988	3rd Year Dec.1988 ~ Dec. 1989	4th Year Dec.1989 ~ Dec. 1990	5th Year Dec.1990 ~ Dec. 1991
<u>1987 Japanese Fiscal Year</u>					
(General)	5,100,000 Y (provided)				
<u>1988 Japanese Fiscal Year</u>					
1) General					
2) Agronomy		7,603,000 Y			
3) Post-Harvest		12,435,000 Y			
4) Microbe		6,392,000 Y (provided)			
<u>1988 Japanese Fiscal Year</u>					
1) General			1,360,000 Y		
2) Agronomy			12,670,000 Y		
3) Post-Harvest			13,140,000 Y		
4) Microbe			16,100,000 Y (provided)		
<u>1989 Japanese Fiscal Year</u>				6,000,000 Y	
<u>1990 Japanese Fiscal Year</u>				(budget)	
Total	5,100,000 Y	26,430,000 Y	42,500,000 Y	6,000,000 Y	

Local cost borne by Japan in Japanese fiscal year 1987, Bahl 3,060,000.- for repairing water reservoir, in 1988 Bahl 484,500.- for modification of the laboratory at Prabodhabat Field Crops Experiment Station and in 1989 Bahl 1,014,139 for roof expansion and storage building was approved.

III. SUMMARY REPORT

(1990.4.)

(JAPANESE TECHNICAL GUIDANCE TEAM)

SUMMARY REPORT OF THE JAPANESE TECHNICAL GUIDANCE TEAM FOR
THE MAIZE QUALITY IMPROVEMENT RESEARCH CENTRE PROJECT

The Japanese Technical Guidance Team for the Maize Quality Improvement Research Centre Project (hereinafter referred to as "the Project") organized by Japan International Cooperation Agency, headed by Dr. Masaru Manabe, visited the Kingdom of Thailand and stayed for 13 days from April 9 to 21, 1990.

The purpose of the team was to review and consult on the present situation of the project activities, based on the Tentative Implementation Programme which was agreed at the Joint Committee Meeting in March 16, 1989.

During stay in Thailand, the team exchanged views and had discussions with the authorities concerned referring to the project activities.

This is a summary report related to the results of the discussions:

A. Research Activities

The main findings and assessments obtained by the three sections are summarized as follows:

1. Agronomy Section

1) Varietal Comparison of Maize Kernel Moisture and its Variation:

According to Different Times of Harvesting.

Code No. I-1-(1)-A, G AG/I/89

Three composite varieties, Suwan 1 (SW 1), Suwan 2 (SW 2), and Suwan 3 (SW 3) and one hybrid variety KU2602 (KU) were utilized for the study. Suwan 3 was new entry this year and others were repeated from 1988. Average kernel moisture content (MC) at 35 days after 50% silking were 36.7%, 35.8%, 36.9% and 35.7% on SW 1, SW 2, KU and SW 3, respectively. There were 24.3%, 20.5%, 23.8% and 24.5% at 64 days after 50% silking, respectively. Similar moisture decrease was observed on SW 1 and KU as 1988. SW 3 started from as low level as SW 2, however decreased slowly to the level of SW 1 and KU at last measurement. The range of moisture content during the observation period were also described by liner regression using averages and standard deviations in respective varieties.

2) Long Term Study on the Relationship of the Environmental Conditions that Cause Aflatoxin Incidence in Maize.

Code No. I-1-(1) -B, E, G, J-(b)AG/II/89

Planting was started from April 14 and ended on September 1 for eleven times at 2 weeks intervals. Growth condition in 1989 was not as good as 1988 on the rainfall especially at early plantings. Planting in April under rainfed condition was tremendous problem to keep maintain plant stand that was achieved lower than 25% of planned density, and yield was low. The highest yield under irrigated condition was around 900kg/rai and around 700kg/rai under rainfed condition.

None aflatoxin contamination was observed at harvest. Harvested ear at 95 days after planting was higher level of contamination than 105 days and 115 days in general. Highest level of aflatoxin contamination was observed on 95 days of April planting as high as 2200 ppb.

3) Effects of Different Harvest Methods, Moisture Conditions and Storage Periods on Aflatoxin Contamination in Maize.

Code No. I-1-(1) -H, G, AG/III/89

Moisture decrease on harvest with husk (+husk) and without husk (-husk) were almost parallel with less than 2% difference. Aflatoxin contamination level in 1989 was much lower than 1988. The highest level was obtained around 400 ppb on 105 days harvested ear after 5 weeks storage without husk this year compared with 1100 ppb in 1988.

No sample from +husk were beyond the 20 ppb and sample from -husk were detected from 2 weeks after harvest, however level of over 20 ppb was obtained from 4 weeks and increased to 8 weeks. Very similar trend of aflatoxin contamination was observed this year as were as 1988.

4) Effect of Plant Density and Nitrogen Application on Aflatoxin Contamination.

Code No. I-1-(1) -D AG/IV/89

Yield response to nitrogen was significant, however on density treatment was not significant due to plant stand. Plant stands at harvest were 98.8%, 88.9% and 76.7% of planned densities on D1 (4260 plants/rai), D2 8533 plants/rai) and D3 (12266 plants/rai) plots, respectively.

Aflatoxin was not detected at harvest and very low level of toxin (with the level from 2 to 9 ppb) were observed from 25% of sample at 2 weeks after harvest.

5) Effect of Crop Rotation on Aspergillus spp. in the Soil .

Code No. I-1-(1) -C, B, AG/V/89

The pattern of crop rotation were applied to study movement of A. flavus population in the soil. Soil samples were taken before and after every cropping. The range of population was 10 to 1000 colonies in 1 gram soil. A. flavus population tend to decrease from the 1st crop to 2nd crop which was opposite result from 1988.

6) Effect of Nitrogen Regarding Prevention of Aflatoxin Contamination by the Inoculation Method.

Code No. I-1-(1) -D AG/VI/89

Yield from nitrogen plots were 478.4, 880.4, 910.0, and 875.3 kg/rai on N1, N2, N3 and N4, respectively. Visual check of A. flavus infected ear from inoculated and non inoculated plots were 3.7, 5.4, 1.6 and 5.0% in inoculated plot and 0, 0, 0 and 1.6% in no inoculated plot on N1, N2, N3 and N4. Result of aflatoxin analysis from visual check A. flavus infected ear were 56, 14, 1 and 39 ppb in inoculated plot of N1, N2, N3 and N4 but in no inoculated plot had only on N4 plot and no aflatoxin was detected.

Samples from no visually A. flavus infected were 0, 40, 6 and 3 ppb in inoculated plot on N1, N2, N3 and N4, however, nothing was detected inoculated plots.

7) Identification of Insects and the Types of Damage They Inflict on

the Maize Kernel.

Code No. I-1-(1) -F AG/VII/89

One hundred ear samples were taken seven times from August 18 to September 27. A kind of insects appeared in the maize field were observed. Ear samples from damaged and non damaged were examined A. flavus and other fungi. Only 2 samples from the damaged kernels of the ears were found A. flavus at 2% level.

Percentage of damaged ear recorded 2% at beginning and gradually increased to 21 % at last sampling. In contrast, some ears were changed from 84% to 17%.

8) Evaluation of Insect Damage that Occurs under Field Condition

Code No. I-1-(1) -F, B AG/VIII/89

A kind of insect appeared in the field were counted twice a week, 23 times from July 3 to September 18 on furadan applied plot and control plot. Effect of insecticide application was observed on some kind of insect such as corn leaf aphid and ladybird beetle.

No significant response was observed to the insecticide application on aflatoxin contamination and level of toxin was lower than 20 ppb in any detected cases.

9) Relation between Kernel Type and Resistance to Fungus Infection after Inoculation.

Code No. I-1-(1) -A AG/IX/89

This experiment was still going on for preparing the samples. It will be terminated in May.

10) Relation between Environmental Factors and Fungus Infection

Code No. I-1-(1) -J, (b)

Related Code No. III-1-(1)-A+B AG/X/89

Numerous ear samples were taken from maize field, storage place and farmer fields from July to December.

On concerning seasonal samples, percentage of samples observed A. flavus in December (36.7%) was much higher than rainy season (0-9.6%). Fine days (15%) appeared more fungus than cloudy or rainy days (3% and 0%). Wind speed had some effect A. flavus appearance in fields, sampling at over 5.0 m/sec (27.3%) had more than 1.0 - 4.9 m/sec (10.2%) and less than 0.9 m/sec (10.0%).

11) Monitoring Aflatoxin Occurrence in the Major Maize Production Areas.

Code No: I-1-(1) -J, I-3-(1) -A, AG/XI/89

Ear samples were collected from major maize production provinces, Loei, Petchabun, Nakhon Ratchasima, Nakhon Sawan and Lopburi. Total 42 samples consisted of 30 ears were collected through five Field Crop Research Stations and Centers of respective areas. Interpretation for the result of A. flavus infection and aflatoxin contamination are undertaking.

2. Post Harvest Section

< Corn sheller improvement >

- 1) Analysis of the relation between mechanical damage to kernel, machinery design, operational condition and moisture content of maize (1989)

Code No. II-3-(2)-A

Effect of the types of cylinder, mechanical design, operational conditions, moisture content of maize on mechanical damage of kernels was investigated. Seven types of cylinder were tested under different moisture content (16-30%) of maize and different peripheral speed (5-15m/s). Rectangular spike tooth cylinder sheller with 52 mm concave clearance is best among all for the prototype model of corn sheller improvements.

- 2) Study on the relationship between damage on kernel, kernel moisture content and aflatoxin contamination. (1989-1990)

Code No. II-1-(2)-B

Related code No. III-1-(2)

Manually shelled maize with various moisture content (15, 17, 22, 30%) was mixed with impurity (3%, crushed corn cob) or damaged maize (3, 6%) and was stored under humid condition.

After 14 days of storage, all the samples were contaminated by aflatoxin but lower at 17% of moisture level and little at 15%, and highly contaminated at 22% level. Samples were severely infected by A. flavus at 30% level of moisture content.

< Moisture Meter Improvement >

- 3) Improvement and development of moisture meter (1987-1991)

Code No. II-2-(2)

Two types of moisture meter were investigated to determine ear maize moisture content: one with knife blade electrodes and another one with plier type electrodes. Determined values by the former are not stable. Moisture meter with plier type electrodes showed more correlation than former one. But further improvement is necessary to develop an ear maize moisture meter.

- 4) Calibration test of established moisture meter (1988-1990)

Code No. II-2-(2)

This year four moisture meters; 'CTR-160', 'Dole model 400', 'Digital Grain Moisture Meter' and 'Multi Grain Portable' were calibrated to determine moisture content of Suwan 1, Suwan 2, Suwan 3 and KU hybrid maize from various area.

Correlation was rather low between detected values by these meters and determined values by the standard oven method, on the contrary to last year's experiment.

- 5) Standardization of standard oven method (1988-1990)

Code No. II-2-(2)

Effect of predrying in accelerating drying procedure, in determination of moisture content was evaluated. Samples were dried for 4 hrs at 130 °C with or without drying varying sample weight and compared with the standard oven method of 72 hrs 103 °C.

Determined values with predrying are more fluctuated than those without predrying. Quick determination method can not be designed by

predrying.

< Chemical Treatment >

- 6-1) Urea treatment of maize to control Aspergillus spp. and so prevent aflatoxin contamination
Code No. II-3-(2)-C-(a)

For farm scale experiment each 400 kg of ear maize was treated and stored in open and close system and another 400 kg for control.

Ammonia concentration increased more rapid in closed system than in open one and also higher in concentration at its peak, though the transaction afterward is almost similar. Discoloration was observed specially in closed system.

- 6-2) Sulfur dioxide supplemented storage of high moisture maize.
Code No. II-3-(2)-C-(a)

Ear maize was fumigated in corn cribs by burning sulfur cakes. Each one ton of ear maize was treated with 0.05% of sulfur dioxide in weight all in one or divided in three times at one week intervals and covered by plastic films for one day after each treatment. Samples were stored for six weeks. Samples were extracted every one week to determine moisture content.

Data for sulfur dioxide supplemented storage are under processing, but no mold nor discoloration was visually observed.

- 7) Ammonia and sulfur dioxide supplemented ambient air drying of high moisture maize in Thailand (1988-1990)
Code No. II-3-(2)-C-(a)

Each 500 kg of shelled maize was stored in a flat bed drier with ambient air flow rate of 1.0 m³/min. For TAP (Trickle Ammonia Drying Process) 0.5% sample weight of ammonia was supplemented at the rate of 15 l/min. at one time or divided in three times at three days intervals. For TSDP (Trickle Sulfur Dioxide Drying Process) 0.5% of sample weight sulfur dioxide was obtained by burning sulfur cake and treated in one time. Discoloration was examined.

In TAP mold was observed after few days in three times supplement and in TSDP mold was observed three days after treatment. Discoloration was observed in TAP.

< Storage and drying >

- 8) Estimation on increase of damaged kernel ratio during handling (1989-1990)

Code No. II-1-(2)-C-(a), II-1-(2)-B-(c)

Related code No. III-1-(2)

Ear maize sample were (1) not selected, (2) selected by farmers and (3) selected by the researchers. All ear maize samples were kept in gunny sacks and stored for 7 days on a pallet under natural environment.

After 7 days of storage, number of ear maize infected by A. flavus was only 4.2% in the none selection sample while 35.4% and 45.1% in the selected sample by farmers and by researchers respectively. This seem to be caused by damage on seed coat by selection procedure on rough surface of concrete floor. Specially in case of selected sample, most of the A. flavus infected ear had seed coat damage.

9) Study on the improvement of storage facilities for farmers.

Code No. II-3-(2)-C-(c)

Experimental models of the elevated-floor and ground-floor corn cribs with 2.6m x 2.6m x 1.8m (D x W x H) were constructed and each 5.1 tons of maize with 29% kernel moisture content were stored in them.

During storage, temperature and relative humidity of each 50 cm layer inside and at the bottom of storage were investigated. After one month's storage period, moisture content of samples at each measuring point is determined.

10) Development of simple drying method (1989-1991)

Code No. II-3-(2)-B-(b)

Air flow resistance of ear maize bulk and equilibrium moisture content under constant temperature and humidity were investigated as basic data.

Two types of experimental drying unit with solar heat collecting chamber and diesel engine are fabricated. In the experiment which use engine exhaust heat, drying rate was high enough but engine exhaust was gas mixed into air in the drying chamber making maize kernel dirty.

11) Allowable duration for delayed drying in the post-harvest process of maize. (1989 - 1990)

Code No. II-1-(2)

Related code No. III-1-(2)

Each 360 kg of ear maize 'Suwan 1' was kept in a flat bed dryer for 0, 1, 2, 3, 5, 6, 7, 9 and 14 days and then was dried by ambient air till it reached equilibrium. Aflatoxin contamination was determined before and after drying.

Drying rate was rather high because of enough air flow rate and dry ambient air. This year, aflatoxin contamination was a little bit higher after drying than before drying, but still in low level, for this year experiment was done after rainy season finished.

3. Microbe Section

- 1) Studies on the population of A. flavus and aflatoxin contamination of maize cultivated under different conditions.

Code No. III-1-(1) -A & B

Related Code No. I-1-(1) -A ~ J

This subject was studied as a joint research project by the Microbe and Agronomy Sections. The studies on the population of A. flavus in maize, air, soil etc. and aflatoxin contamination of maize have been carried out. Those samples were collected by both groups. The Microbe group has been carrying out the microbiological and analytical studies on the samples. The results are reported in the Agronomy Section.

- 2) Studies on aflatoxin contamination in maize shelled by different types of corn sheller under various operating conditions and moisture content.

Code No. III-1-(2)

Related Code No. II-1-(2) -B-(a) & (b)

This subject was studied as a joint research project by the Microbe and Post-Harvest Sections. The studies on aflatoxin contamination in maize under the various shelling process has been carried out. The maize samples were prepared and collected by the Post-Harvest group. The Microbe group has analyzed aflatoxin content in the samples.

- 3) Studies on A. flavus and aflatoxin contamination routes in maize.

Code No. III-1-(3)

The observation sites for the confirmation of A. flavus infestation route were set up from north to central maize cultivation area. The samples of air and soil in the maize fields, and ear and shelled maize, air, soil and dust in the farmers and middleman's warehouses were collected for microbiological studies. The high population of A. flavus was observed in the soil samples of fields and both warehouses. Freshly harvested ear maize and shelled maize were more clean than long term stored maize. The spore density of A. flavus in the air was very high near by working shelling machine. The shelling process is presumed as a main infestation route by A. flavus.

- 4) Aflatoxin producing ability of the various strains of A. flavus .

Code No. III-1-(1)

One hundred twenty one strains of A. flavus isolated from various samples, such as maize, air, soil and dust in the warehouses etc. were tested on aflatoxin producing ability. Among them about 70% strains were aflatoxin producer and 30% strains were sclerotium formation type. The relation was not clear between aflatoxin producing ability and sclerotium formation.

- 5) Aflatoxin content in stored maize by means of Immunoassay (ELISA).

Code No. III-2-(1) -B

Newly developed analytical method for aflatoxin, ELISA using monoclonal antibody with high specificity against AFB₁ was applied to stored maize samples. It is recognized that ELISA is simple and rapid analytical method and has high sensitivity for AFB₁. However it is costly.

- 6) Water activity of maize and growth of A. flavus.

Code No. III-1-(3)

The maize samples with various moisture content were prepared and measured Water activity (A_w). At the same time, the growth of A. flavus on the maize samples was observed. The critical A_w of maize for growth of A. flavus seems to be ca. 0.85 (nearly 18% moisture content).

7) Equilibrium moisture content of maize and growth of A. flavus.
Code No. III-1-(3)

Wet and dry maize samples were kept in the constant humidity prepared by several kinds of saturated salt solution. Moisture content in maize were periodically determined. By using the data, the figure of maize's equilibrium moisture content curve was made.

B. Tentative Plan for the Project Activities in the 1990 Japanese Fiscal Year

1. Assignment of Japanese Experts

The schedule for the assigned period of Japanese experts is shown in Annex I, of the Progress Report. (Japanese side)

1) Long Term Experts

At present, five experts scheduled under the R/D and Tentative Schedule of Implementation (TSI) have been assigned. This year three of them will come to the expiration of their assigned period. If a vacancy occurs, JICA will arrange to dispatch a successor, considering the smooth implementation of the project.

2) Short Term Experts

The short term experts requested for the project are listed below. JICA will dispatch the experts, considering the requests from Thailand, budget, and so on.

- a) Microbe Section < Aspergillus spp. ecology, 3 months >
- b) Microbe Section < Aflatoxin analysis, 3 months >
- c) Agronomy Section < Photosynthesis, 3 months >
- d) Post-Harvest Section < Improvement of corn sheller, 5 months >
- e) Agronomy Section < Simulation, 3 months >
- f) Agronomy Section < Insect damage & Control, 4 months >
- g) Microbe Section < Microbiology, 1 month >
- h) Post-Harvest Section < Moisture meter for ear maize, 3 months >
- i) Post-Harvest Section < Improvement of drying method, 3 months >
- j) Post-Harvest Section < Ammonia & chemical treatment, 3 months >

2. Training of Thai Counterparts in Japan

The schedule for the training of Thai counterparts, is shown in Annex II, of the Progress Report (Japanese Side). The counterparts in the fields below are requested for training. JICA will accept the counterparts for training, considering the requests from Thailand, budget, and so on.

- a) Division of Agricultural Engineering < one person, 4 months >
- b) Division of Plant Pathology and Microbiology < two persons, 3 months >
- c) Planning and Technical Division < one person, 3 weeks >

3. Provision of Equipment

Equipment worth 60 million Yen will be requested for the project as shown in Annex III, of the Progress Report (Japanese Side). JICA will provide the equipment, considering the request from Thailand, budget, and so on.

4. Research Plan

The Main subjects which will be studied in the 1990 Japanese physical year are as follows:

< Agronomy Section >

- 1) Varietal comparison of maize kernel moisture and its variation according to different times of harvesting.
Code No. I-1-(1) -A, G (1988 - 1990)
- 2) Study on the relationship between environmental conditions and aflatoxin incidence in maize.
Code No. I-1-(1) -B, G, J-(b) (1988 - 1992)
- 3) Effects of different harvest methods, moisture conditions and storage periods on aflatoxin contamination in maize.
Code No. I-1-(1) -H, G
- 4) Effect of plant density and nitrogen application on aflatoxin contamination in maize.
Code No. I-1-(1) -D (1988 - 1989)
- 5) Effect of crop rotation on Aspergillus spp. in the soil.
Code No. I-1-(1) -C, B
- 6) Effect of nitrogen regarding prevention of aflatoxin contamination by the inoculation method.
Code No. I-1-(1) -D (1989 - 1990)
- 7) Identification of maize insects and the types of damage they inflict on the maize kernel.
Code No. I-1-(1) -F, B (1989 - 1990)
- 8) Evaluation of insect damage that occurs under field conditions.
Code No. I-1-(1) -F, B (1989 - 1990)
- 9) Relation between environmental factors and fungus infection.
Code No. I-1-(1) -A
- 10) Large scale practices on concerning harvest methods and aflatoxin occurrence in maize.
Code No. I-3-(1) (1990 - 1991)
- 11) Basic agronomical study for developing the simulation model of maize productivity and its quality.
Code No. I-1-(1) -B, E, G, J -(b) (1990 - 1992)
- 12) Reduction on A. flavus infection and aflatoxin production by potassium application.
Code No. I-1-(1) -D (1990)

< Post-Harvest Section >

- 1) Trickle ammonia drying process and trickle sulfur dioxide drying process for maize.
Code No. II-3-(2) -C-(a) (1988-1990)
- 2) Study on moisture determination of maize:
 - 2-1. Performance test of newly developed moisture meter for ear maize.
Code No. II-2-(2) (1988-1990)
 - 2-2. Calibration of moisture meter.
Code No. II-2-(2) (1988-1990)
 - 2-3. Study on standardization of the oven method in Thailand.
Code No. II-2-(2) (1988-1990)
- 3) Corn sheller improvement.
Code No. II-1-(2) -B-(a) & (b), II-3-(2) -A (1988-1991)
- 4) Delayed drying of the maize for safe storage.
Code No. II-1-(2) -C-(c) (1989-1990)

- 5) Study on improvement of storing facilities for farmers.
Code No. II-3-(2)-C-(c) (1989-1991)
- 6) Ammonia treatment for preservation of maize.
Code No. II-3-(2)-C-(a) (1988-1990)
- 7) Study on the effect of grading ear maize on storage quality.
Code No. II-1-(2)-C-(a) (1989-1990)

< Microbe Section >

- 1) Correlation between cultural practices and aflatoxin contamination.
Code No. III-1-1-(1)
- 2) Correlation between post-harvest storage/processing and aflatoxin contamination.
Code No. III-1-(2)
- 3) Physiological and ecological studies on A. flavus including the infection routes.
Code No. III-1-(3)
 - 3-1. Studies on the infection routes of A. flavus and aflatoxin contamination during pre- and post-harvest of maize.
 - 3-1-1. Studies on the population of A. flavus in the maize plant, air and soil in the cultivation area.
 - 3-1-2. Studies on the population of A. flavus and aflatoxin content in trade maize.
 - 3-2. Physiological and ecological studies on A. flavus in Thailand.
 - 3-2-1. Study on aflatoxin producing ability of the various strain from maize, air and soil in the field.
 - 3-2-2. Studies on the time to keep various moisture content of maize without aflatoxin contamination.
 - 3-2-3. Study on the contamination of A. flavus from insect damaged cobs.
- 4) Development of simple and rapid analytical method of the aflatoxin content in maize.
Code No. III-2-(1)
 - 4-1-1. Improvement of the mini-column method for the aflatoxin content in maize.
- 5) Aflatoxin prevention by controlling A. flavus.
Code No. III-3-(3)
 - 5-1-1. Control of A. flavus and aflatoxin contamination of various moisture content of maize in anaerobic condition.

C. Others

1. Area Expansion of the Center

The area expansion of the center was requested from Thai side.

2. Medical Examination

In Japan, the mycotoxin researchers have medical examinations twice a year. Therefore, the Guidance Team recommend that JICA should consider providing the budget for MQIRC Japanese experts engaging aflatoxin research works to have medical examinations at least twice a year.

It is our pleasure to say that the relationship between Thai side and Japanese side is well established and the project runs successfully. We hope that both side will continue mutual cooperation in order to promote this project. Lastly, we appreciate your kind cooperation during our stay in Thailand.

IV. ABSTRACT OF THE
EXPERIMENT RESULTS

AGRONOMY SECTION

THE MAIZE QUALITY IMPROVEMENT RESEARCH CENTER PROJECT

AG/I/89

Varietal Comparison of Maize Kernel Moisture and its Variation according to Different Times of Harvesting
Code No. I-1-(1)-A, G (1988 - 1989)

Objectives

The object of the study is to examine the varietal characteristics on kernel moisture content shown at different times of harvesting. Moisture distribution as well as its variation by ear and by plot are also to be examined among the varieties.

Materials and Methods

Suwan 1 (SW1), Suwan 2 (SW2), KU2602 (KU) and Suwan 3 (SW3) were been utilized as variety entries. The first three varieties are the same as 1988 and Suwan 3 was decided as the new entry.

The varieties were harvested 4 rows of 8 rows plot at 35(H1), 45(H2) and 55(H3) days after 50% silking for observing the agronomical feature. Kernel moisture was measured by CTR-800 manufactured by Shizuoka Seiki Co.Ltd. in Japan. The measurement was carried on every day from 35th day after 50% silking to 64th day. Ears for the measurement were taken from H1 plot for the days from 35th day to 44th day after 50% silking, and from 45th day to 54th day from H2 plot and from 55th day to 64th day from H3 plot.

5 ears were taken every day from each plot, then morphological observation was practiced. Randomly 3 ears were selected for measure the kernel moisture content and the other 2 ears were observed moisture content of grain and cobs by using oven.

During the growth, germination count, Tasseling count, silking count and pre-harvest agronomical study were practiced.

Summary of the Result

Overall germination was 90.6% at one week after planting and 92.1% at 2 weeks. Plant stand after thinning was 99.5% of planned density on entire plot and variety average were 99.9%, 98.7%, 99.7% and 100% on SW1, SW2, KU and SW3 respectively. It was satisfactory level of plant stand.

Tasseling and silking date of 50% emerge were as follow:

	50% Tasseling	50% Silking
SW1	Jul. 13	Jul. 16
SW2	Jul. 10	Jul. 12
KU	Jul. 12	Jul. 17
SW3	Jul. 15	Jul. 19 (probably 18th but no observation this day)

Yield at each harvest (kg/rai) were as follow:

	H1	H2	H3	AVG
SW1	930.8	968.4	1079.8	993.0
SW2	837.7	839.7	851.0	842.8
KU	645.4	962.0	863.5	823.6
SW3	983.5	845.0	817.9	882.1

Plant stand at harvest were 99.9%, 97.5%, 97.0% and 96.0% of planned density on SW1, SW2, KU and SW3 respectively. Those are quite good stand. Plant height of variety average were 204.6cm, 178.7cm, 179.9cm and 195.9cm and ear height were 102.1cm, 83.3cm, 88.9cm and 98.9cm on SW1, SW2, KU and SW3. Husk cover by CIMMYT method (1:excellent to 5:very poor) was utilized for evaluation. Variety averages were 1.628, 1.599, 1.679 and 1.534 on SW1, SW2, KU and SW3.

Those statistical significance was not yet examine.

Yield of KU was low at harvest HI due to less No. of ear harvested and small ear size at one replication comparing to the other replication.

Statistical analysis was not done yet. Generally, SW1 and SW2 were almost the same level but KU was about 20% lower compare to the year 1988.

Moisture content of kernel from 35 days to 64 days after 50% silking are to be discussed.

Average of 3 ears each measured days were considered here.

Initial average moisture content(MC) of kernel on SW1 was around 36.7% and the final MC at 64 days was 24.3%. On SW2 was 35.8% at 35 days and down to 20.5% at 64 days. On KU2602 was 36.9% to 23.8% and 35.7% to 24.5% on SW3.

Initial MC on SW2 and SW3 were about 1% lower than SW1 and KU, however at 64 days, level of MC of SW2 was 3-4% lower than the other three varieties.

As observed 1988, SW1 and KU seems to behave similar moisture feature compare to SW2.

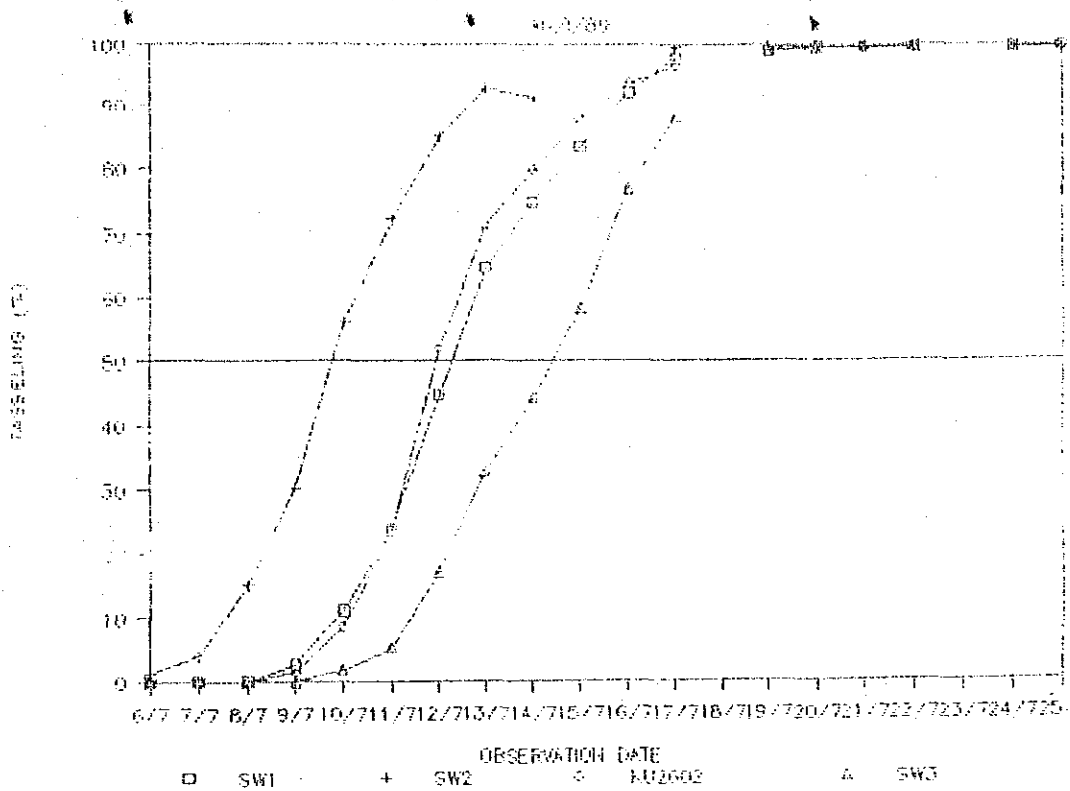
Graphs shown here are daily average MC and calculated upper and lower range from Standard deviation. Then linear regressions are shown each data to observe possible range of MC at harvest date. However the ranger of these graphs are at early days, due to the capacity of the CTR-800 which measures the range of lower than 40%MC. Therefore those days when the kernel more than 40%MC was calculated as 40%.

Future Plan

Variety comparison of kernel MC will be continued with newly released varieties, such as SW3 which is from Pakchong, Kasetsart University and NS1 which is from Nakhon Sawan, DOA, to compare with SW1 as a check.

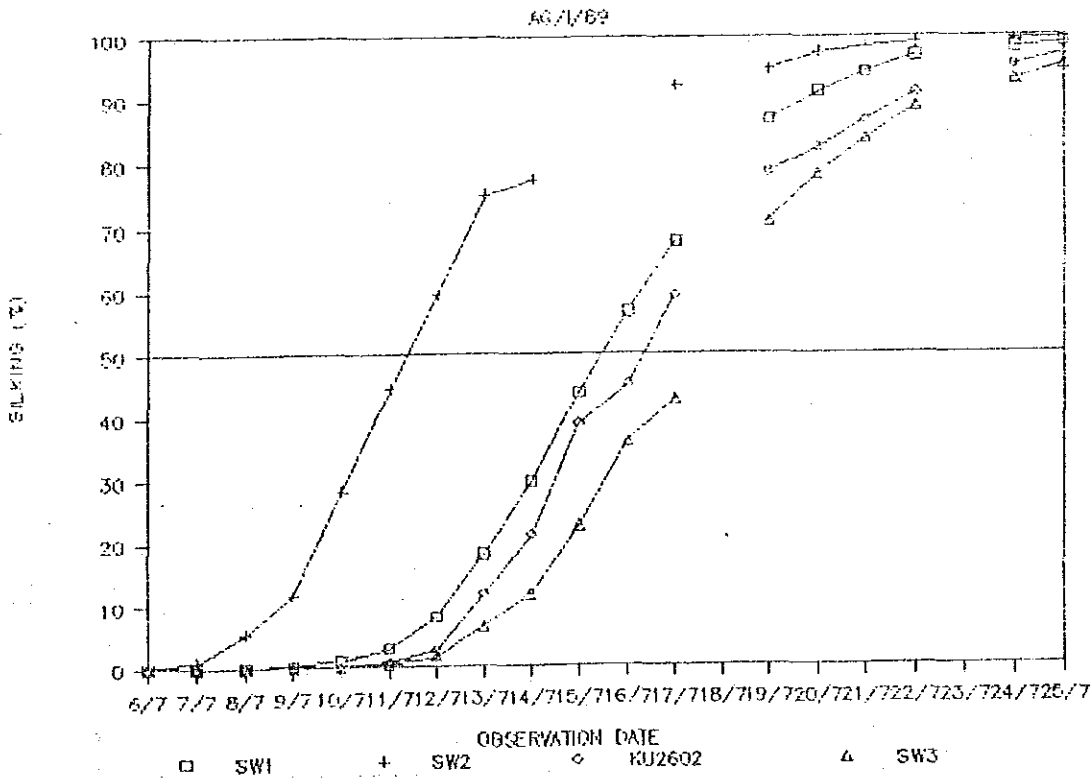
TASSLING OF 4 VARIETIES

AG/69

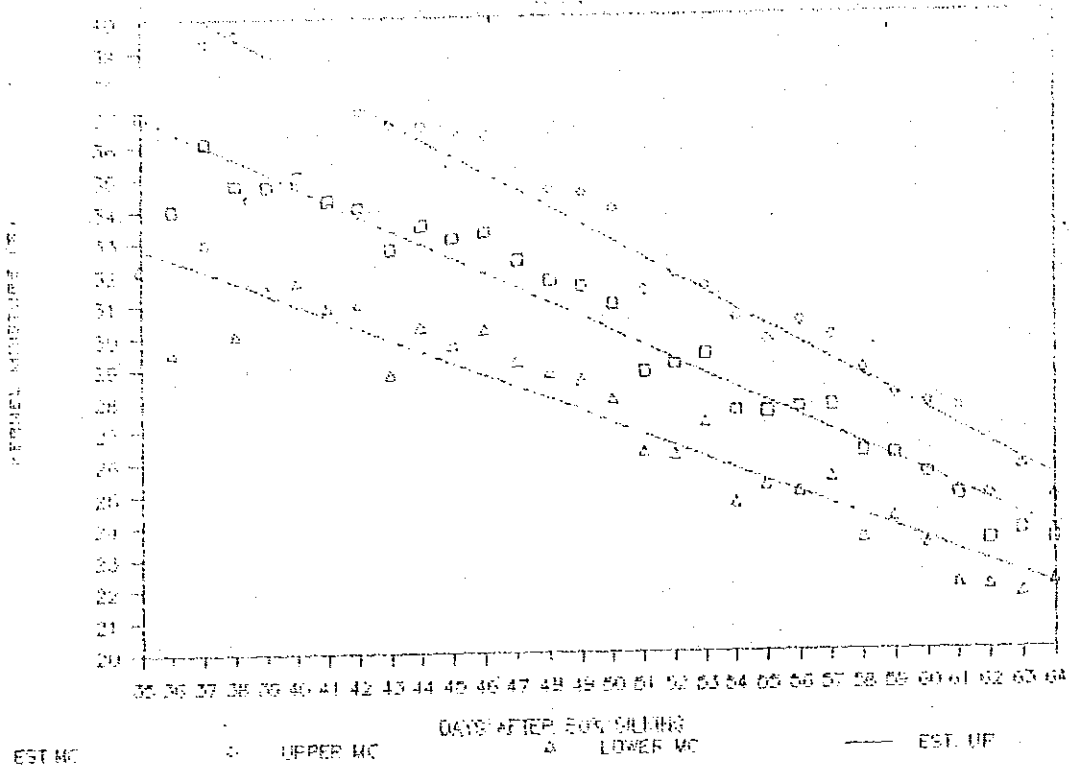


SILKING OF 4 VARIETIES

AG/69

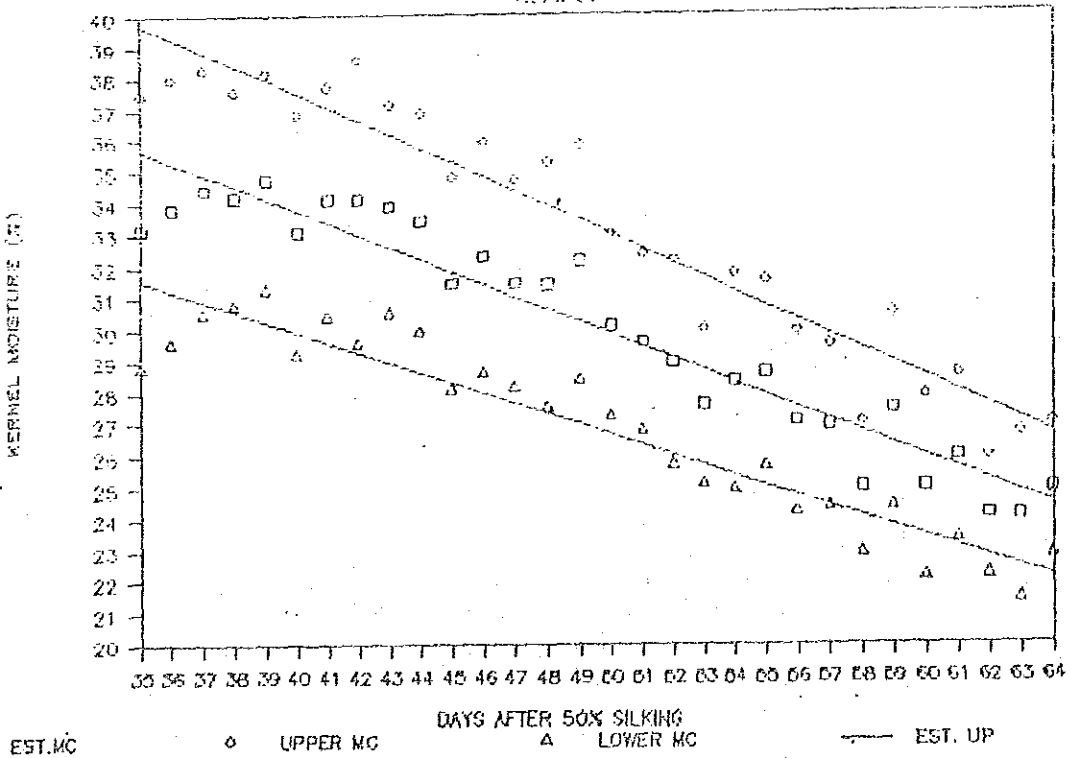


KERNEL MOISTURE CONTENT OF SUWAN 3

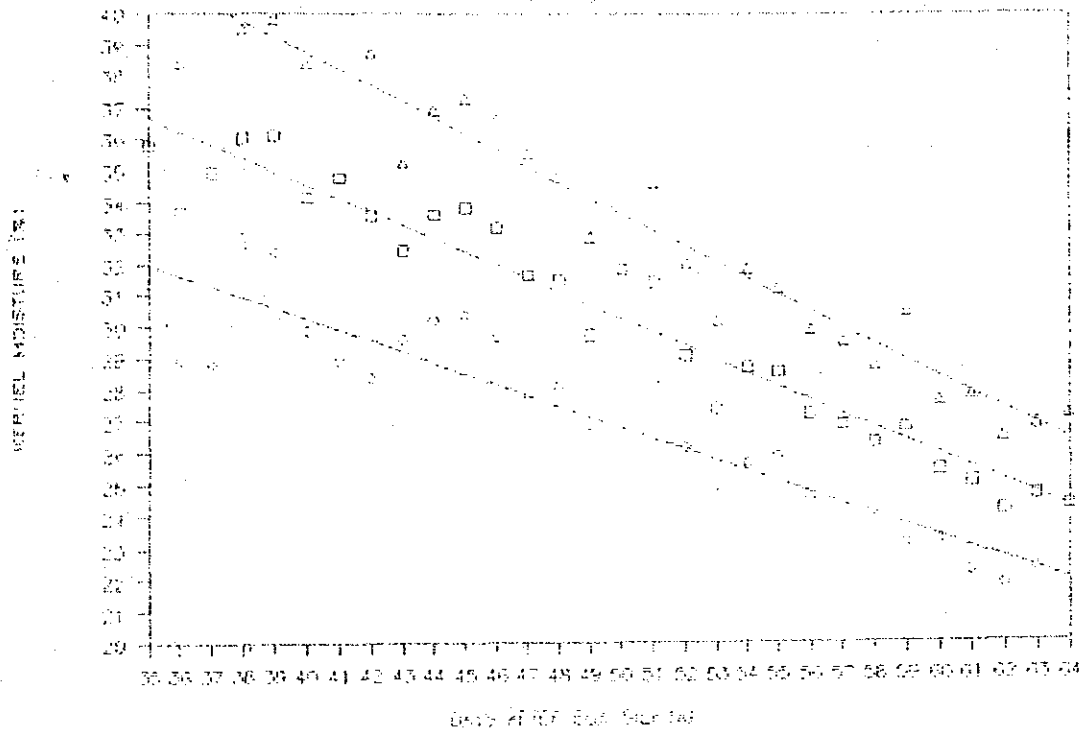


KERNEL MOISTURE CONTENT OF SUWAN 3

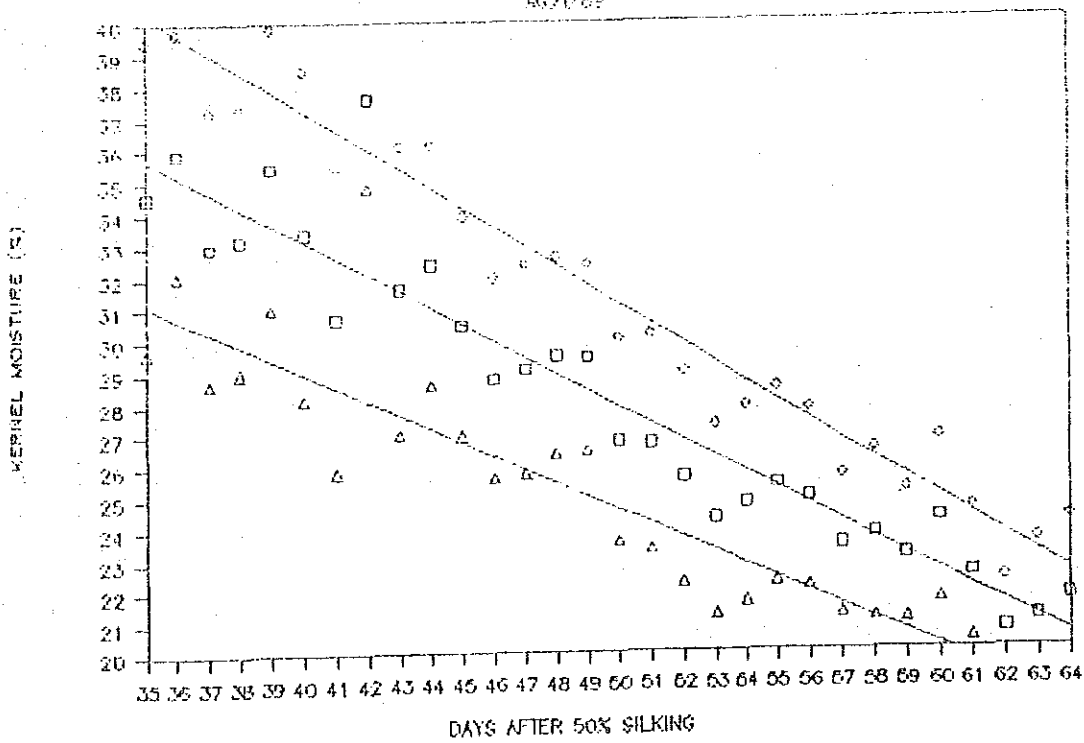
AB/1709



KERNEL MOISTURE CONTENT OF SUWAN 2
50/1/69



KERNEL MOISTURE CONTENT OF SUWAN 2
50/1/69



AG/II/89

Long Term Study on the Relationship of the Environmental Conditions that Cause Aflatoxin Incidence in Maize
Code No. I-1-(1)-B, E, G, J-(b) (1988 - 1992)

Objectives

The object of the study is to clarify the relation between the environmental conditions and productivity and quality of maize.

Materials and Methods

Plots were treated by planting time and water supply. Planting was practiced ten times, every other week, from April 14 until September 1. Water supply was 1)rainfed and 2)adjusted irrigation. Irrigation was adjusted to supply water when the rainfall in the week was lower than the average rainfall over the last ten years. Adjustment was made to bring the water supply up to the same level.

Plot size was 12m x 12m. Spacing was 75cm x 50cm with 2 plants per hill. Maize was harvested 105, 115 and 125 days after planting and then kept in store for 2 months. Sample for aflatoxin analysis were taken at harvest and every other week during storage.

The study will be carried out for five years.

Summary of the Results

Rainfall during the experiment period was shown at (Fig.1). Rainfall distribution in 1989 was not favorable, especially from April to June was serious.

In the early season of the planting had difficult condition to keep maintain the plant stand especially in the rainfed condition by poor germination due to poor rainfall distribution. Germination percentages were 65.1% with 15.8 of Standard Variation (STD) on irrigated condition and 49.9% with 26.7 (STD) on rainfed condition across planting times. Plant stand to planed plant density after thinning were 91.0% with 11.7 (STD) on irrigated condition and 74.2% with 29.6 (STD) on rainfed condition.

Agronomical information before harvest was recorded. Plant stand percentage to planed density were 80.6% on irrigated condition (IR) and 60.4% on rainfed condition (RF). Plant height and ear height on IR were 173.6cm and 88.1cm and 143.2cm and 72.3cm on RF respectively.

Lodging plants were counted. No. of lodged plant at stem were 10.2 on IR and 9.0 on RF in average on each harvest. Lodged at bottom of plant (Root lodging) were 15.8 on IR and 30.3 on RF in average.

No. of insect damage by counting the pinhole on the ear and stem were counted. Average No. on the ear were 11.5 and 8.8 on IR and RF respectively. 8.3 and 6.9 on the stem were observed on IR and RF respectively.

Tendency on planting time or harvesting time for the damage were not clearly recognized.

Husk cover evaluation by CIMMYT method that is 1 as excellent to 5 as very poor, was recorded.

Average across planting time and harvesting time was 1.958 on IR and 2.002 was on RF.

Grain moisture content (MC) percentage was measured by using "Stainlite" moisture meter. (Fig.2)

Average MC at harvested at 95 days (H1) was 33.6% on irrigated condition and 34.6% on rainfed condition. 28.2% and 28.7% on IR and RF was observed on 105 days harvest (H2) and 22.6% and 22.8% on IR and RF was recorded on 115 days harvest (H3).

On April 28 planting extremely lower than other data was observed without knowing the reason. Late planting tend to decrease MC according to the later harvesting time. It may be the reason that lower yield and dry atmosphere.

Average yield of both treatment was 548.7kg/rai and 304.8kg/rai on IR and RF. Very low yield were recorded at early planting of RF plots. (Fig.3)

On June 23 and July 7 plantings were showed higher yield on RF plots, although No. of plant harvested on June 23 plots were much more, and slightly more on July 7 plots on IR plots. MC were measured when sampling were practiced. (Fig. 4-1~3)

Although the results were not completed yet, H1 (Fig.4-1) showed 33.6%, 26.2%, 20.0% and 14.1% on IR, and 34.7%, 24.9%, 18.5% and 13.6% on RF for at harvest, 2 weeks after harvest, 4 weeks and 8 weeks respectively.

On H2 (Fig.4-2) were 28.2%, 22.3%, 18.0% and 14.4% on IR and 28.7%, 20.6%, 16.0% and 14.0% on RF.

On H3 (Fig.4-3) were 22.6%, 18.8%, 16.2% and 13.9% on IR and 22.8%, 16.9%, 14.7% and 12.7% on RF.

None of the sample detected Aflatoxin at harvest in both IR and RF samples.

Toxin analysis was carried out of at the MQIRC Center at Bangkok (Fig.5-1~3). More than 2,000ppb sample were observed from 2 samples of IR H1 on 4 weeks after harvest.

When harvested early (H1) at 2 weeks already Toxin were detected, however on H2 higher level (more than 20ppb) were detected at 4 weeks and H3 were at 8 weeks to the level of more than 20ppb.

Generally, maize produced IR condition had more sample were detected with higher level.

Fig.1

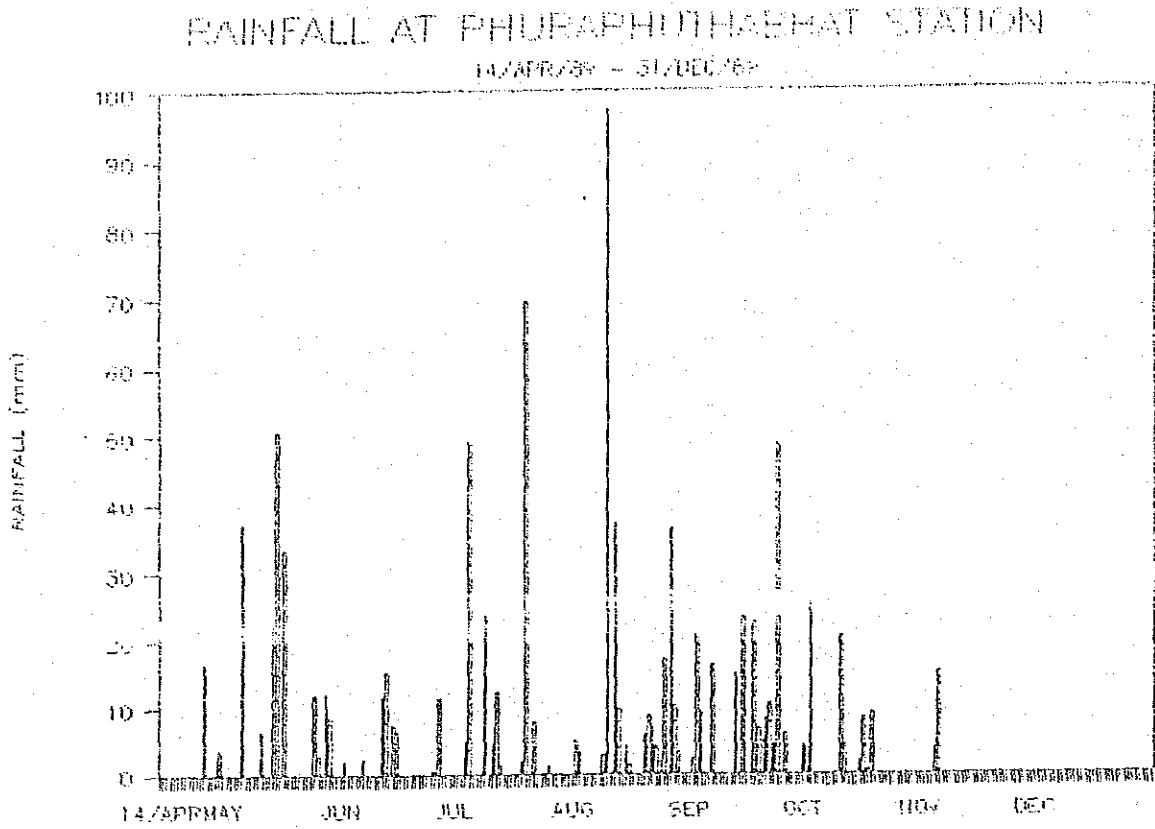
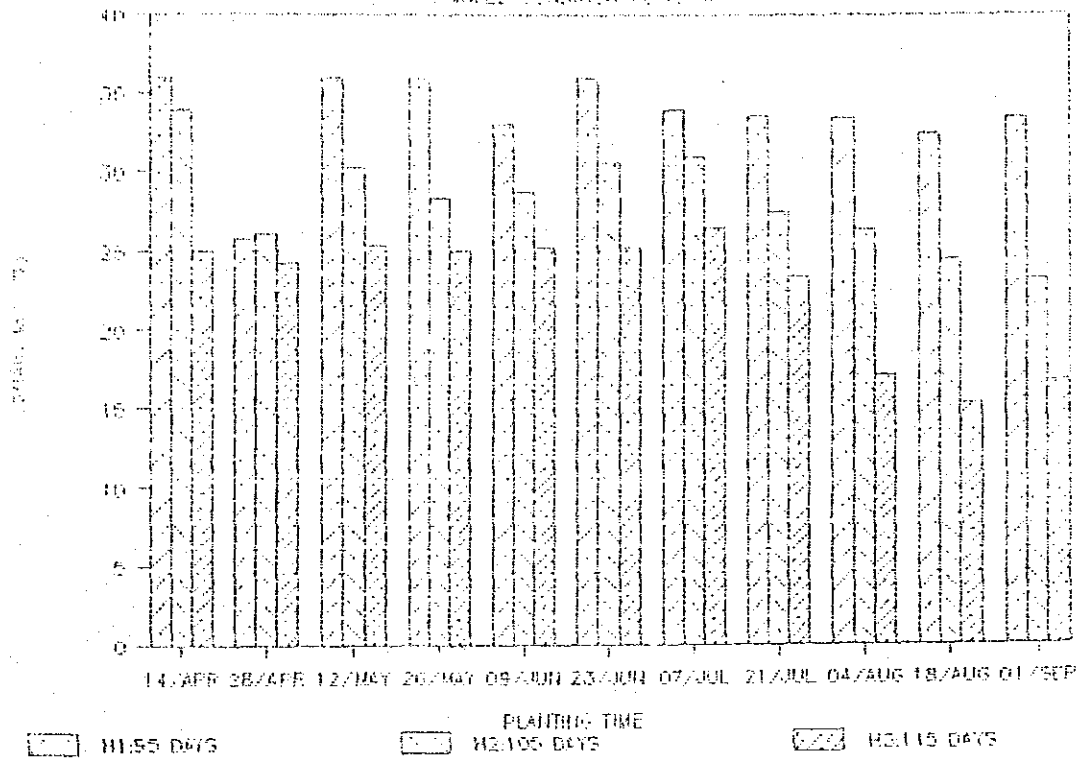


Fig. 2

GRAIN MC (%) AT DIFFERENT HARVEST TIME

RAINFED CONDITION AS/71/79



GRAIN MC (%) AT DIFFERENT HARVEST TIME

RAINFED CONDITION AS/71/79

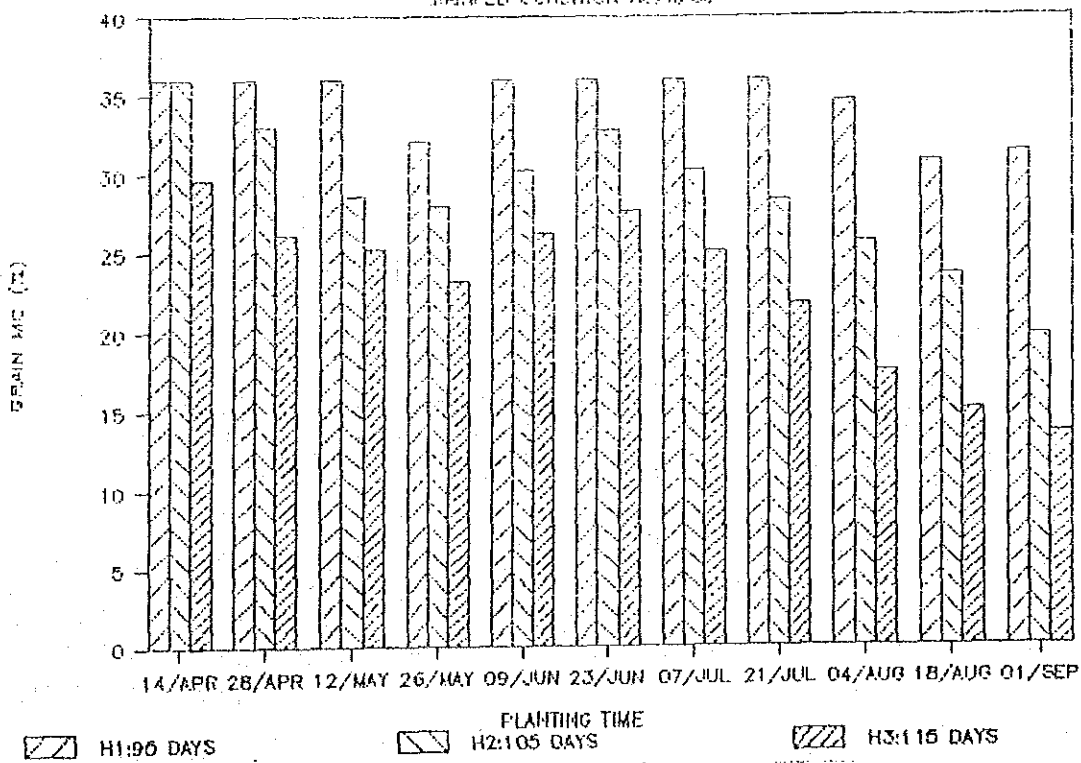


Fig. 3

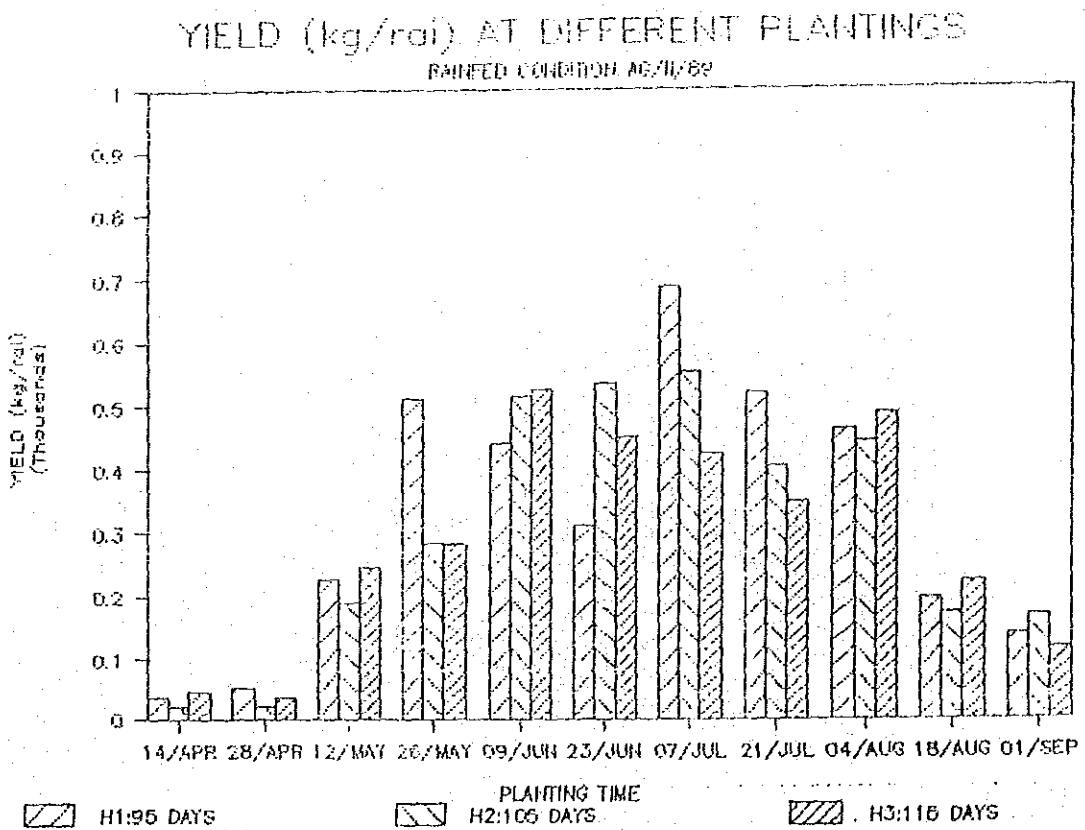
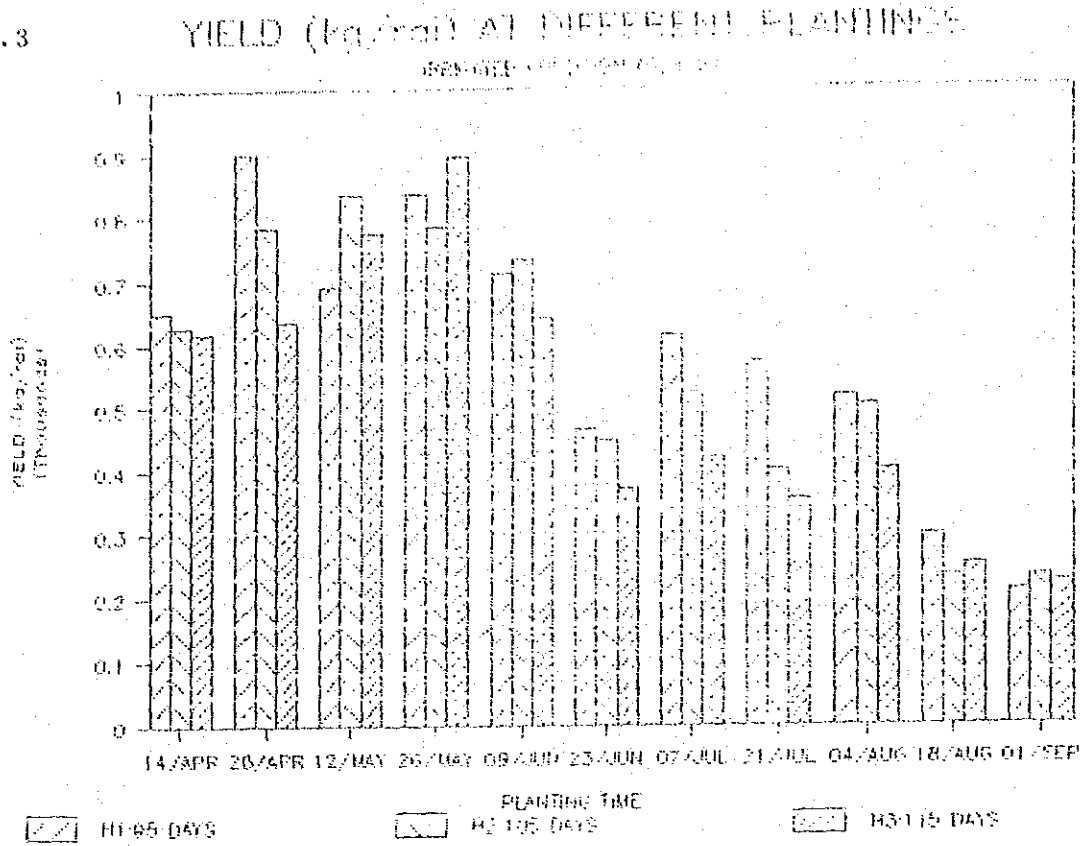
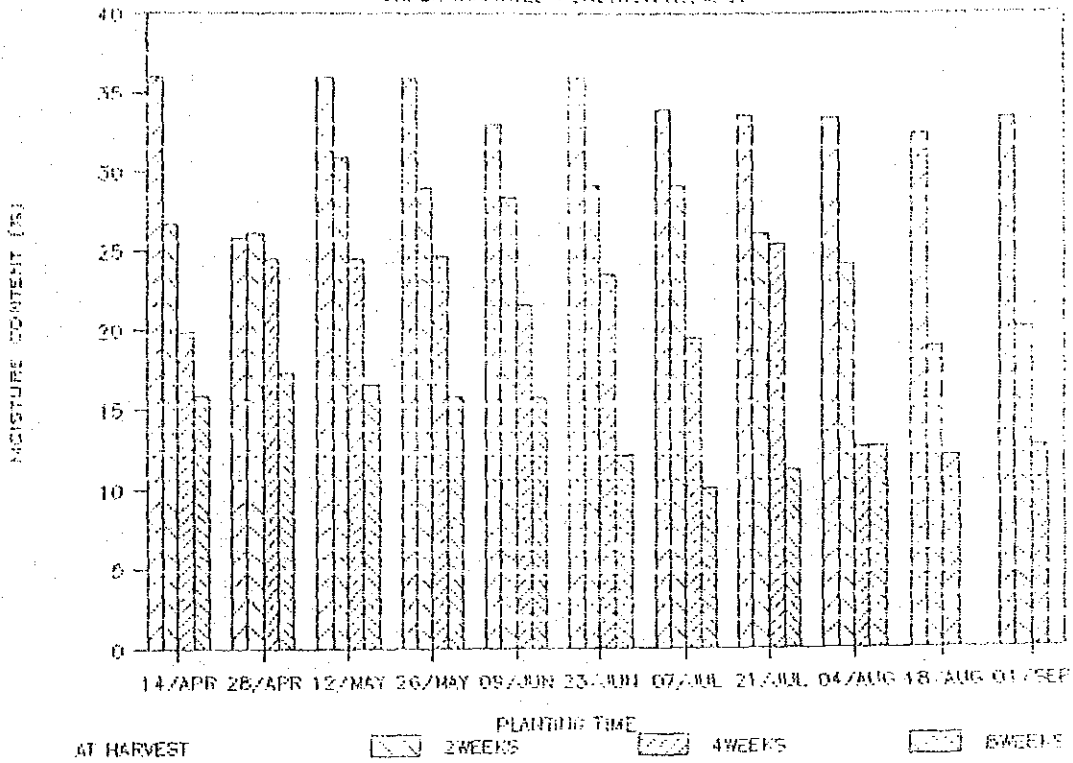


Fig. 4-1

MC(%) AT SAMPLING ON 95 DAYS HARVEST

UNDER IRRIGATED CONDITION AG/II/69



MC(%) AT SAMPLING ON 95 DAYS HARVEST

UNDER RAINFED CONDITION AG/II/69

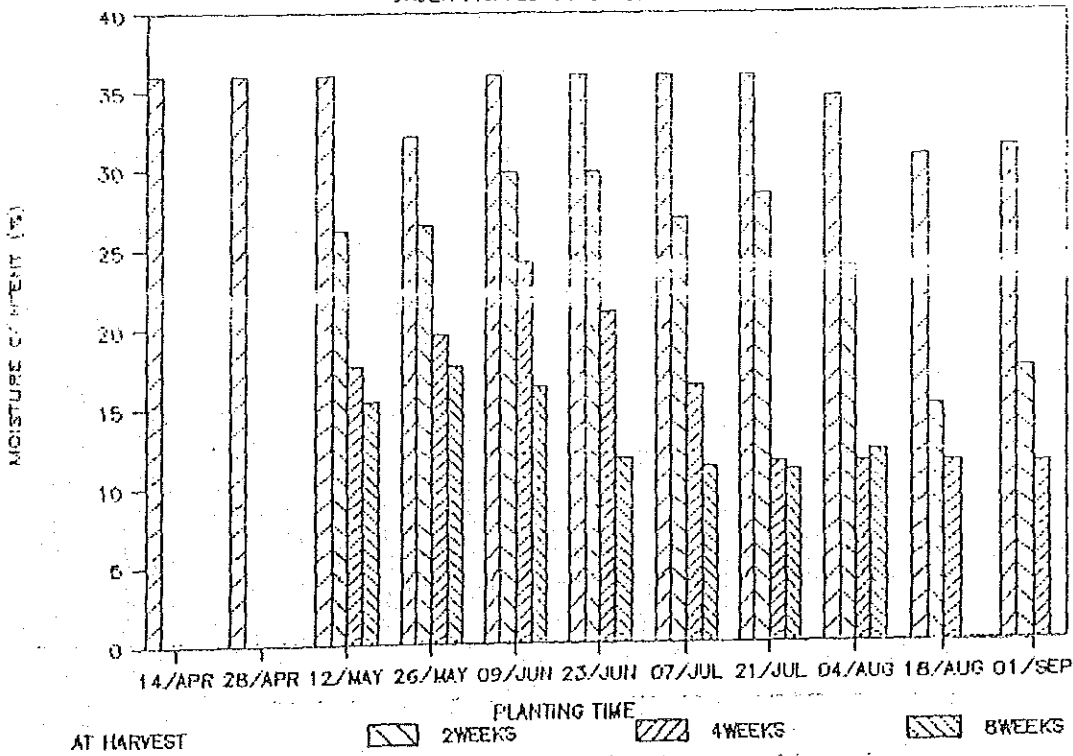
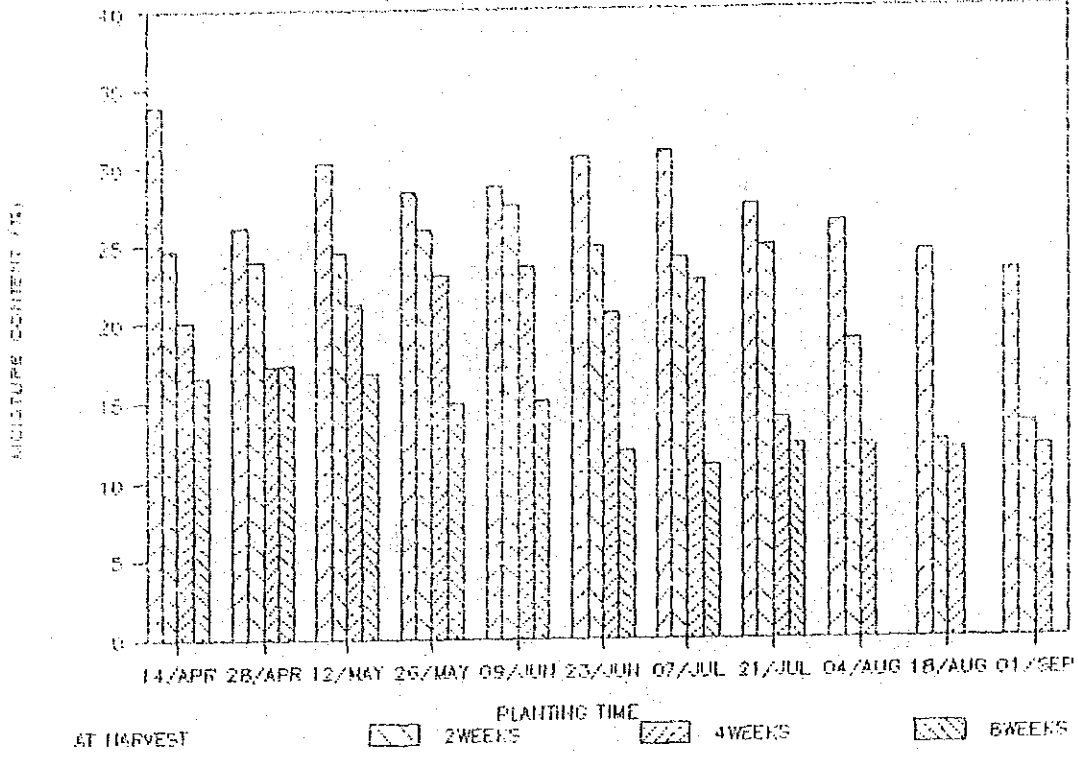


Fig. 4-2

MC(%) AT SAMPLING ON 105 DAYS HARVEST

UNDER IRRIGATED CONDITION AG/PL/89



MC(%) AT SAMPLING ON 105 DAYS HARVEST

UNDER RAINFED CONDITION AG/II/89

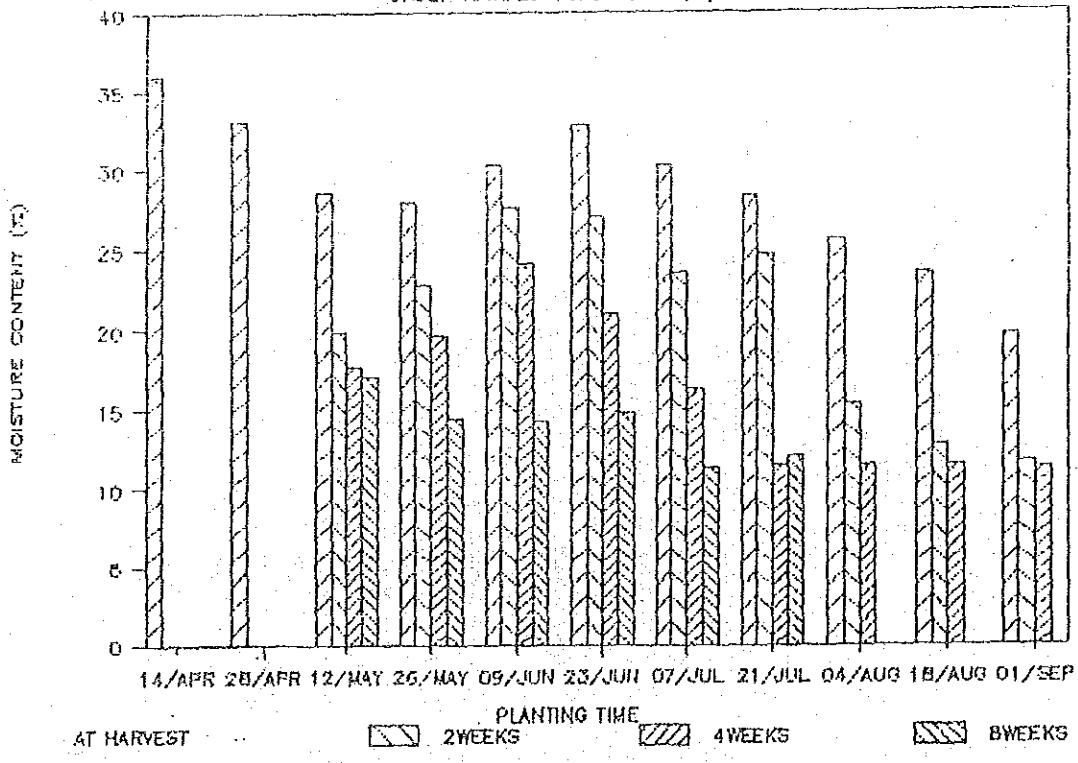
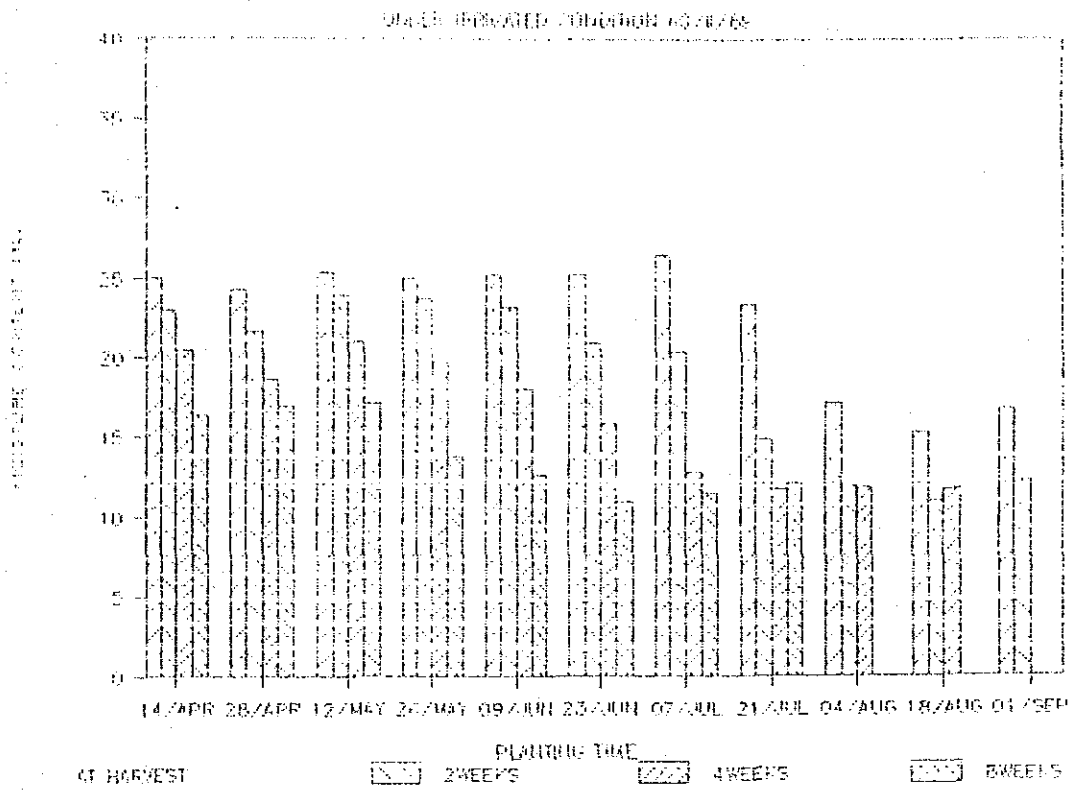


Fig. 4-3 MC(%) AT SAMPLING ON 115 DAYS HARVEST



MC(%) AT SAMPLING ON 115 DAYS HARVEST

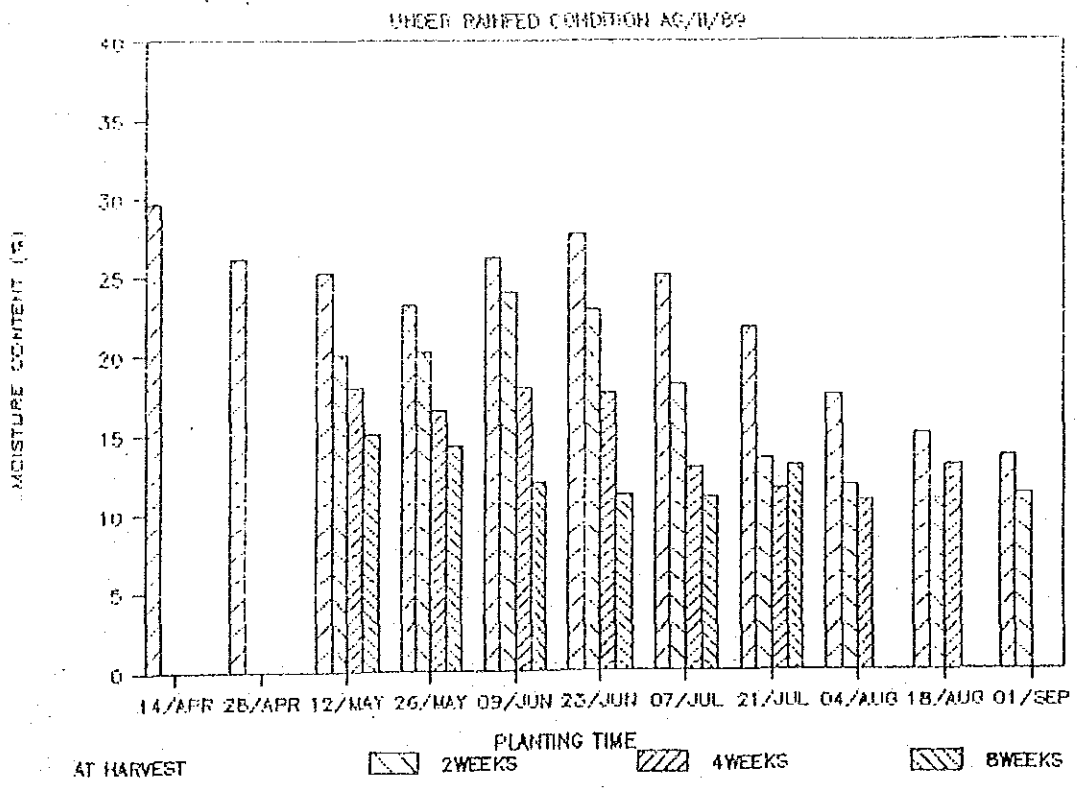
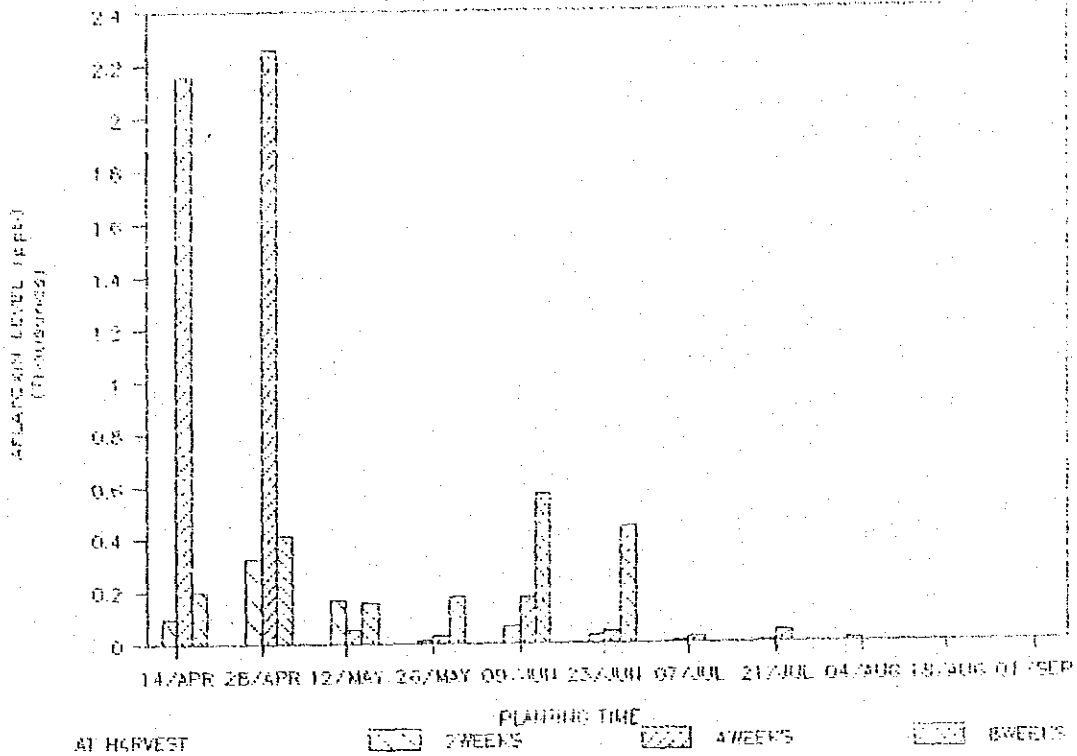


Fig. 5-1

AFLATOXIN LEVEL (ppb) ON 95 DAYS HARVEST

UNDER IRRIGATED CONDITION AG/II/69



AFLATOXIN LEVEL (ppb) ON 95 DAYS HARVEST

UNDER RAINFED CONDITION AG/II/69

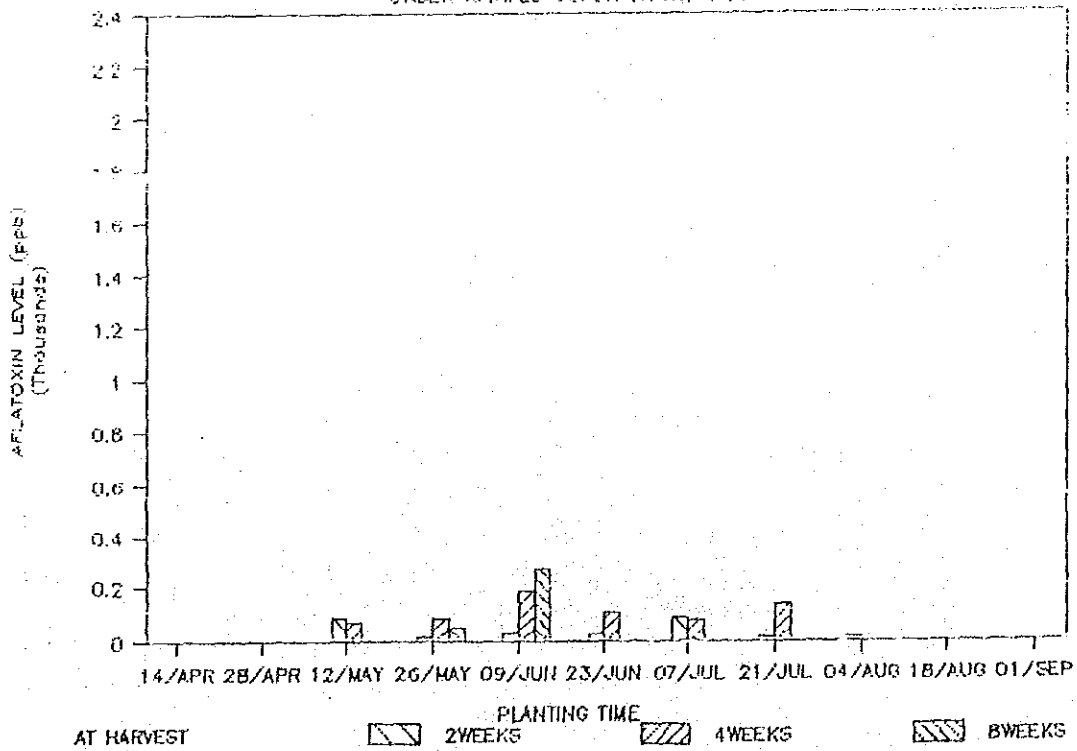
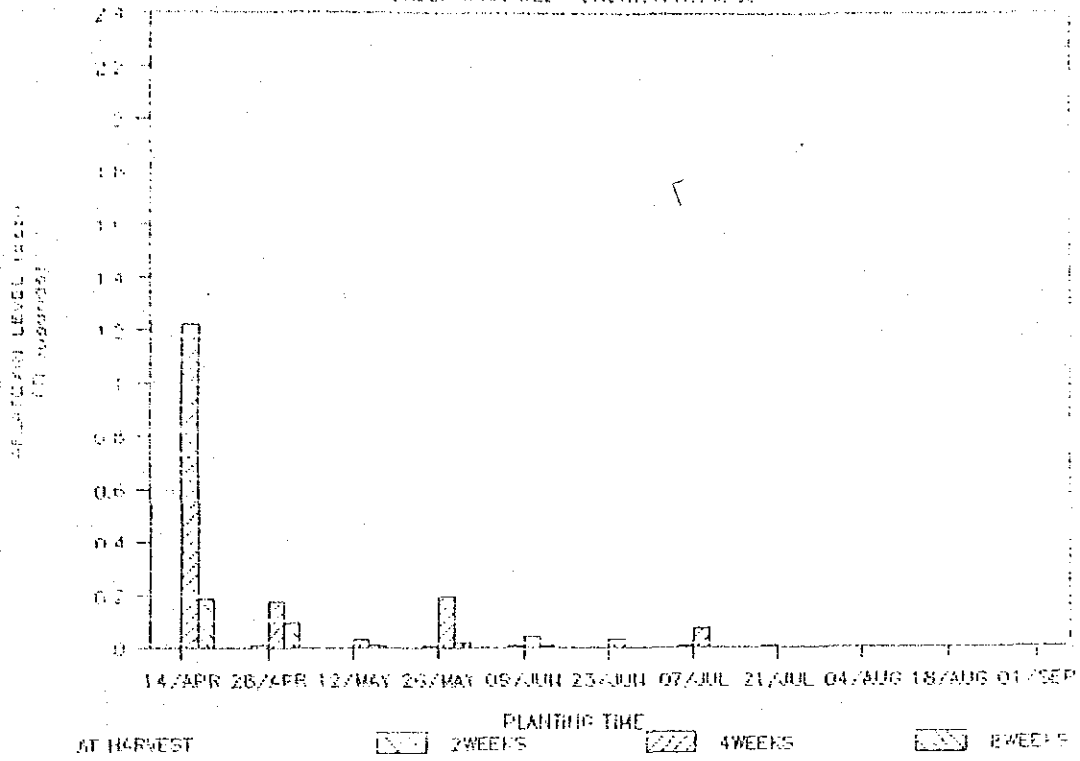


Fig. 5-2

AFLATOXIN LEVEL (ppb) ON 105 DAYS HARVEST

UNDER IRRIGATED CONDITION 05/11/69



AFLATOXIN LEVEL (ppb) ON 105 DAYS HARVEST

UNDER RAINFED CONDITION 06/11/69

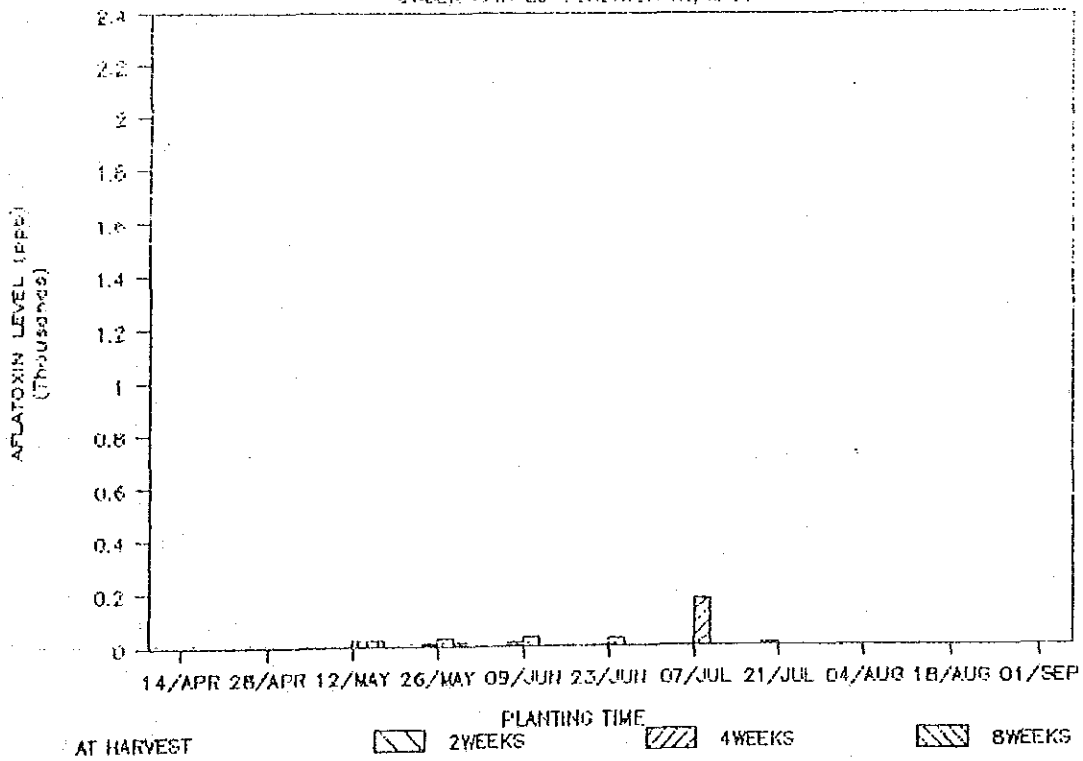
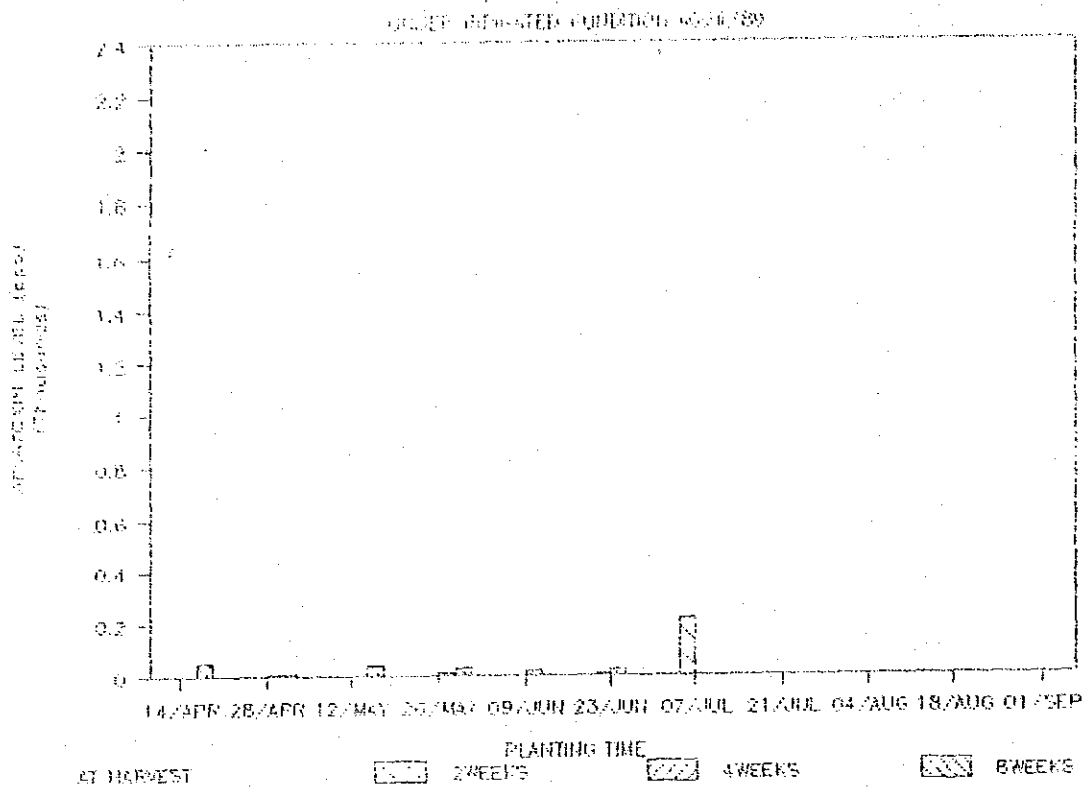
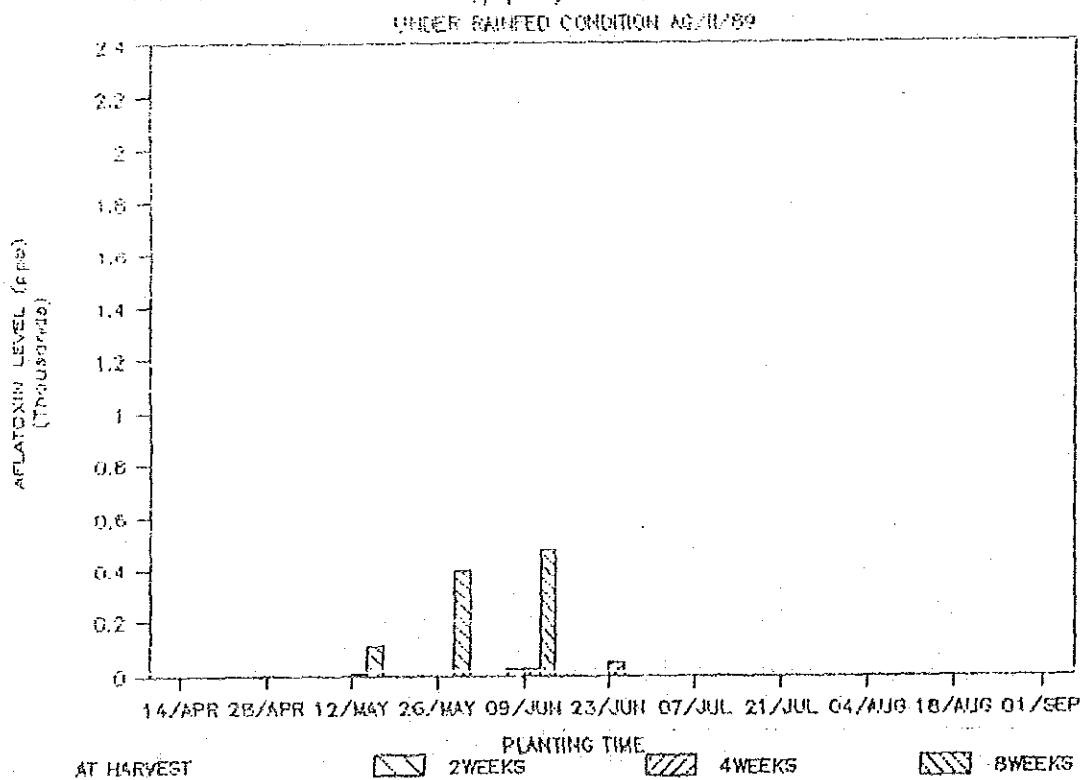


Fig. 5-3 AFLATOXIN LEVEL (ppb) ON 115 DAYS HARVEST



AFLATOXIN LEVEL (ppb) ON 115 DAYS HARVEST



AG/III/89

Effects of Different Harvest Methods, Moisture Conditions and Storage Periods on Aflatoxin Contamination in Maize
Code No. I-1-(1)-H, G (1989)

Objectives

The objectives of the study is to examine aflatoxin contamination by changing the methods of harvest and the time of harvesting.

Materials and Methods

Experiment was planted in one rai plot with standard cultivation method. Spacing was 75cm between rows and 50cm between hills. Seeds was sown 4 seeds per hill then thin to 2 plants at 2 week after planting. When missing hill was observed, compensation was practiced in the adjacent hills up to 3 plants on the hill. Fertilizer was applied at 2 weeks after planting at the same time of thinning.

Prior to harvest, husk cover, insect damage by counting No. of pinhole on the ear and No. of lodged plant in the treatment plots were counted.

Harvest was practiced at 95 days (H1), 105 days (H2) and 115 days (H3) after planting and method was as dehusked in the field for conventional method (-H) and with husk for testing method (+H). Harvesting process of the testing method was that at first held a shank of ear then broke it. Some husk remained enough to cover the whole ear with this method.

Ear was harvested from standing plants for +H and -H by each replication and ear from lodging plant was harvested at treatment plots, however those are not separated by replication. Yield were calculated from only standing plants.

Summary of Results

1) Pre-harvest Record

Average husk cover in the treatment plots were 1.461 and 1.629 at H1 harvest, 1.827 and 1.799 at H2 harvest and 1.836 and 1.757 at H3 harvest on +H and -H harvesting method respectively.

No. of insect attack by counting pinhole on the ear were 98 and 94 at H1, 125 and 123 at H2 and 99 and 132 at H3 on +H and -H respectively.

No. of lodging plant at treatment plots were none at H1, 71 and 60 at H2 and 36 and 49 at H3 on +H and -H respectively.

2) Harvest Record

Yield (Kg/rai) at +H plots were 954.1, 1218.4 and 884.2 and at -H plots were 1034.6, 753.3 and 916.3 on H1, H2 and H3 harvest. There were very good yield in the area.

Grain moisture contents were measured every sampling time by stainlite moisture meter. On H1 harvest maize moisture content started around 33% then decreased 28.9, 25.9 and 19.7 on +H and 27.6, 25.5 and 17.6 on -H at 2 weeks, 4 weeks and 8 weeks after harvest.

On H2 harvest, started around 29%, then decreased 27.6, 25.3 and 16.5 on +H and 27.0, 24.0 and 16.2 on -H. H3 was started from around 25% then 23.9, 21.9 and 15.9 on +H and 23.6, 21.5 and 15.1 on -H. The differences between +H and -H were less than 2% as resulted 1988 trial.

Aflatoxin B1, B2 were analyzed at harvest and 2 weeks, 4 weeks and 8 weeks after harvest.

On H1 harvest, +H did not detected aflatoxin until 2 weeks and at 4 weeks and 8 weeks, 13.5ppb and 12.5ppb were detected. -H found aflatoxin from 2 weeks and 13.0, 90 and 267ppb were in plot average at 2 weeks, 4 weeks and 8 weeks respectively.

On H2, +H observed aflatoxin at 2 weeks and 8 weeks at the average level of 1.0 and 10.5ppb however -H found 2 weeks, 4 weeks and 8 weeks at the level of 4.5, 145 and 416.5ppb.

On H3, aflatoxin detected only 8 weeks as low as 0.5ppb on +H, on the other hand -H was found on 2 weeks, 4 weeks and 8 weeks at 3.0, 36 and 47ppb.

Sample were taken also from the ear of lodging plant as the same treatments but no replication. These samples showed no detection on +H of H1, however on -H of H1, aflatoxin on 2 weeks, 4 weeks and 8 weeks were observed 7.0, 119.0 and 19.0ppb.

On H2 plots, +H was observed at 4 weeks and 8 weeks at the level of 1.0 and 23ppb and 2 weeks, 4 weeks and 8 weeks on -H was found 3.0, 78 and 72ppb.

On H3 harvest, none of aflatoxin was observed on +H, however on -H was detected at 2 weeks, 4 weeks and 8 weeks at the level of 2.0, 2.0 and 4.0ppb.

The level of aflatoxin last year was not as high as 1988 in general. Maximum level of year 1988 were 1100ppb comparing to 416ppb this year. However the similar tendency to the treatments was obtained except on H3, -H treatments, 3.0, 36.0 and 47.0ppb were detected at 2 weeks, 4 weeks and 8 weeks instead of none was detected at respective time.

Future Plan

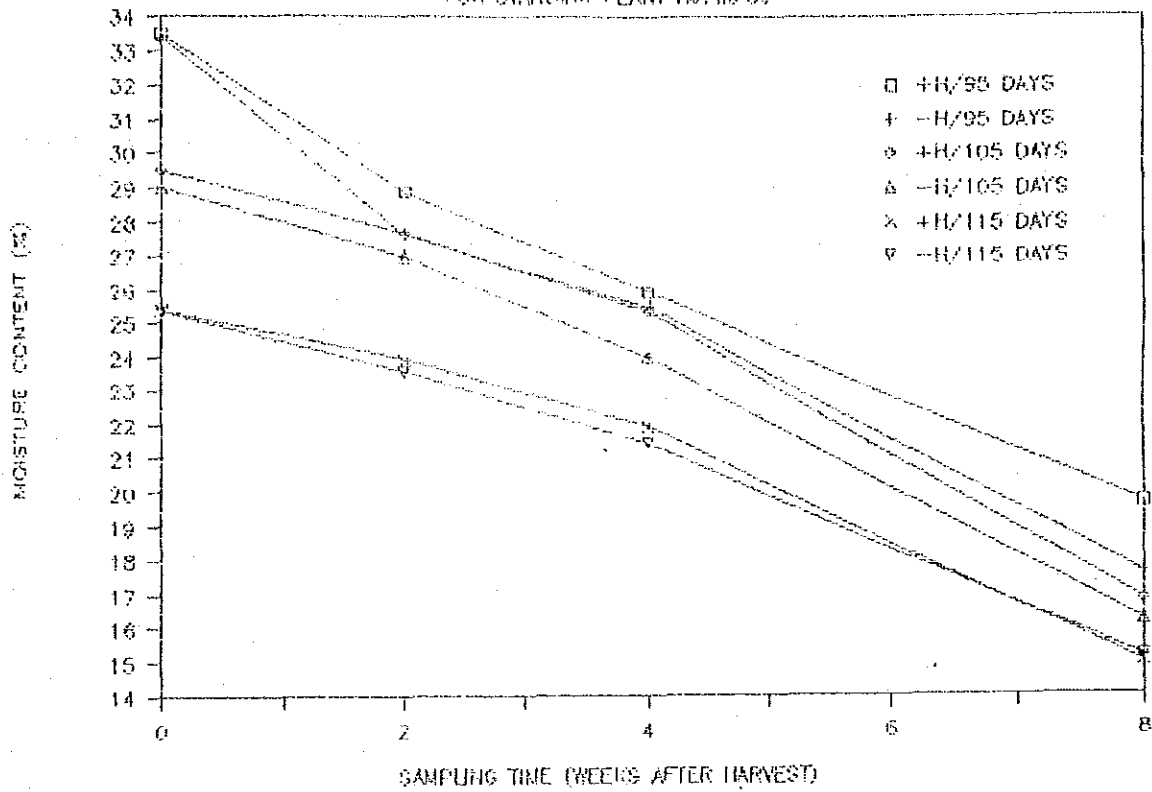
The same experiment will be conducted to examine the annual variation at the station field this year.

The proposal for large scale trial on this subject was approved by the research committee. The plan is to conduct at farmer's fields with using the farmer's storing facilities. The size of the area of the field is to be around 25 rai as the standard of the area of the station.

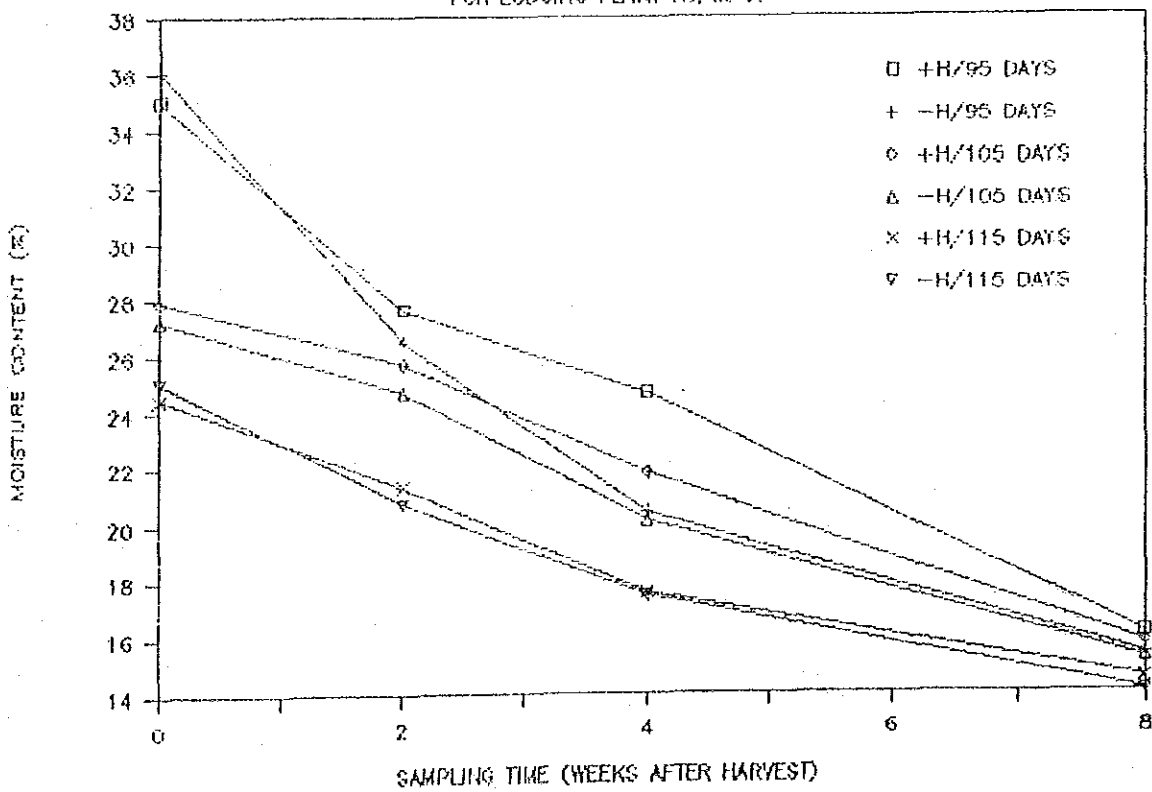
The other plan is to prove the reason of the difference between the treatments. It may be the physical damage on the surface of the kernel or moisture and humidity dynamic.

Shelling work will be the largest bottleneck when harvest with husk is applied in practice. For this work, cooperation of post-harvest section will be essential.

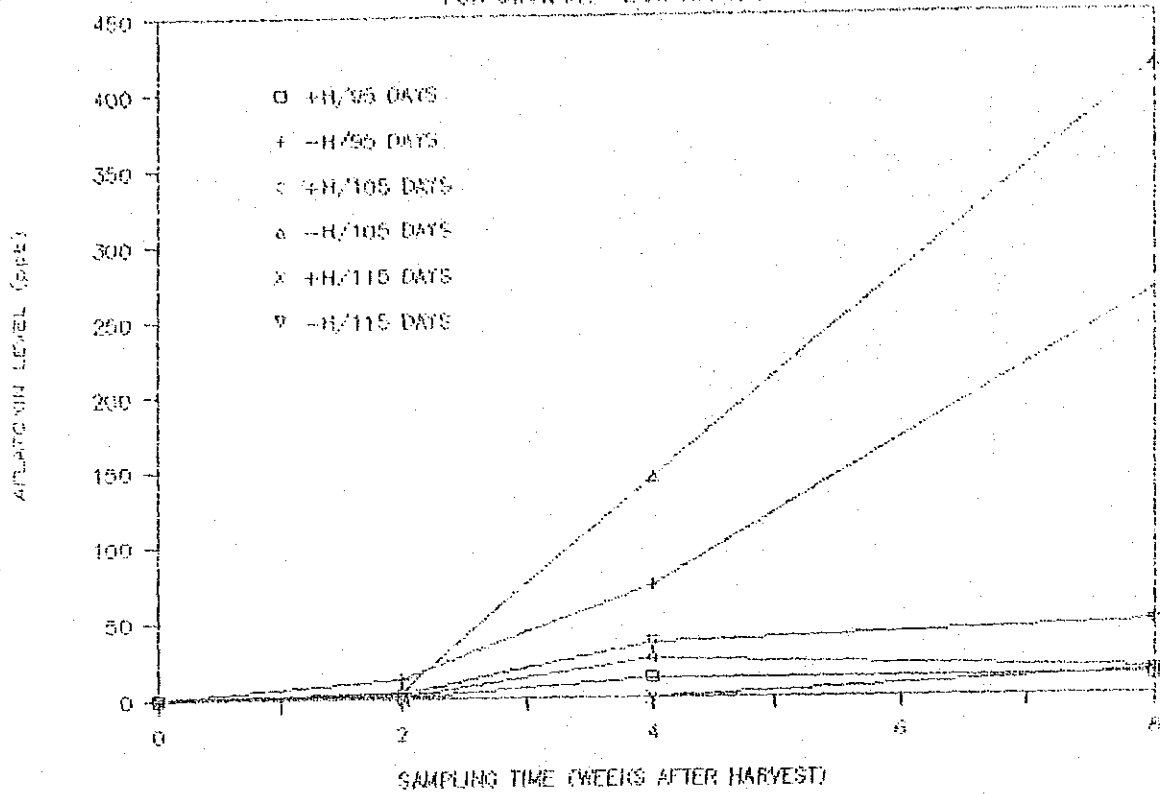
GRAIN MOISTURE CONTENT AFTER HARVEST FOR STANDING PLANT AG/III/69



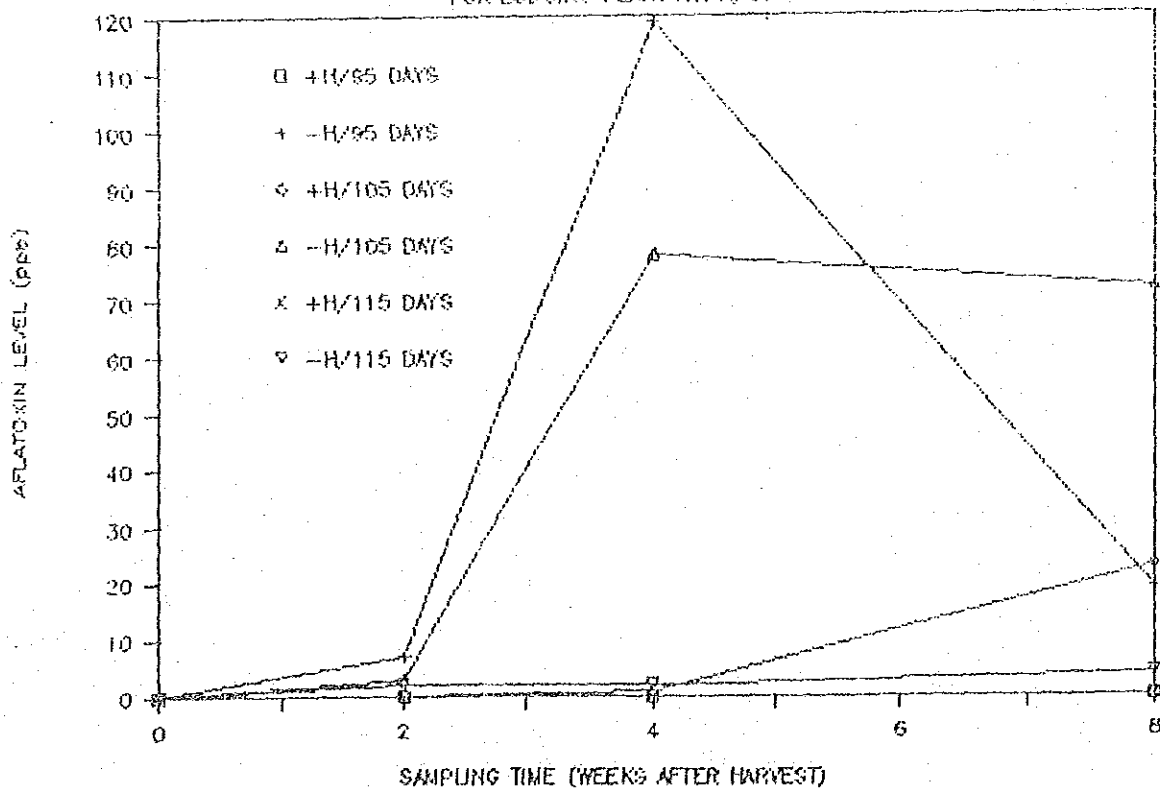
GRAIN MOISTURE CONTENT AFTER HARVEST FOR LODGING PLANT AG/III/69



AFLATOXIN (B1+B2) ppb AFTER HARVEST
FOR STANDING PLANT AG/III/89



AFLATOXIN (B1+B2) ppb AFTER HARVEST
FOR LOGGING PLANT AG/III/89



AG/IV/89

Effect of Plant Density and Nitrogen Application on Aflatoxin Contamination

Code No. I-1-(1)-D (1988 - 1989)

Objectives

The object of the study is to examine productivity and quality concerning aflatoxin contamination in relation to plant density and nitrogen application.

Materials and Methods

Three levels of plant density : 4266(D1), 8533(D2) and 12266 (D3) plants per rai and four levels of nitrogen application : 0(N1), 10(N2), 20(N3) and 30(N4)kg/rai were applied. Maize was harvested at 105 days after planting and kept in storage for 2 weeks.

Grain samples for moisture measurement and aflatoxin contamination analysis were taken at harvest and 2 weeks after harvest.

Soil samples for analyzing fertility were taken before planting and after harvest.

Summary of the result

Germination count and plant stand after thinning were practiced. Overall average of the germination percentage at one week after planting was 59.1% and 60.9% at 2 weeks after. There was no significant difference among the treatment plots neither densities nor Nitrogen levels.

Plant stand percentage to the planed densities in whole plot was 94.3%. Plant stand percentage on Density plot across Nitrogen level were 99.7%, 94.8% and 88.4% on D1, D2 and D3 respectively. Statistical significance is to be examined. On Nitrogen plots across densities were 95.3%, 93.7%, 93.8% and 94.8%.

Statistical analysis for pre-harvest records are not done yet.

Plant stand at harvest time was 98.8%, 88.9% and 76.7% to plant densities on D1, D2 and D3, and 83.1%, 83.2%, 84.5% and 86.3% on N1, N2, N3 and N4 respectively.

Plant height was 161.4cm, 159.7cm and 161.3cm on densities and 153.3cm, 161.9cm, 165.1cm and 163.0 on Nitrogen levels. Ear height was 83.9cm, 84.1cm and 87.6cm on densities and 77.9cm, 84.5cm, 88.8cm and 89.6cm on Nitrogens.

Total No. of lodged plants on stem and root were 163, 122 and 367 on densities and 69, 103, 372 and 108 on Nitrogens. Husk cover by CIMMYT Method was 2.016, 1.960, 1.906 on densities and 1.998, 1.994, 1.879 and 1.973 on Nitrogens.

No. of insect pinhole on the ear was 122, 168 and 168 on densities and 104, 97, 102 and 155 on Nitrogens.

No. of insect pinhole on stem was 78, 120 and 371 on densities and 51, 134, 289 and 95 on Nitrogens.

Yield(kg/rai) on density levels were 387.7, 431.2 and 447.9 on D1, D2 and D3 and there is no significance was recognized.

On Nitrogen levels of yield were 336.7, 406.7, 462.1 and 483.6 on N1, N2, N3 and N4 and significant difference was observed at 1% level between N4 and others, however among the N1, N2 and N3 was no different. Aflatoxin at harvest was not contaminated in any sample. However at 2 weeks after harvest, very low level of toxin was detected from 25% of sample. Level of aflatoxin was at the range of 2 to 9ppb. 16.7% of D1, 41.7% of D2 and 16.7% of D3 samples and 22.2% of N1, N2 and N3 and 33% of N4 samples were detected aflatoxin.

AG/V/89

Effect of Crop Rotation on Aspergillus spp. in the Soil
Code No. I-1-(1)-C, B (1988 - 1990)

Objective

As a part of A. flavus infestation route, population of the fungus in the soil is an important information to understand the situation.

Some of the observation on the aflatoxin contamination said that A. flavus population in the soil may change by crop rotation. This study objected to investigate the population movement in the soil by the cultural practice.

Materials and Method

The pattern of crop rotation were planed as 1988 by the possible farmer's practice on maize cultivation. Cropping pattern and calendar were as follows :

		Planting	Harvesting	Duration (days)	
1.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Soybean	14/Sep/89	24/Nov/89	72
2.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Mungbean	14/Sep/89	30/Nov/89	78
3.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Sorghum	14/Sep/89	25/Dec/89	103
4.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Peanut	14/Sep/89	25/Dec/89	103
5.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Fallow			
6.	1st	Soybean	25/May/89	07/Aug/89	75
	2nd	Maize	08/Aug/89	22/Nov/89	106
7.	1st	Mungbean	25/May/89	19/Jul/89	56
	2nd	Maize	28/Jul/89	22/Nov/89	118
8.	1st	Sesami	25/May/89	02/Aug/89	70
	2nd	Maize	28/Jul/89	17/Nov/89	113
9.	1st	Peanut	25/May/89	29/Aug/89	97
	2nd	Maize	01/Sep/89	03/Jan/90	125
10.	1st	Maize	25/May/89	05/Sep/89	104
	2nd	Maize	14/Sep/89	12/Jan/90	121

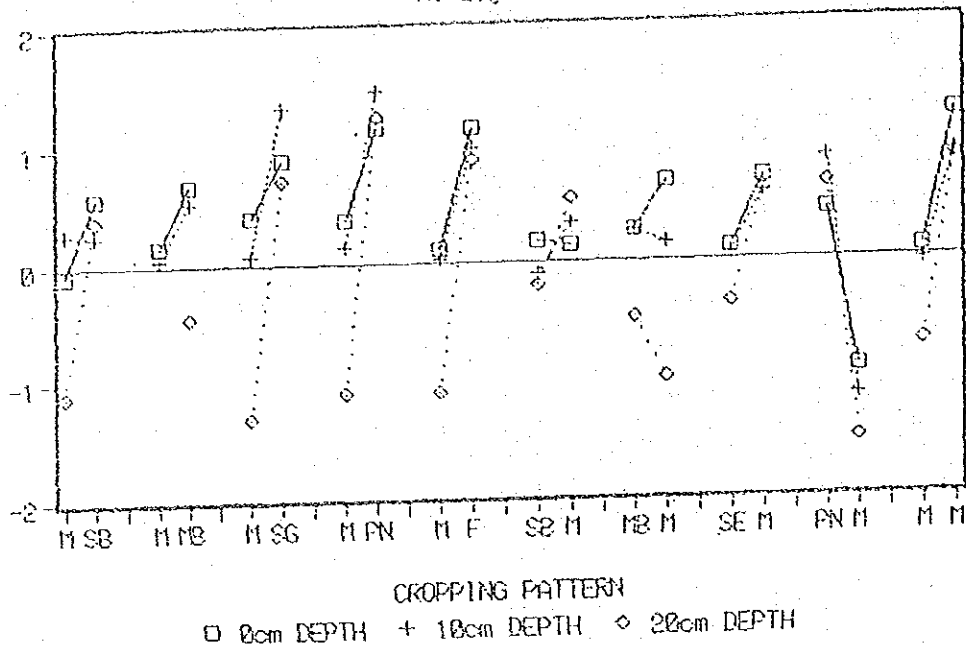
Soil samples for A. flavus count in 1grm soil were taken 3 times on the plots planted maize as second crop at before 1st crop planting, after 1st crop harvesting and after 2nd crop harvesting and on the plots planted maize as the first crop at before and after 1st crop, after ploughing for 2nd crop and after 2nd cropping. The samples were taken at 0cm and 10cm depth from 9 points in each plot then mixed as plot samples.

Summary of Result

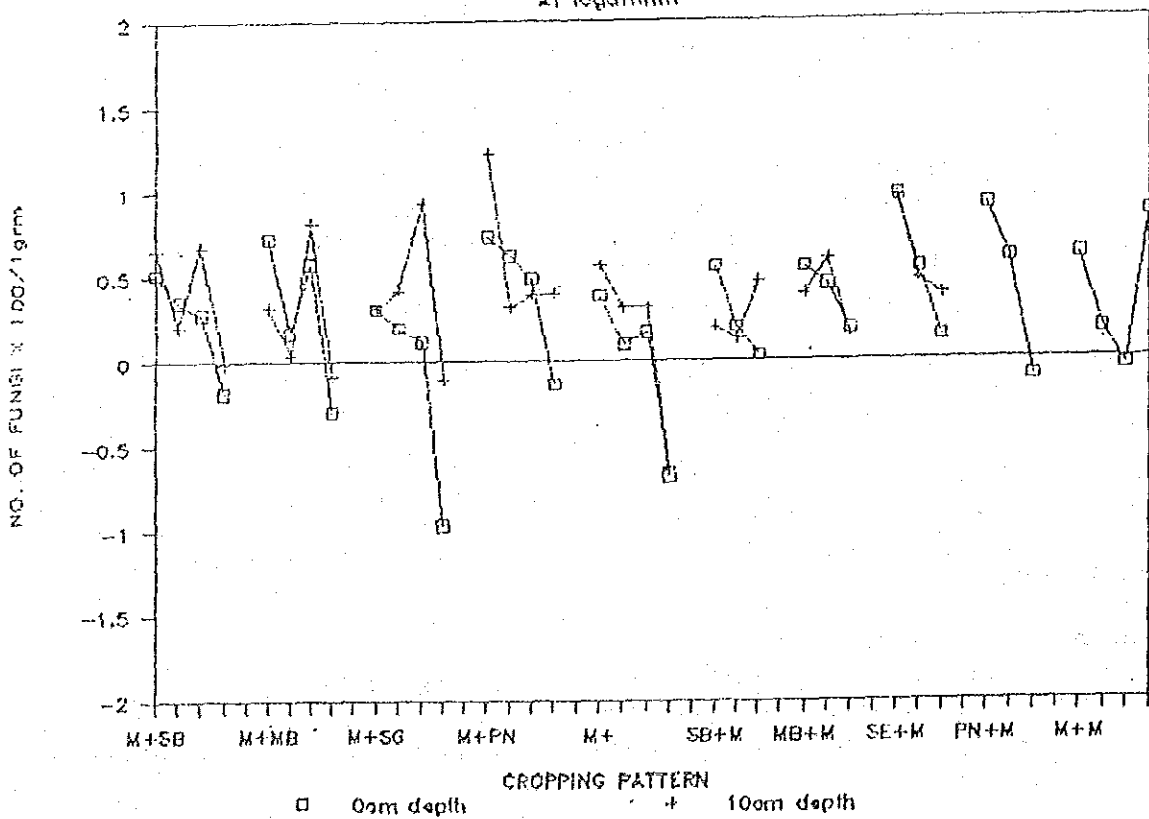
Population of A. flavus was generally lower comparing to 1988. Population changes on 1989 was quite differed from 1988. It is difficult to interpretation for those phenomena.

NO. OF FUNGI X 100 / 1 GRM

NUMBER OF A. FLAVUS in 1gm DRY SOIL 1968
At Logarithm



NO. OF A. FLAVUS in 1gm DRY SOIL 1989
AT logarithm



AG/VI/89

Effect of Nitrogen Regarding Prevention of Aflatoxin Contamination by the Inoculation Method

Code No. I-1-(1)-D (1989 - 1990)

Objective

To clarify the effect of nitrogen application on aflatoxin contamination in maize.

Materials and Methods

Prior to planting, soil sample from every plot to know the existing nitrogen levels and to determine the level of Nitrogen fertilizer application.

Planting was practiced as standard method 75cm x 50cm with 2 plants per hill.

Nitrogen levels were calculated from analysis result and applied as normal practice which is band application beside the planting rows.

Plots were 22 rows x 14 hills, and 1 to 6 rows and 19 to 22 rows were border rows and center's 12 rows were for treatment. Treatment of inoculation by spore suspension 10ml of 1,000,000 concentration through silk was applied on 7, 9, 11, 13, 15 and 17 rows. The other rows of even number were as no inoculated as a check.

Inoculation was practiced 2 weeks after 50% silking. At harvest, ear was harvested by row and visual check of A. flavus infestation on the kernel was carried out. Samples for analysis aflatoxin and A. flavus check on the surface of the kernel were done at the Bangkok Center.

Summary of the result

Average plant height(cm) were 155.8, 173.2, 171.7 and 166.7, and ear height(cm) were 68.6, 81.9, 83.8 and 80.0 on 0kgN (PL1), 10kgN (PL2), 20kgN (PL3) and 30kgN (PL4) respectively.

Total plant lodging at stem and root on entire plot were 181, 207, 348 and 240 on PL1, PL2, PL3 and PL4.

Husk cover by CIMMYT method was 2.05, 1.79, 1.75 and 1.85, total No. of insect pinhole on the ear were 25, 41, 9 and 17 and 8, 7, 30 and 7 on the stem were observed.

Yield(kg/rai) record was calculated from the harvest of 1, 2 and 3 rows, and 478.4, 880.4, 910.9 and 875.8 on PL1, PL2, PL3 and PL4 were obtained. Nitrogen response on PL4 was unexpected result therefore further investigation was needed.

Results of treatments on visual A. flavus infection by No. of ear was as follow ;

PL	Inoculated Plot Infection			Non Inoculated Plot Infection		
	+ <u>A. flavus</u>	- <u>A. flavus</u>	%	+ <u>A. flavus</u>	- <u>A. flavus</u>	%
1	2	52	3.7	0	50	0
2	3	53	5.4	0	56	0
3	1	60	1.6	0	56	0
4	3	57	5.0	1	60	1.6

From those which infested ears were analyzed toxin level.

Aflatoxin level (ppb) from A. flavus infested ears

Plot	Inoculated	Non Inoculated
1	56	-
2	14	-
3	1	-
4	39	ND

The other ears which visual A. flavus infestation were not recognized and also examined A. flavus and toxin in the Center.

A. flavus on the kernel (%)

Plot	Inoculated	Non Inoculated
1	0	0
2	4	0
3	2	1
4	1	0

Aflatoxin (B1 + B2) from the visually non A. flavus infected ears

Plot	Inoculated	No Inoculated
1	ND	ND
2	40	ND
3	6	ND
4	3	ND

Tendency to the treatment was no recognized and A. flavus infestation on inoculated rows were very low. I might need to consider the inoculation method to examine clear response to the Nitrogen level.

AG/VII/89

Identification of Insects and the Types of Damage They
Inflict on the Maize Kernel

Code No. I-1-(1)-F (1989 - 1990)

Objective

To identify maize insect and the types of their damage on the maize ear in relation to the A. flavus infection.

Materials and Methods

Maize was planted in 1 rai plot according to the standard cultivation method. 11 types of insect were counted during the growing period at randomly selected 10 rows x 20 hills (75sqr.m.). Insect trap with phyramon was set at 4 points and No. of moth caught in the trap was recorded. A. flavus was checked from the samples of damaged and no damaged kernel.

Summary of the result

Insect count is shown on Table 1. Early growing season was observed more insect than later. Stem borer stated a little earlier than ear worm appeared.

Sample from damaged ear and no damaged ear by insect were examined A. flavus and other fungi. Only 2 samples from the damaged kernels of damaged ears were found A. flavus at 2% level. The other samples which were sound kernels of damaged ear and sound kernel of no damaged ear was not found any.

Percentage of damaged ear of 7 times sampling and record of insect trap are not summarized yet.

INSECT RECORD ON AVAIL: 69

DATE	MONTH	DAY	A	B	C	D	E	F	G	H	I	J	K	L	M
1	JUL	3	0	1000	0	0	0	0	0	0	2	2	0	210	0
2	JUL	4	0	1000	0	0	0	0	0	0	1	0	101	0	0
3	JUL	11	0	2000	0	0	0	0	0	0	0	4	0	0	0
4	JUL	13	0	20040	0	0	0	0	0	0	0	11	0	0	0
5	JUL	17	0	1000	0	0	0	0	0	0	1	61	0	0	0
6	JUL	20	0	100	0	0	0	0	0	0	0	101	0	0	1
7	JUL	24	0	0	0	0	0	0	1	0	0	0	0	0	0
8	JUL	27	0	0	0	0	0	0	0	22	0	0	17	0	10
9	JUL	31	0	0	0	0	0	0	0	12	0	4	0	0	0
10	AUG	3	0	0	0	0	0	0	0	0	2	0	0	0	0
11	AUG	7	0	0	0	0	0	0	1	0	0	10	0	0	0
12	AUG	10	0	75	0	0	0	0	2	0	0	11	0	0	0
13	AUG	14	0	10	0	0	0	0	0	0	0	10	0	0	0
14	AUG	17	0	0	0	0	0	0	1	0	0	5	0	0	0
15	AUG	20	0	70	0	0	0	0	0	0	0	0	0	1	0
16	AUG	24	0	30	0	0	0	0	0	0	0	0	0	0	0
17	AUG	28	0	14	0	0	0	0	0	0	0	0	0	0	0
18	AUG	31	0	5	0	0	0	0	0	0	0	0	0	0	0
19	SEP	4	0	20	0	0	0	0	0	0	0	2	0	0	0
20	SEP	7	0	0	0	0	0	0	0	2	0	0	0	0	0
21	SEP	11	0	0	0	0	0	0	0	1	0	1	0	1	1
22	SEP	14	0	0	0	0	0	0	0	0	0	1	0	0	0
TOTAL			0	26100	0	0	14	0	42	25	6	330	100	253	8

NOTE

A : Common name = Corn Thrips
 : Scientific name = *Franklinella williamsi* Hood.
 : (Family : Order) = (Thripidae : Thysanoptera)

B : Common name = Corn leaf aphid
 : Scientific name = *Rhopalosiphum maidis* (Fitch)
 : (Family : Order) = (Aphididae : Homoptera)

C : Common name = Rose beetle
 : Scientific name = *Adoretus compressus* Water
 : (Family : Order) = (Rutelidae : Coleoptera)

D : Common name = Egg of corn stem borer
 : Scientific name = *Ostrinia furnacalis* (Guenee)
 : (Family : Order) = (Pyralidae : Lepidoptera)

E : Common name = Larvae of corn stem borer
 : Scientific name = *Ostrinia furnacalis* (Guenee)
 : (Family : Order) = (Pyralidae : Lepidoptera)

F : Common name = Egg of corn ear-worm
 : Scientific name = *Heliothis armigera* Hubner
 : (Family : Order) = (Noctuidae : Lepidoptera)

G : Common name = Larvae of corn ear-worm
 : Scientific name = *Heliothis armigera* Hubner
 : (Family : Order) = (Noctuidae : Lepidoptera)

H : Common name = Corn armyworm
 : Scientific name = *Mythimna separata*
 : (Family : Order) = (Noctuidae : Lepidoptera)

I : Common name = Green stink bug
 : Scientific name = *Nezara viridula* (L.)
 : (Family : Order) = (Pentatomidae : Hemiptera)

J : Common name =
 : Scientific name = Lady bird beetle
 : (Family : Order) =

K : Common name = Froghopper or Spittle bug
 : Scientific name = *Callitettix versicolor* F.
 : (Family : Order) = (Cercopidae : Homoptera)

L : Unknown

M : Unknown

RECORD SAMPLING AG/VII/89

DATE	NO. OF DAMAGED EAR	NO. OF TRACE EAR	NO. OF NON-DAMAGE EAR
16/08/89	2	14	84
23/08/89	4	34	62
29/08/89	4	34	62
05/09/89	7	51	42
13/09/89	8	65	42
20/09/89	13	63	24
27/09/89	21	62	17

AG/VIII/89

Evaluation of Insect Damage that Occures Under Field Conditions

Code No. I-1-(1)-F, B (1989 - 1990)

Objective

Evaluation of insect damage occurs in the field condition.

Materials and Methods

Maize was planted in 1 rai plot. 5 treatment plots were set in 1 rai for insecticide application (A) and natural condition (B) which were 10 rows and 20 hills each. Insect count was practiced 23 times for July 3 to September 18. Aflatoxin was checked after harvest.

Summary of the Result

Insect count at early growing stage in July had effected of insecticide application in A plots on corn leaf aphid, however Ladybird beetle which is predator of aphid also decreased as well. Egg and larvae of corn stem borer and corn ear worm seems to be no effect.

Result of aflatoxin analysis was low level as follows ;

Aflatoxin B1 level(ppb) on insect effect ear condition

Ear condition	Plot	Sample	1	2	3
Damaged	A		NA	4	16
	B		NA	4	4
Trace	A		NA	12	12
	B		NA	2	ND
Sound	A		NA	8	ND
	B		NA	1	16

Note

Trace : not able to distinguish cause of damage

No significant tendency was not observed on aflatoxin level.

PILOT A: FRESHLY APPLIED IN WGRU

SAMP	MONTH	DAY	A	B	C	D	E	F	G	H	I	J	K	L	M
1	JUN	3	0	184	9	0	0	0	0	0	0	0	0	0	11
2	JUN	6	0	166	0	0	0	0	0	0	0	0	3	1	5
3	JUN	10	0	202	0	0	0	0	0	0	0	0	0	0	9
4	JUN	15	0	303	0	0	29	0	0	0	0	0	0	0	2
5	JUN	17	0	303	0	0	0	0	0	0	0	103	0	0	0
6	JUN	20	0	50	0	0	0	0	0	0	0	0	18	25	0
7	JUN	24	0	0	0	0	0	0	0	0	0	0	0	0	4
8	JUN	27	0	0	0	0	0	0	0	0	0	0	0	0	0
9	JUL	31	0	0	0	0	0	0	0	0	0	0	0	0	0
10	AUG	3	0	0	0	0	0	0	0	0	0	0	0	0	0
11	AUG	7	0	29	0	0	0	0	0	0	0	0	0	0	0
12	AUG	10	0	101	0	0	0	0	0	0	0	0	0	0	0
13	AUG	14	0	0	0	0	0	0	0	0	0	0	0	0	0
14	AUG	17	0	0	0	0	0	0	0	0	0	0	0	0	0
15	AUG	21	0	0	0	0	0	0	0	0	0	0	0	0	0
16	AUG	24	0	0	0	0	0	0	0	0	0	0	0	0	0
17	AUG	28	0	0	0	0	0	0	0	0	0	0	0	0	0
18	AUG	31	0	0	0	0	0	0	0	0	0	0	0	0	0
19	SEP	4	0	0	0	0	0	0	0	0	0	0	0	0	0
20	SEP	7	0	0	0	0	0	0	0	0	0	0	0	0	0
21	SEP	11	0	0	0	0	0	0	0	0	0	0	0	0	0
22	SEP	14	0	0	0	0	0	0	0	0	0	0	0	0	0
23	SEP	18	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL			0	2102	0	0	29	0	0	0	0	0	0	0	54

PILOT B: CONTROL

SAMPLE	MONTH	DAY	A	B	C	D	E	F	G	H	I	J	K	L	M
1	JUL	3	0	24	0	0	0	0	0	0	0	0	0	0	4
2	JUL	6	0	1423	0	0	0	0	0	0	0	0	0	0	0
3	JUL	10	0	1832	0	0	0	0	0	0	0	0	0	0	0
4	JUL	13	0	27016	0	0	0	0	0	0	0	0	0	0	0
5	JUL	17	0	19860	0	0	0	0	0	0	0	0	0	0	0
6	JUL	20	15	1420	0	0	0	0	0	0	0	0	0	0	0
7	JUL	24	0	0	0	0	0	0	0	0	0	0	0	0	0
8	JUL	27	0	0	0	0	0	0	0	0	0	0	0	0	0
9	JUL	31	0	0	0	0	0	0	0	0	0	0	0	0	0
10	AUG	3	0	0	0	0	0	0	0	0	0	0	0	0	0
11	AUG	7	0	149	0	0	0	0	0	0	0	0	0	0	0
12	AUG	10	0	47	0	0	0	0	0	0	0	0	0	0	0
13	AUG	14	0	0	0	0	0	0	0	0	0	0	0	0	0
14	AUG	17	0	0	0	0	0	0	0	0	0	0	0	0	0
15	AUG	21	0	0	0	0	0	0	0	0	0	0	0	0	0
16	AUG	24	0	0	0	0	0	0	0	0	0	0	0	0	0
17	AUG	28	0	0	0	0	0	0	0	0	0	0	0	0	0
18	AUG	31	0	0	0	0	0	0	0	0	0	0	0	0	0
19	SEP	4	0	0	0	0	0	0	0	0	0	0	0	0	0
20	SEP	7	0	0	0	0	0	0	0	0	0	0	0	0	0
21	SEP	11	0	0	0	0	0	0	0	0	0	0	0	0	0
22	SEP	14	0	0	0	0	0	0	0	0	0	0	0	0	0
23	SEP	18	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL			15	6792	0	0	29	0	0	0	0	0	0	0	54

NOTE

- A: Common name = Corn Thrips
 Scientific name = *Franklinella williamsi* Hood
 (Family : Order) = (Thripidae : Thysanoptera)
- B: Common name = Corn leaf aphid
 Scientific name = *Rhopalosiphum maidis* (Fitch)
 (Family : Order) = (Aphididae : Homoptera)
- C: Common name = Rose beetle
 Scientific name = *Adoretus compressus* Weber
 (Family : Order) = (Rutelidae : Coleoptera)
- D: Common name = Egg of corn stem borer
 Scientific name = *Ostrinia furnacalis* Guenee
 (Family : Order) = (Pyralidae : Lepidoptera)
- E: Common name = Larvae of corn stem borer
 Scientific name = *Ostrinia furnacalis* (Guenee)
 (Family : Order) = (Pyralidae : Lepidoptera)
- F: Common name = Egg of corn ear-worm
 Scientific name = *Heliothis armigera* Hubner
 (Family : Order) = (Noctuidae : Lepidoptera)
- G: Common name = Larvae of corn ear-worm
 Scientific name = *Heliothis armigera* Hubner
 (Family : Order) = (Noctuidae : Lepidoptera)
- H: Common name = Corn armyworm
 Scientific name = *Mythimna separata*
 (Family : Order) = (Noctuidae : Lepidoptera)
- I: Common name = Green stink bug
 Scientific name = *Nesara viridula* (L.)
 (Family : Order) = (Pentatomidae : Hemiptera)
- J: Common name = Lady bird beetle
 Scientific name =
 (Family : Order) =
- K: Common name = Froghopper or Spittle bug
 Scientific name = *Deltilletria versicolor* F.
 (Family : Order) = (Cercopidae : Homoptera)
- L: Unknown
- M: Unknown

AG/IX/89

Relation between Kernel Type and Resistance to Fungus
Infection after Inoculation

Code No. I-1-(1)-A (1989)

AG/X/89
 Relation between Environmental Factors and Fungus
 Infestation
 Code No. I-1-(1)-J-(b) (1989 - 1991)

Objectives

The work of collecting air sample in the field was a part of the study to clarify fungus infestation to the maize product.

Materials and Methods

Air sample was taken in the maize experiment field by using the SAS portable air sampler. Addition to the field, some samples were taken storage place of the project material and some place where shelling was carried on. Amount of air sample was adjusted to the dial 3 of the machine which is 90 liters/minute. Sampling was continued from July to December. Each sample recorded time, plot, temperature, relative humidity, wind direction, wind speed and weather. Sampled dish was kept in the normal temperature room and counted A. flavus and other fungi 3-5 days after. Total 240 samples were collected during the period. Most of the samples found A. flavus were sent to Microbe to examine toxin production.

Summary of the Result

Sample collected by months and A. flavus observation are following :

Field Sample

Month	Total Sample	No. of Sample <u>A. flavus</u>	%
July	31	2	6.5
August	60	2	3.3
September	10	0	0.0
October	52	5	9.6
December	30	11	36.7

Storage place

September	4	1	25.0
October	26	6	16.7
December	15	10	66.7

Shelling place

September	7	1	14.3
October	4	2	50.0

By sample collecting places are ;

Irrigated plots	92	11	12.0
Rainfed plot	92	9	9.8
Storage place	45	27	60.0
Shelling place	11	3	27.3

	Total Sample	No. of Sample <u>A. flavus</u>	%
By weather conditions are ;			
Fine	101	16	15.8
Cloud	75	4	5.3
Rain	11	0	0.0

By wind directions are ; (only in the field)

South west	72	4	5.6
North east	67	15	22.4
West	8	1	12.5
South	32	0	0.0
North	6	0	0.0
East	2	0	0.0

*South west wind is in the rainy season and Northeast wind is in dry season.

By wind speed ; (m/sec.)

~ 0.9	60	6	10.0
1.0 ~ 4.9	11	1	10.2
5.0 ~	3	1	27.3

AG/XI/89

Monitoring Aflatoxin Occurrence in the Major Maize Production Area

Code No. I-1-(1)-J, I-3-(1)-A (1989 - 1991)

Objective

To observe aflatoxin occurrence in the maize production area and areal differences of its incidence.

Materials and Methods

Sample collecting were requested to the Field Crop Research Center and Stations in the 5 provinces where were major maize production area in Thailand.

Provinces were Loei, Petchabun, Nakhon Ratchasima, Nakhon Sawan and Lopburi.

Sample requested to collect was 10 samples, around 30 cars each.

Questionnaire was also attached to know the background of maize sample.

Summary of the result

Sample was collected 5 provinces as follow ;

<u>Provinces</u>	<u>No. of sample</u>
1. Loei	7
2. Petchabun	13
3. Nakhon Ratchasima	10
4. Nakhon Sawan	10
5. Lopburi	2
Total	42

The background of sample and interpretation of the result of A. flavus infection and aflatoxin contamination are undergoing.

Problem

We have faced the difficulty of collecting the sample at proper time and process.

However the importance of the subject is recognized to understand the country's situation.

IV. ABSTRACT OF THE
EXPERIMENT RESULTS

POST-HARVEST SECTION

THE MAIZE QUALITY IMPROVEMENT RESEARCH CENTER PROJECT

II. Post-Harvest Studies

Study on the relationship between damage on kernel, kernel moisture content and aflatoxin contamination. (1988-1989)
Code No. II-1-(2)-B

Objectives

This experiment was conducted to investigate the effect of broken kernel and impurity mixed in intact kernel on aflatoxin contamination during storage period.

Methods and Procedures

Manually shelled ear maize sample, variety Suwan 1, were prepared to have 4 different ratio of breakage and impurity as following percentage of 0:0, 0:3, 3:0 and 6:0. Moisture content of the samples were 15, 17, 22 and 30%. Each sample was weighed 1kg and packed in perforated plastic container and stored in a high humidity cabinet arranged at random.

Results

- 1) Aflatoxin contamination was detected on the 7th day of storage and more on the 14th day.
- 2) At 22% level of moisture content, all samples were highly contaminated by aflatoxin, while samples with 30% level of moisture was found contaminated lower in the moisture of 0:0 and 0:3, but severely infected by A. flavus in the mixture of 3:0 and 6:0.
- 3) After 14 days of storage, all the samples were contaminated by aflatoxin, lower at 17% of moisture level and much lower at 15% of moisture level.

Problems and Future Plans

- 1) In the mixture of 0:3, samples were already highly contaminated on the 7th day of storage. It is necessary to prolong storage duration and chase the profile of aflatoxin contamination.
- 2) moisture content should be prepared by artificial drying to make sample moisture content more equal.
- 3) Effect of scratch on seed coat should be investigated together with other effects.

Table 1 Aflatoxin (B1 in ppb) contamination of shelled maize during storage.

Storage period (days)	Kernel moisture content (%wb)	Type of mixture							
		A		B		C		D	
		repl	rep2	repl	rep2	repl	rep2	repl	rep2
3	15	0	0	0	0	0	0	0	0
3	17	0	0	0	0	0	0	0	0
3	22	0	0	0	0	0	0	0	0
3	30	0	0	0	0	0	0	0	0
7	15	0	0	0	0	0	0	0	0
7	17	0	0	29	28	0	0	7	0
7	22	579	434	481	478	464	355	467	447
7	30	0	0	3	6	***	***	***	***
14	15	10	na	0	6	6	0	4	na
14	17	48	43	363	309	45	31	13	56
14	22	205	164	926	912	900	820	874	904
14	30	30	12	29	29	***	***	***	***

A contains 0 % broken and 0 % impurity.

B contains 0 % broken and 3 % impurity.

C contains 3 % broken and 0 % impurity.

D contains 6 % broken and 0 % impurity.

Remark: - *** means sample severely infested by A. flavus.

- na means missing data.

Estimation on increase of damaged kernel ratio during handling
Code No. II-1-(2)-C-(a) and II-1-(2)-B-(c)

Objectives

This experiment was conducted to investigate the effect of pre-storage quality of ear maize on aflatoxin prevention during storage period and to estimate the effect of rejecting damaged ear maize on aflatoxin prevention.

Methods and Procedures

Freshly harvested ear maize was prepared 3 levels of quality through selecting procedure: (1) non-selection, (2) farmers' selection and (3) and researchers' selection. All ear maize samples were kept in gunny sacks and stored for 7 days on a pallet under natural environment. After storage, all the stored samples were inspected on the relationship between A.flavus infected kernels and those damage cause.

Summary of the results

After 7 days storage, number of ear maize infected by A.flavus was only 4.2% in the non-selection sample, while 35.4% and 45.1% in the selected samples by farmers and by researchers respectively. This seems to be caused by damage on seed coats by selection procedure on rough surface concrete floor, and it suggested that even a small scratch on seed coat may induce A.flavus infection. Most of ears infected by A.flavus had seed coat damage, 50.4% in the non-selection samples and 86.4-87.9% in the selected samples.

Problems and Future Plans

(1) To evaluate the effect of each kind of damage on aflatoxin contamination, samples should be collected according to their cause of damage and be stored for investigating relationship between damage and aflatoxin contamination.

(2) Effect of moisture content also should be concerned.

(3) It should be inspected how the dropped kernels are infected by A.flavus ; ① by small scratches on seed coats, ② through black layers, ③ effect of maturity of kernels.

(4) To look for selection methods which would not induce another damage on kernels, effect of selection on the field should be investigated as a practical method.

TABLE 1 SAMPLE SIZE AND RATE FOR SELECTING OPERATION

TREATMENT	STORED EAR	REJECT	TOTAL	REJECTION	TIME	RATE FOR
				RETIO		SELECTION
REP.1	REP.2					
CONTROL	58.89	59.47	118.36	-	-	-
F-1	59.50	56.00	115.50	4.0	13.97	516.80
F-2	54.60	55.30	110.00	7.5	14.77	482.71
F-3	52.75	53.30	106.05	10.2	19.33	356.52
F-4	56.35	54.50	110.85	7.9	13.27	544.30
F-5	53.80	51.70	105.50	11.6	15.15	472.48
F-6	53.60	54.90	108.50	7.7	12.47	505.99
GOOD EAR	49.90	52.55	102.45	42.3	-	-

TABLE 2 CAUSE OF REJECTION OF THE REJECTED SAMPLES (IN PERCENTAGE)

TREATMENT	A. FLAVUS	OTHER	SLIGHTLY	SEVERE	CRACK/	MECHANICAL	GERMINATE	NO/FEW	NORMAL
	INFESTED	FUNGUS	DAMAGED	INSECT	INJURE	DAMAGE	INJURE	KERNELS	EAR
	INFESTED	BY	DAMAGE	DAMAGE	OH	OH	OH	OH	OH
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
F-1	12.18	36.75	0.00	12.61	6.20	0.00	8.97	20.73	2.56
F-2	4.11	23.71	0.00	19.55	9.15	0.00	23.00	4.81	15.25
F-3	6.85	22.80	0.00	9.19	22.70	0.00	10.51	13.04	14.32
F-4	5.22	14.88	0.00	12.66	17.62	0.00	18.02	15.80	15.80
F-5	2.93	17.11	0.00	9.86	15.48	0.00	5.70	22.58	26.32
F-6	3.70	23.58	0.00	16.30	8.40	0.00	7.16	29.51	11.36
GOOD EAR	1.25	13.31	6.36	5.55	2.22	22.62	13.03	3.73	31.88

(III PERCENTAGE)

Table 4 A. FLAVUS INFECTION RETIO AFTER ONE WEEK STORAGE

TREATMENT	CAUSE OF DAMAGES										TOTAL INFECTION	NO A. FLAVUS INFECTION
	INSECT GERMINATION FUNGUS	OTHER GERMINATION	HANDLING (SEED COAT)	INSECT GERMINATION + HANDLING	INSECT GERMINATION + HANDLING	NATURAL CRACKAGE	IMMATURE	DAMAGE ON THE SURFACE	INFECTION	NO		
CONTROL 1	1.33	0.00	0.62	0.44	0.00	0.00	0.60	0.00	0.44	3.43	96.57	
CONTROL 2	0.72	0.00	0.09	3.78	0.00	0.00	0.11	0.00	0.25	4.95	95.05	
F-1-1	1.50	0.00	0.00	15.70	0.73	0.44	0.52	0.62	0.65	20.16	79.84	
-2	1.99	0.00	0.00	27.67	0.83	0.00	0.00	0.49	2.08	33.06	66.94	
F-2-1	0.46	0.00	0.42	30.21	1.21	0.40	0.27	0.24	0.55	33.82	66.18	
-2	1.85	0.00	1.75	23.56	0.96	0.00	0.11	0.96	0.28	29.47	70.53	
F-3-1	2.53	0.00	0.00	25.05	0.21	0.00	0.00	0.00	0.99	29.78	70.22	
-2	0.75	0.00	0.00	23.22	3.21	0.41	0.00	0.41	1.55	29.54	70.46	
F-4-1	1.71	0.00	1.03	26.11	6.93	0.59	1.14	0.57	1.41	39.49	60.51	
-2	2.70	0.00	0.36	21.88	3.14	1.17	0.69	0.00	2.37	32.31	67.69	
F-5-1	1.48	0.00	0.40	34.89	2.25	0.00	0.00	0.72	1.69	41.44	58.56	
-2	3.90	0.00	0.21	36.75	4.53	0.21	1.71	0.00	1.52	48.84	51.16	
F-6-1	5.79	0.00	1.29	35.34	2.76	0.60	0.00	0.91	0.27	46.97	53.03	
-2	1.84	0.00	2.08	33.48	1.58	0.31	0.00	0.00	0.70	40.01	59.99	
GOOD EAR 1	1.04	0.00	0.82	35.12	0.00	1.42	0.00	0.00	1.23	39.64	60.36	
GOOD EAR 2	0.96	0.00	0.41	33.48	8.15	1.13	0.96	1.54	3.99	50.52	49.38	
=====												
AVERAGE OF												
CONTROL	1.02	0.00	0.36	2.11	0.00	0.00	0.26	0.00	0.34	4.19	95.81	
FARMERS	2.24	0.00	0.62	27.82	2.41	0.35	0.37	0.41	1.17	35.41	64.59	
GOOD EAR	1.00	0.00	0.62	34.30	4.08	1.27	0.48	0.77	2.61	45.13	54.87	

Table 5 COMPONENTS OF STORED SAMPLES (IN PERCENTAGE)

TREATMENT	A. FLAVUS		OTHER FUNGUS		INFESTED		SLIGHTLY SEVERE DAMAGE		SEVERE DAMAGE		SLIGHTLY SEVERE DAMAGE		INSECT		MECHANICA GERMINATED		NATURAL IMMATURE		NO OR FEW DROPPED		NO DAMAGED		
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
CONTROL 1	0.25	3.33	2.80	6.30	1.12	3.58	6.66	0.46	0.53	0.22	0.54	74.21											
CONTROL 2	0.00	4.36	3.25	6.96	1.11	5.22	9.77	0.77	0.12	1.68	0.72	66.03											
F-1-1	3.00	3.03	1.21	12.92	0.51	18.63	3.70	0.54	0.28	0.00	1.33	54.25											
-2	1.90	5.65	2.12	13.92	1.77	13.13	5.76	1.69	0.37	0.00	0.83	52.85											
F-2-1	3.82	6.87	2.12	13.63	0.00	21.17	4.56	1.26	0.61	0.00	1.09	44.86											
-2	3.17	6.58	0.00	9.25	0.78	13.38	5.79	0.00	0.40	0.00	1.16	59.48											
F-3-1	1.42	2.65	0.00	6.19	0.00	17.40	3.07	0.21	0.32	0.00	0.71	68.04											
-2	6.87	3.47	1.95	11.46	0.00	36.40	2.28	1.39	0.27	0.00	1.54	34.36											
F-4-1	4.57	4.16	1.66	11.35	1.04	23.61	6.16	0.35	0.00	0.00	0.82	46.28											
-2	3.88	4.41	0.00	9.43	0.00	26.88	6.63	0.72	0.00	0.00	1.24	46.57											
F-5-1	2.73	4.28	2.20	11.02	0.00	24.06	3.69	0.32	0.00	0.00	1.11	50.60											
-2	7.61	5.67	0.53	17.60	0.57	37.20	5.98	0.96	0.00	0.00	0.76	23.12											
F-6-1	2.05	3.77	0.00	9.93	0.44	15.39	4.08	0.13	0.66	0.00	0.88	62.69											
-2	4.70	2.42	1.59	11.52	0.79	18.56	7.76	0.20	0.24	0.00	1.02	51.20											
GOOD EAR 1	0.00	0.00	0.00	0.00	0.00	33.07	0.00	0.00	0.00	0.00	0.00	66.93											
GOOD EAR 2	0.00	0.00	0.00	0.00	0.00	15.55	0.00	0.00	0.00	0.00	0.00	84.45											
AVERAGE OF																							
CONTROL	0.13	3.65	3.03	6.63	1.12	4.40	8.21	0.62	0.32	0.95	0.63	70.12											
FARMERS	3.61	4.41	1.11	11.52	0.49	22.15	4.97	0.65	0.26	0.00	1.04	49.58											
GOOD EAR	0.00	0.00	0.00	0.00	0.00	24.31	0.00	0.00	0.00	0.00	0.00	75.69											

Analysis of the relation between mechanical damage to kernel, machinery designs, operational condition and moisture content of maize. (1989)
Code No. II-3-(2)-A(II-1-(2)-B-(a)&(b))

Objectives

This experiment was conducted to clarify the relationship between the type of cylinder, machinery design, operational conditions, moisture content of maize and mechanical damage of kernels, to get data for making up a proto-type of improved corn sheller for high moisture maize.

Methods and Procedures

Seven types of cylinders were tested to evaluate their efficiency at MQIRC. Ear maize samples of Suwan 1 were prepared to have moisture content ranging from 16 to 32%, and were shelled at five different peripheral speed from 5 to 15m/s. And rate of breakage and power consumption was investigated.

Results

All factors, Peripheral speed, kernel moisture content and types of cylinder have effect on rate of breakage in order of degree. The effect of speed on broken kernel rate decreased as the moisture level increased. At 16% moisture, rate of broken kernel rose from 1.4% to 8.9% while it increased from 4.3% to 5.8% at 30% moisture when speed changed from 5 to 15m/s. Type of shelling cylinder will more affect the broken rate if moisture level and speed increase.

Problems and Future Plans

- (1) From the analysis of this experiment, No.6 the rectangular-spike tooth cylinder with concave clearance of 52mm seems to be the best type for proto-type cylinder.
- (2) Design of the Proto-type corn sheller should be modified from the result of other 6 cylinders.
- (3) Simulation of shelling process is worth conducting.
- (4) According to the Agronomy Section of this project, corn sheller for ear maize with husks should be investigated.

Table 1.1 DMRT for percent (%) total broken kernels for various shelling cylinders at different speeds.

MC (%)	Speed (m/s)	Cylinder Stat. 1	Sig. 1	Cylinder Stat. 2	Sig. 2	Cylinder Stat. 3	Sig. 3	Cylinder Stat. 4	Sig. 4	Cylinder Stat. 5	Sig. 5	Cylinder Stat. 6	Sig. 6	Cylinder Stat. 7	Sig. 7
16	5.0	1.7551	a	1.2154	a	1.2945	a	1.4592	a	1.3359	a	1.1865	a	1.7036	a
16	7.5	2.1650	ab	1.5293	ab	2.0279	a	1.8745	a	1.9703	ab	2.3256	bc	2.0941	a
16	10.0	3.1676	c	2.1423	bc	2.2160	a	2.4545	a	3.5490	b	2.0166	b	2.7748	ab
16	12.5	2.9054	bc	2.4804	cd	3.5656	b	5.7270	a	5.5243	c	3.1302	c	6.8573	b
16	15.0	5.9529	d	4.3797	d	8.6031	c	10.9367	b	13.7187	d	3.0210	c	15.9561	c
22	5.0	2.6363	d	2.5507	a	2.2485	a	3.2705	a	-	-	2.4448	a	2.4900	ab
22	7.5	2.2701	d	2.8082	a	2.4215	a	2.9316	a	-	-	2.7459	a	1.7612	a
22	10.0	3.1720	d	3.2959	ab	3.0071	ab	3.5195	ab	3.0436	a	3.0649	a	3.5033	b
22	12.5	3.5395	a	3.9760	bc	2.9863	ab	3.0301	a	3.4618	a	2.6314	a	3.6856	b
22	15.0	4.1120	d	4.5596	c	3.9785	b	5.0023	b	4.6870	b	-	-	6.3902	c
26	5.0	2.5121	a	3.0615	a	3.4705	a	3.4833	a	2.7831	a	1.6927	a	3.6376	a
26	7.5	3.1284	a	3.1880	a	2.8165	a	3.9248	ab	3.7503	ab	2.7501	b	3.9549	a
26	10.0	3.2316	a	4.5411	a	3.6955	a	4.2439	ab	4.7030	bc	2.9535	b	3.6396	a
26	12.5	3.4217	a	3.5884	a	3.4438	a	3.6628	a	5.4831	c	3.7516	b	4.7245	a
26	15.0	5.0622	b	4.1832	a	4.2903	a	4.9096	b	5.7213	c	3.4789	b	5.3064	a
30	5.0	4.4413	a	4.9176	a	5.0128	a	3.3431	a	3.2614	a	4.6453	a	4.1842	a
30	7.5	4.3054	a	5.0720	a	4.0937	a	4.3023	a	2.9372	a	5.2880	a	6.1350	b
30	10.0	4.8499	a	5.1244	a	5.6144	a	4.2519	a	3.7303	ab	5.5205	a	5.6877	ab
30	12.5	5.3432	a	6.2706	a	5.7306	a	4.4959	a	4.7290	bc	4.5454	a	6.8408	bc
30	15.0	5.0176	a	6.5552	a	5.9043	a	4.3692	a	5.5026	c	5.7888	a	8.0239	d

** ANALYSIS OF VARIANCE **

Broken kernels
by Type of cylinder
Moisture content
Peripheral speed

Source of Variation	Sum of Squares	DF	Mean Squares	F
Main Effects	806.469	13	62.036	64.476
Type	92.769	6	15.462	16.070**
MC	168.034	3	56.011	58.215**
Speed	510.037	4	135.009	140.319**
2-way interactions	676.212	54	12.522	13.015
Type MC	159.801	18	8.878	9.227**
Type Speed	159.481	24	6.645	6.906**
MC Speed	353.869	12	29.449	30.607**
3-way interactions	211.869	69	3.071	3.191
Type MC Speed	211.869	69	3.071	3.191**
Explained	1694.55	136	12.460	12.950
Residual	260.745	271	0.962	
Total	1955.294	407	4.804	

12 Cases were missing. * Significant at 5% ** Significant at 1%

Analysis of Variance for Broken Kernels

MC (%)	Type of Cylinder	Speed (m/s)	5.0m/s x Type	7.5m/s x Type	10.0m/s x Type	12.5m/s x Type	15.0m/s x Type
16	2.2930*	31.2767**	1.2125 ^{NS}	0.6660 ^{NS}	4.8139**	5.3146**	9.2061**
22	1.4342 ^{NS}	24.1116**	1.7563 ^{NS}	2.8748 ^{NS}	0.1905 ^{NS}	0.9869 ^{NS}	3.1092 ^{NS}
26	4.3210**	11.2162**	2.7145 ^{NS}	2.6180 ^{NS}	2.4744 ^{NS}	2.5270 ^{NS}	3.2095*
30	7.5624**	5.7494**	2.9801*	3.5371*	3.5737*	2.6673 ^{NS}	5.6119**

Urea treatment of maize to control Aspergillus spp. and so prevent aflatoxin contamination
Code No. II-3-(2)-C-(a)

Objectives

This experiment was conducted to develop urea treatment method of maize without discoloration for preventing aflatoxin contamination.

Methods and Procedures

At first, small scale experiment was conducted to determine the concentration of urea solution, ratio of water and soybean for enzyme emulsion and soaking time of ear maize into urea solution.

Then effect of farm scale treatment was investigated using non-treatment, open-air system and closed-air system covered with plastic films. Each 400kg of sample was stored in a farm scale storage model for six weeks. Amount of ammonia release from urea solution is investigated everyday in early first week and every one week later. Oxygen consumption was determined every week instead of aflatoxin contamination.

Results

(1) From small scale experiment, it is optimum urea treatment to soak ear maize into 30% of urea solution for 20 minutes using 1:3 water-soybean solution extracted 24hours as enzyme solution. And urea treatment in open-air system is more acceptable for discoloration level of grain.

(2) In farm scale experiment, ammonia concentration was a little bit more slowly increased in open-air system. Oxygen consumption rate was higher in urea treated maize than control, and it shows that microbacterial activities are higher because of higher moisture content. Yeast and fungus were observed after treatment, and more during storage period of six weeks in urea treated samples. Widespread discoloration was observed after all treatments and the color changed from orange to light darker orange.

Problems and Future Plans

(1) Maize get moisture by urea solution soaking treatment and would be infected by fungus or mold more than untreated maize during treatment and storage.

(2) Discoloration still occurs in urea treated maize and color changes darker in storage.

(3) Residue of urea under soaking treatment should be examined.

Urea treatment on farm system

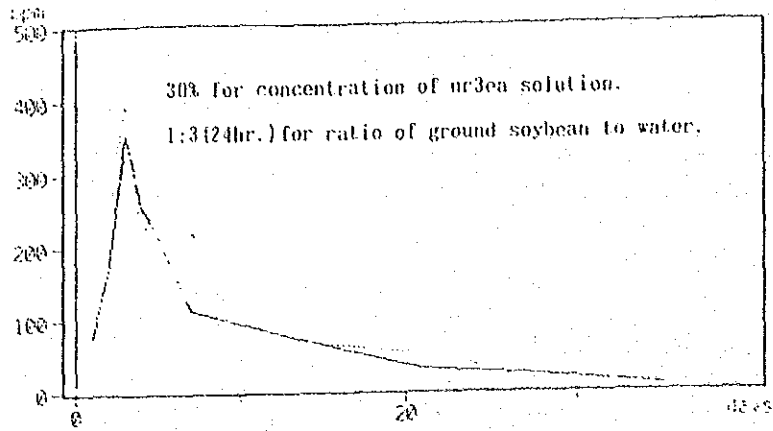


Fig. 11. Ammonia releasing time
— open system - - - close system

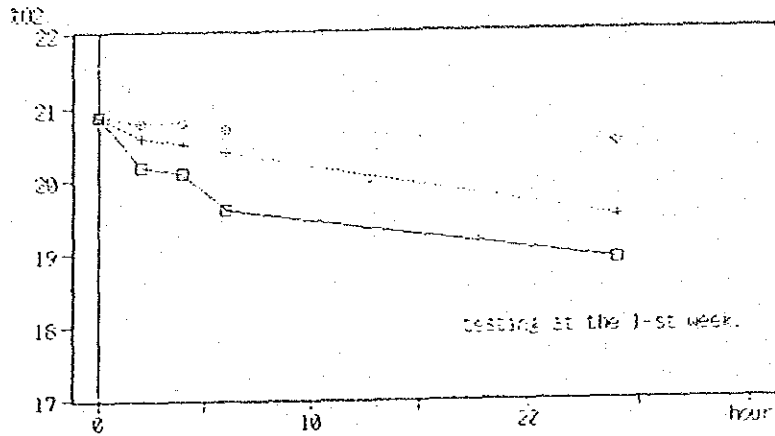


Fig. 12. SO₂ consumption in kernels
□ open sys. + close sys. ○ control

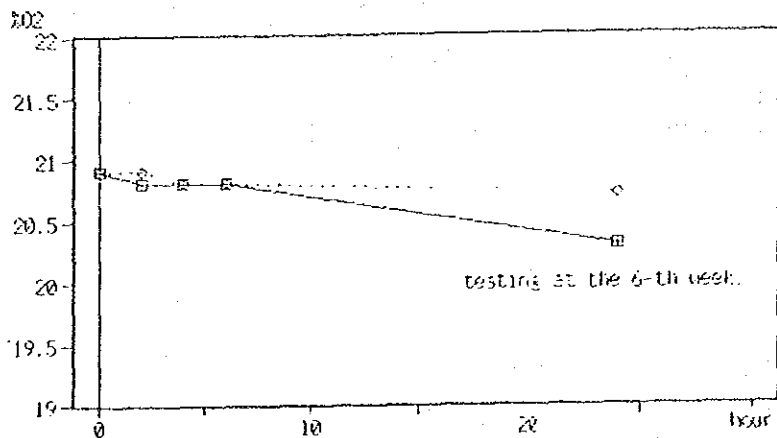


Fig. 13. SO₂ consumption in kernels
□ open sys. + close sys. ○ control

Ammonia and sulfur dioxide supplemented ambient air drying of high moisture maize in Thailand
Code No. II-3-(2)-C-(a)

Objectives

This experiment was conducted to evaluate the potential of TAP and TSDP in Thai maize

Methods and Procedures

Manually harvested ear maize, variety Suwan 3, was shelled by Plate-tooth cylinder sheller and each 500kg was stored in dryers and dried by ambient air with flow rate of 1.0m³/min. For TAP, 0.5% of the initial sample weight of ammonia was injected in one operation or divided in three, with flowing rate of 15l/min. For TSDP, 0.5% of the initial sample weight of sulfur dioxide was added in one operation by burning sulfur cakes. Discoloration was examined by colormeter and fungus infection was examined by oxygen consumption.

Results

In TAP with one operation, mold was observed inside storage sample and discoloration was observed. And in TAP with three operation, less discoloration was observed, but mold infection occurred on the surface of storage after 2 or 3 days of operation. In TSDP, discoloration was not observed, but 2 or 3 days after injection mold infected from edges or corners of the storage.

Problems

- (1) Though treated with continuous air flow, discoloration still occurs in ammonia treatment.
- (2) Air flow rate may be too slow to adapt TAP or TSDP for Thai climate or Thai maize conditions.
- (3) In TSDP, distribution of sulfur dioxide may not be sufficient, because mold occurred from the edges or corners of the drying storage.
- (4) Effect of treatment does not last long.

Future Plans

- (1) Air flow rate should be arranged in higher level to make TAP or TSDP more effective for Thai maize.
- (2) Relation between effect of chemicals and maize moisture content levels should be investigated to design injection time or rate.
- (3) TAP treated maize should be evaluated by middlemen or traders to prove their color change is still in safe level.
- (4) Residue of chemicals should be inspected to prove the treatment safe.

Sulfur dioxide supplemented storage of high moisture maize.
Code No. II-3-(2)-C-(a)

Objectives

This experiment was conducted to investigate effects of sulfur dioxide on storage of ear maize.

Methods and Procedures

After arranging sulfur cake for fumigation of ear maize, each 1 ton of ear maize was stored in a corn crib and treated by sulfur dioxide. Total volume of sulfur dioxide treatment is 0.05% of the initial sample weight. Distribution of sulfur dioxide was detected by gas detector. Ear maize was treated in one operation or three at one week intervals. After treatment, corn crib was covered with plastic films for one day. Ear maize was stored for 6 weeks and sample was extracted every week to examine oxygen consumption, moisture content and discoloration.

Results

Composition of sulfur cake is determined 100:10:10:50 as sulfur:coconut fiber:burnt rice husk:starch. There were no problems in the distribution of smoke for ear maize storage by visual observation, and the data for distribution by gas detector are under analysis. Data for sulfur dioxide supplemented storage are under processing, but no discoloration was visually observed.

Problems

(1) Sulfur cakes sometimes do not burn stable and catch fire.

Future Plans

(1) Composition of sulfur cake should be improved to make calm smoke.
(2) Residue of sulfur dioxide should be inspected to prove the treatment safe.

Study of the improvement of storage facilities for farmers(1989-1991)
Code No. II-3-(2)-C-(c)

Objectives

This investigation was conducted to clarify the changes inside of the storage facility during ear maize storage period to improve farmers' storage facilities.

Methods and Procedures

Experimental models of the elevated-floor and ground-floor corn cribs with 2.6m x 2.6m x 1.8m (D x W x H) were constructed and each 5.1 tons of maize with 29% kernel moisture were stored in them.

During storage, temperature and relative humidity of each 50cm layer inside and at the bottom of storage were investigated. After one month's storage period, moisture content of samples at each measuring point is determined.

Results

The results are under processing.

Future Plans

- (1) Possibility of estimating kernel moisture content through relative humidity of air surrounded by ear maize.
- (2) According to the results of this year's investigation, modification of storage available for farmers should be taken and its effect should be investigated.

Development of simple drying method(1989-1991)
Code No. II-3-(2)-B-(b) (II-1-(2)-C-(c))

Objectives

This experiment was conducted to develop a cheap and less energy-intensive method to dry maize for farmers.

Methods and Procedures

At first, air flow resistance of ear maize was determined using a wind channel partly filled with ear maize, and moisture content under constant temperature and humidity condition was investigated.

Two types of experimental drying unit was fabricated. One is with 10Hp diesel engine with solar heat collecting chamber and the other is with 15Hp diesel engine with dehumidifier in the solar heat collecting chamber. The drying chamber is 1150 x 1150 x 1320mm(W x D x H) size and with acyl plastic side walls. Ear maize sample were dried with this unit and weight decrease of the whole batches, temperature and humidity of inlet, outlet and inside of it were measured.

Results

Problems

- (1) These models are too expensive for each farmers.
- (2) For rainy season, it takes too long to dry products by solar system due to lower sunshine energy and high relative humidity content.
- (3) In solar system, discolorstion was observed when engine exhaust heat was supplemented. This may be caused by engine exahaust gas together with high temperature.

Future Plans

- (1) Portable system adaptable for farmers' storage is preferable if it should be continued.
- (2) Furthur analysys for basic data of car maize drying procedure is necessary.

Allowable duration for delay of drying in the post-harvest process of maize
Code No. II-1-(2) - (C-(c))

Objectives

This experiment is conducted to know the allowable delay of drying concerning aflatoxin contamination.

Methods and Procedures

Each 360kg of ear maize "Suwan 1" with different moisture content was loaded into a flat bed dryer and was dried from 0,1,2,3,5 and 7 days after loading till it comes to the equilibrium moisture content. Temperature profile at the bottom and top layer of the batch, outlet air and relative humidity profile of outlet air were measured. Samples were extracted to determine moisture content twice a day. Aflatoxin contamination was determined before and after drying.

Results

Drying rate was rather high, because of enough air flow rate and because it was not in rainy season. Drying rate and the profile of temperature and relative humidity would be shown later.

Aflatoxin contamination levels became a little bit higher after drying, but still in low levels.

Problems and Future Plans

- (1) This experiment needs to be conducted also in rainy season to get data of the first crop maize in rainy season and of high moisture ambient air.
- (2) Air flow rate should be varied in some degrees to estimate the effect of drying rate.
- (3) Basic data is better to be collected on relationship among drying rate, humidity, temperature and air flow rate.
- (4) Delayed period should be prolonged to investigate the maximum delay in each environmental conditions.
- (5) If possible, the effect of drying ear/maize which is already A. flavus infected but not yet aflatoxin contaminated is to be investigated.

Effect of Pre-Drying Delay on Aflatoxin Contamination
Aflatoxin Content Before and After Drying under Different Delay

Delay (days)	Moisture 23%		Moisture 24%		Moisture 33%	
	Pre-Dry	Post-Dry	Pre-Dry	Post-Dry	Pre-Dry	Post-Dry
0	ND	ND	ND	ND	ND	3
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	--	--	ND	4
5	ND	ND	--	--	ND	2
6	--	--	ND	ND	--	--
7	ND	ND	--	--	3	7
9	--	--	ND	3	--	--
14	--	--	3	7	--	--

ND: Not Detected, --: Not Examined, Number: Aflatoxin in ppb

Standardization of the standard oven method(1988-1989)
Code No. II-2-(2)

Objectives

This investigation was conducted to standardize oven method in this project.

Method and Procedures

(1) Effect of absolute humidity was investigated under Thai and USDA climate. Both 72 hrs 103°C with 15g of whole kernel and 4 hrs 130 °C with 5g ground sample was investigated.

(2) To evaluate the effect of predrying in accelerating drying procedure, estimation of procedure was done by comparing moisture contents between 100g whole kernel 72 hrs at 103°C method, 15g whole kernel 72 hrs at 103 °C methods, 5g whole kernel 4 hrs at 130 °C methods with and without predrying, 5g ground kernels 4 hrs at 130 °C methods with and without predrying and 15g 4 hrs at 130°C methods with and without predrying.

(3) To evaluate quick method for moisture content determination, the 4 hrs at 130°C method with ground and whole kernel of 5 or 10 g were investigated the correspondence to the 15g whole kernel 72 hrs at 103°C method.

Results

(1) (Data would be shown later)

(2) Predried determination showed lower correlation with the USDA method, so it cannot accelerate moisture determination procedure.

(3) Predried-ground method showed linearity to the other un ground methods, but did not parallelism. And standard deviation is higher. So not applicable for this purpose.

Problems

(1) The USDA and Thai method shows clear linearity, but indicated moisture content differs more than 2%.

(2) Sample population does not seem to be large enough.

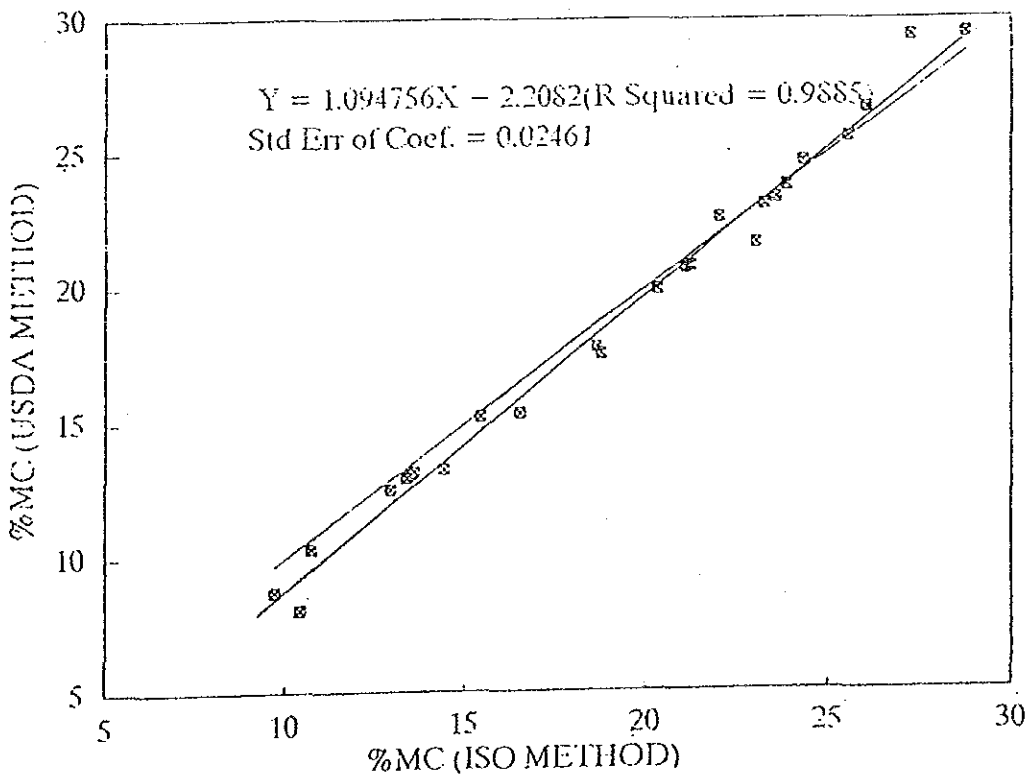
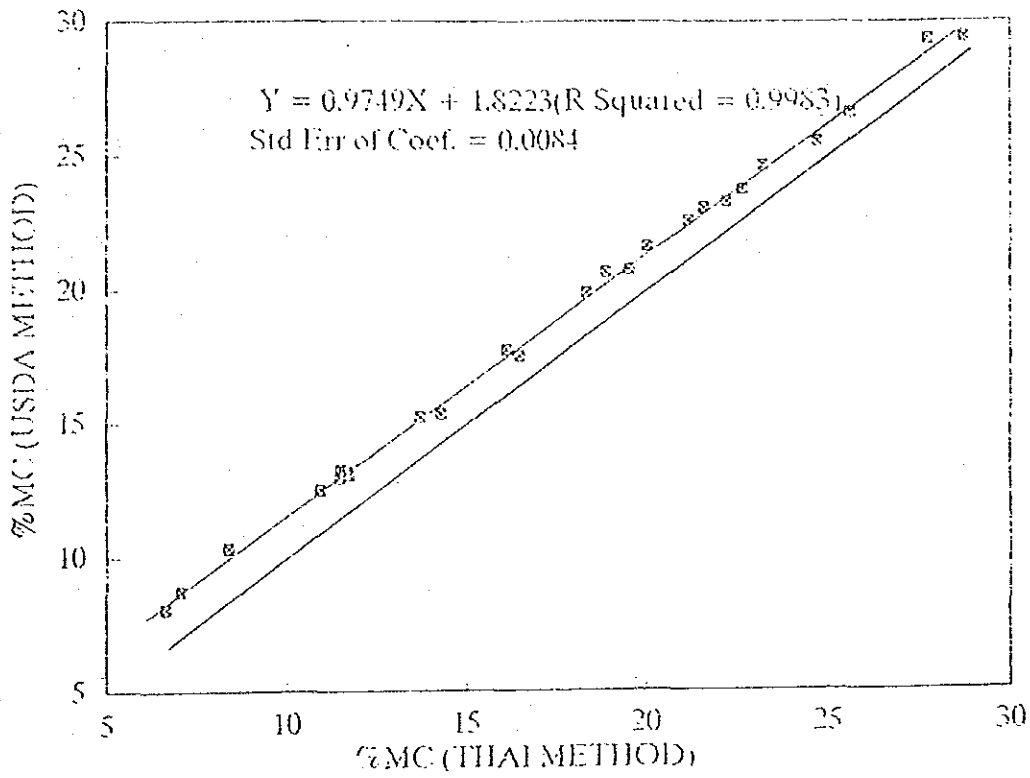
(3) Effect of the absolute humidity in ambient air for Thai method has not yet been investigated.

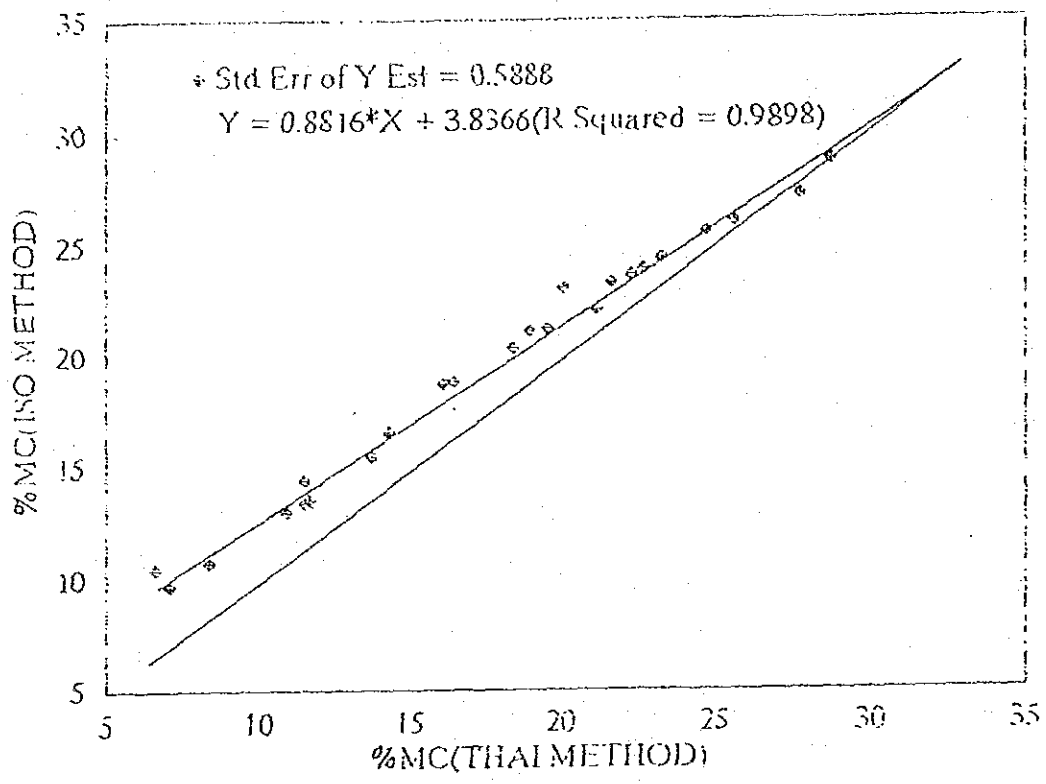
Future Plans

(1) Effect of the absolute humidity in ambient air for Thai method should be investigated.

(2) For Calibration of Thai method, more samples varied in variety is necessary to be examined.

(3) If it is available, drying time should be longer to make better correlation with USDA method.





Calibration test of established moisture meter (1988-1990)
Code No. II -2- (2)

Objectives

This investigation was conducted to calibrate existing moisture meters to be available to use for Thai maize

Method and Procedures

Four moisture meters, "CTR-160", "Dole model 400", "Digital Grain Moisture Meter" and "Multi Grain Portable", were compared with the standard oven method. Varieties of the samples were "Suwan 1", "Suwan 2", "Suwan 3" and "KU hybrid". Samples were collected from the experimental field of the Agronomy Section with different varieties and planting time. Samples were also collected from farmers' field and middlemen's storage.

Results

Calibration lines by generalized least square were presented in figures. However, we could not get enough correlations between them this year.

Problems

Correlations are low for calibration between the standard oven method and other meters.

Future Plans

- (1) Effect of maturity, variety and harvested area should be investigated, if samples are available.
- (2) Effect of conditioning should be investigated.
- (3) It may be necessary to test more than two units for each meter.

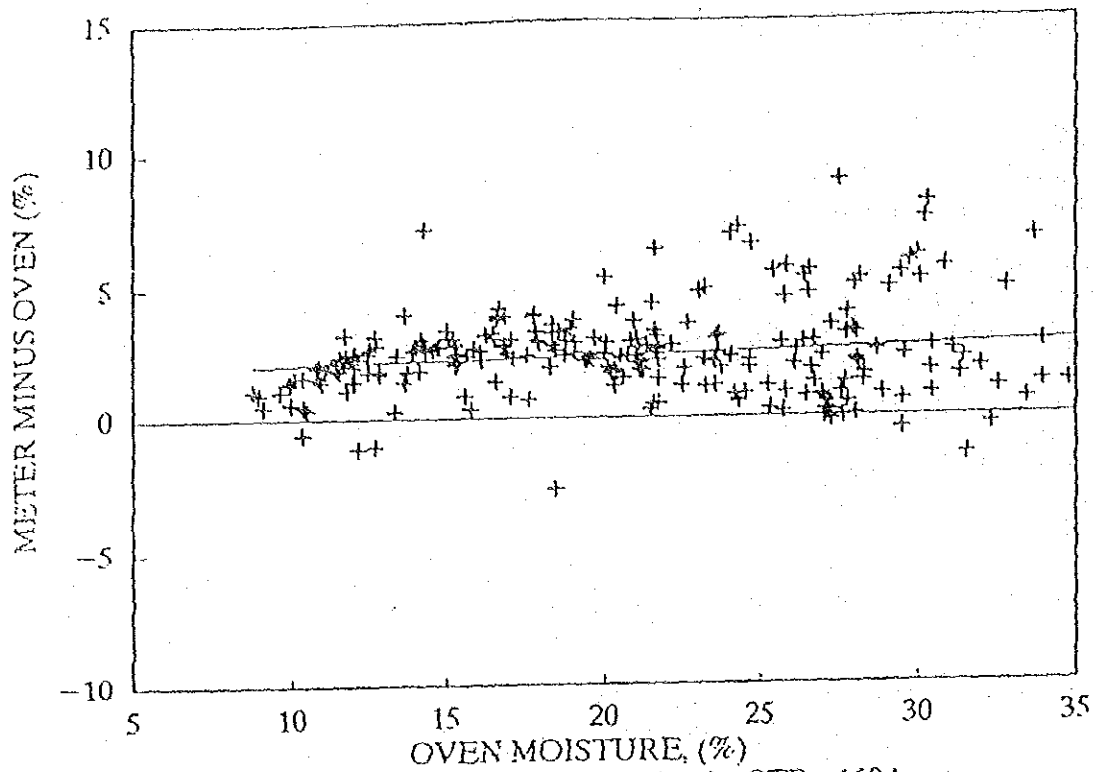


Fig.0 Moisture meter bias versus oven moisture for the CTR-160A.
(r square=0.0174)

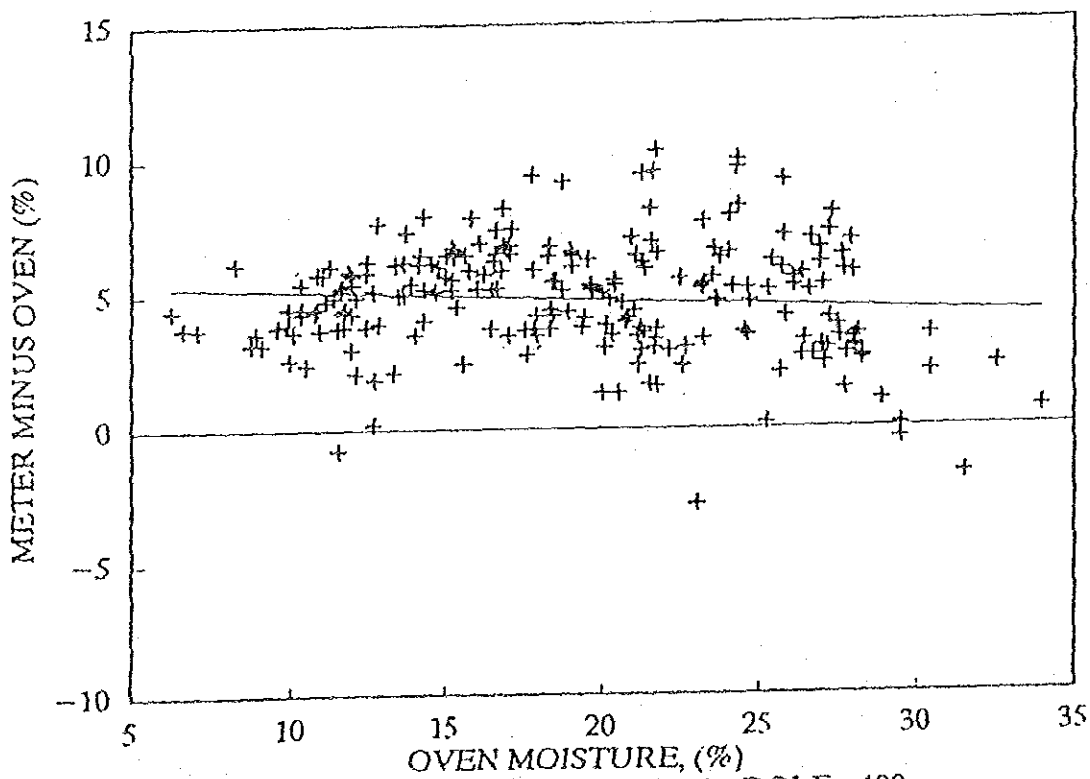


Fig.1 Moisture meter bias versus oven moisture for the DOLE-400.
(r square=0.01314)

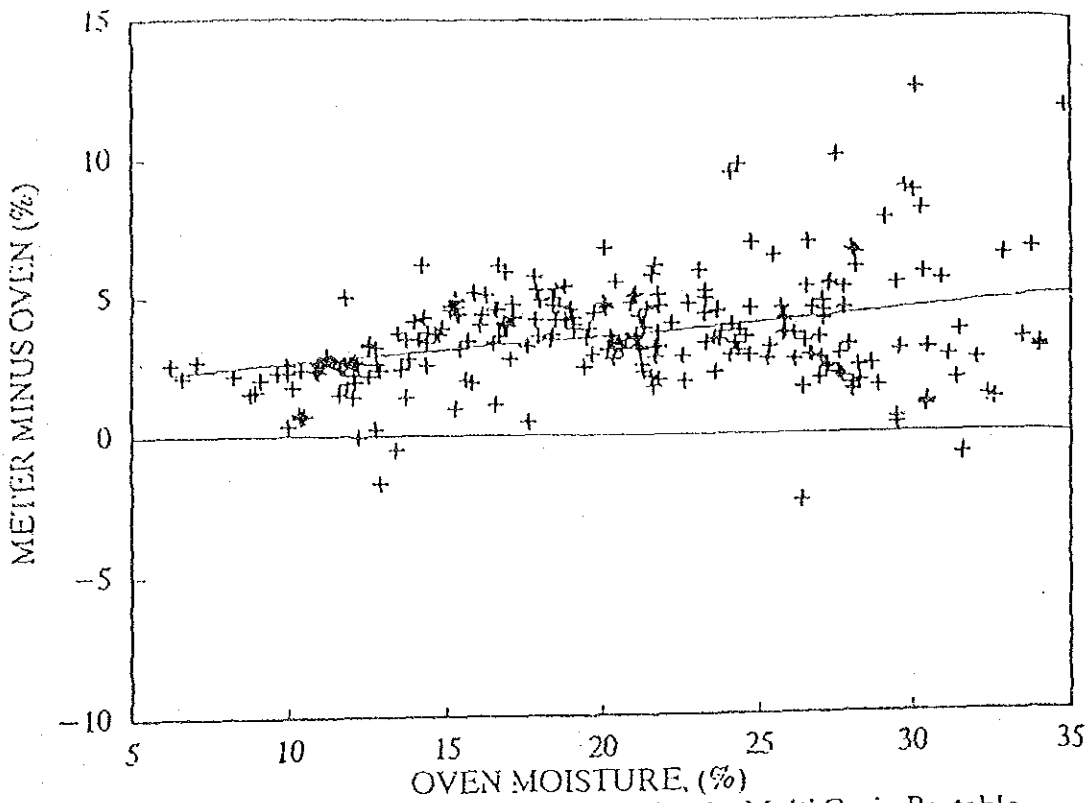


Fig.2 Moisture meter bias versus oven moisture for the Multi Grain Portable.

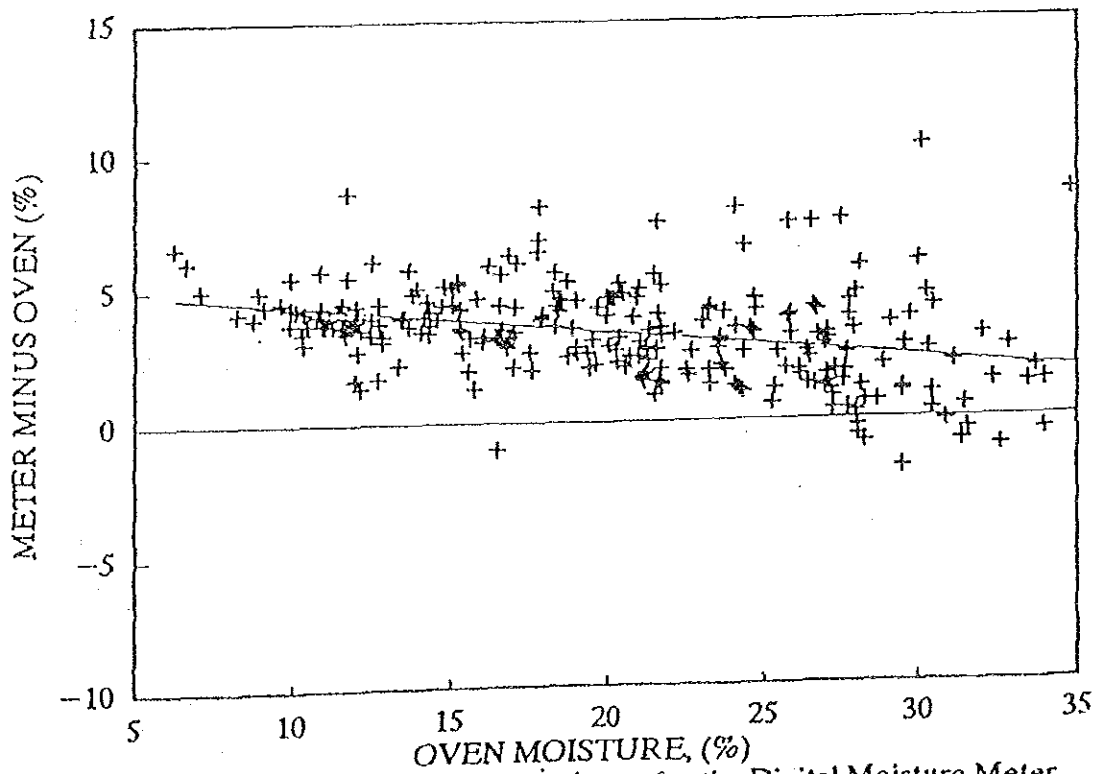


Fig.3 Moisture meter bias versus oven moisture for the Digital Moisture Meter.
(r square=0.1301)

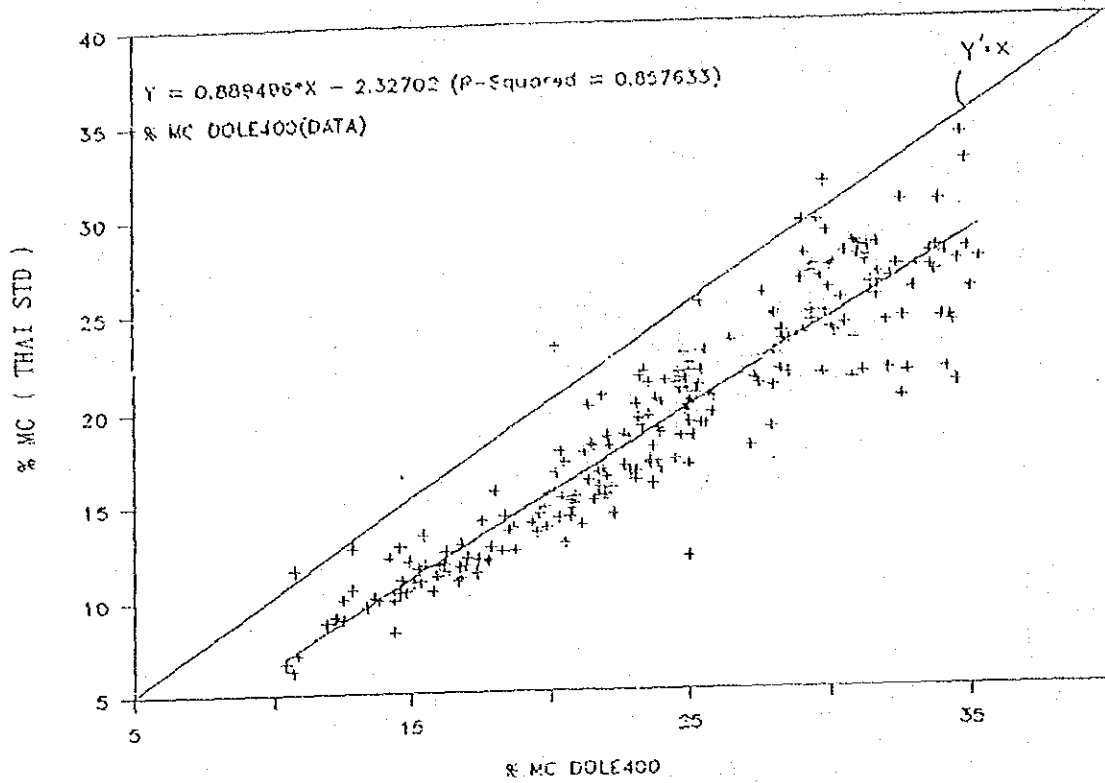


Fig.14 Distribution of Moisture Determination by "Dole 400".(1989)

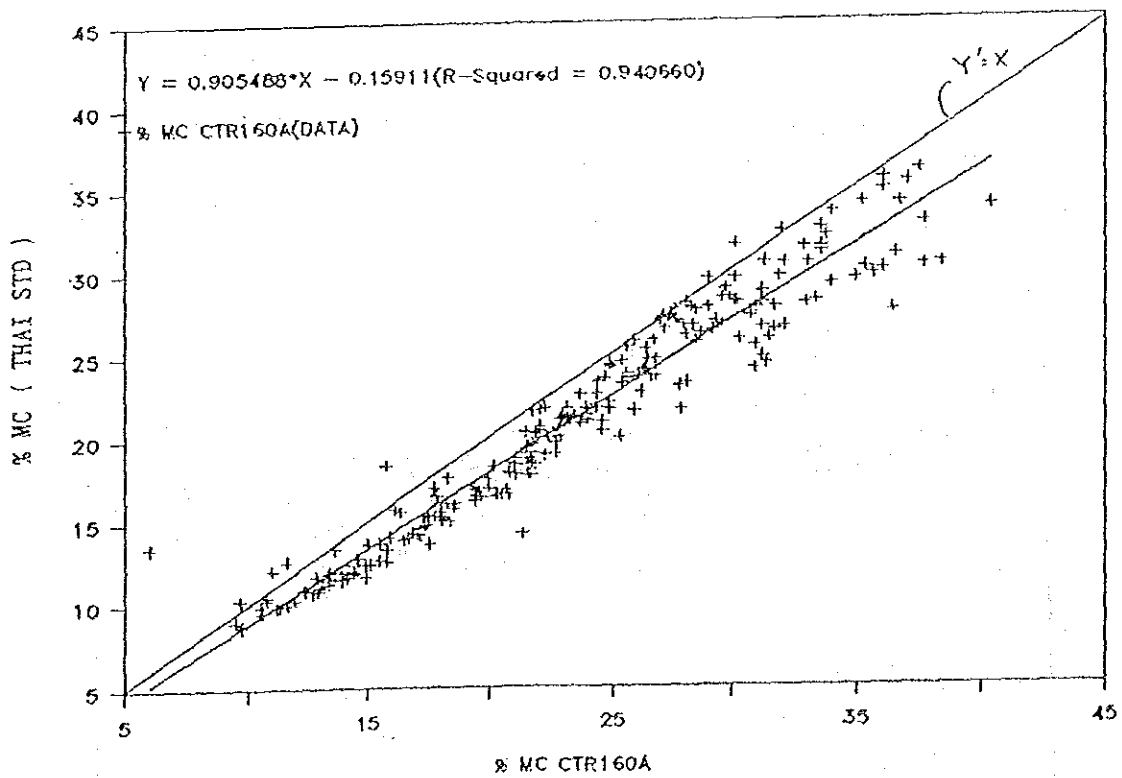


Fig.15 Distribution of Moisture Determination by "CTR-160". (1989)

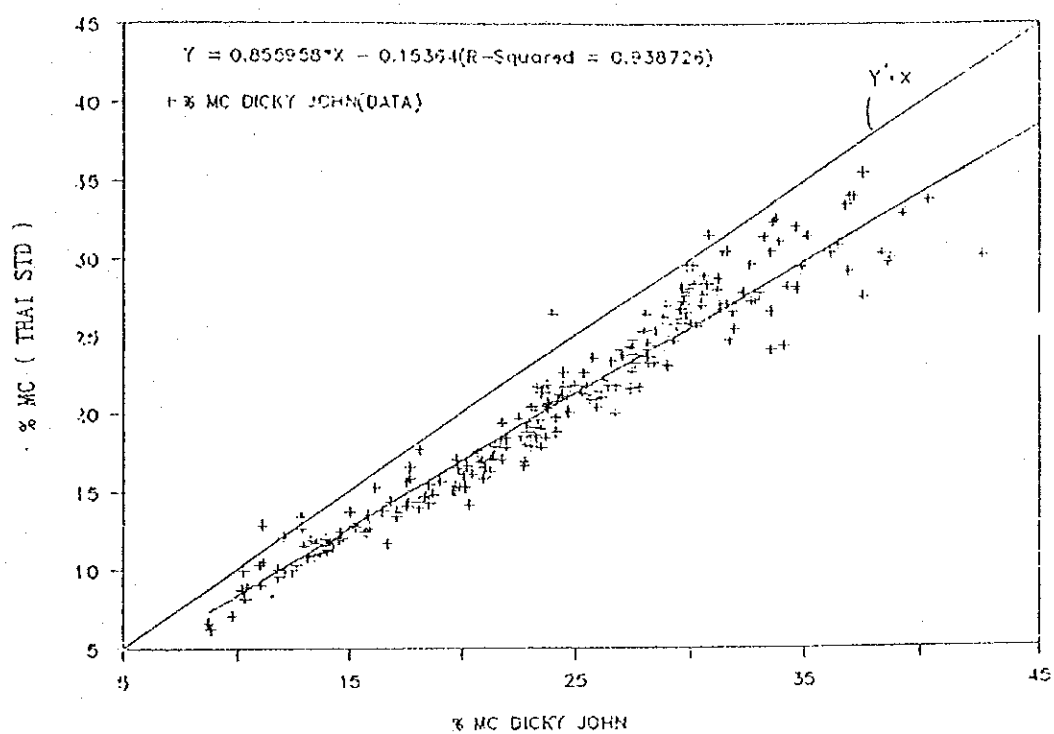


Fig.16 Distribution of Moisture Determination by "Multi Grain Portable". (1939)

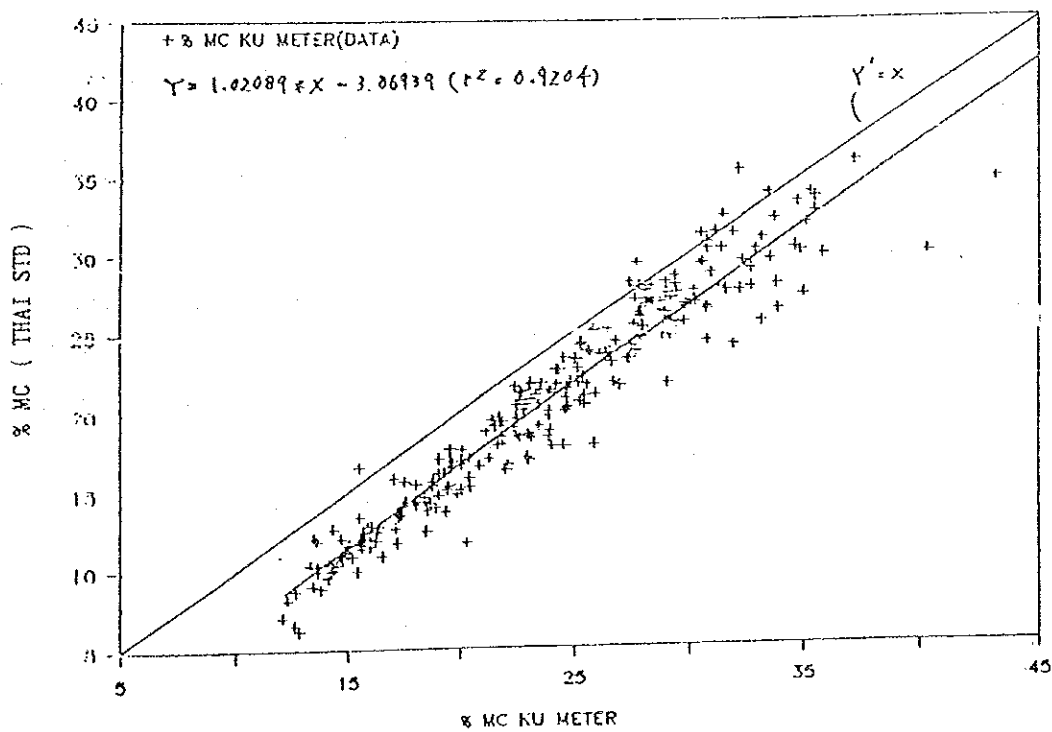


Fig.17 Distribution of Moisture Determination by "Digital Grain Moisture Meter" (1989)

Improvement and development of moisture meter (1987-1991)
Code No. II-2-(2)

Objectives

This research was conducted to develop an moisture meter for ear maize

Methods and Procedures

Prior to develop a moisture meter, distribution of kernel moisture content within each ear was examined.

"CD-2L" moisture meter was adapted as a prototype of this meter.

(1) Ear maize moisture meter with knife blade electrodes.

Knife blade electrodes were connected with calculating circuit of "CD-2L". Electrodes were inserted into shelled kernels from three directions and calibration line was investigated.

(2) Ear maize moisture meter with plier electrodes.

Electrodes were made of screws with 9mm diameters and arranged parallel with 4mm distance, and were connected with CD-2L. On determination of moisture content, ear maize was pressed at 27kg, and compared with the values determined by CTR-800. Varieties of maize are "Suwan 1", "Suwan 2", "Suwan 3" and "KU 2602".

Results

(1) Kernel moisture distribution in each ear is not significantly different among columns, but among rows. Kernel moisture is relatively low in the butt and tip of an ear and higher in the middle part.

(2) With knife blade electrodes, determined value of one kernel varies according to directions of insert. So these electrodes are not suitable for moisture meter.

(3) With screw electrodes, determined value of ear maize showed some correlation between value of CTR-800, but still not enough for calibration.

Problems and Future Plans

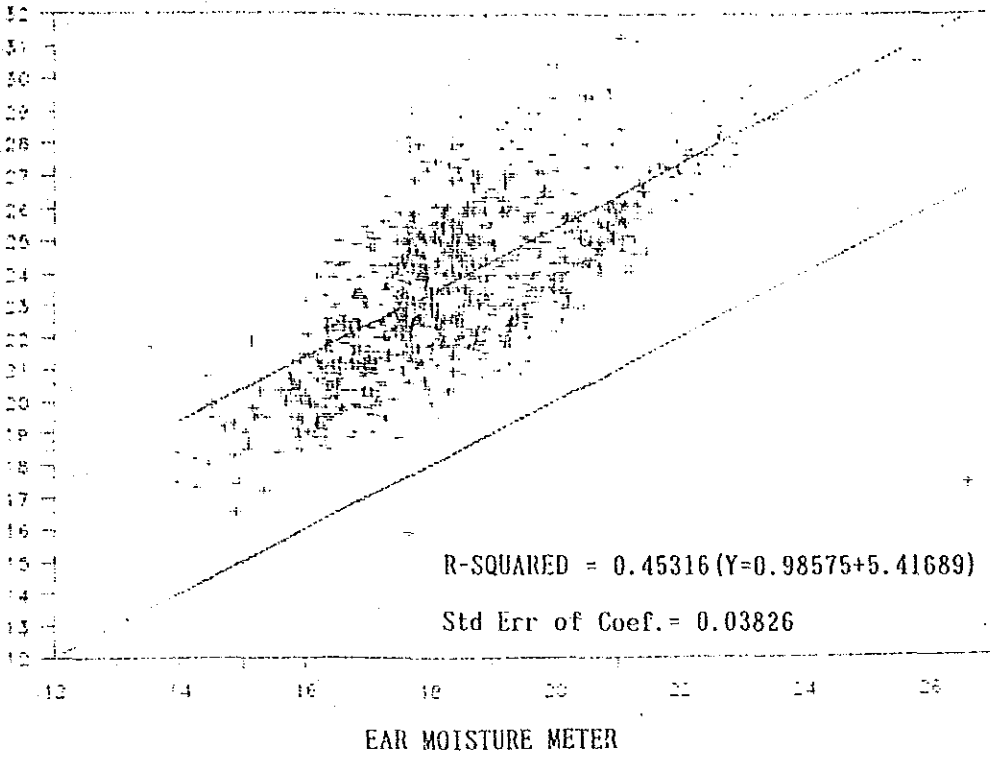
(1) By using plier type meter, only middle part of ear maize can be detected. So determined value does not always represent a whole ear. Calibration also may be taken with comparison of corresponding part of the ear.

(2) Distance of electrodes may be arranged to make stable relation with kernel columns.

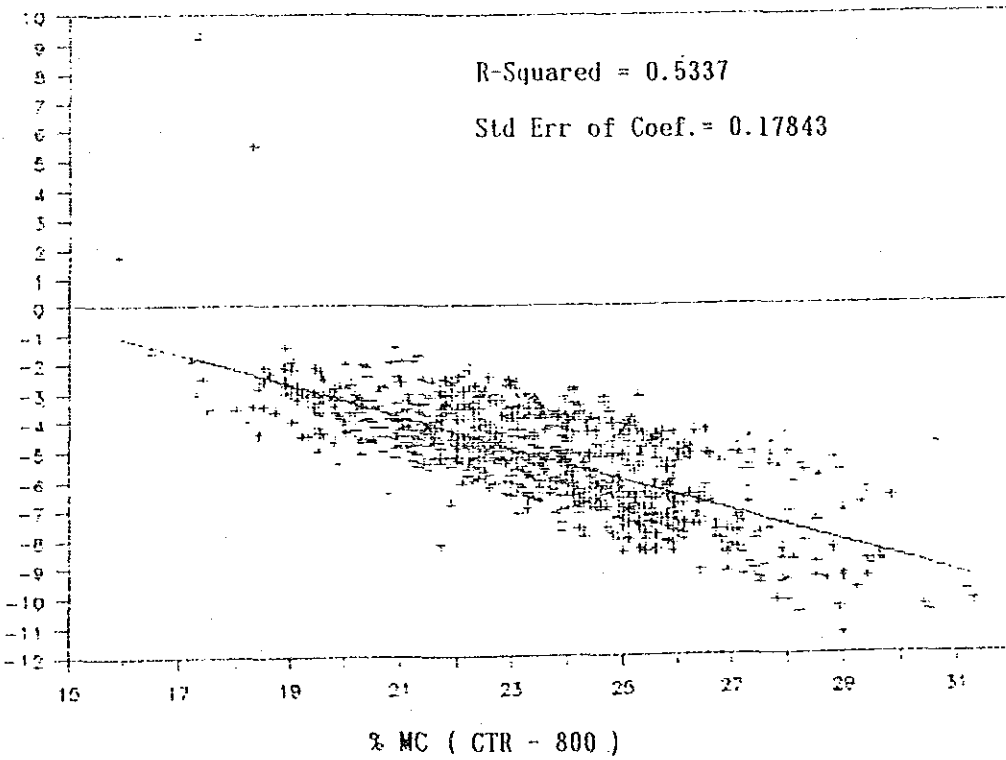
(3) Effect of conditioning should be investigated.

(4) Resistance value of calculating circuit should be arranged if possible.

CTR - 800



EAR METER - CTR 800



IV. ABSTRACT OF THE
EXPERIMENT RESULTS

MICROBE SECTION

THE MAIZE QUALITY IMPROVEMENT RESEARCH CENTER PROJECT

Studies on the A.flavus and aflatoxin contamination routes in Thai maize
Geographical and seasonal differences of A.flavus population in air, soil and maize, and aflatoxin content in maize.
Code No. III-1-(3) (MIC/III/89)

In the preliminary experiments, A.flavus was frequently found in both ear and maize kernels but also in maize plant, air, soil in maize field, farmers' store house, merchants' godown. However, main infection route is still not clear in Thailand. Accordingly, the microbe group of the MQIRC set the observation site in major maize production area, Chiang Rai, Nakhon Ratchasima, Lopburi and Saraburi provinces, to collect air, soil and maize samples periodically and analyzed the population of A.flavus and aflatoxin content in maize. Details of the results are shown in Table 1 (air, soil) & 2 (maize), and summarized in Figure 1~3. In addition, A.flavus strains separated from those samples were tested on the aflatoxin productivity (see aflatoxin productivity of A.flavus)

Materials and methods

Air sample was collected from maize fields, farmers' and merchants' store houses and a silo in the observation site by means of "SAS" Bacteriological Air Sampler onto the surface of PDA-Rose Bengal media, then incubated at 27 C for 3 - 5 days. Soil sample in maize field was collected from surface and 10 cm depth and kept in the clean plastic bag. Three grams of soil was weighed into 100 ml volume of erlenmyer flask having glass stopper and mixed well with 50 ml of sterilized water. Aliquote volume of soil suspension, 0.5, 1.0 and 2.0 ml. was taken to the petri dishes by pipett in duplicate respectively. Mix with malt agar media containing NaCl, then incubated at 27 C for 3 - 5 days. Number of A.flavus colony was expressed as in one gram of soil. Maize sample was collected from farmers' fields and store houses, merchants' godowns and a silo, both ear and shelled maize. Samples of ear maize were shelled by clean hands or small type corn sheller. A hundred gram of kernel of each sample was taken into 300 ml volume of erlenmyer flask having glass stopper and sterilized with 3% NaOCl solution for one minute by vigorous shaking. Discard NaOCl solution and wash the kernels with sterilized water for five times in the same manner by NaOCl sterilization. A hundred sterilized kernel was placed on the petri dishes containing PDA-Rose Bengal media (5 kernels in a petri dish), then incubated at 27 C for 3 - 5 days. Count number of infected kernels by A.flavus and other fungi, expressed as percent.

Aflatoxin B1, B2, G1, G2 in maize samples were determined by TLC method by means of TLC scanner, CS-920 of Shimadzu, on the extract with chloroform refined by Sep Pak silica gel cartridge followed by BGYF test for corn grit (see studies on aflatoxin content in stored wet maize).

Results and discussion

1) A.flavus in air

A.flavus population in air at the maize fields were always low, min. 0 - max. 5×10^4 /unit (301/unit) by SAS air sampler in any observation sites through year. It is presumed that contamination by A.flavus on maize in the field might be rare through air. A.flavus population in the air of merchants godown were fructuated ranging from 0-innumerable. Generally, low population was observed in late dry season to beginning of wet season. However, some abnormal A.flavus population was observed in an off-crop season. This may be due to agitated air in the godown by trading (other commodities such as mung bean, sorghum, etc.) In September at Nakhon Ratchasima, very high A.flavus population was observed in the air near the merchants' godown where shelling of ear has been done on that day. This suggest that shelled and damaged maize kernels might be contaminated seriously by A.flavus spore during shelling process.

Where A.flavus come from ?

In the parts of maize plant, stem, leaves, husks, tassel and silk were usually contaminated by A.flavus more or less. A.flavus contamination in the ear covered with husk is usually very rare except insect damaged portion. However, head of ear is usually exposed in the air which may be contaminated by A.flavus through air or transported by insects in the maize field. In shelling process, the head of ear is also thrown in the shelling machine where maize kernels might be contaminated by A.flavus spore.

A.flavus population in the farmers' store house is usually low in an off-crop season but it seems to be increased when harvest and storing of maize started.

The air in silo is always contaminated by A.flavus. This might be occurred or come from the commodity of maize contaminated by A.flavus.

2) A.flavus in the soil

Clear relations of A.flavus population were not found between depth of soil in the farmers' field. Accordingly, observations were made only surface soil in the fields. Although there are some exceptional result owing to observation site, it seems to be low A.flavus population in dry season but increased when wet season started.

A.flavus population in the soil or dust in the farmers' and the merchants' were always dirty. Unexpected difficulties were, sometimes, happened in sampling of soil, such as changing maize cultivation to another crops due to economical reason, harvest baby corn due to low precipitation, rejected sampling by merchants, etc.

3) A.flavus and aflatoxin in maize

Various degree of A.flavus contamination and AF content were observed depending on the form of maize, moisture content, freshness, sampling place and season.

Maize from Farmers

(1) Chiang Rai

Both ear and grain maize were collected during December, 1988 - October, 1989. The moisture content were ranging from 25 - 35 %, and 11 - 16 % in stored ear and grain maize. A.flavus infection to the kernels and AF contamination were very low in case of fresh ear maize. However, high AF contamination was found in 4 months stored grain maize harvested in the late wet season.

(2) Nakhon Ratchasima

Only ear maize samples were collected during Dec. 1988 - Oct. 1989. The moisture content in fresh maize were ranging from 20 to 27 %, 18 to 20 % in one or two days stored and 15 to 17 % in one week stored. A.flavus contamination was found in both fresh and stored maize. AF contamination were also not found in the almost all samples.

(3) Lopburi

Only ear maize samples were collected during Dec. 1988 - Oct. 1989. The moisture content of fresh maize (2nd crop, cultivated in the late wet season and beginning dry season) was ranging from 17 to 24 % and 16 to 21 % in 3 days stored. Little A.flavus contamination was observed in the samples collected on Dec. 1988, and 3 days stored maize collected in Aug. - Oct. 1989. AF contamination was not found in the almost all samples.

Maize from middlemen and silo

(1) Chiang Rai

Both ear and grain maize were collected during Dec. 1988 - Oct. 1989. The moisture content in ear maize was ranging from 24 to 35%, and 13 to 19% in grain maize. High A.flavus infection was found in the 3 months stored grain maize. Five grain maize stored in cotton bags, collected in Mar. 1988, showed high A.flavus infection, but AF contents were rather low level except one (42 pbb). Although various AF contamination level were found in the maize from merchants, usually new crops contain lower AF than long term stored maize.

(2) Nakhon Ratchasima

Only grain maize was collected during Dec. 1988 - Oct. 1989. The moisture content were ranging from 10 to 25%, mainly about 13%. Various grade of maize were collected in both A.flavus infected and AF levels. Extraordinary high AFB1 was detected in grain maize collected in Dec. 1989 which was harvested in the late wet season and stored for 3 to 4 months. In spite of highly infected by A.flavus sometimes low level AF in maize were found. The samples collected in May and Jun. 1989 showed rather high AF content which may be suspected as old crop because May and Jun. are a little bit early for new crop season.

(3) Lobburi

Only grain maize was collected during Dec. 1988 - Oct. 1989. The moisture content was ranging from 13 to 26% in which 15 among 24 samples showed higher than 20%. Twenty two samples collected in Aug. to Oct. 1989 were almost infected by A.flavus, 8 - 83%. Well dried maize showed low A.flavus infection, but one day dried maize containing 16 - 26% of moisture content showed high infection.

Maize from silo

(1) Saraburi

Only grain maize was collected during May - Sep. 1989. The moisture content were ranging from 9 to 27%, mainly about 13%. The samples collected in May and June showed rather high A.flavus infection. They may be presumed as old crop expecting price up in trade. AF content in maize collected in May and June were ranging from 2 to 140 ppb, but samples collected in Jul., perhaps new crop, showed very low level, 0 - 4 ppb.

Table 1 A. flavus population in the air and soil collected in different area

1-1

Air	Chiang Rai	Farmer's field Merchant's warehouse Farmer's storage										MONTH	
		12/88	1/89	2	3	4	5	6	7	8	9		10
FARMER'S FIELD													
No. of samples	10			10	10	10	10			20	10	10	
A. flavus	0			1	0	0	0			0	0	1	$\times 10^1$
A. niger	0			0	0	0	0			0	0	0	
Other f	16			5	0	0	2			10	80	5	
MERCHANT'S WAREHOUSE													
No. of samples	10			8	10	10	10			10	10	10	
A. flavus	7			5	1	4	0			>>	7	>>	$\times 10^1$
A. niger	0			0	0	0	0			1	3	3	
Other f	11			>>	11	2	3			>>	6	3	

Air Nakhon Ratchasima Farmer's field
Merchant's Warehouse
Farmer's storage

Air	Nakhon Ratchasima	Farmer's field Merchant's Warehouse Farmer's storage										MONTH	
		12/88	01/89	2	3	4	5	6	7	8	9		10
FARMER'S FIELD													
No. of samples	19			10	20	20	20	20	5				
A. flavus	0				0	0	0	0	0				
A. niger	0				0	1	0	0	1				$\times 10^2$
Other f	1				0	0	>>	0	2				
MERCHANT'S WAREHOUSE													
No. of samples	10			10	20	20	20	10	5				
A. flavus	88				0	2	1	0	0				
A. niger	40				0	4	0	0	2				$\times 10^2$
Other f	1				0	4	>>	0	>>				

Air

Lopburi
Saraburi

Farmer's field
Merchant's warehouse
Silo

	12/8801/89	2	3	4	5	6	7	8	9	10	11	
FARMER'S FIELD												
No. of samples	5			10	30	20	20	10				
A. flavus	0			0	0	0	0	0				x 10 ²
A. niger	0			0	0	0	0	0				
Other f.	0			0	0	2	0	>>				
MERCHANT'S WAREHOUSE												
No. of samples	6			10	20	10	10	10				
A. flavus	0			0	5	>>	2	1				x 10 ²
A. niger	1			0	4	0	16	0				
Other f.	1			1	>>	>>	>>	>>				

Air Chiang Rai Farmer's field (1)
Merchant's warehouse
Farmer's storage

	12/88	1/89	2	3	4	5	6	7	8	9	10	11			
				FARMER'S STORAGE											
No. of samples	4			10	20	20	20		6	10					
A. flavus	1			0	1	0	0		0	7			x10 ³		
A. niger	1			0	0	1	1		0	1					
Other f.	11			20	22	2	3		4	29					

No. of colonies/unit

Air Nakhon Ratchasima (2)

	12/88	01/89	2	3	4	5	6	7	8	9	10	11		
				FARMER'S STORAGE										
No. of samples	10			10				4	20					
A. flavus	0			0				12	13				x10 ³	
A. niger	0			0				12	8					
Other f.	3			0				>>	4					

No. of colonies/unit

Air Lopburi (2)
Saraburi

	12/89	01/89	2	3	4	5	6	7	8	9	10	11		
				SILO										
No. of samples						16	10	10	10					
A. flavus						>>	>>	2	>>				x10 ³	
A. niger						>>	>>	4	>>					
Other f.						1	>>	2	>>					

No. of colonies/unit

Soil Chiang Rai Farmer's field Merchant's warehouse

		MONTH												
		12/88	01/89	2	3	4	5	6	7	8	9	10	11	
FARMER'S FIELD														
No. of samples	6			12	20	20	20			20				same farm.
A. flavus														$\times 10^2$
0 cm	0.34			0.2		1.12	2.14			1.4	0.4	1.7		
10 cm	0.6			0.2		1.07	1.4			1.6	0.2	0.7		
Other f.														
0 cm	88.1			79.4	21.9	>>	47.9			90.6	52.3	62.5		
10 cm	74.2			92.6	38.2	>>	76.3			116.2	84	65.7		
MERCHANT'S WAREHOUSE														
No. of samples	5			5	10	10	10			10				
A. flavus	>>			>>	>>	>>	>>			>>	>>	512		
Other f.	>>			>>	>>	>>	>>			116.4	>>	1006		

No. of colonies / g. soil
>> countless

Soil Nakhon Ratchasima (Pakchong) Farmer's field Merchant's warehouse

		MONTH												
		12/88	01/89	2	3	4	5	6	7	8	9	10	11	
FARMER'S FIELD														
No. of samples	40			20	40		40	20	30					2 farmers FA, B
	FA, B			FA, B	FA									
A. flavus														$\times 10^2$
0 cm	1.51			-	-		0.5	0.5	0.2					
10 cm	1.30			-	-		1.02	0.5	1.0					
Other fungi														
0 cm	25.5			54.7	42.1		32.5	35	33.0					
10 cm	40.0			42	42.8		23.0	27.5	41.5					
MERCHANT'S WAREHOUSE														
No. of samples	5			10	15	10	20	10						3 merchant MA, B, C
	MA			MA	MA	MA	MA	MB						
A. flavus	>>			9.3	9	>>	>>	>>						$\times 10^2$
Other fungi	>>			6.37	>>	>>	>>	>>						

No. of colonies/g soil
>> countless

Soil Lodburi Farmer's field
 Saraburi Merchant's warehouse

	12/88	01/89	2	3	4	5	6	7	8	9	10	11	
FARMER'S FIELD													
No. of samples	20				20	40	40	20	30	20	40		
	FA				FB	FB	FB	FB	FB				farmer; FA & FB
A. flavus					-	4.84	2.5	1.14	1.0	0.32	0.9		x10 ²
0 cm	1.91				-	2.4	1.1	2.1	1.3	0.18	0.48		
10 cm	4.27												FA
													Change crop ind. (Noinan)
Other fungi													
0 cm	16.7				29	54.8	29.3	41.6	45.6	41.6	35.7		
10 cm	22.7				32.4	66.3	44.6	41	65.3	46	44.5		No rain in 7.8
MERCHANT'S WAREHOUSE													
No. of samples	2					15	10	10	10	10	10		
													Same merchant x10 ²
A. flavus	>>					9	106.7	>>	158.9	72.5	100		
Other fungi	>>					28.9	162.8	>>	89.3	220	946		

No. of colonies/g soil
 >> countless

Soil Lodburi Silo
 Saraburi

	12/88	01/89	2	3	4	5	6	7	8	9	10	11	
SILO													
No. of samples						20	10	10	10	10	10		
A. flavus						36.4	>>	>>	>>	>>	334		
Other fungi						24.7	>>	>>	>>	>>	1376		

No. of colonies/g soil
 >> countless

Table 2 A. flavus population in the maize collected in different area and godown.

2-1

Maize Farmer Chiang Rai

No.	Code No.	Sample date	Ear/ grain	M/C	Stor/ Harv.	AF(ppob)				Fungi			Remarks
						B1	B2	G1	G2	A.f.	A.n.	other	
1	M 9-1	21Dec88	G	16.2	S4m.	189	7	-	-	-	9	9	G
2	10-1	21Dec88	E	26.5	H	-	ND	-	-	-	-	-	H
3	2	"	"	25.3	"	"	"	"	"	"	"	"	"
4	3	"	"	27.0	"	"	"	"	"	"	"	"	"
5	4	"	"	27.3	"	"	"	"	"	"	"	"	"
6	5	"	"	25.0	"	"	"	"	"	"	"	"	"
7	6	"	"	24.9	"	"	"	"	"	"	"	"	"
8	7	"	"	24.9	"	"	"	"	"	"	"	"	"
9	8	"	"	26.7	"	"	"	"	"	"	"	"	"
10	9	"	"	26.0	"	"	"	"	"	2	2	2	"
11	10	"	"	26.1	"	"	"	"	"	"	"	"	"
12	13-1	17Mar89	E	11.6	S15m.	-	ND	-	-	33	12	45	A
13	2	"	"	11.9	"	"	"	"	"	36	2	38	"
14	3	"	"	11.6	"	"	"	"	"	53	-	53	"
15	14-1	18Mar89	E	14.3	S2m.	-	ND	-	-	36	11	47	B
16	2	"	"	13.9	"	"	"	"	"	31	10	41	"
17	3	"	"	14.1	"	"	"	"	"	21	8	29	"
18	16-1	16Apr89	E	11.7	S2.5m	-	ND	-	-	2	-	2	A
19	2	"	"	11.0	"	"	"	"	"	2	-	2	"
20	3	"	"	12.0	"	"	"	"	"	4	-	4	"
21	4	"	"	12.6	"	"	"	"	"	3	-	3	"
22	5	"	"	12.0	"	"	"	"	"	6	-	6	"
23	M 17-1	"	"	12.7	S3m.	-	ND	-	-	4	-	4	B
24	2	"	"	13.7	"	"	"	"	"	2	-	2	"
25	3	"	"	12.6	"	"	"	"	"	-	-	-	"
26	4	"	"	13.2	"	"	"	"	"	7	-	7	"
27	5	"	"	13.3	"	"	"	"	"	6	-	6	"
28	25-1	16Aug89	"	>30	S3day	-	ND	-	-	-	17	17	C
29	2	"	"	>30	"	-	ND	-	-	-	1	26	"
30	26-1	16Aug89	"	>35	H	-	ND	-	-	-	-	35	"

31	2	"	"	"	"	"	"	"	"	"	"	101	101	"
32	3	"	"	"	"	"	"	"	"	"	"	86	88	"
33	4	"	"	"	"	"	"	"	"	"	"	13	16	"
34	5	"	"	"	"	"	"	"	"	"	"	44	45	"
35	32-1	13Sep89	"	24.2	S10d.	"	ND	"	"	"	"	39	39	D
36	2	"	"	23.6	"	"	"	"	"	"	"	34	34	"
37	34-1	13Sep89	G	25	S10d.	"	"	"	"	"	"	11	14	"
38	2	"	"	24.8	"	"	"	"	"	"	"	12	7	"
39	44-1	10Oct89	"	17.0	S40d.	19.8	0.4	"	"	"	"	12	17	E
40	45-1	"	"	"	S	101.2	1.8	"	"	"	"	21	1	"
41	2	"	"	"	"	32.4	0.4	"	"	"	"	8	2	"
42	3	"	"	21.1	"	54.2	1.3	"	"	"	"	36	1	"
43	46-1	11Oct89	E	"	H	1.6	"	"	"	"	"	1	99	F
44	2	"	"	"	"	"	ND	"	"	"	"	"	91	"
45	3	"	"	"	"	"	"	"	"	"	"	"	100	"
46	4	"	"	"	"	"	"	"	"	"	"	"	100	"
47	5	"	"	"	"	"	"	"	"	"	"	"	100	"
48	M 47-1	11Oct89	E	S6w.	"	"	ND	"	"	"	"	"	100	D
49	2	"	"	"	"	"	ND	"	"	"	"	"	100	"
50	3	"	"	"	"	8.2	0.1	"	"	"	"	"	100	"

Maize

Farmer

Nakhon Ratchasima

No.	Code NO.	Sample date	Ear/ grain	M/C	Stor/ Harv.	AF(ppb)				Fungi			Remarks	
						B1	B2	G1	G2	A.f	A.n	other		total
1	M 3-1	8Dec88	E	22.7	H	-	ND	-	-	-	-	22	22	A
2	"	"	"	22.8	"	"	"	"	"	"	"	14	14	"
3	"	"	"	24.5	"	"	"	"	"	"	"	26	26	"
4	"	"	"	23.8	"	"	"	"	"	"	"	21	21	"
5	"	"	"	22.9	"	"	"	"	"	"	"	11	11	"
6	"	"	"	23.0	"	"	"	"	"	"	"	10	10	"
7	"	"	"	25.3	"	"	"	"	"	"	"	13	13	"
8	"	"	"	24.3	"	"	"	"	"	"	"	21	23	"
9	"	"	"	23.8	"	"	"	"	"	2	-	20	20	"
10	10	"	"	23.7	"	"	"	"	"	1	-	22	23	D
11	4-1	8Dec88	E	16.0	store	-	ND	-	-	-	-	28	28	"
12	2	"	"	16.7	1week	"	"	"	"	"	"	27	27	"
13	3	"	"	15.7	"	"	"	"	"	"	"	31	31	"
14	4	"	"	16.8	"	"	"	"	"	"	"	19	19	"
15	5	"	"	15.9	"	"	"	"	"	"	"	27	27	"
16	6	"	"	15.8	"	"	"	"	"	"	"	27	27	"
17	7	"	"	14.7	"	"	"	"	"	"	"			"
18	8	"	"	17.3	"	"	"	"	"	"	"			"
19	9	"	"	16.1	"	"	"	"	"	"	"	20	20	"
20	10	"	"	16.9	"	"	"	"	"	"	"	26	26	"
21	5-1	8Dec88	E	18.5	S/H	-	ND	-	-	-	-	30	30	C
22	2	"	"	19.2	"	"	"	"	"	"	"	13	13	"
23	3	"	"	19.3	"	"	"	"	"	"	"	35	35	"
24	4	"	"	20.3	"	"	"	"	"	"	"	20	20	"
25	5	"	"	19.2	"	"	"	"	"	"	"	25	25	"
26	M 8-1	09Dec88	E	20.3	H	-	"	"	"	"	"	8	8	B
27	2	"	"	21.9	"	"	"	"	"	1	-	9	10	"
28	3	"	"	19.6	"	"	"	"	"	"	"	6	6	"
29	4	"	"	23.5	"	"	"	"	"	"	"	15	15	"
30	5	"	"	20.5	"	"	"	"	"	"	"	15	15	"

31											1	-	10	10	
32				"			"	"			-	-	11	12	"
33				"			"	"			-	-	8	8	"
34				"			"	"			-	-	3	3	"
35				"			"	"			2	-	3	5	"

S/H after harvest 1~2 days stored
in the farmers store house

Maize

Farmer

Lopburi
Saraburi

No.	Code No.	Sample date	Ear/ grain	M/C	Stor/ Harv.	AV(ppb)				Fungi			Form	
						B1	B2	G1	G2	A.f	A.n	Other		Total
1	M 1-1	8Dec88	E	19.0	H	-	ND	-	-	-	-	21	21	A
2	"	"	"	18.8	"	"	"	"	"	-	-	16	16	"
3	"	"	"	18.4	"	"	"	"	"	-	-	29	33	"
4	"	"	"	17.6	"	"	"	"	"	3	-	12	12	"
5	"	"	"	21.2	"	"	"	"	"	-	-	9	9	"
6	"	"	"	19.2	"	"	"	"	"	-	-	17	17	"
7	"	"	"	21.7	"	"	"	"	"	-	-	23	23	"
8	"	"	"	23.0	"	"	"	"	"	-	-	21	22	"
9	"	"	"	19.8	"	"	"	"	"	1	-	27	27	"
10	"	"	"	19.4	"	"	"	"	"	-	-	50	52	C&D
11	29-1	18Aug89	E	>25	S	S	ND	-	-	1	1	111	116	"
12	36-1	15Sep89	E	20.5	S	"	"	"	"	1	4	15	55	"
13	"	"	"	19.9	"	"	"	"	"	18	22	110	111	"
14	"	"	"	21.5	"	"	"	"	"	1	-	17	51	"
15	39-1	18Sep89	E	21.1	S	"	ND	-	-	8	26	51	55	"
16	"	"	"	21.2	"	"	"	"	"	3	1	46	46	"
17	"	"	"	21.3	"	"	ND	-	-	-	-	55	55	"
18	40-1	18Sep89	E	21.3	S	"	"	"	"	-	-	108	108	"
19	"	"	"	21.1	"	"	"	"	"	-	-	99	101	"
20	"	"	"	20.3	"	"	"	"	"	-	-	99	102	"
21	42-1	8Oct89	E	19.0	S	1.8	"	"	"	3	-	100	102	"
22	"	"	"	19.0	"	7	"	"	"	3	-	100	102	"
23	"	"	"	16.4	"	-	ND	-	-	2	-			"

Maize

Middleman

Chiang Rai

No.	Code No.	Sample date	Ear/ grain	M/C	Stor/ Harv.	AF(ppb)				Fungi			Remarks	
						B1	B2	G1	G2	A.f	A.n	other total		
1	M 11-1	22Dec88	G	15.9	S	-	ND	-	-	2	-	29	31	same
2	"	"	"	15.1	"	273	13			-	-	28	28	merchant
3	"	"	"	15.2	"	308	16			3	-	31	34	"
4	"	"	"	15.0	"	79	4			-	-	39	39	"
5	12-1	17Mar89	G	14.9	S	-	ND	-	-	32	-	2	34	"
6	"	"	"	15.5	"	42	0.2			40	-	10	50	"
7	"	"	"	15.8	"	-	ND	-	-	31	-	3	34	"
8	"	"	"	15.5	"	-	ND	-	-	43	-	2	45	"
9	"	"	"	15.5	"	8				24	-	29	53	"
10	18-1	28Apr89	G	12.7	S	4				2	-	9	11	"
11	24-1	16Aug89	E	>35	S2day	1				4	-	9	142	"
12	"	"	"	"	"	-	ND	-	-	5	1	138	94	"
13	"	"	"	"	"	2.8				11	2	88	103	"
14	"	"	"	"	"	1.4				7	1	90	96	"
15	"	"	"	"	"	-	ND	-	-	4	-	88	92	"
16	"	"	"	"	"	0.5				1	-	97	98	"
17	"	"	"	"	"	-	ND	-	-	16	1	59	76	"
18	"	"	"	"	"	1				4	1	92	97	"
19	"	"	"	"	"	1.4				2	-	85	87	"
20	"	"	"	"	"	-	ND	-	-	8	-	52	70	"
21	M 31-1	12Sep89	E	24.4	S		"			5	-	119	124	same merch
22	"	"	"	23.9	"		"			2	-	108	110	"
23	"	"	"	26.2	"		"			1	-	104	105	"
24	33-1	13Sep89	G	19.8	"		"			38	1	12	51	"
25	"	"	"	19.5	"		"			22	4	13	39	"
26	43-1	10Oct89	"	14.2	"	22.1	0.9			9	-	19	28	"
27	"	"	"	14.2	"	21.9	0.9			2	1	65	68	"
28	"	"	"	18.8	"	18.5	1.0			6	-	15	21	"
29	"	"	"	16.4	"	9.9	0.2			3	-	100	103	"
30	"	"	"	12.9	"	35.2	2.3			1	4	19	24	"

Maize

Middleman

Nakhon Ratchasima

No.	Code No.	Sample date	Ear/ Grain	M/C	Stor/ Harv.	AF(ppb)			Fungi			Remarks		
						B1	B2	G1	G2	A.f	A.n		other	total
1	M 6-1	8Dec88	G	15.0	S	561							A	
2	"	"	"	15.1	"	598				2	1	37	40	"
3	"	"	"	15.2	"	610				100	-	2	102	"
4	"	"	"	15.8	"	488				40	1	97	138	"
5	"	"	"	14.4	"	625				30	2	110	142	"
6	7-1	8Dec88	G	17.7	S	-	ND	-	-	-	-	9	9	A
7	"	"	"	19.0	"		"			6	-	16	22	"
8	"	"	"	19.0	"		"			-	-	12	12	"
9	"	"	"	20.1	"		"			-	-	6	6	"
10	"	"	"	18.8	"		"			-	-	33	33	"
11	15-1	Mar89	G	12.9	S	-	ND	-	-	26	-	4	30	A
12	"	"	"	12.8	"		"			25	-	4	29	"
13	"	"	"	12.4	"		"			38	-	1	39	"
14	"	"	"	12.9	"		"			29	-	-	29	"
15	"	"	"	12.0	"		"			29	-	5	34	"
16	"	"	"	13.5	"		"			17	-	9	26	"
17	"	"	"	13.1	"		"			35	-	3	38	"
18	"	"	"	12.0	"		"			19	-	1	20	"
19	"	"	"	12.1	"		"			18	-	5	23	"
20	10	"	"	13.0	"		"			29	-	2	31	"
21	20-1	23May89	G	13.2	S	-	ND	-	-	-	-	-	-	B
22	"	"	"	14.3	"	9	1							"
23	"	"	"	13.8	"	-	ND	-	-					"
24	"	"	"	13.5	"		"							"
25	"	"	"	14.6	"	2								"
26	M 20-6	23May89	G	12.6	S	170	23	-	-					B
27	"	"	"	12.1	"	181	16	3	-					"
28	"	"	"	11.8	"	34	8	4	-					"
29	23-1	20Jul89	G	19.9	S					5	3	21	29	B
30	"	"	"	13.2	"	6	-	-	-	5	2	24	31	"

31	"	"	"	13.1	"	14	-	-	-	4	-	13	20	"
32	"	"	"	11.1	"	8	-	-	-	6	-	13	20	"
33	"	"	"	12.2	"	45	1	-	-	2	2	19	23	"
34	"	"	"	23.7	"	10	-	-	-	5	3	22	30	"
35	"	"	"	18.9	"	6	-	-	-	5	4	41	50	"
36	"	"	"	23.8	"	160	7	40	20	7	3	21	31	"
37	"	"	"	10.6	"	40	-	-	-	3	-	15	18	"
38	"	"	"	10.6	"	62	1.4	-	-	-	-	-	-	"
39	30-1	18Aug89	G	14.8	S					14	-	83	97	B
40	2	"	"	14.4	"					17	9	80	106	"
41	3	"	"	25.7	S	0.2	-	-	-	8	2	97	107	"
42	4	"	"	25.2	"	-	ND	-	-	33	6	72	111	"
43	5	"	"		SSund	188	14	-	-	17	8	79	104	"
44	6	"	"		"	10	-	-	-	19	6	88	113	"
45	7	"	"		"	7.8	-	-	-	20	6	86	112	"
46	8	"	"		"					17	7	90	114	"
47	9	"	"		"					14	5	99	118	"
48	10	"	"		"	6	-	-	-	8	6	94	108	"
49	M 41-1	20Sep89	G		S	20	5	-	-	2	3	96	101	"
50	2	"	"		"		ND	-	-	22	4	91	117	"
51	3	"	"		"	9	-	-	-	18	5	93		"
52	4	"	"		"	10	2	-	-	76	14	39		"
53	5	"	"		"	7	1	-	-	37	14	74		"
54	48-1	23Oct89	"	14.4	Dryid	8.7	-	-	-	21	1	76	88	"
55	2	"	"	13.7	"	5.4				14	3	80	97	B
56	3	"	"	17.0	"	37.7				4	1	94	99	"
57	4	"	"	13.7	"	5.4				2	-	99	101	"
58	5	"	"	14.2	"	17.7				10	5	92	107	"

Maize

Middleman

Lopburi
Saraburi

No.	Code No.	Sample date	Ear/ Grain	M/C	Stor/ Harv.	AF(pbb)				Fungi			Remark	
						B1	B2	G1	G2	A.f	A.n	Other		Total
1	M 2-1	8Dec88	G	15.6	S	3.3				1	-	49	50	
2	" 2	"	"	15.7	"	1.8				-	-	2	2	
3	27-1	18Aug88	"	25.4	S	0.9				83	16	50	149	
4	" 2	"	"	25.4	dry-					82	45	58	185	
5	" 3	"	"	26.2	ing					79	20	50	149	
6	" 4	"	"	25.6	"					83	25	66	174	
7	" 5	"	"	25.3	"					70	8	84	162	
8	" 6	"	"	25.2	"					67	21	73	161	
9	" 7	"	"	25.6	"					79	18	70	167	
10	" 8	"	"	25.1	"					54	13	89	156	
11	" 9	"	"	25.5	"	0.6				70	5	68	143	
12	" 10	"	"	25.9	"	1.6				81	8	61	150	
13	35-1	15Sep88	"	16.3	S					44	6	66	116	
14	" 2	"	"	15.8	S					25	18	73	116	
15	37-1	15Sep88	"	22.7	S					48	7	67	102	
16	" 2	"	"	22.5	dry-					33	15	83	131	
17	" 3	"	"	25.6	ing					47	35	71	153	
18	" 4	"	"	23.6	"					49	21	85	155	
19	" 5	"	"	15.4	"					25	9	14	49	
20	50-1	24Oct88	"	13.8	S	14.7	0.9			8	7	89	104	
21	" 2	"	"	13.3	"	76.7	5.6			20	19	84	123	
22	" 3	"	"	13.0	"	-	ND			18	9	70	97	
23	" 4	"	"	13.6	"	18.7	0.8			20	10	84	114	
24	" 5	"	"	14.1	"	11.1	1.1			13	9	73	95	

Maize

Silo

Saraburi

No.	Code No.	Sample date	Ear/ grain	M/C	Stor/ Harv.	AF(ppb)					Fungi			Remark
						B1	B2	G1	G2	A.f	A.n	Other	Total	
1	M 19-1	17May89	G	12.8	S	3	3	-	-	4	2	7	13	
2	"	"	"	12.3	"	2	-	-	-	6	-	10	16	
3	"	"	"	12.2	"	64	8	-	-	-	-	12	12	
4	"	"	"	13.3	"	26	2	11	-	6	1	21	28	
5	"	"	"	13.5	"	3	-	-	-	1	1	13	15	
6	"	"	"	12.9	"	19	2	-	-	1	-	14	15	
7	"	"	"	12.9	"	16	2	-	-	12	2	7	21	
8	"	"	"	12.5	"	92	12	-	-	2	-	7	9	
9	"	"	"	13.0	"	2	-	-	-	4	-	13	17	
10	"	"	"	13.1	"	4	-	-	-	1	1	6	8	
11	21-1	21Jun89	G	12.6	S	76	5	1	-	11	2	24	37	
12	"	"	"	13.0	"	93	11	26	-	3	3	5	11	
13	"	"	"	12.0	"	40	3	-	-	2	2	64	68	
14	"	"	"	9.0	"	140	13	-	-	41	-	2	43	
15	"	"	"	11.2	"	44	5	-	-	2	1	15	18	
16	"	"	"	9.3	"	32	4	-	-	2	3	13	18	
17	"	"	"	13.8	"	71	5	5	-	-	-	-	-	
18	"	"	"	11.6	"	85	11	1	-	-	-	-	-	
19	"	"	"	13.3	"	44	4	9	-	-	-	5	5	
20	"	"	"	11.6	"	98	6	-	-	-	-	-	-	
21	22-1	19Jul89	G	13.7	S	0.2	-	-	-	15	1	4	20	
22	"	"	"	12.3	"	4	-	-	-	-	-	7	7	
23	"	"	"	13.1	"	0.3	-	-	-	-	-	full	full	
24	"	"	"	12.7	"	1	-	-	-	17	3	5	25	
25	"	"	"	13.7	"	1.4	-	-	-	-	-	-	-	
26	"	"	"	15.0	"	1	-	-	-	2	-	5	7	
27	"	"	"	12.0	"	1.3	-	-	-	2	-	3	5	
28	"	"	"	14.4	"	0.2	-	-	-	1	-	6	7	
29	"	"	"	12.3	"	0.1	-	-	-	-	-	-	-	
30	"	"	"	10.5	"	-	ND	-	-	1	-	5	6	

31	28-1	18Aug89	G	14.2	S	-	-	-	96	7	32	135
32	2	"	"	15.2	"	-	ND	-	36	10	34	80
33	3	"	"	14.4	"	-	"	-	36	14	68	118
34	4	"	"	14.8	"	-	-	-	31	7	66	104
35	5	"	"	21.5	"	2	-	-	68	21	86	173
36	6	"	"	21.8	"	1	-	-	63	17	89	169
37	7	"	"	22.9	"	4.2	0.3	-	66	9	74	149
38	8	"	"	22.8	"	1	-	-	64	8	83	155
39	9	"	"	23.2	"	1.2	-	-	57	6	83	146
40	10	"	"	22.2	"	0.6	-	-	47	14	65	126
41	38-1	16Sep89	G	14.5	S	6	1	-	4	-	84	88
42	2	"	"	13.5	"	7	1	-	3	-	63	66
43	3	"	"	14.5	"	14	2	-	7	1	79	87
44	4	"	"	21.8	"	7	-	-	25	10	85	120
45	5	"	"	27.8	"	2	-	-	13	24	81	118
46	M 49-1	24Oct89	G		S	3.8	0.1	-	11	11	93	115
47	2	"	"		"	12.7	0.6	-	11	5	99	115
48	3	"	"		"			-	96	6	63	165
49	4	"	"		"	8	0.2	-	14	1	97	112
50	5	"	"		"			-	7	-	95	102
51	6	"	"		"	7.4	0.2	-	10	-	82	92
52	7	"	"		"	4	-	-	5	-	96	101

Figure 1

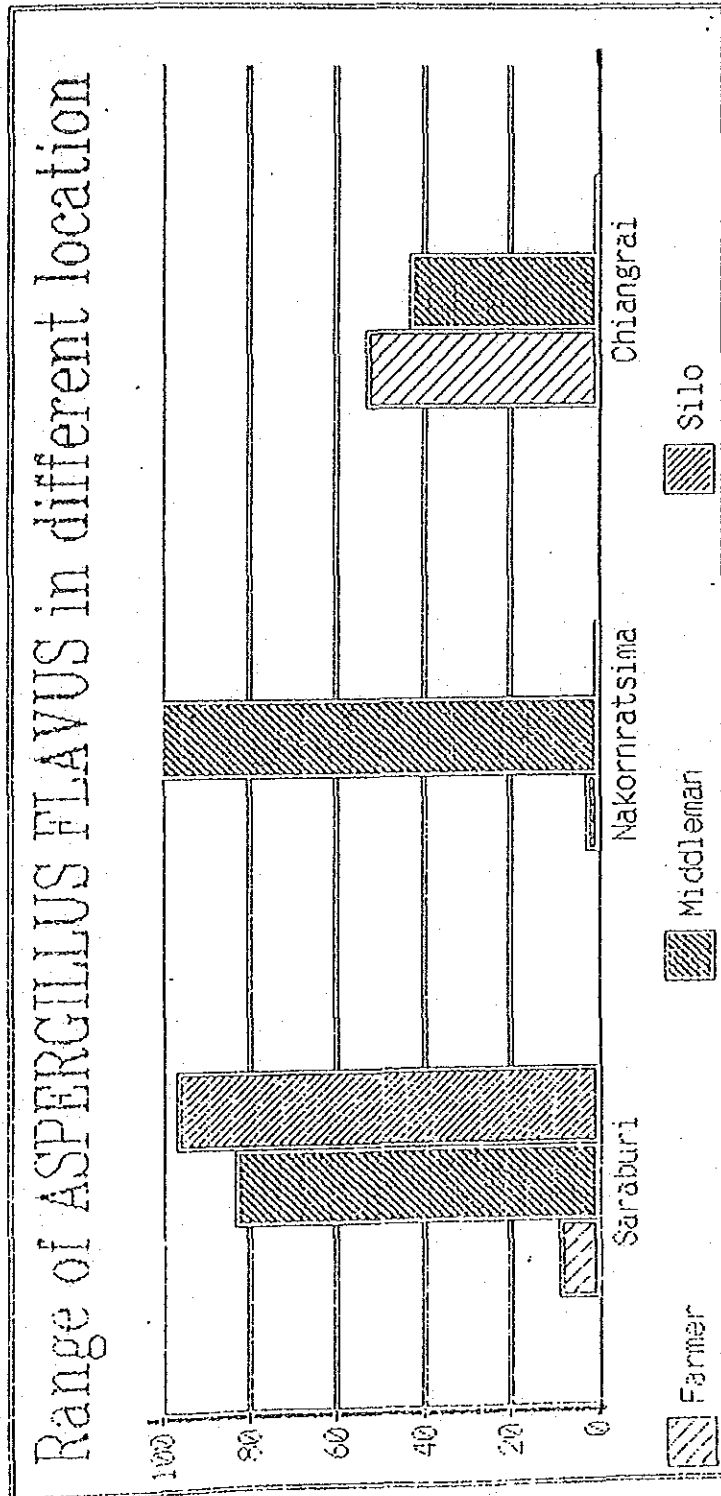


Figure 2

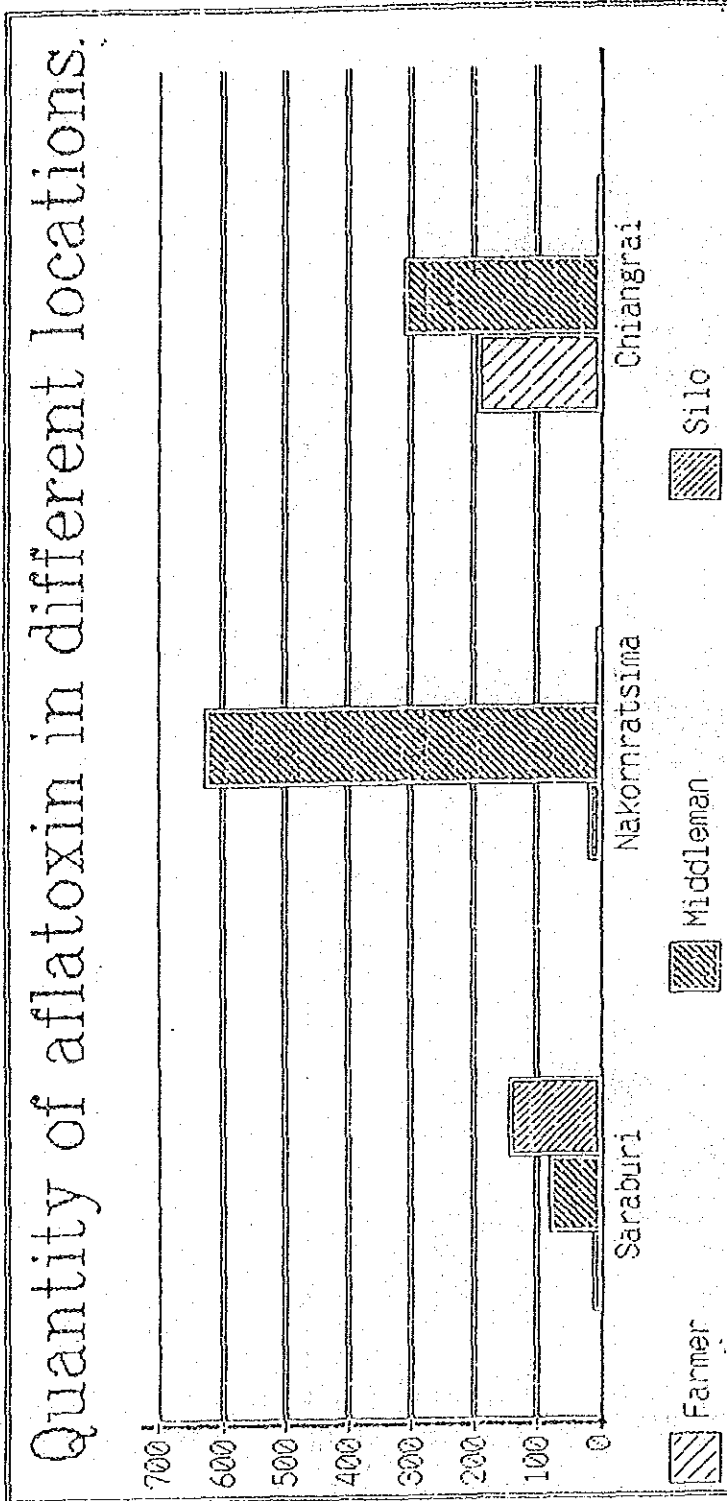
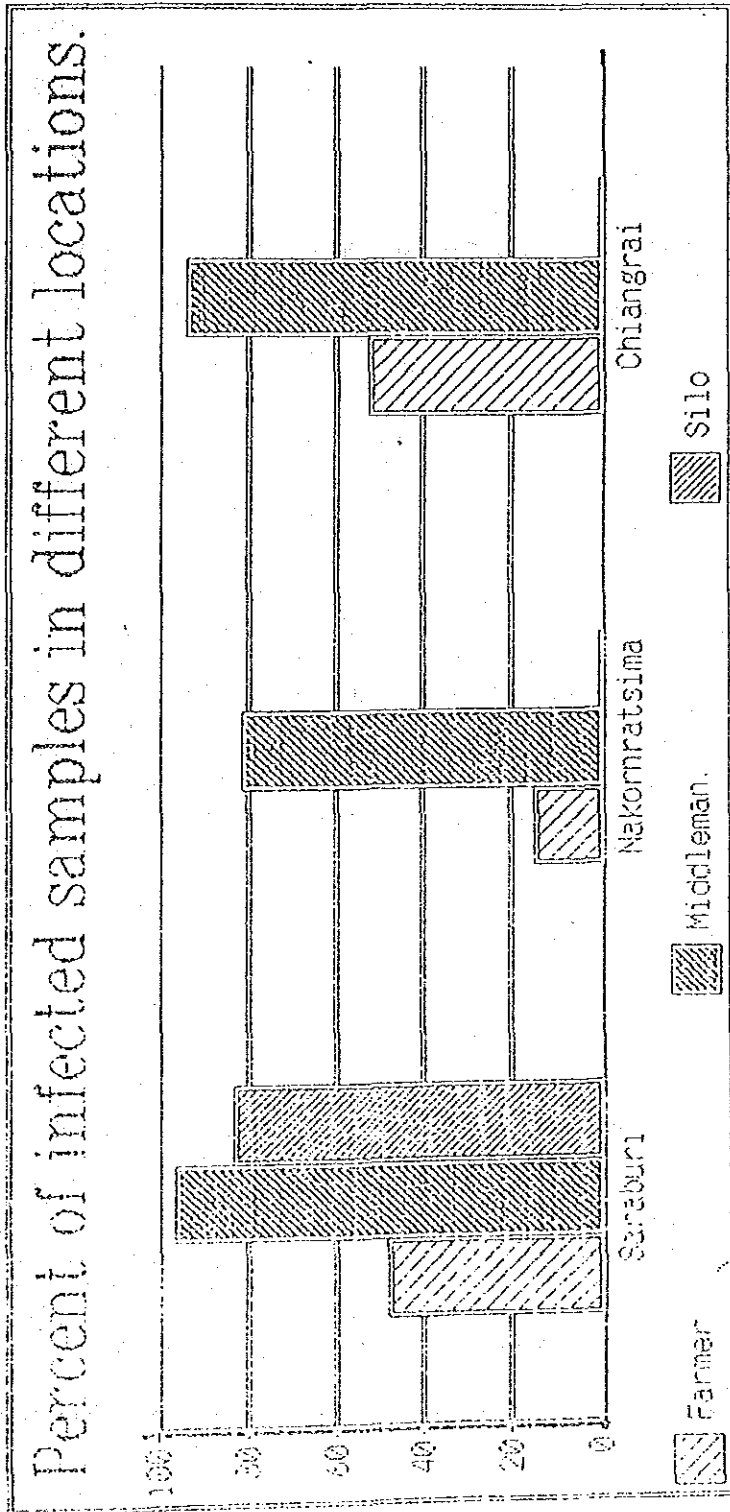


Figure 3



Aflatoxin productivity of A.flavus in Thailand
Code No. III-1-(3), (MIC/III/89)

A.flavus is one of common fungi in Thailand. It is frequently found in maize, both ear and kernels but also in maize plant, air and soil in maize field, air, soil or dust in farmers' or merchants' store house, silo, etc. However, it is presumed that there would be slight differences in morphological or toxicological characteristics among the strains of A.flavus, viz. color of colony, sclerotium formation, aflatoxin productivity, etc.

In this study, the relations between aflatoxin productivity of A.flavus collected from air, soil and maize in main maize production area in Thailand were examined.

Materials and method

Hundred twenty one strains of A.flavus were isolated from air, soil and maize collected in Chiang Rai, Nakhon Ratchasima, Lopburi, Saraburi and Phraputtabat FCES. A.flavus collected from the colony of each sample was transplanted to the slant culture containing PDA (Potato Dextrose Agar) media and incubated at 27 C for one or two weeks. Then, observe the color of colony, to confirm presence of sclerotium in the colony. Samples of aflatoxin productivity test were prepared as follows: Each strains were cultivated in GY (Glucose Yeast Extract) and YES (Sugar - Yeast Extract) liquid medium and AFPA (special media for A.flavus and A.parasiticus) media.

After 7 days incubation, both GY and YES liquid medium were sterilized in the autoclave at 121 C, for 15 minutes, then examined aflatoxin productivity by means of Immunoassey (ELISA : Enzyme Linked Immunosorbent Assay using UBE EIA KIT-AFB1, made in Japan) for the GY media and TLC qualitative method for YES media. For AFPA media, A.flavus spore was inoculated in the center of petri dish, then incubated at 27 C. Observe the color of reverse side of colony, measure the diameter at 42 and 120 hours.

In addition, to observe the presence of fluorescent materials of reverse side, the colony was exposed under the ultraviolet ray, 365 nm, in the dark box.

Results and discussion

Table 1 shows the morphological characteristics of A.flavus strains, aflatoxin B1 (AFB1) quantity (by ELISA), qualitative estimation of aflatoxin (by TLC) and degree of colony pigmentation, diameter of colony in AFPA media.

The colonies in the slant culture show the various in color ranging from white to dark green, but mainly greenish color. However, two strains show the whitish color of colony (No. 12 and 32). Fifty seven among 121 strains are sclerotium formation type. Results of ELISA showed that 80 strains were AFB1 producer among all.

Table 2 shows the ratio of AFB1 producer and sclerotium formation

type strains in which 86 strains (72%) are AFBI producer and 39 strains (32%) were sclerotium formation type, while 35 strains are non AFBI producer but 18 strains were sclerotium formation type.

Table 3 shows the number of aflatoxin producer and sclerotium formation type in 24 strains known the background among 121 strains. Fourteen strains (59%) are AFBI producer but 10 strains were not. No clear relations were observed between AFBI productivity and sclerotium formation. However, special observation between AFBI were paid on two abnormal strains in white color in which both strains showed very high AFBI productivity determined by ELISA. They are also sclerotium formation type. Though ELISA, UBE EIA AFBI - KIT, can detect only aflatoxin B1, TLC qualitative test also showed very interesting results. These two have the strong fluorescent of aflatoxin and produce not only B1 and B2 but also G1 and G2. Aflatoxin productivity of A. flavus by AFPA media, observing colony pigmentation, could not identified the producer exactly. Although Pitt et. al. reported that aflatoxin producer of A. flavus and A. parasiticus in AFPA media show orange yellow, almost all strains, both producer and non producer, showed orange yellow, pale to dark, except No. 11, in this study. The sclerotium formation in the AFPA media was, also not same as in the PDA slant culture. Observation of fluorescent in the reverse side of colony under UV ray, 365 nm, in the dark box has been tried. Some aflatoxin producer showed fluorescence in the reverse side of colony and disappeared the shape of colony. However, there was no significant correlations between aflatoxin producer and fluorescent. The colony pigmentation and fluorescent methods will be continued using Na-desoxycholate.

Table 1 Morphological Characteristics and Aflatoxin Productivity of *A. flavus* in Thailand

No.	Source	Morphology*	Afla(m/m)		AFLA medium*		UV-FL	Immuno assay (ppb)	TLC qual. test	Remarks
			40	41	color	Sclero.				
1	A-1	Whitish green	40	41	OY	++	-	>>80	+++ B1 B2	Phrapht, field, air, Jul/89
2	A-2	Sclerotium(+++) green	39	38	OY	-	+	22	ND	Phrapht, field, air, Sep/89
3	A-3	green	39x38	38	OY	-	+	ND	ND	
4	A-4	green	38	38	OY	+++	+	ND	ND	
5	A-5	green	33	38	OY	-	-	ND	ND	
6	S-1	green	39	42	OYD	++	±	>>80	** B1 B2	
7	S-2	green	41	39	OYP	-	+	>>80	ND	
8	S-3	green	36	36	OY	-	+	19	ND	
9	S-4	dark green	42	36	OYD	-	±	ND	ND	
10	S-5	green	39	35	OY	-	+	ND	ND	
11	S-6	white-yellow	38	38	OY	-	+	>>80	** B1 B2	
12	S-7	white-yellow	38	39	OYB	-	-	>>80	+++B1B2 G1G2	
13	S-8	green	44	46	OY	-	-	>>80	** B1 B2	
14	S-9	green	36	39	OY	-	±	30	ND	
15	S-10	green	32	35	OY	-	+	29	ND	
16	S-11	green	43	41	OYD	-	±	25	ND	
17	S-12	dark green	38	42	OYD	+	-	>80	+ B1	
18	S-13	dark	39	41	OYP	-	+	ND	ND	
19	S-14	green	35	37	OYP	-	+	ND	ND	
20	S-15	green	40	37	OYD	-	+	19	ND	

No.	Source	Morphology	AFPA medium*		UV-FL	Immuno assay	TLC gualt.	Remarks
			OYP	OY				
21	S-16	green SC(+)	36 37	OYP OY	± +	22	ND	
22	S-17	green				19	ND	
23	S-18	green SC(+++)	42 45 45	OY OYD OYD	+ - -	>>80	+ B1	
24	S-19	pale green SC(+++)	35 32	OY OY	+ +	39	ND	
25	S-20	white mycelium green SC(+)	39 36	OY OY	+ +	20	ND	
26	S-21	green SC(+)	39	OY	±	ND	ND	
27	S-22	dark green SC(+)	40x36	OY	+	52	ND	
28	S-23	white-green	39	OYD	±	>80	** B1 B2	
29	S-24	green SC(++)	39	OYD	-	ND	ND	
30	S-25	dark green	39	OY	±	>>80	** B1 B2	
31	S-26	dark green	39	OY	+	>>80	** B1 B2	
32	S-27	white-yellow SC(++)	38 34	OY Brown	+ -	>>80	+++B1B2 G1G2	
33	S-28	dark green	35	Brown	-	>80	ND	
34	S-29	green SC(++)	39 38	OYD OYP	- +	27	ND	
35	S-30	dark green SC(++)	41 42	OY OY	+ +	>80	** B1 B2	
36	S-31	dark green	31	Br	-	ND	ND	
37	S-32	dark green	35 37	OY OY	+ +	36	ND	
38	S-33	dark green	36 34	OYP OY	- -	29	ND	
39	S-34	dark green SC(++)	36 33	OYD OYP	± +	51	ND	
40	S-35	green SC(++)	41	OY	+	>80	+ B1	

No.	Source	Morphology**	AFPA medium*			UV-FL	Immuno assay	TLC result.	Remarks
			41	40	39				
41	S-36	Green SC(+++)	OY +++	OYD +++	+	18	ND		
42	S-37	dark green	OYP	OYP	+	ND	ND		
43	S-38	Green	OY	OY	-	3	ND		
44	S-39	Green SC(+++)	OYD	OYD	-	>>80	+++ B1 B2		
45	S-40	Green	OYP	OYP	+	ND	ND		
46	S-41	Green	OY	OY	+	>>80	+++B1B2 G1G2		
47	S-42	Green SC(+)	OYD	OYD	-	ND	ND		
48	S-43	dark green	OYP	OYP	+	>>80	ND		
49	S-44	Green	OY	OY	+	5	+ B1		
50	S-45	dark green	OY	OY	+	3	ND		
51	S-46	dark green	OYP	OYP	+	>>80	ND		
52	S-47	dark green SC(+)	OY	OY	±	>80	ND		
53	S-48	dark green	OYD	OYD	-	3	ND		
54	S-49	dark green	OYD	OYD	+	ND	ND		
55	S-50	Green	OY	OY	+	12	+ B1		
56	M-1 (Maiz)	white mycerium. green SC(+)	OY	OY	+	>80	ND		
57	M-2	green	OY	OY	-	>>80	+ B1		
58	M-3	dark green SC(+)	OY	OY	+	3	ND		
59	M-4	dark green	OY	OY	+	>80	+		
60	M-5	dark green	OY	OY	±	>>80	+++B1B2 G1G2		
			OY	OY	+				