Pesticide Residues -- Their Nature, Distribution, and Persistence in Plants, Animals, and Soils

L. A. Carruth

Objectives

1. To determine the nature and extent of pesticide contamination of animal feeds (particularly baled forage), animals, and animal products which are in marketing channels.
2. To investigate practical and accurate methods, suitable for use during the marketing period, for measuring and evaluating chlorinated hydrocarbon pesticide residues in the above commodities.

Summary of progress

This new project was begun in 1965.

Sampling methods for pesticide residues on growing alfalfa, windrowed alfalfa, and baled alfalfa hay were compared. The analytical results are now complete only for the baled hay. A three-fold range around a mean of 0.07 ppm and a ten-fold range around a mean of 0.004 ppm was determined for a method involving a high intensity of sampling by coring. The levels detected were in a range where the analytical method is marginal and analytical errors contribute to the apparent sampling error, and studies need to be made at moderate and higher levels. Sampling of milk from a single cow in relation to day-to-day and morning-evening variation was studied. The data do not reveal any inherent variation in pesticide levels. Results from these studies are important for providing sampling procedures which will give uniform results without a gross oversampling effort so that producers and processors may conduct adequate surveillance of their products.

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E. DISEASE CONTROL

Diseases Cause Cotton Losses

A. D. Davison

Cotton diseases caused an estimated 10.28% loss to cotton farmers in Arizona during 1965. A breakdown of the estimate is as follows:

Disease	Organism	Estimated % Loss
Verticillium Wilt	*Verticillium albo-atrum*	5.54
Bacterial Blight	*Xanthomonas malvacearum*	Trace
Root Rot	*Phymatotrichum omnivorum*	.83
Seedling Diseases	Various organisms	1.10
Boll Rots	Various organisms	.75
Root Knot	*Meloidogyne* sp.	1.01
Southwestern Cotton Rust	*Puccinia stakmanii*	1.05
	Total	10.28

In terms of value a 10% loss is approximately 74,000 bales worth about $150.00 each for a total of approximately 11 million dollars. To this figure must be added the cost of control measures where they were used.

In order to combat the losses due to disease members of the Department of Plant Pathology are working on specific cotton diseases. Each member also has freedom to develop an in-depth program in some basic area. Thus Dr. Alcorn is concerned with the vascular wilts, Dr. Boyle with biological control of soil-borne pathogens, Drs. Bloss and Gries with the biochemistry of disease resistance and Dr. Nigh with nematology. All are using common and important cotton diseases as one of their experimental tools.

New projects have been initiated during the year by Dr. Alice Boyle who is investigating the possibilities of controlling seedling diseases of cotton by antibiosis and by Dr. H. E. Bloss who is studying the occurrence of mycotoxins in cotton seed, meal and other plant products. In addition, Drs. Gries and Streets are responsible for the service aspects of cotton disease research and are expected to keep abreast of developments in this area. The talents of all staff members are available for the solution of new problems as they arise.

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Physiology Growth Survival and Parasitism of Verticillium Wilt

Stanley M. Alcorn, Lee Stith, and L. M. Blank, ARS, USDA

The objectives of this project are as follows:

a. To determine mechanisms of survival (in an arid environment; emphasis on microsclerotial production) and pathogenesis and to determine the influence of soil depth, season and cropping practice on the inoculum potential.

b. To determine the variation in metabolism of susceptible and tolerant plants (i.e. modes of resistance).

c. To provide plant breeders with reliable methods of inducing Verticillium wilt in the greenhouse or field.

Objectives 'a" and "b" are attained through bioassays of plant exudates, sterile fungus filtrates, soil samples and fungal and plant extracts; qualitative separation and detection of pertinent components of these materials are made by chromatographic and electrophoretic techniques. Since knowledge of the mechanism of microsclerotial formation could facilitate control procedures, particular research emphasis is being placed on this phase of the life cycle of Verticillium.

Emphasis is placed on the utilization of plants and fungal cultures of varying physiological ages. Standard inoculation techniques are used to determine the susceptibility of native plants. The influence of microbial complexes on plant susceptibility to Verticillium are determined in the greenhouse using combinations of organisms (at varying concentration of initial inocula), temperatures, soil types, physiological ages of plants, and by analysis of field samples from Verticillium infested soils both treated with nematocide and untreated.

Objective "c" is being obtained by trial and error studies combining inoculum concentrations, inoculation techniques and hosts of varying physiological ages.

Progress

Early studies indicate that formation of microsclerotia (Carry over stage of the fungus) is inhibited by certain chemicals and exudates from certain plants.

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Isolation of Biochemical and/or Morphological Characters Related to Tolerance to Verticillium Wilt in the Genus Gossypium.

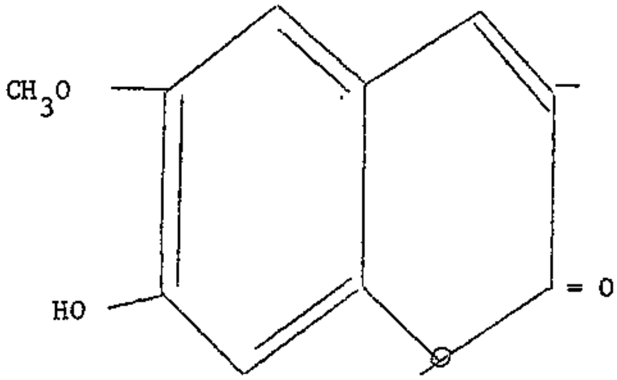
Lee S. Stith, S. M. Alcorn and A. R. Kemmerer

The objectives of this project supported by the Cotton Producers Institute are to: (1) determine whether tolerance or resistance to *Verticillium albo-atrum* can be related to specific metabolites inherently present in the host, or whether such metabolic products may develop under the influence of the pathogen, and (2) to develop a technique that can be used in the seedling stage that will identify genotypes of *G. hirsutum* L. and *G. barbadense* L. tolerant to the pathogen, *Verticillium albo-atrum*.

Progress

In the initial phases of this project, investigations centered around the identification of an amino acid that might serve as the "chemical fingerprint" to identify genotypes possessing tolerance or resistance. In a paper entitled "A Qualitative Chemical Difference In Cotton In Response to Infection By *Verticillium albo-atrum*" (presented at the National Cotton

Council Conference January 1966), we indicated that a phenolic compound, identified as Scopoletin, can be found in plants infected with the wilt pathogen. Tests are now underway to attempt to relate the presence of Scopoletin directly with specific genotypes known to possess some tolerance to the disease. The structural formula for Scopoletin is:



Okra, safflower, and squash were infected with Verticillium albo-atrum when planted after cotton in the field. Saguaro cactus and mesquite seedlings are also susceptible. Susceptibility to Verticillium appears to vary with the age of cotton plants. In the study to determine the variation in metabolism between susceptible and tolerant plants some differences in chemical composition have been noted. An attempt is being made to determine if consistent chemical differences can be found between susceptible and tolerant plants. If such differences can be found, it will be useful in the breeding program by affording a rapid method of screening for resistance.

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Soil Fumigation for Control of Verticillium Wilt

G. A. Gries, Henry Chavez, and A. D. Davison

Several compounds have been evaluated for their efficiency in controlling Verticillium albo-atrum in the field. Replicated plots were established at the Marana Experiment Station and data obtained on the incidence and severity of Verticillium Wilt.

Telone and Vorlex at rates of 50 and 75 gallons per acre delayed the onset of Verticillium Wilt; however, they also delayed maturation of the plant. Work will continue on chemical control in an effort to find a suitable material or a rate which would be economically feasible.

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Disease Complex Studies

E. L. Nigh, B. A. Tait, R. W. Miller and Stanley M. Alcorn

The relationships between nematodes and Verticillium wilt are being investigated. Field plots were established near Marana where several nematocides (SD7727, DBCP, DD and Telone) were used to control nematodes. The field also was uniformly infested with Verticillium albo-atrum.

Observations indicate that Verticillium wilt symptoms appear earlier in fumigated soil. Approximately 85% of the plants were wilted in September in all fumigated treatments compared to less than 20% of the plants exhibiting wilt symptoms in the non-treated control plot. The nematode population apparently had an indirect influence on the appearance of Verticillium wilt by suppressing the disease for approximately 30 days. This may be due to an increase or decrease of minerals available to the cotton plant or the physiological age of the plant may be reduced thereby making it more susceptible to Verticillium wilt. Also, soil temperatures may be increased due to the number and size of the plants and therefore influence the development of the fungus. Endo and ectoparasitic nematodes were present throughout the growing season on all treatments indicating further study is needed before a complex can be demonstrated between the two pathogens.

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Cropping Sequences & Nitrogen Fertilization in Relation to Verticillium Wilt.

Lester H. Blank, A.R.S., U.S.D.A. and Curt Tucker

Wilt was observed for the first time on the Cotton Research Center, Phoenix, in 1957. Since that time the various fields have been checked each year they are in Upland cotton to determine the spread of wilt and the intensity of damage. Special attention has been given to the experimental plots conducted by the Department of Agricultural Chemistry and Soils, where various cropping systems are used in conjunction with the addition of various rates of nitrogen fertilizer. Sampling in each replicate of each treatment is done near the end of the crop season by cutting of the stalks near the ground line and evaluating these stalks as to internal evidence of wilt.

It has been found that the incidence and intensity of wilt is greatest in cropping systems where cotton occurs most frequently and that wilt is intensified by the addition of manure and by high rates of nitrogen fertilizer. Rotation with other crops is helpful in retarding the build-up of Verticillium wilt. Grains and sorghums are more effective than alfalfa.

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The Effect of Phymatotrichum Root Rot of Cotton on Crop Residues

G. A. Gries and H. B. Chavez

The role of crop residues in the control of Phymatotrichum root rot is being investigated. The primary interest is in the mechanism of control by the addition of organic matter to the soil. It was shown some years ago by Dr. R. B. Streets and others that cotton root rot was controlled when papago pea residue or manure were incorporated in the soil. The mechanism of such control is not understood.

Organic matter of various composition is grown on and/or incorporated into infested field soil. Changes in soil microflora and progress of disease are determined as well as the respiration and growth of the various micro-organisms of the soil. Results of field studies have established that both winter cropping of barley and papago pea and the incorporation of the organic residues into the soil contribute to the control of root rot in cotton.

The reduction in disease resulting from organic matter additions does not seem to be a function of the chemical composition (species of plant) but rather a function of the amount of residue added. Populations of micro-organisms at time of planting are much higher in soils that have been cropped than in those fallowed during the winter. A higher percentage of the species of fungi from cropped soils than from fallowed soils were inhibitory to *Phymatotrichum* in culture.

In conjunction with these studies field plots were treated with a number of fungicides and fumigants. Telone and Vorlex at 50 and 75 gallons per acre gave significant control of the organism. Work will continue in an effort to find an economical rate or material for commercial use or application to other high value crops.

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Nature of Resistance to *Phymatotrichum omnivorum* (Cotton Root Rot)

H. E. Bloss and G. A. Gries

In general, monocotyledonous plants such as corn and grasses are resistant to the fungus causing cotton root rot while nearly all dicotyledonous plants such as cotton are susceptible. This difference in susceptibility and the reasons for it are the subject of the investigations.

If the mechanism of this resistance or susceptibility could be determined it would be a big step forward in understanding the nature of resistance and would be very useful to the plant breeder. Studies of this type are basically chemical in nature and are conducted in the laboratories and greenhouses.

It has been found that corn roots less than two weeks old are completely susceptible to the fungus but by the time the roots are four weeks old they become immune. Qualitative and quantitative differences in the phenolic fractions of corn and cotton have been demonstrated.

In supporting studies done by Dr. R. M. Hosford the nuclear history of the fungus was examined. A possible mechanism for variation within the genetic system of the fungus has been found which relates to the work or susceptibility or resistance of hosts. The research on the nature of disease resistance will be continued.

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Evaluation of Soil Fumigants for Control of Cotton Root-Knot Nematodes Attacking Irrigated Cotton

Edward L. Nigh and Bernard A. Tait

Several soil fumigants are being evaluated for their effectiveness in controlling cotton root-knot nematode. In the field experiments conducted near Marana, fumigants were injected immediately prior to planting using one shank per row in a randomized design. Fumigated treatments were compared with non-treated controls.

A root-knot index made 60 days following planting indicated no significant differences between fumigated treatments in degree of root-knot nematode control. Results from the first picking representing approximately 95% of the total crop showed no significant differences between fumigated treatments but yield from all fumigated treatments were significantly greater than the untreated control.

Yield Data from Fumigation Trials

Treatment	Total Seed Cotton	Bales/Acre (Calculated)
7 gal. DD	1553a[1]	2.050
7 gal. Telone	1579a	2.084
1 gal Fumazone (86%)	1497a	1.976
1-1/2 gal. Fumazone (86%)	1524a	2.012
Control	1255b	.814

1) Like letters indicate no significant differences as determined by Duncans Multiple Range.

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Nematode Control

Harold W. Reynolds, ARS, USDA

Experiments established in 1963 and 1964 indicated that a non-volatile, residual, experimental nematocide - 2,4-dichloro-phenyl-methanesulfonate (SD 7727) had a high degree of activity in controlling root-knot nematodes on Deltapine cotton. The chemical was broadcast as a granular application (10 and 15 lb/A active ingredient) on the soil surface and mixed into the soil with a disk harrow to a depth of 7-8 inches before the seed beds were prepared. Treated plots yielded 20% to 100% more cotton than nontreated control plots, depending on the soil texture and the number of nematodes present at cotton planting time. SD 7727 increased yields 15% to 35% over plots which were treated with the volatile, non-residual nematocides currently used in the cotton fumigation program.

In 1965, one of the experiments established in 1964 was again planted to Deltapine cotton and the other to Pima S-2, without retreatment with SD 7727. The 1964 untreated control plots were also reserved for yield comparison in the 1965 crop.

SD 7727 treated plots yielded 13% and 20% more cotton for Deltapine Smoothleaf and Pima S-2 varieties respectively for the second crop following treatment.

In 1965, additional plots were established to study rates and methods of application of SD 7727. These treatments consisted of granular application at rates of 10 and 15 pounds active ingredient per acre and SD 7727 as an emulsifiable concentrate. All were applied on the soil surface and mixed to a depth of 7-8 inches with a disk harrow before the seed beds were prepared. Deltapine Smoothleaf cotton was planted five weeks after the chemicals were applied. For comparison, a volatile, non-residual nematocide and untreated controls were included in the experiment.

SD 7727 treatments gave significant increases in yield of 11% to 13% over the control. The volatile non-residual nematocide did not show a significant increase in yield over the control.

In another experiment, SD 7727 was applied in the water with the preplanting irrigation. The chemical was applied as an emulsifiable concentrate at the rate of 9 pounds of active ingredient per acre by means of a constant head vessel into each row furrow during the last five hours of the preplanting irrigation.

In this experiment the chemical increased the yield significantly or 13% over the untreated controls.

Conclusions

The experiments have shown that SD 7727 is a highly effective nematocide for controlling the root-knot nematode in Deltapine Smoothleaf and

Pima S-2 varieties. Because of its non-volatile and residual nature, control is extended over a long period and is effective in the second year following an initial treatment. Usually the volatile, non-residual nematocides have not been entirely effective in controlling root-knot nematodes on these two varieties of cotton. They dissipate rapidly and the surviving nematodes increase in numbers sufficient to cause damage to these cotton varieties several weeks before they are mature.

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Deep Placement Fumigation for Root-knot Nematode Control

James Hazlitt, William Larsen and Arlen D. Davison

Deep placement of a nematocide was the subject of a field test established on the Roy Black farm near Yuma. The objectives were to compare the effect of deep chiselling and deep placement of soil fumigant and to determine if the growth response was attributable to chiselling alone or deep placement of a nematocide. The treatments were randomized in the field. Adjacent to but not incorporated with in the experiments were plots treated with Telone (6 gal./A) injected at a depth of 10-12 inches. Chiselling and deep placement of Telone (7 gal./A) were at the depth of 20-22 inches.

There was a noticeable growth response during the entire season in favor of the deep placement of Telone as compared to chiselling alone. The stand within the fumigated plots was noticeably better than in the checks. Yield data confirms that the observed growth response was due to the deep placement of the fumigant.

Yield Data
20-22" Deep Placement Trial

Deep Placement (Telone - 7 gpa)		Chiselled Only (Check)
Rep	lbs. Lint cotton/A	lbs. lint cotton/A
1	2058	617
2	1795	897
3	1821	1013
4	1608	739
5	1768	739
6	1979	949
Total	11,029	4954
Ave.	1,838	825
Bales/A	3.67	1.65

10-12" Placement Trial

Fumigated (Telone - 6 gpa)		Check
Rep	lbs. lint cotton/A	lbs. lint cotton/A
1	1847	908
2	1662	886
Total	3509	1794
Ave.	1754	897
Bales/A	3.50	1.79

The yield results from both experiments would tend to indicate that either deep or shallow placement of Telone was equally effective in controlling root-knot nematodes. Apparently, deep chiselling alone was not of appreciable benefit under the conditions of this trial.

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Seedling Disease Control

Lester M. Blank, ARS, USDA

Investigations on cotton seedling disease control in Arizona are being conducted. Members of the Department of Plant Pathology cooperate in these studies.

Seed-treatment fungicides were compared as to their relative effectiveness in control of seedling disease in tests at CRC, Phoenix, and at Marana. The fungicides were applied to acid-delinted seed of var. Stoneville 213 as single treatments or in combinations. Emergence, disease loss and final stands were recorded and the data analyzed. The surviving stands in the Phoenix test ranged from 43 to 63%, based upon number of seed planted, and at Marana the range was from 51 to 80%. As in previous years, post-emergence seedling loss was lowest and final stands highest in the "combination" treatments involving a standard mercurial fungicide + PCNB. The treatment currently suggested for use in Arizona is Panogen + PCNB, and it ranked second in final stand at CRC and third at Marana in the 16-entry tests.

In-furrow addition of soil fungicides at time of planting for control of seedling diseases was investigated in tests at two locations in 1965, using acid-delinted seed treated with Panogen + PCNB. Variety DPL Smooth Leaf was planted in the Phoenix test and DPL 5540 in the trial at Marana. No benefits resulted from the in-furrow addition of any of the four soil fungicides used in these tests. Similar results have been recorded for several years and it appears that the in-furrow addition of fungicides is a costly and unnecessary practice when used with seed treated with Panogen + PCNB.

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Southwestern Cotton Rust

Lester M. Blank, ARS, USDA

Southwestern cotton rust has occurred in Arizona for many years. For the past several years investigations have been conducted on the control and life history of the causal agent.

A study of the several spore stages of the cotton rust fungus is being done in the laboratory with the purpose of determining more exactly the environmental factors influencing their germination. In greenhouse tests new fungicides are screened for their effectiveness in control of rust, and the better ones are carried into field trials.

The severe outbreak of rust in southern Arizona in 1965 was due in part to the favorable build-up of the overwintering stage on grama grass at the end of the 1964 season. Under the above-average rainfall in July and early August, 1965, the conditions were favorable for the repeated release of the spores which infect cotton. Damage to the cotton plants varied from almost none to severe, depending upon the intensity of infection, the degree of defoliation of infected plants, and the injury to squares and young bolls.

The damage was greatest in Santa Cruz, Pima, and Cochise counties with trace to moderate damage in fields in Pinal, Graham, and Greenlee. Comparisons of the effectiveness of several protectant fungicides were made in field trials near Tumacacori and Continental, in Santa Cruz and Pima counties, respectively. The fungicides were applied on July 8 and 21 and August 4 at both locations, using 1.5 lbs. active ingredient per 40 gal. per acre. The yield data are summarized in the accompanying table.

Fungicide	Active ingredient per acre	Yield, lbs. seed cotton per acre: Continental	Yield, lbs. seed cotton per acre: Tumacacori
Zineb	1.5	3164	1240
R&H M-45	1.5	3010	1396
R&H M-45	1.0	3210	1156
Nia Polyram	1.5	3144	620
DAC 2787	1.5	2894	554
GC 9832		2174	392
None-control	0	2000	300
Diff. required, at .05% level		500	172

Several of the protectant fungicides gave significant increases in yield over that of the untreated plots. Zineb, the only one cleared for use as a foliar spray on cotton, was one of the better treatments at both locations. Under conditions of moderately severe rust at Continental, yields were higher and more of the fungicides were effective than under the conditions of extremely severe rust at Tumacacori. The cost of materials and three applications by ground-rig equipment is about $11 per acre, and it is obvious that the increased yields in the 1965 test actually paid the cost of control many times over.

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