

THE ROLE OF BIRDS IN SPREADING THE
CYLINDROCLADIUM BLACK ROT OF PEANUTS,

by

Richard Berton Hiller

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Approved:

P.F. Scanlon, Chairman

K.H. Garren

R.L. Kirkpatrick

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INTRODUCTION

In 1965, a new disease of peanuts was discovered in Georgia. The disease, *Cylindrocladium* Black Rot (CBR), was named after the causal fungus *Cylindrocladium* *crotalariae* (Bell and Sobers 1966). The disease was found in South Carolina in 1968, Japan and Virginia in 1970, and North Carolina in 1971 (Garren et al. 1972). The spread in Virginia has been particularly rapid (Table 1). CBR has not been found in the western peanut growing states of Texas and Oklahoma. The disease has developed into a severe threat to the peanut crop, and has also been shown to be pathogenic to soybean and tobacco (Powell et al. 1976). It has been estimated that the crop in Virginia is reaching only 85 percent of its potential due to CBR (Garren : personal communication). That amounts to a loss of over \$10 million, based on the figures in Table 2, in the state of Virginia alone.

The peanut industry is a large scale business in most of the states where the crop is grown. This is evident from the dollar values of the 1976 peanut crop in 7 peanut growing states as seen in Table 2. These figures do not include the multiple economic benefits derived from the peanut crop , such as the equipment sales and employment.

Table 1. Occurrence of *Cylindrocladium* Black Rot (CBR) in Virginia peanut fields from 1970 to 1975 (Garren and Coffelt 1976).

Year	Number of fields
1970	1
1971	2
1972	9
1973	24
1974	125+
1975	epidemic

Table 2. Dollar values of the 1976 peanut crop
in seven states (Anon. 1977).¹

State	Dollar values
Alabama	105,686,240.
Georgia	320,559,072.
North Carolina	96,717,600.
Oklahoma	50,128,650.
South Carolina	460,350.
Virginia	64,780,000.
Texas	90,255,750.
Total	728,587,622.

¹ Florida contributed an additional \$33,564,000
in sales during 1976.

The incidence pattern of the disease over the peanut growing states on the Atlantic coast of the United States has created speculation as to the method of dispersal of the causal fungus. The disease can be spread short distances by wind and mechanical means (Rowe et al. 1974; Krigsvold et al. 1977). Patterns of spread over short distances are shown clearly in Fig. 1.

Wind and mechanization explain how the disease can spread locally, but not over long distances, which was the case with CBR. As mentioned earlier, the disease was found first in Georgia in 1965. It was not found again until 1968 in South Carolina and in 1970 was found in Virginia. North Carolina's first diseased field was found in 1971. Outbreaks of the disease were clearly separated by relatively long periods of time and over great distances. The progression northward from Georgia was also discontinuous, reaching Virginia prior to North Carolina. It is conceivable however that many fields were diseased before their discovery and diagnosis. It is therefore possible that the spread northward was indeed in a continuous pattern over relatively short distances. Circumstantial evidence however tended to lean towards some means of long distance dispersal.

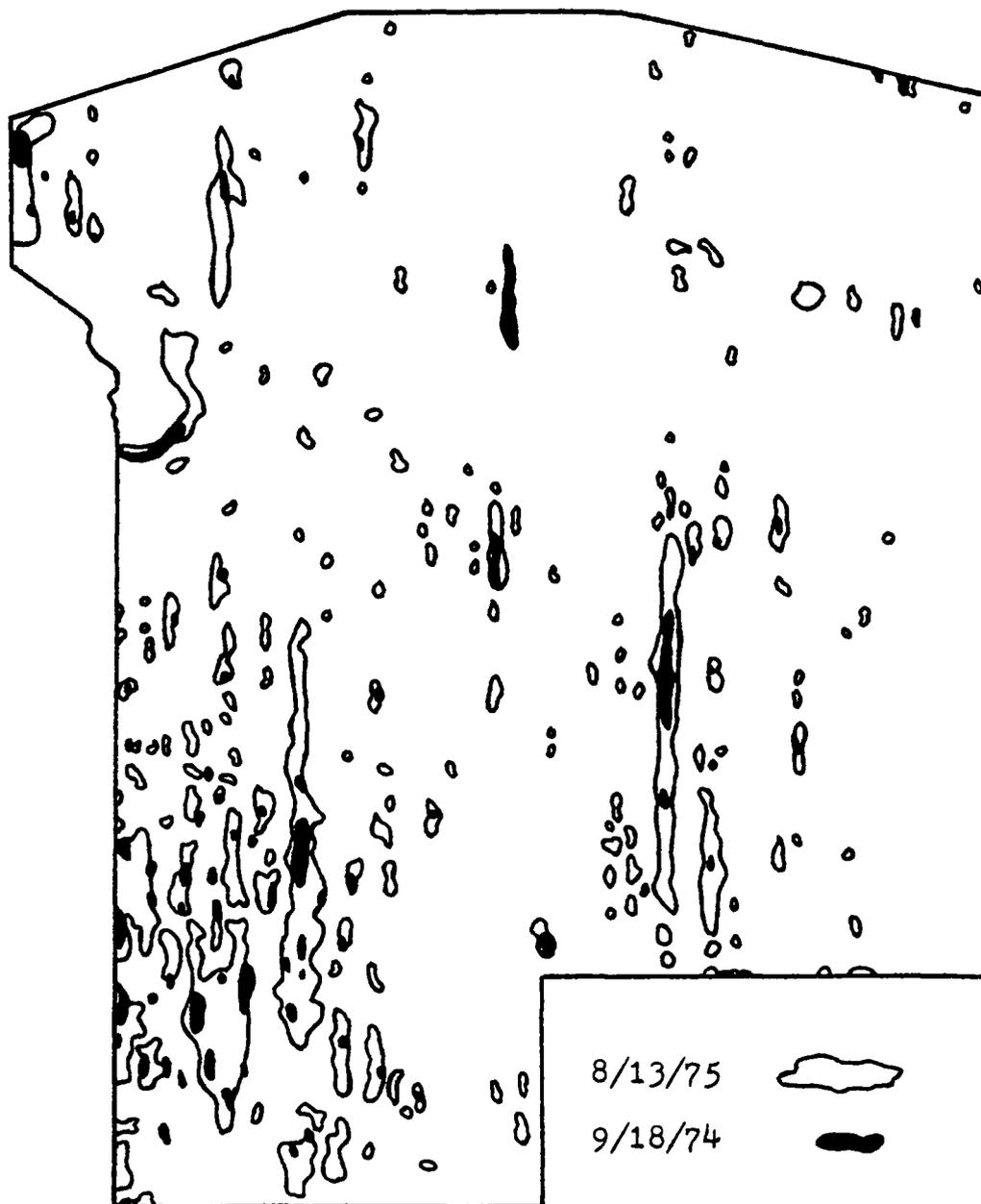


Fig. 1. Two superimposed infrared aerial photographs of the same peanut field one year apart (9/18/74 and 8/13/75) indicating the presence and spread of *Cylindrocladium* Black Rot of peanuts (After Powell et al. 1977).

Birds seemed obvious candidates for long distance dispersal of CBR. Migrating birds especially fit the pattern of incidence along the east coast. It is generally known that birds disperse plant seeds over great distances. The proven instances of dispersal of plant pathogens and other plant parts are much more rare. The literature review section following goes into depth on the proven instances to date. The hypothesis developed in this research project, was that birds in general, migratory birds more specifically, were carrying the causal fungus and therefore spreading the disease. The actual mode of transport was speculated to be of one or more types. Soil containing the fungus could be carried on the feet of birds, or infected plant parts or peanuts could be ingested with fecal deposition causing the necessary inoculation elsewhere.

Objectives were established in hopes of supporting or refuting the circumstantial evidence. In general, the role of birds in spreading the plant disease, *Cylindrocladium* Black Rot, was to be investigated. Five specific objectives were developed as follows:

- 1) To determine what bird species were utilizing peanut fields at all times of the year.
- 2) To determine the migratory or movement patterns of species observed using peanut fields.

3) To determine if soil on the feet, ingested material, or crop contents of birds collected in peanut fields contained the fungus. As part of the third objective, soil from a blackbird roost was analysed for the fungus also.

4) To determine whether or not the fungus could survive passage through the gastrointestinal tract of birds held in captivity.

5) To devise a control program if birds were positively implicated in the spread of the disease.

The importance of the peanut crop to southeastern states and the potential severity of CBR to that crop justifies research towards answering questions concerning CBR. This is especially relevant as control procedures have not been found for the disease at present.

LITERATURE REVIEW

Bird Utilization Of The Peanut Crop

This research project involves the use of peanut fields by birds. Some known instances of this type of use are listed. According to Martin et al. (1951), peanuts are used by some birds and mammals, probably due to the nutritious nature of the seed. He noted that Mourning Doves (Zenaida macroura), Bobwhite Quail (Colinus virginianus), Turkeys (Meleagris gallopavo), and Common Flickers (Colaptes auratus) utilized peanuts as food to varying degrees. Graham (1941) listed the peanut as an important food of Bobwhite Quail and Turkeys, while it was found in the stomachs of the Common Crow (Corvus brachyrhynchos), Boat-Tailed Grackle (Cassidix mexicanus), Mourning Dove, Red-Winged Blackbird (Agelaius phoeniceus), House Sparrow (Passer domesticus), and possibly woodpeckers. The Canada Goose (Branta canadensis) has changed its migratory patterns recently, staying further north. This has been attributed to changing agricultural practices, which have made the peanut more attractive to geese (Hankla et al. 1967). Beal (1900:71) found traces of the pulp of some large seed or nut (peanuts ?) in Boat-Tailed Grackles, Bobolinks (Dolichonyx

eryzivorus), Brown-Headed Cowbirds (Molothrus ater), Red-Winged Blackbirds, Rusty Blackbirds (Euphagus carolinus), and Common Flickers. Crebbs (1960:92) found that Red-Winged Blackbirds and Common Grackles (Quiscalus quiscula) fed on peanuts heavily, while Starlings (Sturnus vulgaris) and Brown-Headed Cowbirds only showed traces of peanuts in their diets. All species, however, did feed in the fields extensively with other materials taken. Much the same results were found in follow up studies to Crebbs' work, as peanuts were found to be the largest component for all blackbird species combined (Hardy 1961; Lefebvre 1961).

Birds As Vectors Of Plant Parts

Dispersal of Seeds by Birds

Birds are well known dispersers of many different plant seeds. Janzen (1971) gives a broad review of seed predation by both birds and mammals. He cites several examples of dispersal of seeds by birds. One aspect of seed dispersal is the benefit to seed of some species of passage through the gastrointestinal tract of vertebrates. This enhances germination of seeds in some cases (Krefting and Roe 1949; Olson and Blum 1968). Small scale dispersal is shown by Fordham (1969).

Examples of long distance dispersal of seeds by birds are numerous. Cleland (1952) found several plant species in Australia had been spread via seeds in birds. Viable seeds were found in the gizzard of Snow Buntings captured on a new volcanic island where no growth had occurred to produce seeds (Sigurdsson and Fridriksson 1969). Proctor (1968) found that several factors were involved with the dispersal of seeds, such as size of the seed, digestive action and diet. He found seeds retained up to 200 or 300 hours in the gastrointestinal tract. Proctor also found some seeds were regurgitated. It has been suggested that this regurgitation may be a possible method of dispersal for propagules that cannot survive passage through the entire tract (Malone 1966). Falla (1960) mentioned several pelagic bird species as dispersal agents. Besides internal transport of seeds, Cruden (1966) reviewed the possibilities of seeds being carried in mud on the feet of birds. He cited an article by Darwin in 1859 and several others which alluded to this possibility.

A few instances of detrimental seed dispersal are also known. Seeds of the mistletoe were shown to be spread by birds in the southwest United States (Bray 1910). Zilka and Tinnin (1976) suggested that 10 out of the 80 bird species they studied could be important vectors of dwarf mistletoe.

The sticky seeds were found on feathers, but not in droppings.

Dispersal of Other Plant Parts by Birds

Sometimes other organisms and plant parts along with seeds are transported or moved about by birds. Plankton and the eggs of brine shrimp were found viable in the fecal contents of Mallards (Anas platyrhynchos; Malcne 1965). A species of spartina was found over 85 km from its nearest point of establishment in the fjords of Denmark, strongly suggesting bird dispersal (Adersen 1974). Schlichting (1960) discussed the role of waterfowl in carrying algae between bodies of water, as did Proctor (1959). Proctor found the algae with simpler cells were viable in the gastrointestinal tract, while the complex types were not. Twenty eight genera of algae were present in one species. De Vlaming and Proctor (1968) found several species of aquatic organisms viable after passage through Killdeer (Charadrius vociferus) and Mallards.

A case of short distance dispersal of plant parts involved moss and several bird species associated with beech forest in England (Davison 1976). Apparently various sized pieces of moss were dislodged and spread about during foraging by bird species. Davison also suggested that

either pieces of moss or spores could be carried on the feet or in the feathers of birds, efficiently spreading the moss greater distances.

Pathogenic Fungi In Seeds

With the high incidence of seed dispersal by birds, the possibility of spreading a pathogenic fungus via seeds seems great, especially when a great number of fungi are seed-borne. The literature contains many instances of fungi inhabiting seeds of many plants, particularly domesticated plant species. This would be expected, as crops and their welfare are of great interest to man and are more thoroughly researched. A few cases where seeds of various plants have been found to contain pathogenic fungi are presented.

Species of Botrytus and Sclerotinia were isolated from chickpea seeds by Cothier (1977). Both are important pathogenic genera. Nik and Parbery (1977) isolated 42 different species of fungi from the seed of 25 legumes. Ameson and Stiers (1977) isolated Cephalosporium gramineum from seeds, and Drechslera spp. were identified as seed-borne pathogenic fungi isolated from cereal seeds in New Zealand by Sheridan (1977).

In studying the possibility of Cylindrocladium crotalariae being seed-borne, Garren et al. (1972) succeeded in isolating the fungus from freshly dug peanuts, (Arachis hypogaea L.), but not from peanuts subjected to normal on-farm processing. This was true even when peanuts were hand picked from 100 percent diseased plants and then processed for market. This process involves drying in the field while exposed to depredation by birds. Hanlin (1973) reported a total of 200 species (110 genera) of fungi were recovered from all parts of the peanut plant, including the seed.

Dispersal Of Fungi And Other Organisms

Only a few kinds of plant pathogens have any independent movement, which in any case is insignificant for long distance dispersal. Thus, all pathogens depend on agents other than themselves for dissemination. These agents are wind, water, insects, man and other animals (Agrios 1969:23). To discuss in detail these aspects is beyond the scope of this review. A few relevant instances of dispersal by factors other than birds will be given.

Conventional Dispersal

Wind has played a major role in the spread of pathogenic fungi and other organisms throughout the world. Christensen (1942) explained the method by which spores are carried up to several thousand miles in the atmosphere. Factors such as spore size, density, and shape, as well as wind velocity and relative humidity were discussed. Another review of spore dispersal due to wind and water was given by Hirst (1959). A few specific examples of wind and rain dispersal involved species of Fusarium from corn fields (Ooka and Kommedahl 1977) being carried 300-400 km from the point of inoculum, and the spread of coffee rust uredospores by wind (Martinez et al. 1977). Levels of rust inoculum in the atmosphere over Canada have been surveyed for several years by Green (1976), but only one article of his was reviewed. With regard to the fungus under study, Cylindrocladium crotalariae, it was found that the propagules (microsclerotia) could be spread a few km by wind and mechanical means (Rowe et al. 1974). This was postulated to be important in local spread of the disease caused by the fungus. Krigsvold et al. (1977) found viable spores in soil taken from equipment used in fields known to have the disease caused by C. crotalariae. The results of this type of mechanical spread were shown in Fig. 1. Deep

bark canker of walnuts was shown to be spread by the mechanical harvester in much the same way (Kado 1977).

Dispersal by Animals

Several instances of animals, primarily birds, carrying fungi have been documented. These involve the presence of fungi only, and do not necessarily imply the spread of a disease caused by those fungi. However, the possibility of their being disease vectors is obvious.

Besides giving a general review on dispersal of fungi Ingold (1953) mentions the dispersal of freshwater aquatic fungi by waterfowl. Extensive work on birds and their association with fungi either in feathers, nests, or soil has been done by Pugh (1964, 1965a, 1965b, 1966a, 1966b, 1972), and Pugh and Evans (1970). European birds were regularly shown to carry fungi, some of which were pathogenic. The nests of birds also contained certain types of fungi frequently. Cooney and Emerson (1965) also found fungi (possibly pathogenic) in birds' nests, as did Apinis and Pugh (1967), who isolated 27 species from plant debris found in the nest itself. Another researcher has shown high levels of occurrence of fungi on feathers and bird nests also (Hubalek 1972, 1974, 1976). One instance of other animal involvement with fungi concerned the isolation of

fungi from the nests of 5 Muridae rodent species in Europe. Pollen along with fungal spores were shown to be carried by birds in the work done by Ash et al. (1961). Several genera of fungi, including pathogenic forms, were isolated from the feet of birds by Evans and Prusso (1969), who believed their results warranted further studies. Fungi were also isolated from the throats and feathers of wild Pink-Footed Geese (Anser brachyrhynchos) sampled in Scotland and Iceland (Sladen and Austwick 1955). Seventeen different genera of fungi associated with these geese were determined to have been picked up in fields by the geese.

An in-depth study into the ability of birds to carry fungi was carried out by Warner and French (1970). They applied spores of 2 fungi to the plumage of 149 birds of 31 species and recovered viable spores 3 to 45 days later. They also isolated fungi of 39 genera, some of which were pathogenic, from the feathers of 248 birds collected in parts of Mexico, Texas and Minnesota. Their work also showed Common Grackles spread 2 diseases of oats from field plants to healthy plants under greenhouse conditions. Monga (1972) isolated several genera of pathogenic fungi from 60 of 233 wild birds he collected.

Birds, especially woodpeckers, were shown to be involved in the economically important spread of chestnut-

blight, caused by Endothia parasitica (Heald and Studhalter 1914). In similar work, Tiffany et al. (1955) attempted to isolate the causal fungus of oak wilt from birds. Although he failed to isolate that particular fungus, he found 41 different genera of fungi associated with birds. Magpies (Pica pica) were implicated in the spread of a fungus causing dieback of citrus trees (Kouyeas and Anastassiadis 1962). The birds built nests from twigs killed by the fungus and thus caused its spread to healthy trees.

There are several cases where birds were suspected of spreading a disease by carrying fungi. Lachmund (1929) suggested that birds were involved in the spread of the sweetfern rust. Migratory birds were considered to be the agent of transfer of blister-blight of tea from Ceylon to Sumatra (Reitsma and Van Emden 1949). Birds may also be the dispersal agent of blister-blight of tea in New Guinea (Shaw 1965). Nielson (1929) suggested that birds aided in the distribution of the potato wart disease fungus.

Dispersal of Organisms Other than Fungi

Birds have been shown to transport or be involved in the spread of other organisms besides plants and fungi, some being of a pathogenic nature. The spread of plant viruses by birds has been shown in several instances (Broadbent and Martini 1959; Broadbent 1964, 1965).

Yeasts have been carried by birds (Kocan and Hasenclever 1974), and Tiunina (1931) suggested that birds may contribute to the spread of yeasts in French vineyards.

Plant pathogenic bacteria carried by birds were involved with a coconut bud-rot (Johnston 1912). Babbar et al. (1976) isolated avian mycoplasma from plants, suggesting bird involvement.

Of a non-pathogenic nature but with interest to this research project, crustacean eggs were recovered in a viable condition from the cloaca of ducks by Proctor (1964). This and all instances mentioned earlier involving the gastrointestinal tract of birds raises questions about the environment encountered by organisms when ingested. The following section deals with this aspect.

Characteristics of the Bird Digestive System

A review of Sturkie (1976) shows that the rate of passage through the digestive tract of birds depends on the consistency, hardness, water content, and the amount of food consumed. Chromic oxide, an indicator chemical, was detected as early as 2.5 hours after feeding, with most of it passed within 24 hours in some species. The hydrogen ion concentration (or pH) found in the tract is dependent on the

amount of HCl secreted in the proventriculus, and the action of bile and pancreatic juice, which tend to neutralize acid. Most workers agree that all parts of the tract are acid, with the highest pH recorded in the intestines, and the lowest in either the gizzard or the proventriculus. Sturkie (1976:202) gives values of the avian gastrointestinal tract pH as ranging from 1.0 in the proventriculus to 7.1 in the lower intestine. According to Sturkie the pH is not significantly influenced by diet, but will increase with high ingested levels of basic salts or milk. Digestive processes listed include swallowing; maceration; grinding in the gizzard; subjection to digestive enzymes from saliva, stomach, intestines, and pancreas; action of bile from the liver; action of HCl from the stomach; and bacterial action.

A review of Farner and King (1972) yields much the same information. They state that HCl is generally found in birds, and that pure gastric juice may have a pH as low as 0.2. Farner and King (1972:380) give details of the avian digestive tract and the pH of its components. Another interesting fact mentioned in their work was that the ceca are emptied no more than once in a 24 hour period in the grouse and domestic chicken (Gallus domesticus). This storage function could have implications in long distance dispersal of organisms.

Fungal Spore Passage Through Various Organisms

Recorded instances of fungi surviving passage through the gastrointestinal tract of organisms are few. Heald (1943) believed that few if any propagules of corn smut could survive passage through the gastrointestinal tract of horses or cattle. Peplinski (1974) failed to isolate viable propagules of Ceratocystis fagacearum in the frass of 2 species of beetles associated with the death of red oaks. However, spores of the pathogenic fungus, Colletotrichum lagenarium, passed through the gut of a snail and were viable for several days, causing anthracnose of melon (Hasan 1976). Similarly, Gilliam (1972) isolated fungi from the intestinal contents of worker honeybees.

METHODS AND MATERIALS

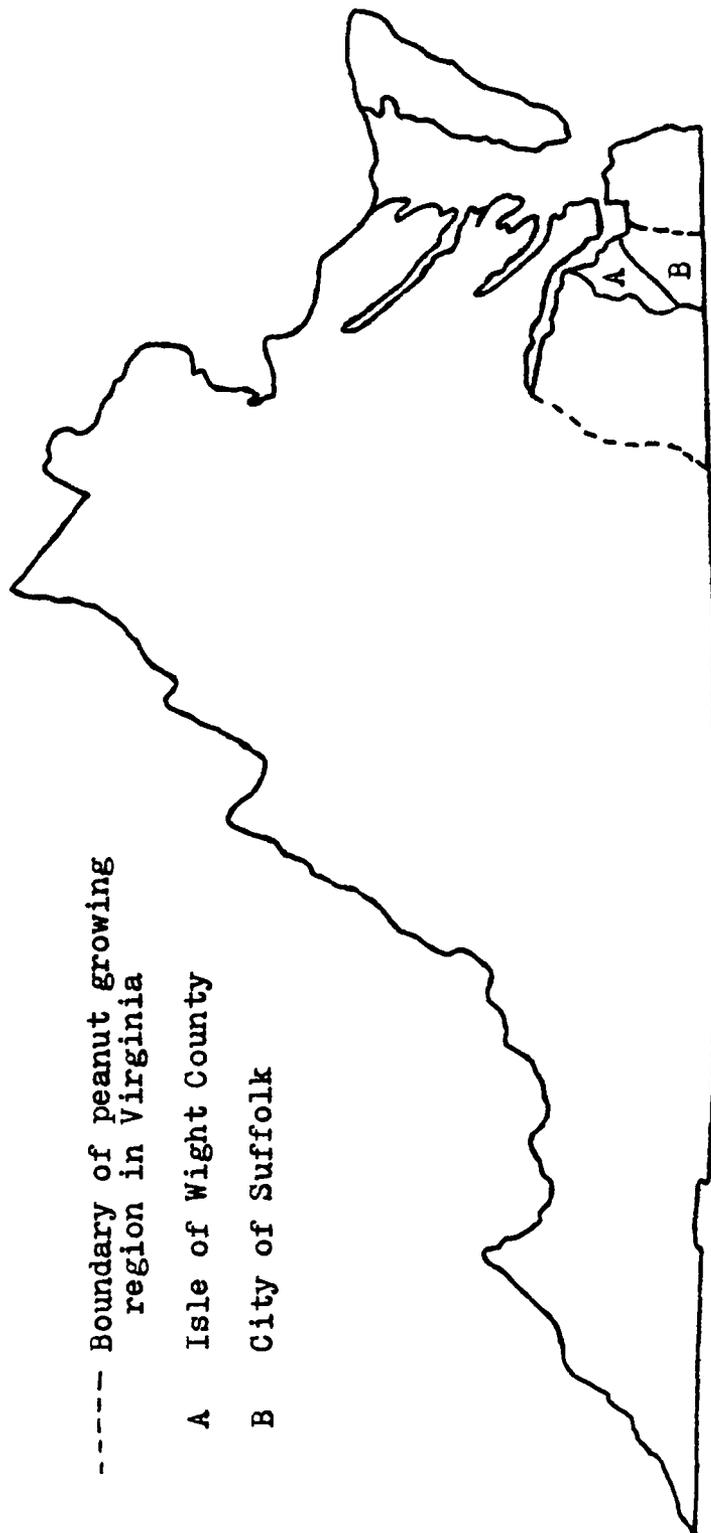
Observation of Peanut Fields

Eleven peanut fields near Smithfield and 9 fields near Suffolk in southeastern Virginia (Figs. 2 and 3) were observed for a period of one year. Bird species, number per species, approximate weather conditions, time of day, and field condition were recorded for each observation period. Fields were observed once a month, except during harvest and fall migration, when observations were made every 2 weeks. Each field in an area was observed for approximately one half hour, with the aforementioned conditions and parameters recorded. The following morning the other area was observed in the same fashion. For bird identification, 7 X 50 binoculars were used.

The fields in an area were chosen for their relatively close proximity to one another. This was done to allow for more observation time per field during the high usage periods, i.e. early morning.

Bird Banding Information

Banding data on 17 of the bird species observed utilizing peanut fields was requested from the Patuxent Bird



----- Boundary of peanut growing region in Virginia

A Isle of Wight County

B City of Suffolk

Fig. 2. Counties where studies were conducted in relation to peanut growing region in Virginia.

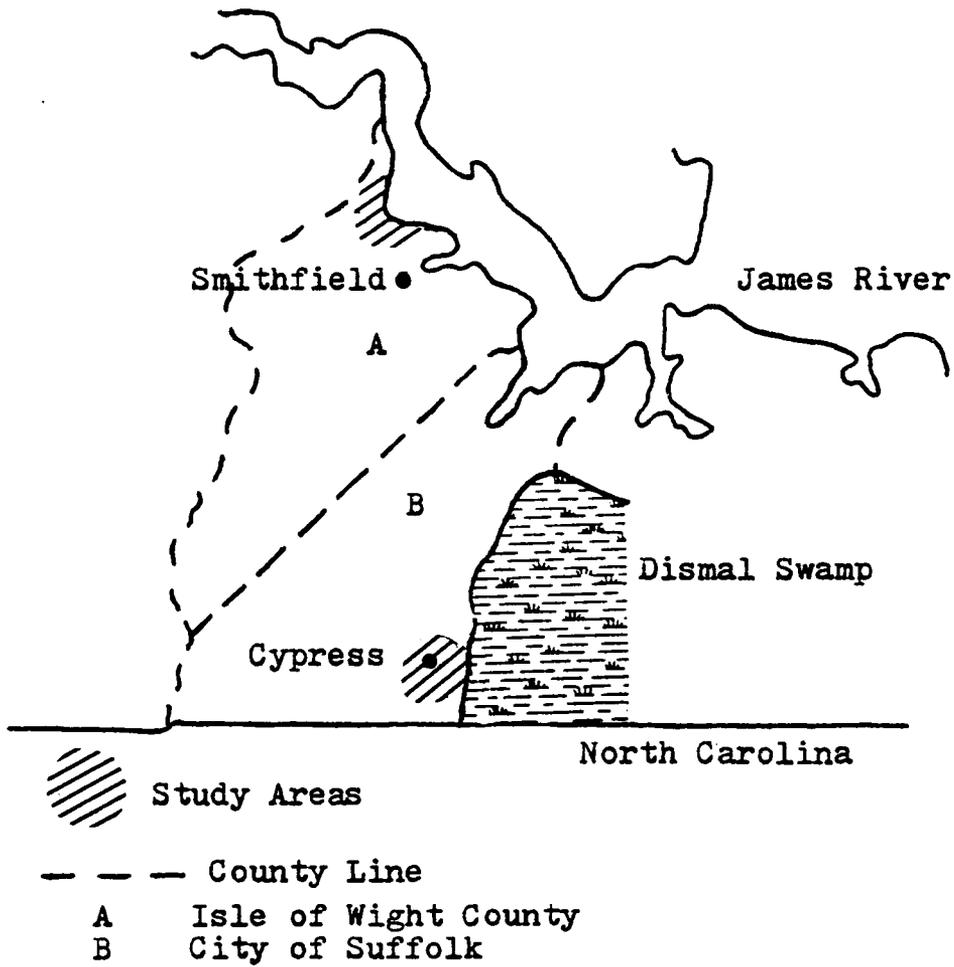


Fig. 3. Locations of study areas in Isle of Wight County and the City of Suffolk.

Banding Laboratory (United States Fish and Wildlife Service) located in Laurel, Maryland.

Longitude and latitude of the study areas were determined, and records of the original banding location of members of the 17 species recaptured or recovered in the study area were extracted. The objective was to determine the past migration or movement patterns of birds observed using peanut fields during the study period. For each species on which data was requested and extracted, points were plotted on a map indicating place of original banding or recapture.

These 17 species were chosen due to their migratory habits or the likelihood of their being vectors of CBR.

Media Preparation

Microsclerotia of Cylindrocladium crotalariae were needed in large quantities for the various experiments performed. One of the best methods for acquiring large amounts of microsclerotia is to grow them in a medium that favors the development of primarily microsclerotia. The medium used commonly with this fungus for growth of microsclerotia in pure culture is Hunter's medium (Hunter 1970).

Hunter's Medium

The constituents (Appendix Table I) were added in the proper amount, while stirring, to the correct amount of distilled water in a large flask. The best way to do this involved making several liters at one time.

The medium was then poured into smaller flasks using a funnel. The small flasks used were 250ml Erlenmeyer flasks with wide mouths (stopper size 8). Approximately 100ml of the medium was poured into each 250ml flask.

A plug was made to fit snugly in the neck of each 250ml flask. Each plug consisted of a ball of cotton wrapped in a square of cheesecloth. The loose ends of cheesecloth pulled together pointed out of the flask. The plug was snug enough if the flask could be picked up by the ends of cheesecloth only. A square of heavy duty aluminum foil was placed over the top of each flask. Each sealed flask was then autoclaved at 121C for 15 minutes. After removal from the autoclave, the flasks were allowed to cool to room temperature.

Each flask was then inoculated. The inoculations were performed under a hood using aseptic techniques. The foil and plug were removed from a flask, and a small piece of agar containing Cylindrocladium crotalariae mycelium was placed in the flask. Agar chunks were taken from cultures of C. crotalariae grown in Petri dishes.

The plug and foil were then replaced in each flask as inoculation was completed under the hood. All flasks were dated and placed in a dark chamber at room temperature for three months. This storage was undertaken to ensure the development of large numbers of mature microsclerotia in Hunter's medium. A total of 60 flasks were prepared in this fashion.

Harvesting microsclerotia from Hunter's medium. Two 250ml flasks containing Hunter's medium and Cylindrocladium crotalariae growth were used for each experiment requiring microsclerotia. Both flasks were poured into a Waring blender and blended at high speed for a total of approximately 20 minutes. To prevent heating of the mixture, and possible damage to the microsclerotia, blending was done for short periods of time to total 20 minutes.

After blending, the mixture was poured into a 250ml flask and agitated by hand, then left to stand for about one minute. The flask was then decanted, removing the upper half of the liquid. This procedure removed the lighter materials, leaving the heavier microsclerotia in the bottom of the flask. This decanting procedure was repeated 5 times by adding additional water each time after half the liquid was drained. After the last decanting, the flask contained almost entirely microsclerotia.

The resultant microsclerotia were then separated by size by washing them with a squirt bottle onto a sieve combination of 100 mesh per inch (150um) sieve over a 500 mesh per inch (25um) sieve. This was done to prevent the use of overly large, unnatural microsclerotia that commonly develop in the Hunter's medium. The microsclerotia collected on the 500 mesh sieve were then utilized for the various experiments.

Sucrose - TBZ Medium

Detection of Cylindrocladium crotalariae microsclerotia in various materials is enhanced by the use of a selective medium. The one used throughout this project was called Sucrose - TBZ (Krigsvold and Griffin 1975), however, a more selective medium has since been developed, called Sucrose - QT medium (Griffin 1977).

The procedure for preparing Sucrose - TBZ (Appendix Table II) was more involved than that for Hunter's medium, as certain chemicals had to be added after autoclaving. This medium was prepared in 4 liter batches (2 liters in each of two 3 liter flasks). Sucrose, peptone, potassium phosphate, magnesium sulfate, and agar were added to distilled water while stirring with a magnetic stirrer. These solutions were then autoclaved for approximately 20 minutes. While autoclaving, the proper amounts of

pentachloronitrobenzene (PCNB), oxgall, streptomycin, chlortetracycline HCl and thiabendazole (TBZ) were weighed out. All chemicals were weighed with a triple-beam balance or a Mettler analytical balance. The TBZ was dissolved in acetone, and the streptomycin and chlortetracycline HCl were dissolved in sterile water. The autoclaved solutions were placed in a 50-55C water bath to cool to handling temperature. This usually took 20 to 30 minutes. After sufficient cooling, the remaining chemicals, which were heat labile, were added while stirring. The pH of each flask was adjusted to pH 4 using HCl.

All plates were poured under a hood using sterilized 600ml beakers. The plates used were disposable plastic. Plates were left to cool overnight, and then placed in a refrigerator until used.

Each 4 liter batch yielded approximately 150 plates of Sucrose - TBZ medium, and required 3.5 to 4 hours of laboratory time for preparation. Approximately 3000 plates were made for the various experiments.

Field CollectionsBirds Collected From Peanut Fields

Several bird species were collected by shooting during the fall and spring. Attempts at cannon netting were unsuccessful. All bird species shot were previously observed in a peanut field. Collected birds were placed individually in plastic bags that were not airtight. The bags were then placed in a styrofoam cooler for transport to the laboratory.

From each bird, crop contents, fecal material, and soil scraped from feet were collected separately. Each sample was then analysed for the presence of viable microsclerotia by the following methods. The sample was briefly mixed with water in a sterile blender top, then washed onto a sterile sieve combination. The top sieve was of large mesh to prevent splashing. The bottom sieve was 500 mesh per inch (25um). Each sample was washed with tap water for 5 minutes before being washed into a 150ml beaker using sterile water in a squirt bottle. The volume was brought to approximately 50ml using sterile water. This mixture was agitated with a magnetic stirrer while 5 1ml aliquots were drawn off with a pipette and spread on 5 Sucrose - TBZ plates. The plates were then left to incubate at room temperature.

Soil Collected From a Blackbird Roost

A blackbird roost in the vicinity of peanut fields in southeastern Virginia (Fig. 4) was located with the help of an employee of the Dismal Swamp National Wildlife Refuge. One hundred soil samples were taken at random from the roost. Each sample was placed in an unsealed plastic bag, which was then placed in a styrofoam cooler. Samples were kept in a moist environment until analysed to prevent drying and possible loss of any microsclerotia present in the sample. An average soil moisture was determined by oven-drying 20 11g samples taken from the original 100 samples. Drying took place at 105C for 24 hours. Moisture was determined so the proper amount of undried soil could be analysed to give the number of colonies per gram of soil.

The following procedure for analysis of soil is adapted from Krigsvold and Griffin (1975). Samples were not treated with sodium hypochlorite (NaClO), as this treatment has been shown to be detrimental to the survival of microsclerotia (Griffin 1975, 1977; Krigsvold and Griffin 1975). From each original soil sample taken from the roost, an 11g sample was taken. This 11g sample was placed in a sterile blender top with water and briefly mixed. This mixture was poured onto a sterile sieve combination of a large mesh over 500 mesh. Each sample was washed for 5 minutes under tap water, then

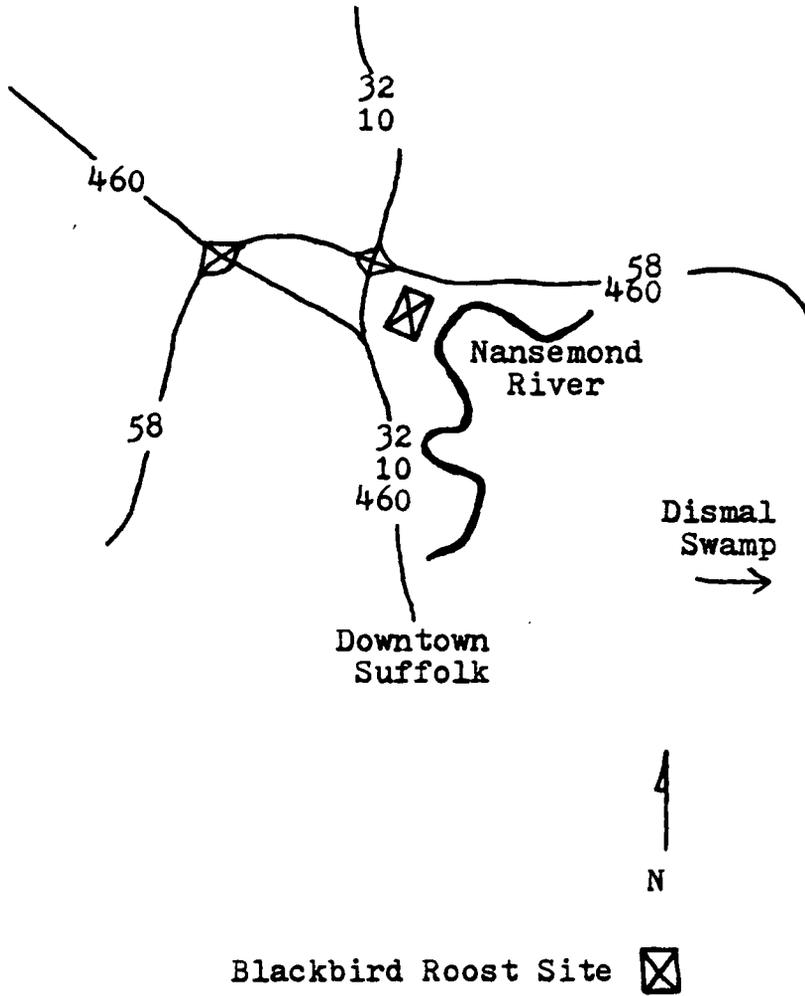


Fig. 4. Location of blackbird roost site, near Suffolk, Virginia, used for soil collections.

washed into a 150ml beaker with a squirt bottle containing sterile water. The volume of water and soil was brought to 40ml. The soil was then brought into suspension by the use of a magnetic stirrer. One ml aliquots were placed on 10 Sucrose - TBZ plates with a pipette. Each 1ml of suspension represented 0.25g of soil. All plates were then incubated at room temperature.

Subculture of suspected colonies. After incubation for 5 to 14 days, all plates were observed. Colonies of fungi which might be Cylindrocladium crotalariae were transferred aseptically under a hood to another plate of Sucrose - TBZ to allow for better expression of characteristics under less competitive conditions. These subcultured plates were also incubated at room temperature.

The use of soil containing a known population as a check. Peanut soil with a known concentration of microsclerotia per gram of soil was used to check the sensitivity of the soil analysis procedure used. Twenty 11g samples were analysed exactly like the blackbird roost soil samples. After incubation at room temperature for 5 days, colonies of Cylindrocladium crotalariae present were counted. Questionable colonies were wet mounted and observed

microscopically for presence of characteristic C. crotalariae structures.

The observable colony count was then compared to the known estimated population in the original soil sample. This procedure was done to check the sensitivity of the soil analysis procedure.

Passage of Microsclerotia Through the Gastrointestinal Tracts of Birds

Cylindrocladium crotalariae microsclerotia were forced to three bird species, Canada Goose, Bobwhite Quail, and Japanese Quail (Coturnix coturnix japonica), by various methods to determine whether microsclerotia could remain viable after passage through the gastrointestinal tract (GI tract). The methods and materials varied slightly for the different species and as techniques were improved. Therefore the following experiments are treated separately.

Germination Trials

Whenever microsclerotia were harvested from pure culture and used in an experiment, germinability studies were performed. As mentioned in the harvesting of microsclerotia section, 2 flasks of pure culture were mixed for each experiment. Water (1ml) containing harvested

microsclerotia was pipetted onto each of 2 Sucrose - TBZ plates for every 2 flasks mixed. Approximately 36 hours after pipetting onto the plates, each plate was observed under a microscope at low power (4X). One hundred microsclerotia were counted per plate, differentiating between those that germinated and those that failed to germinate. The percentage of germinating microsclerotia was then taken directly from the count of each plate.

Test for Effects of Feed on Microsclerotia

Both water and the feed used for the experiments were tested for their effects on microsclerotia. A 10 ml solution of water containing microsclerotia was added to each of 3 screw cap 125ml flasks. One flask contained 50ml of sterile water and no feed. The second flask contained 50ml of sterile water and 5g of feed. The last flask contained 50ml of sterile water and 10g of feed. Three 1ml aliquots were drawn off daily from each flask and plated on Sucrose - TBZ medium for incubation at room temperature. This was done for 6 consecutive days.

Canada Geese

Twelve Canada Geese were captured from the Virginia Polytechnic Institute and State University pond for use in

various experiments. They were caught in a small funnel type net (Fig. 5) during the relatively flightless phase of primary feather replacement. The geese, as were all species of birds used, were fed a standard ration (Table 3) throughout the experiments.

Force feeding two levels of microsclerotia and feces collection. Two groups of 6 geese each were force fed 1ml at 100 and 300 microsclerotia per ml levels. A 60cm long flexible but firm clear plastic tube (2mm o.d.) was fitted onto the end of a Manostat syringe apparatus. The suction intake tube of the Manostat syringe was placed in a beaker containing water and microsclerotia. The beaker also contained a magnetic stirring bar for suspension of the microsclerotia.

The 2 levels of microsclerotia were made in several steps. Water was added to bring harvested microsclerotia to a 100ml volume. The concentration was then determined by counting microsclerotia per ml in 2 separate milliliters. Dilutions to 100 and 300 microsclerotia per ml were then made and checked by counting as above. Concentrations were also checked using the actual application procedure to ensure proper levels being delivered to each goose.

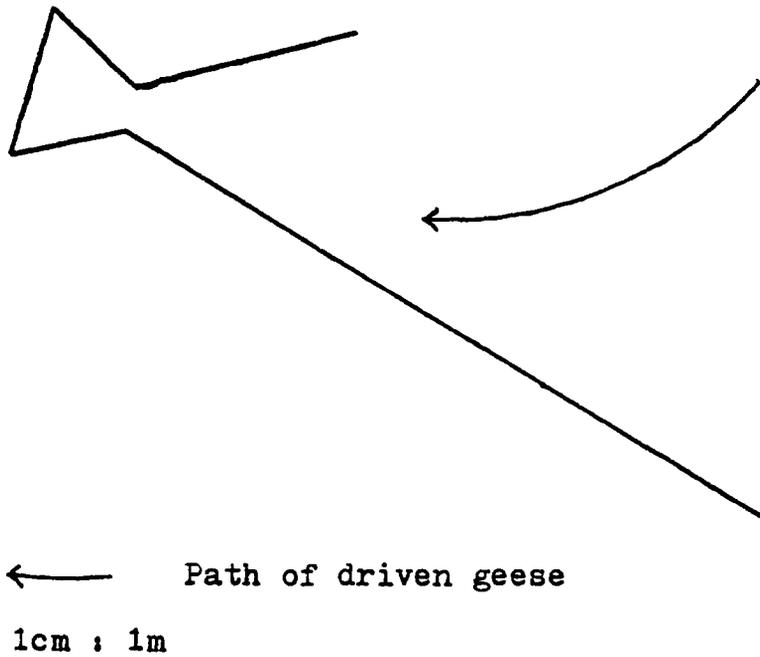


Fig. 5. Diagram of net used to catch Canada Geese during primary feather development.

Table 3. Ingredients of rations used to feed
birds of all species on experiment.

Ingredient	Pounds per ton
Ground yellow corn	880
Stabilized fat	40
Dehulled soybean meal	700
Menhaden fish meal	100
Meat and bone meal	100
Dehydrated alfalfa meal	40
Ground limestone	90
Defluorinated phosphate	30
Salt (NaCl)	8
DL - methionine	1
Trace mineral mix	1
Vitamin premix 3	10
Total	2000

For each goose, the tube attached to the Manostat was cleaned and moistened with water before being inserted half way down the esophagus. One ml of either the 100 or 300 microsclerotia per ml level solution was given to each goose. The solutions were being stirred with a magnetic stirrer while the 1ml aliquots were drawn off for each goose.

All 12 geese were penned individually 1 day prior to and 4 days after being force fed microsclerotia. Feces were collected daily from each goose on paper placed beneath each pen. The first collection was at the time of force feeding of the microsclerotia, 1 day after being placed in the pens. Collections were made on each of the next 4 days. Pens were cleaned and disinfected daily to prevent contamination of the next days' collection. The feet of all geese were washed prior to being placed in a clean pen each day.

Feces were taken off the collection paper for each goose and placed in a sterile jar. Feces collected from all 12 geese were then taken to the laboratory and processed to determine presence of viable microsclerotia. This procedure involved materials and methods similar to those used for the harvesting of microsclerctia. Each feces sample was briefly mixed with water in a sterile Waring blender and then placed on a sterile sieve combination for washing with tap water.

A large meshed sieve was placed over a 500 mesh sieve to prevent splashing from the bottom sieve. Each sample was washed for approximately 5 minutes with tap water. The material remaining in the sieve after washing was then washed into a sterile 150ml beaker with a squirt bottle containing sterile water, and brought to a 50ml volume. The solution was then agitated using a magnetic stirrer. One ml was removed using a soil pipette and placed on a Sucrose - TBZ plate. This was done 3 times for each collection of feces. Plates were then stored at room temperature for incubation and subsequent observation.

Force feeding concentrated microsclerotia and feces collection. Twelve geese were force fed approximately 5000 microsclerotia in 1ml of water. This procedure was similar to that used when force feeding 2 levels of microsclerotia. Harvested microsclerotia were placed in a small volume of water containing the intake tube of a Manostat syringe. As each 1ml aliquot was delivered to a goose, the microsclerotia to be force fed to the next goose were allowed to settle near the end of the clear plastic applicator tube. This procedure allowed for a visual check on the amount force fed to each goose, and the counts done prior to the actual feeding provided further checks.

The 12 geese had been penned separately in sterile cages 1 day prior to and 3 days after the above force feeding took place. Feces were collected daily on paper placed under the cages which were disinfected daily. The feet on all geese were washed with water as cages were disinfected. The first collection ended prior to force feeding of the microsclerotia. Three subsequent collections followed the force feeding at 24 hour intervals. Feces were analysed in the same manner as those in the 2 level force feeding experiment, except 5 1ml aliquots were plated on Sucrose - TBZ medium instead of 3.

Force feeding of a porous bag containing microsclerotia.

Fifteen geese were force fed a small, tight meshed, porous bag containing approximately 5000 microsclerotia. A bright red synthetic cloth material was cut into 3cm discs. Microsclerotia were pipetted from a flask and allowed to settle in the tip of the pipette. The end of the pipette was then touched to the center of the cloth, depositing approximately 5000 microsclerotia. The number was estimated by microscopically counting 5 similar depositions on slides. With the use of forceps and a hemostat, a piece of surgical thread was then used to draw the cloth together into a bag tied at one end. The loose ends were trimmed with scissors.

A 30 cm tube (3mm i.d.; 7mm o.d.) was used to administer the bags to geese. A bag was placed in the tip of the tube prior to being inserted part way down the esophagus of each goose. By blowing sharply, but briefly, into the outer end of the tube, the bag was deposited in the goose upon removal of the tube. A squirt bottle containing water assisted in moistening the tube and the swallowing process by the geese.

The geese were penned individually over paper after being fed the bag containing microsclerotia. Feces were then collected and sieved at the end of 14 hours and daily thereafter until the bag was found. The sieve used had mesh approximately one third the size of the bags, approximately 1mm. Running tap water was used for sieving. Upon recovery, bags were cut open, if not already open, and the contents washed onto a Sucrose - TBZ plate using a squirt bottle containing sterile water. Three plates were used per recovered bag, with the bag itself placed on the third plate. Plates were then placed at room temperature to incubate for later observation.

To check the effect of the material on the viability of microsclerotia, 6 identical bags to those force fed were placed in an airtight 125ml screw cap flask filled with sterile water. One bag was removed daily for 6 days and plated on 3 plates by washing with a squirt bottle, as were

the force fed and recovered bags. The bag itself was placed on the third plate with microsclerotia. Plates were left to incubate at room temperature.

Feeding inoculated peanuts and feces collection. Raw peanuts were shelled and then placed briefly in warm water to remove the skins. The skinned peanuts were then surface sterilized for 30 seconds in water containing 1 percent sodium hypochlorite (NaClO). After rinsing with tap water, the peanuts were left to dry at room temperature. Approximately 10 peanuts were then placed in a petri dish with a small amount of water. Each plate was then inoculated with 3 separate pieces of Cylindrocladium crotalariae mycelium grown on Sucrose - TBZ medium. All transfers were performed aseptically under a hood. The plates, totalling 50, were then placed in a dark moist chamber at room temperature for 3 months to grow microsclerotia.

At the end of 3 months, the peanuts were briefly ground in a blender and then mixed 1:1 with quail feed. This mixture was given along with peanut foliage to 6 geese penned individually. Feces were collected on paper daily for 3 days after introducing the inoculated feed mixture. Cages were cleaned and disinfected daily at collection

times. The feet of geese were washed also at collection time. Care was taken in collection to prevent any spilled feed from contaminating the sample.

Feces were treated as they were in the concentrated force feeding experiment, with 5 plates being used per day per sample. Plates were then incubated at room temperature.

Japanese Quail

Japanese Quail (Coturnix coturnix japonica) were acquired from the Poultry Science Department of Virginia Polytechnic Institute and State University. Adults were used in the experiments.

Force feeding concentrated microsclerotia and feces collection. Twelve Japanese quail were force fed a concentrated dose of Cylindrocladium crotalariae microsclerotia. The birds were caged individually in the laboratory in clean cages with feed and water. Force feeding of microsclerotia took place 1 day after the birds were individually penned. Microsclerotia for force feeding were placed in a small volume of water in a beaker. Approximately 5000 microsclerotia were delivered to each bird in 1ml of water. A small syringe with an intubation needle was used to administer the microsclerotia into the crop of the quail.

Feces were collected every 24 hours for 4 days, including the day prior to force feeding. Collections were made on paper. The quail were moved to clean cages daily, at which time their feet were washed.

All the fecal samples were briefly mixed with water in a sterile blender top and then washed onto a sterile sieve combination. The top sieve was of large mesh to prevent splashing, the bottom sieve was 500 mesh. Each sample was washed into a 150ml beaker using a squirt bottle and sterile water. The volume was brought to approximately 50ml. The mixture was agitated using a magnetic stirrer to achieve a uniform suspension. Five 1ml aliquots were drawn off and plated on Sucrose - TBZ medium for each sample analysed. The plates were incubated at room temperature.

Bobwhite Quail

Bobwhite Quail were raised from hatching and used in this experiment as adults. The original eggs were from the Iowa Giant strain. A portable automatic incubator was obtained from the Poultry Science Department at Virginia Polytechnic Institute and State University. Adult birds were all fed the same standard ration as the Canada Geese and Japanese Quail.

Force feeding concentrated microsclerotia and feces
collection. Twelve Bobwhite Quail were treated identically
in all respects to the Japanese Quail. All procedures were
the same, as were materials and analyses.

RESULTS

Observation of Bird Use in Peanut Fields

Twenty peanut fields in Virginia, 11 near Smithfield (Isle of Wight County) and 9 fields near Cypress (City of Suffolk) were observed for one full year (May 1977 to April 1978) after preliminary observations were made in March and April of 1977. The results of these observations were tabulated by month to allow for better differentiation between observation periods. At least 30 bird species were observed using peanut fields at some stage of the year. Of these species 15 probably utilize peanuts as a food source. Data for each month included the field conditions and stage of the peanut crop, species of birds observed that month, and the number per species. Numbers in excess of 20 for any species were estimated to the nearest 10 birds. Daily data sheets with numbers per species in each field are presented in Appendix Table III, and are summarized in Tables 4 and 5. Fields in one area were observed one morning and the fields in the other area were observed the following morning. This pattern was maintained for each trip to the study areas.

The 20 fields observed are briefly described in Table 6. Scientific names for birds observed during the year are

Table 6. Brief description of the 20 peanut fields observed for one year, 4/77 to 4/78, in southeastern Virginia.

Field Number	Area	Size Ac (Ha)	Shape	Nearby Land Features
1	Smithfield	60 (24)	Square	Fields
2	Smithfield	20 (8)	L Shaped	Woods, road, house
3	Smithfield	25 (10)	Rectangle	Fields, house
4	Smithfield	50 (20)	Rectangle	Woods, road
5	Smithfield	10 (4)	Square	Fields, road
6	Smithfield	30 (12)	Square	Woods, road, house
7	Smithfield	30 (12)	Rectangle	Pond, fields, road
8	Smithfield	10 (4)	Square	Pond, woods, field
9	Smithfield	30 (12)	Rectangle	Pond, woods, field
10	Smithfield	60 (24)	Square	Pond, fields, road
11	Smithfield	10 (4)	Square	Pond, woods, road
12	Suffolk	10 (4)	L Shaped	Pond, woods, road
13	Suffolk	20 (8)	Rectangle	Woods, field, road
14	Suffolk	5 (2)	Square	Woods, field, house
15	Suffolk	5 (2)	Square	Fields, house
16	Suffolk	35 (14)	Rectangle	Woods, fields
17	Suffolk	15 (6)	Square	Woods, fields
18	Suffolk	60 (24)	Square	Fields, road
19	Suffolk	15 (6)	Rectangle	Woods, fields
20	Suffolk	30 (12)	L Shaped	Woods, fields

listed in Table 7. In Table 7, note that both the Common Crow (Corvus brachyrhynchos) and the Fish Crow (Corvus ossifragus) are listed. This was done as the study area was within the range of both species and because field observations did not permit differentiation between the 2 species. Therefore the possibility of both species being observed in peanut fields exists. It should be noted also that the Rock Dove (Columba livia) is commonly referred to as the domestic pigeon. Table 8 contains the general field conditions and stage of the peanut crop throughout the observation period (05/77 to 04/78).

Birds were observed utilizing peanut fields in various ways. Nesting behavior, loafing, incidental peripheral visits, passage to adjacent areas, and feeding activity were observed. Feeding activity was most common, with peanut foliage, seed peanuts, drying peanuts, waste peanuts, insects, and cover crop acting as attractants to various species. A portion of the feeding activity was directed towards attractants other than the peanut crop itself. A few of these exceptions were cover crops serving as attractants to Canada Geese, and a worm (fall army worm?) infestation in August attracting a relatively large number of House Sparrows (Appendix Table III, 08/24/77). The only

Table 7. Common and scientific names of birds observed
in peanut fields in southeastern Virginia, from
5/77 to 4/78.

Common Name	Scientific Name
Canada Goose	<i>Branta canadensis</i>
Pigeon Hawk	<i>Falco columbarius</i>
Bobwhite	<i>Colinus virginianus</i>
Killdeer	<i>Charadrius vociferus</i>
Upland Sandpiper	<i>Bartramia longicauda</i>
Ring-Billed Gull	<i>Larus delawarensis</i>
Rock Dove	<i>Columba livia</i>
Mourning Dove	<i>Zenaida macroura</i>
Common Flicker	<i>Colaptes auratus</i>
Red-Bellied Woodpecker	<i>Centurus carolinus</i>
Red-Headed Woodpecker	<i>Melanerpes erythrocephalus</i>
Horned Lark	<i>Eremophila alpestris</i>
Barn Swallow	<i>Hirundo rustica</i>
Purple Martin	<i>Progne subis</i>
Blue Jay	<i>Cyanocitta cristata</i>
Common Crow	<i>Corvus brachyrhynchos</i>
Fish Crow	<i>Corvus ossifragus</i>
Mockingbird	<i>Mimus polyglottos</i>
Brown Thrasher	<i>Toxostoma rufum</i>
Robin	<i>Turdus migratorius</i>
Eastern Bluebird	<i>Sialia sialis</i>
Starling	<i>Sturnus vulgaris</i>
House Sparrow	<i>Passer domesticus</i>
Eastern Meadowlark	<i>Sturnella magna</i>
Red-winged Blackbird	<i>Agelaius phoeniceus</i>
Common Grackle	<i>Quiscalus quiscula</i>
Brown-Headed Cowbird	<i>Molothrus ater</i>
Cardinal	<i>Richmondia cardinalis</i>
Indigo Bunting	<i>Passerina cyanea</i>
Savannah Sparrow	<i>Passerculus sandwichensis</i>

Table 8. Approximate stages of cultivation and peanut growth in fields throughout the year (4/77 to 4/78), in southeast Virginia.

Month	Description
April	Unbroken, with/without cover crop
May	Planted to peanuts, plants breaking surface
June	Small plants, soil still exposed
July	Larger plants, soil still exposed
August	Soil covered by maturing plants
September (late)	Start of digging, plants drying
October (early)	Fields undug, drying, or harvested
October (late)	Almost all harvested, some still drying, cover crop growing
November	Cover crop or bare soil
December to February	Mature cover crop or bare (most have a cover crop)
March (late)	Most fields broken, some planted to corn
April	Most fields planted to corn

sighting of Canada Geese actually in a study field occurred in early October, however, other peanut fields in the area contained many Geese feeding on cover crops. Ring-Billed Gulls were observed feeding in freshly plowed fields, probably on grubs or earthworms. The Purple Martin observed on 04/22/78 was subsequently stooped upon and killed in field 9 by a Pigeon Hawk (Appendix Table III, 04/22/78).

Bird Banding Records

The records of 17 out of the 30 species observed utilizing peanut fields were requested from the bird banding laboratory. Appendix Table IV contains the species requested and the number of records received for analysis. The number of records found of birds that were banded south of the study area and later recovered in the study area are also listed in Appendix Table IV. Appendix Table V contains the coordinates of the records plotted in Figures 6 and 7. These figures give the number banded per location of the 6 species later recovered in the study area, along with possible movement patterns to the study area.

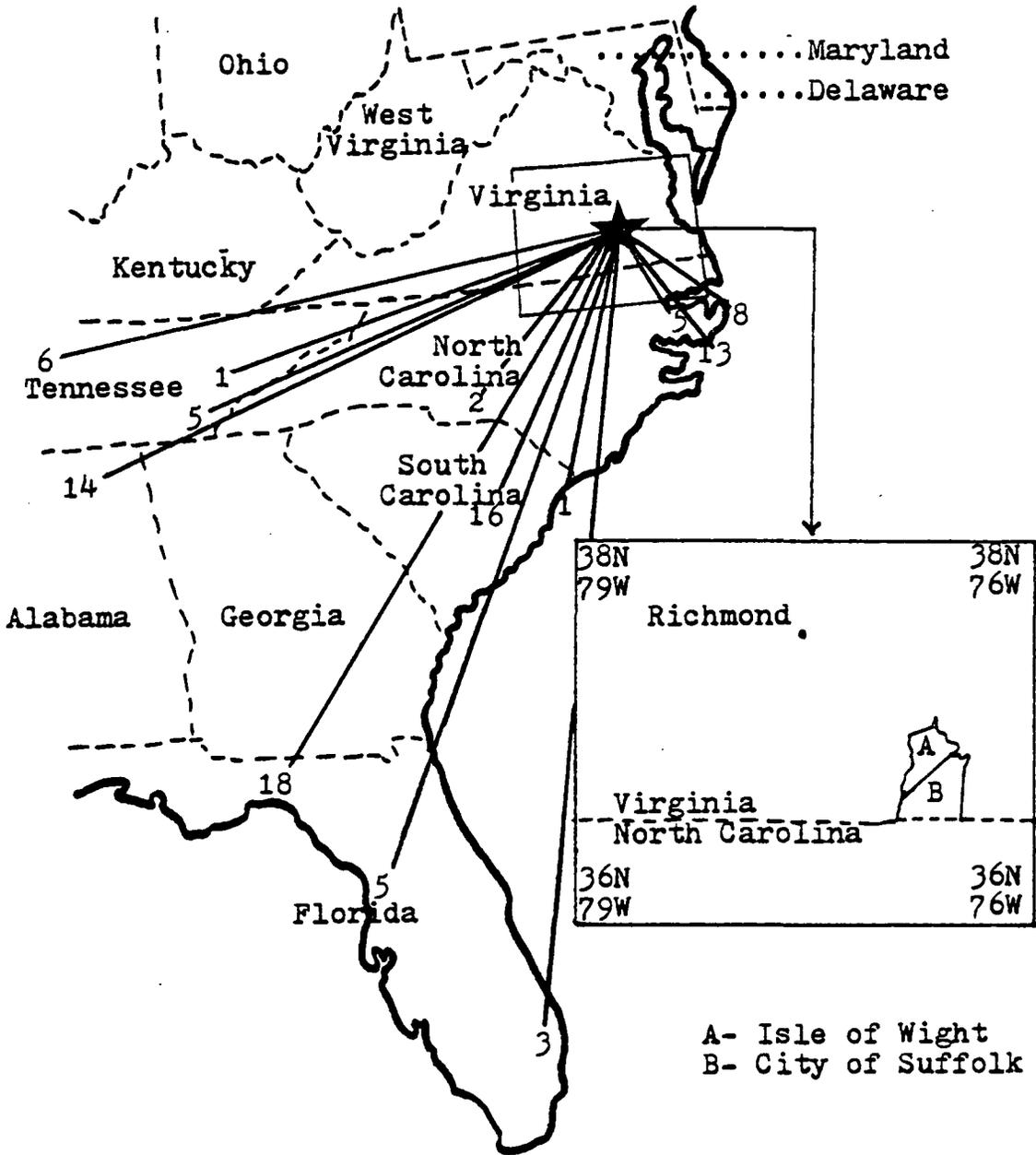


Fig. 6. Original banding sites (South of latitude 36N) of Canada Geese that were subsequently recovered in the study area outlined. Numbers indicate the number of Canada Geese from that site.

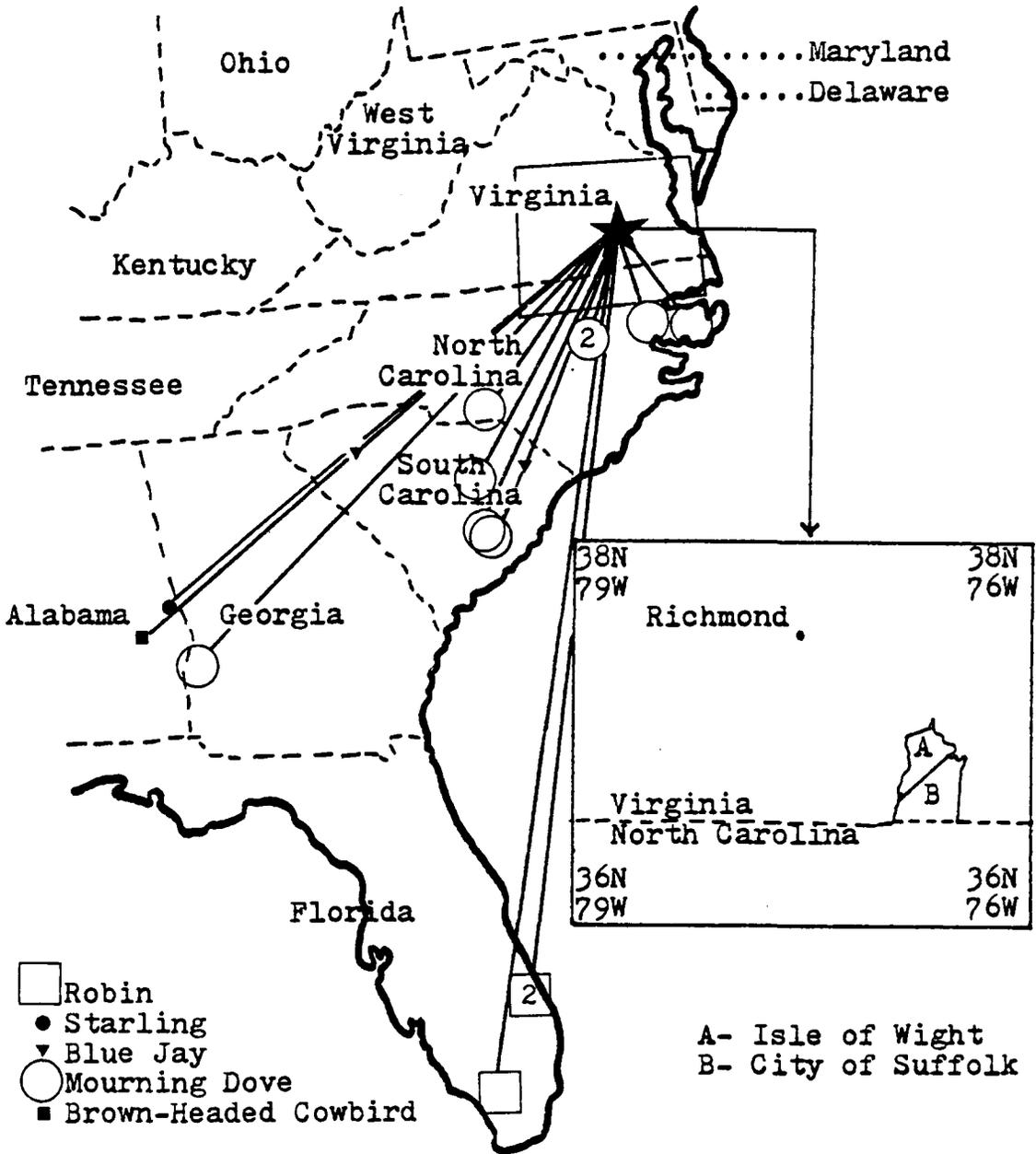


Fig. 7. Original banding sites (South of latitude 36N) of five species of birds that were subsequently recovered in the study area outlined. Numbers in excess of one record per species are indicated.

Analyses on Field Collected Birds and SoilBirds

Fall 1977. A total of 20 birds of 6 species were shot over peanut fields during the fall of 1977. Three Mourning Doves, 2 Brown-Headed Cowbirds, 5 Starlings, 2 Rock Doves, one Common Grackle, and 7 Red-Winged Blackbirds were the birds taken. Three hundred Sucrose - TBZ plates were used to analyse the crop contents, fecal material, and soil from feet for the presence of viable microsclerotia. Plates were observed beginning at day 5 for 9 days. No colonies of Cylindrocladium crotalariae were found on any of the 300 plates.

Spring 1978. A total of 41 birds of 2 species (one Mourning Dove and 40 Rock Doves) were shot over peanut fields during the spring of 1978. Collections were made during the plowing and planting period. In all, 615 Sucrose - TBZ plates were used to determine the presence or absence of viable microsclerotia in the crop contents, fecal material, and soil taken from feet. Plates were observed beginning at day 5 for 9 days. No colonies of Cylindrocladium crotalariae were found in any of the 615 plates.

Blackbird Roost Soil

One thousand plates containing soil samples taken from a blackbird roost were observed for the presence of Cylindrocladium crotalariae colonies. A total of 70 unidentified colonies were taken from the 1000 plates and subcultured back onto fresh Sucrose - TBZ plates. These 70 colonies were observed for 2 weeks beginning at day 3 of incubation. None of the 70 colonies proved to be C. crotalariae.

Test on Soil Analysis Procedure

A peanut field soil known to contain approximately 26 microsclerotia per quarter gram (104 per gram) was used to determine the sensitivity of the soil analysis procedure. This population was determined by using Sucrose - QT medium. Twenty samples at 11g each were treated identically to the blackbird roost soil, and plated on 200 Sucrose - TBZ plates. This procedure indicated approximately 18 microsclerotia per quarter gram (72 per gram) of soil, compared to the known population of approximately 26 (104 per gram). These figures correspond well with those found by Griffin (1977) in comparing Sucrose - TBZ and Sucrose - QT media without NaClO treatment.

Due to the low number of Sucrose - QT plates used in determining the population of microsclerotia contained in the soil, the means of the 2 methods above proved to be not significantly different ($p > 0.2$).

The reason for using the Sucrose - TBZ without the NaClO treatment, which permits greater interference on the plates, was to avoid killing of any microsclerotia that may have been present. The benefits derived from NaClO treatment did not offset the potential loss of microsclerotia, as few if any were expected to be present before treatment.

Survival of Microsclerotia in the Gastrointestinal Tract of Birds

Germinability of Microsclerotia

A total of 20 tests were run on the germinability of microsclerotia used in the experiments. An overall average was calculated from the 40 plates tested, each having 100 microsclerotia counted. The average overall was approximately 94 percent of the microsclerotia observed having germinated at the end of 36 hours. The range of germination rates was 90 to 99 percent.

Effects of Feed and Water on Microsclerotia

Two levels of feed mixed with water and water only were tested to determine if any reduction in germinability could be attributed to the feed or water. The germinability of the microsclerotia used for the tests was approximately 92 percent. After 6 days, the germinability of microsclerotia from the water test was difficult to determine exactly, as microsclerotia had started to germinate in the water prior to plating on Sucrose - TBZ medium. The percent germinated was approximately 88 percent. No significant difference was found between these 2 rates using A Chi-Square test ($P > .05$). The germinability of microsclerotia taken from the feed tests was difficult to quantify due to the large amount of extraneous material present. However, colonies of Cylindrocladium crotalariae grew from all plates, from day 1 to day 6.

Canada Geese

Force feeding of two levels of microsclerotia. A total of 12 Canada Geese were force fed 2 levels of microsclerotia. Six geese were fed 100 microsclerotia per 1ml of water, and 6 were given 300 microsclerotia per 1ml of water. Fecal samples taken over each of 5 days were analysed on a total of 180 Sucrose - TBZ plates. None of the 180 plates

contained observable colonies of Cylindrocladium crotalariae.

Force feeding of concentrated microsclerotia. A total of 12 Canada Geese were force fed a concentrated level (5000) of microsclerotia. Feces collected over each of 5 days were processed and analysed using 240 Sucrose - TBZ plates. No visible colonies of Cylindrocladium crotalariae were found on the plates after incubation.

Force feeding of microsclerotia in porous bags. Fifteen geese were each force fed a small porous bag containing approximately 5000 microsclerotia. Thirteen bags were recovered by sieving feces collected, 2 were not recovered within 4 days. Of the 13, 3 were recovered after 14 hours, 7 were recovered after 38 hours, 2 more after 62 hours, and 1 was recovered 86 hours after force feeding. The 3 bags recovered after 14 hours were relatively intact and still contained microsclerotia. The other 10 bags were damaged to varying extents and contained few or no microsclerotia. All bags and their contents were plated on Sucrose - TBZ medium.

Two of the 3 bags collected at 14 hours contained viable microsclerotia that grew when plated. None of the other bags collected showed any growth of Cylindrocladium

crotalariae. The 2 bags containing viable microsclerotia contained only 2 and 3 out of approximately 5000, respectively. The 5 colonies were subcultured on Acid Potato Dextrose Agar (APDA) to promote conidial growth for microscopic confirmation. In total, 5 out of approximately 15,000 microsclerotia from the 3 bags collected at 14 hours proved to be viable after passage through the gastrointestinal tract of Canada Geese.

The test on the bag material showed no observable effect on the viability of microsclerotia, as the germinability of the microsclerotia was similar to that of the microsclerotia taken from the 6th day of the water test described above.

Feeding of CBR infected peanuts. Ninety plates of Sucrose - TBZ medium were used to check for the presence of viable microsclerotia contained in feces collected over 3 days from 6 Canada Geese which were fed infected peanuts. None of the plates contained observable colonies of Cylindrocladium crotalariae after incubation and observation for 2 weeks.

Japanese Quail

Twelve Japanese Quail were each force fed approximately 5000 microsclerotia. Feces collected over four days from the 12 birds were plated on 240 Sucrose - TBZ plates. After

incubation and observation for 2 weeks, no observable colonies of Cylindrocladium crotalariae could be found on any of the 240 plates.

Bobwhite Quail

Feces collected for 4 days from 12 Bobwhite Quail were analysed and plated on 240 Sucrose - TBZ plates. Each bird had been force fed approximately 5000 microsclerotia at the time of the first feces collection. None of the 240 plates showed growth of Cylindrocladium crotalariae after incubation for a total of 2 weeks. The plates had been observed from day 5 to 14.

DISCUSSION

Some trends are evident in the use of peanut fields by birds throughout the year. Certain periods of the year had relatively little use. The months of July, August, and September in both areas had few species and few birds per species utilizing the fields. This was not unexpected, as fields hold no real attraction for the species of birds present in the area at that time. The worm infestation in August probably attracted the Starlings and House Sparrows to the field near Cypress. Except for the large numbers of Rock Doves in the Smithfield area, relatively few birds of any species were seen in the fields during May and June. However, a much larger diversity of species existed at this time. This corresponds to the active mating period and rearing of young for many species of birds. Many of the birds were observed gathering insects and seeds at the edges of fields. Territorial constraints probably limited the numbers of individuals from each species using the areas during the breeding season.

There was an apparent buildup of bird numbers, starting with the month of October and lasting through the winter months. The large numbers were due mainly to the blackbird species (Red-Winged Blackbirds, Common Grackles, and Starlings).

March and April appeared to be transition months, as there were fewer numbers, but an increase in diversification. In April the largest number of species (18) was observed. During this time the northern migration of birds would be expected to be in progress for some species, such as the Canada Goose, Mourning Dove, and Robin. This period also corresponds to low levels of inoculum in the fields, reducing the possibility of birds picking up microsclerotia on their migration northward.

Individually, Crows were observed more often in the two areas combined than any other, being seen in 21 of the 26 observation periods. Several other species (Mourning Dove, Starling, and Eastern Meadowlark) were seen over half of the observation periods. The Upland Sandpiper, Canada Goose, Red-Headed Woodpecker, Horned Lark, Purple Martin, Brown Thrasher, Eastern Bluebird, Indigo Bunting, and the Savannah Sparrow were only sighted in a study field on one occasion each.

There were also a few notable differences between the study areas concerning species. Canada Geese were seen in abundance in fields other than the study fields near Smithfield, while none was seen in fields near Suffolk. This probably was due to the close proximity of the Smithfield area to the James River. Rock Doves were also

more abundant in the Smithfield area, probably due to the proximity to the town of Smithfield and the liberty ships moored in the James River. Killdeer were sighted twice as often in Suffolk as in Smithfield, though no significance is attached to this. Other species were commonly seen in both areas.

Taking everything into account, the use of fields was relatively similar, with 21 species being observed near Smithfield, and 25 species in the City of Suffolk. With the exception of limited use by Canada Geese and Rock Doves the use by birds of the study areas was probably representative of bird use of peanut fields in Virginia.

Based on the analysis of bird banding data, several of the bird species observed utilizing peanut fields have records of travel between the peanut growing area in Virginia and peanut growing areas further south along the Atlantic coast and could conceivably be vectors of CBR. However, banding south of the study area and recovery in the study area is not necessarily indicative of direct travel between these points as considerable time may have elapsed between banding and recovery and birds may have migrated by routes other than the most direct. Canada Geese appear to have the greatest potential as vectors. The large number of banding records is probably due to the status of the species

as a game bird. As mentioned earlier, many of the species were not actually utilizing the peanut crop, but were in the fields for other reasons. That does not preclude those species as possible vectors, however, as soil could still be picked up on the feet, or microsclerotia could become lodged in the feathers. Amounts of soil taken from the feet of birds collected in the field were small. The soil itself in most cases was relatively dry, possibly resulting in the desiccation of any propagules present in the soil. Plumage of birds collected in peanut fields was not analysed for microsclerotia during this project, as it was hypothesised that viable microsclerotia were more likely to be found in the materials analysed (soil from feet, crop contents, and fecal material) and time and materials necessary for analysis were limited. Since no microsclerotia were found in the materials tested, further research involving possible CBR transport by birds, if deemed necessary, should include the analysis of plumage from all birds collected, and an increase in the number of birds and bird species collected. An effort to identify fields with a high inoculum level during periods of heavy bird use, with intensive bird collections carried on at those times and places would be helpful in determining if birds indeed do carry this fungus.

Results from the present project indicate that birds are not CBR vectors. Birds collected did not yield viable microsclerotia from crop contents, feces, or soil on their feet. Also, almost all microsclerotia fed as a contaminant of feed did not survive passage through the gastrointestinal tract. Such negative results cannot be absolutely conclusive. There may only be a few instances of birds dispersing the fungus in nature, and the chances of collecting one of those birds is small. However, it would only take one or a few such instances for the disease to become established in an area, from which it could spread to nearby fields by known methods (Rowe et al. 1974; Krigsvold et al. 1977).

The status of CBR in the fields from which birds were collected was not known. However, some of the fields were suspected of containing low levels of CBR (Personal communications from farmers).

Although no cultures of Cylindrocladium crotalariae were found in the blackbird roost soil analyses, the potential for inoculation of soil appears great. Many blackbirds die each winter in roosts, which would allow propagules on the feet or feathers to reach the soil. Infected peanuts not totally digested by birds could collect on the surface below the roost, increasing the chance of

soil inoculation. This last method of possible inoculation was tested. However, a roost can contain several thousand times the number of birds (therefore increasing chance of spread) that were laboratory tested.

A possible experiment for future research would involve a field known to contain CBR, a large net, and a captive flock of birds. After the net was placed in the field, birds could be introduced inside of the net and left for a period of time. Subsequent analysis of feet (soil), feathers, and ingesta after removal of the birds from the net and for several days thereafter may reveal viable microsclerotia had been picked up by the birds. This experiment would give more conclusive results, even if no microsclerotia were found in a viable condition.

The question arises as to whether or not the class Aves could be significant in the spread of CBR even if they were shown to carry the fungus from field to field. Other means of dispersal such as wind, may actually be more important. Granted, microsclerotia are large and heavy structures as far as most spores are concerned. Christensen (1942), however, mentions some spores that are just as large as microsclerotia being transported many miles via wind. Conceivably, small microsclerotia could become airborne during harvest operations and be blown further than has been

reported by Rowe et al. (1974). The period of harvest coincides with high levels of microsclerotia, and high bird use of fields.

For future work with this fungus, the use of Sucrose - QT medium (Griffin 1977) is strongly recommended. There is a substantial benefit derived by the use of this selective medium, as colony recognition is easier, and interference from other organisms is negligible. This medium was not used after it was developed due to difficulty in obtaining chemicals, cost, and analysis procedures had already begun using Sucrose - TBZ medium. The use of Sucrose - QT also allows the avoidance of NaClO treatment and possible loss of viable microsclerotia of Cylindrocladium crotalaria. As mentioned earlier in the methods section, NaClO was not used, even with the Sucrose - TBZ medium, because the potential loss of microsclerotia was thought to be a greater risk than interference by other organisms.

The test to determine the sensitivity of the soil analysis procedure yielded expected results. As the known population was determined by a better method (Sucrose - QT), the lower numbers found by Sucrose - TBZ medium were not surprising. This small test emphasized the greater selectivity and potential for future use of Sucrose - QT medium.

The first test run on captive birds by force feeding microsclerotia was structured to mimic naturally occurring levels of C. crotalariae. Either 100 or 300 microsclerotia were given to each Canada Goose, and levels of microsclerotia per gram of soil can fall into that range. Numbers of microsclerotia on plant parts can be higher. With the failure to isolate viable microsclerotia in feces at levels of 100 and 300, concentrated levels were used to increase the probability of determining whether or not microsclerotia could survive passage of the gastrointestinal tract (GI tract).

Approximately 5000 microsclerotia were used in subsequent experiments, with the same results. Not until the force feeding of a porous bag containing microsclerotia were there viable microsclerotia isolated from feces after passage. This method was employed to ensure the finding of microsclerotia, so they could be plated. Previous methods did not guarantee getting microsclerotia onto the medium. This method was only used on Canada Geese, due to the problem of size of the porous bags. The use of 5000 microsclerotia for force feeding was probably a higher level ingested than any encountered by birds in the field, but this high level employed was used to increase the odds of plating a viable propagule.

The fact that only 5 microsclerotia were found to survive passage through the GI tract is not surprising in view of the environment encountered. A very low pH, anaerobic conditions, physical grinding in the gizzard, and enzymatic activity may be, and in all probability are detrimental to the viability of microsclerotia. The bag may have provided some protection to those microsclerotia which survived. However, the fact that 5 did survive does show that it may be possible for Canada Geese, and other species, to carry microsclerotia and deposit them elsewhere in a viable condition. The survival rate of microsclerotia may actually be greater (or less) in the wild, as birds would be on a different diet than that fed during the experiments. The chances of a bird ingesting 5000 microsclerotia at any one time are probably remote. This fact alone would lead one to speculate on whether or not the odds of birds spreading CBR via feces was very high. Those microsclerotia which survived passed through the GI tract in 14 hours or less. The rapid rate of transport of foodstuffs through the avian GI tract probably limits the risk of spread of disease entities by this route. Normal passage of a meal is completed in less than 24 hours. Material such as grits are retained longer in the gizzard which would tend to delay such materials as the porous bag used in feeding

microsclerotia. Grit is actively picked up by birds in nature, and may be a method of picking up microsclerotia. Those bags that took longer than 14 hours for passage failed to yield viable microsclerotia. This may be due to the bags being damaged (possibly by gizzard action) in passage. This fact again minimizes the risk for long distance dispersal or transport of materials taking longer than 14 hours to pass through the GI tract.

Several species of birds were observed utilizing peanut fields at some time of the year. Of these, several are known to migrate through the peanut growing region of the southeast United States, including Virginia. Based on the banding data analysed, it appears that a small proportion are recaptured or recovered in the peanut growing region of Virginia. When these facts are coupled with the laboratory results of failure to isolate the fungus from birds collected both fall and spring, failure to isolate the fungus from roost soil, and failure to isolate the fungus after passage through the GI tract of birds (except in 2 cases where the microsclerotia were enclosed in a porous bag), there seems little reason to implicate birds in the spread of CBR.

Should it be deemed appropriate to control bird use of peanut fields some reduction ought to be effected by a

better harvesting procedure that would leave fewer peanuts as waste. Turning under waste peanuts would also reduce the attractiveness of fields to birds. These procedures would further minimize the possibility of birds spreading CBR.

SUMMARY AND CONCLUSIONS

A total of 30 bird species was observed utilizing peanut fields in southeastern Virginia over a one year period. Activities included feeding, loafing, nesting behavior, peripheral visits, and passage to adjacent areas.

Seventeen of the 30 species were deemed sufficiently important to request bird banding data from the Fish and Wildlife Service. Six of the 17 species on which data were requested had been recovered in the study area after being banded further south, indicating northward movement by the species, though this was not necessarily directly between peanut growing regions.

Individuals of six species were shot and collected (both fall and spring) from the peanut fields under study and analysed for Cylindrocladium crotalariae. The fungus was not isolated from crop contents, fecal material, or the soil taken from the feet of the birds.

Soil from a blackbird roost located in the peanut growing region of Virginia was analysed for CBR, with none of the fungus being isolated.

Canada Geese were force fed microsclerotia of Cylindrocladium crotalariae at rates of 100, 300, or 5000 in several different ways. Only in two of 15 geese fed

microsclerotia in porous bags were viable microsclerotia found to survive passage through the gastrointestinal tract. In each case 2 of 5000 microsclerotia, and 3 of 5000 microsclerotia, respectively survived passage through the GI tract and that only within 14 hours of feeding.

Japanese Quail were force fed microsclerotia at a concentrated level (5000), with no positive isolations of Cylindrocladium crotalariae being made from the fecal material collected after force feeding.

No positive cultures of the fungus were found in the feces after Bobwhite Quail had been force fed 5000 microsclerotia in a manner similar to the Japanese Quail.

Based on the failure to isolate Cylindrocladium crotalariae from the crop contents, soil from feet, and fecal material of birds collected from the field, and the failure to isolate the fungus from either blackbird roost soil or feces of birds force fed microsclerotia (except in 2 cases where the microsclerotia were enclosed in a porous bag), there seems little reason to implicate birds in the spread of CBR.

REFERENCES CITED

- Aderson H. 1974. *Spartina* (Vadagraes) i Horsens Fjord. (New site for spartina in Denmark). *Flora Fauna* 80(2):37-42. (In *Biol. Abstr.* 59(3):1343).
- Agrios, G.N. 1969. *Plant pathology*. Academic Press. New York. 629 pp.
- Ameson, E., and D.L. Stiers. 1977. *Cephalosporium gramineum*: A seed-borne pathogen. *Plant Dis. Rep.* 61(8):619-621.
- Anon. 1977. *Virginia-Carolina Peanut News*. 23(2):10.
- Apinis, A.E., and G.J.F. Pugh. 1967. Thermophilic fungi of birds' nests. *Mycopathol. Mycol. Appl.* 33:1-9.
- Ash, J.S., P.H. Jones, and R. Melville. 1961. The contamination of birds with pollen and other substances. *Brit. Birds* 54:93-100.
- Babbar, O.P., U.S. Shukla, V.P. Agnihotri, and K. Singh. 1976. Some new aspects of mycoplasmal infections in plants. *Proc. Indian Natl. Sci. Acad. Part B, Biol. Sci.* 41(4):373-378. (In *Biol. Abstr.* 63(8):4617).
- Beal, F.E.L. 1900. Food of the Bobolink, blackbirds and Grackles. *Bull.* 13, U.S.D.A., Div. Biol. Survey. Washington, D.C. 77 pp.
- Bell, D.K., and E.K. Sobers. 1966. A peg, pod, and root necrosis of peanuts caused by a species of *Calonectria*. *Phytopathology* 56:1361-1364.
- Bray, W.L. 1910. The mistletoe pest in the southwest. U.S. Dept. Agr. Bur. Plant Ind. Bull. No. 166. 39 pp.
- Broadbent, L., and C. Martini. 1959. The spread of plant viruses. *Advance. Virus Res.* 6:93.
- Broadbent, L. 1964. Control of plant virus disease. pp. 330-364. In *Plant virology*. M.K. Corbett and H.D. Sisler (eds.). Univ. Florida Press. Gainesville. 527 pp.
- Broadbent, L. 1965. The epidemiology of tomato mosaic. VIII-IX. *Am. Appl. Biol.* 55(1):57-69.

- Christensen, J.J. 1942. Long distance dissemination of plant pathogens. In Aerobiology. F.R. Mculton (ed.). Washington, D.C. 289 pp.
- Cleland, J.B. 1952. The dispersal of plants by birds. South Australian Ornith. 20:72-77. (In Biol. Abstr. 27:205).
- Cooney, D.G., and R. Emerson. 1965. Thermophilic fungi. W.H. Freeman and Co., San Francisco. 188 pp.
- Cother, E.J. 1977. Isolation of important pathogenic fungi from seeds of Cicer arietinum. Seed Sci. Technol. 5(3):593-598.
- Crebbs, T.C., Jr. 1960. Blackbird ecology and their relationship to agriculture in southeastern Virginia. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg. 153 pp.
- Cruden, R.W. 1966. Birds as agents of long-distance dispersal for disjunct plant groups of the temperate western hemisphere. Evolution 20:517-532.
- Davison, G.W.H. 1976. Role of birds in moss dispersal. Brit. Birds 69(2):65-66.
- De Vlaming, V., and V. Proctor. 1968. Dispersal of aquatic organisms: viability of seeds recovered from the droppings of captive Killdeer and Mallard ducks. Am. J. Bot. 55:20-26.
- Evans, R.N., and D.C. Prusso. 1969. Spore dispersal by birds. Mycologia 61(4):832-835.
- Falla, R.A. 1960. Oceanic birds as dispersal agents. Roy. Soc. London Proc. Ser. B, Biol. Sci. 152(949):655-659.
- Farner, D.S., and J.R. King. 1972. Avian biology. Vol. II. Academic Press, Inc., New York. 612 pp.
- Fordham, A.J. 1967. Seed dispersal by birds and animals in the Arnold Arboretum (Harvard University). Arnoldia 27(10/11):73-84.
- Garren, K.H., M.K. Beute, and D.M. Porter. 1972. The Cylindrocladium black rot of peanuts in Virginia and North Carolina. Am. Peanut Res. Educ. Assoc., Inc. 4(1):67-71.

- Garren, K.H., and T.A. Coffelt. 1976. Reaction to Cylindrocladium black rot in Virginia-type peanut cultivars. *Plant Dis. Rep.* 60(2):175-178.
- Gilliam, M. 1972. Fungi isolated from the intestinal contents of foraging worker honeybees, Apis mellifera. *J. Invertebr. Pathol.* 20(1):101-103.
- Graham, E.H. 1941. Legumes for erosion control and wildlife. U.S.D.A. Misc. Pub. No. 412. Washington, D.C. 153 pp.
- Green, G.J. 1976. Airborne rust inoculum over western Canada in 1976. *Can. Plant Dis. Surv.* 56(4):117-118.
- Griffin, G.J. 1975. Cylindrocladium crotalariae populations in naturally infested peanut field soil. *Proc. Am. Phytopathol. Soc.* 2:27 (Abstr.).
- Griffin, G.J. 1977. Improved selective medium for isolating Cylindrocladium crotalariae microsclerotia from naturally infested soils. *Can. J. Microbiol.* 23(6):680-683.
- Hankla, D.J., and R.R. Rudolph. 1967. Changes in the migration and wintering habits of Canada Geese in the lower portion of the Atlantic and Mississippi flyways - with special reference to National Wildlife Refuges. *Proc. Southeastern Assoc. Game and Fish Commissioners* 21:133-144.
- Hanlin, B.T. 1973. The distribution of peanut fungi in the southeastern U.S.A. *Mycopathol. Mycol. Appl.* 49(4):227-241.
- Hardy, J.W. 1961. Resident and migrant blackbirds in southeastern Virginia, agricultural deprecations and winter roost locations. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg. 96 pp.
- Hasan, S. 1976. Study of the passage of a plant pathogen fungus, Colletotrichum lagenarium through the gut of Euparypha pisana. *Ann. Zool. Ecol. Anim.* 8(2):221-230. (*In Biol. Abstr.* 63(7):4061).
- Heald, F.D. 1943. Introduction to plant pathology. McGraw-Hill. New York. 603 pp.

- Heald, F.D., and R.A. Studhalter. 1914. Birds as carriers of the chestnut-blight fungus. *J. Agric. Res.* 11:405-422.
- Hirst, J.M. 1959. Spore liberation and dispersal. pp. 529-538. *In* *Plant pathology, problems and progress*. C.S. Holton (ed.). Univ. Wisconsin Press. Madison. 588 pp.
- Hubalek, Z. 1972. Keratinophile pilze an freilebenden Voegeln. (Keratinophilic fungi on wild birds). *Mykosen.* 15(5):207-211. (*In* *Biol. Abstr.* 55(8):4527).
- Hubalek, Z. 1974. Dispersal of fungi of the family Chaetomiaceae by free-living birds: I. A survey of records. *Ceska. Mycol.* 28(2):65-79. (*In* *Biol. Abstr.* 58(12):6984).
- Hubalek, Z. 1976. Seasonal distribution of fungi on House Sparrows. *Trans. Br. Mycol. Soc.* 66(3):509-516.
- Hunter, B.B. 1970. Nutritional and environmental factors affecting growth, sporulation, and sclerotial formation of species of Cylindrocladium. Ph.D. Dissertation. West Virginia University. Morgantown. 161 pp.
- Ingold, C.T. 1953. Dispersal of fungi. Oxford University Press. London. 197 pp.
- Janzen, D.H. 1971. Seed predation by animals. *Ann. Rev. Ecol. Systematics* 2:465-492.
- Johnston, J.R. 1912. The history and cause of the coconut bud-rot. U.S.D.A. Bur. Plant Ind. Bull. No. 228. 175 pp.
- Kado, C.I., and J.M. Gardner. 1977. Transmission of deepbark canker of walnuts by the mechanical harvester. *Plant Dis. Rep.* 61(4):321-325.
- Kocan, R.M., and F. Hasenclever. 1974. Seasonal variation of the upper digestive tract yeast flora of feral pigeons. *J. Wildl. Dis.* 10:263-266.
- Kouyeas, V., and B. Anastassiadis. 1962. Dissemination of Deuterophoma tracheiphila Petri by the Common Magpie (Pica pica L.). *Kiphissia. Ann. Inst. Phytopathol. Benaki, N.S.* 4:52-55.

- Krefting, L.W., and E.I. Roe. 1949. The role of some birds and mammals in seed germination. *Ecol. Monogr.* 19:269-286.
- Krigsvold, D.J., and G.J. Griffin. 1975. Quantitative isolation of Cylindrocladium crotalariae microsclerotia from naturally infested peanut and soybean field soils. *Plant Dis. Rep.* 59:543-546.
- Krigsvold, D.J., K.H. Garren, and G.J. Griffin. 1977. Importance of peanut field cultivation and soybean cropping in the spread of Cylindrocladium crotalariae within and among peanut fields. *Plant Dis. Rep.* 61(6):495-499.
- Lachmund, H.G. 1929. Cronartium comptoniae (Arth.) in western North America. *Phytopathology* 19:453-466.
- Lefebvre, P.W. 1961. Blackbirds in southeastern Virginia nesting productivity, depredations and damage control methods. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg. 106 pp.
- Malone, C.R. 1965. Dispersal of plankton: rate of food passage in Mallard ducks. *J. Wildl. Manage.* 29(3):529-533.
- Malone, C.R. 1966. Regurgitation of food by Mallard ducks. *Wilson Bull.* 78(2):227-228.
- Martin, A.C., H.S. Zim, and A.L. Nelson. 1951. American wildlife and plants. McGraw-Hill Book Co. New York, New York. 500 pp.
- Martinez, J.A., D.A. Palazzo, and M. Karazawa. 1977. Importance of the wind on the liberation and propagation of Hamileia vastatrix Berk and Br. spores. *Fitopatol. Bras.* 2(1):35-42. (*In Biol. Abstr.* 65(1):423).
- Monga, D.P. 1972. Prevalence of pathogenic fungi in wild birds. *Indian J. Med. Res.* 60:517-519.
- Nielson, O. 1929. Bekampfung des Kartoffelkresbsses (Synchytrium endobioticum) in Danemark. *Nord. Jordbrugsforsk* 7:549. (*In Rev. Appl. Mycol.* 9:670).
- Nik, W.Z., and D.G. Parbery. 1977. Studies of seed borne fungi of tropical pasture legume species. *Aust. J. Agric. Res.* 28(5):821-842.

- Olson, S.L., and K.E. Blum. 1968. Avian dispersal of plants in Panama. *Ecology* 49(3):565-566.
- Ooka, J.J., and T. Kommedahl. 1977. Wind and rain dispersal of Fusarium moniliforme in corn fields. *Phytopathology* 67(8):1023-1026.
- Peplinski, J.D., and W. Merrill. 1974. Nonsurvival of Ceratocystis fagacearum in frass of oak bark beetles and ambrosia beetles. *Phytopathology* 64(12):1528-1530.
- Powell, N.L., G.J. Griffin, K.H. Garren, and D.E. Pettry. 1976. Use of aerial photography to detect diseases in peanut fields II. Cylindrocladium Black Rot. *Peanut Sci.* 3(1):25-29.
- Powell, N.L., K.H. Garren, and G.J. Griffin. 1977. Cylindrocladium black rot disease development in two peanut fields as monitored by infrared aerial photography. *Plant Dis. Rep.* 60(12):1003-1007.
- Proctor, V.W. 1959. Dispersal of freshwater algae by migratory water birds. *Science* 130(3376):623-624.
- Proctor, V.W. 1964. Viability of crustacean eggs recovered from ducks. *Ecology* 45(3):656-658.
- Proctor, V.W. 1968. Long distance dispersal of seeds by retention in digestive tract of birds. *Science* 160(3825):321-322.
- Pugh, G.J.F. 1964. Dispersal of Arthroderma curreyi by birds, and its role in the soil. *Sabouraudia* 3:275-278.
- Pugh, G.J.F. 1965a. Fungi recorded on birds from Stockholm. *Rep. Stockholm Bird Observ.* pp. 21-24.
- Pugh, G.J.F. 1965b. Cellulytic and keratinophilic fungi recorded on birds. *Sabouraudia* 4:85-91.
- Pugh, G.J.F. 1966a. Associations between birds' nests, their pH, and keratinophilic fungi. *Sabouraudia* 5:49-53.
- Pugh, G.J.F. 1966b. Fungi on birds in India. *J. Indian Bot. Sci.* 45(3/4):296-303. (In *Biol. Abstr.* 50(23/24):12367).
- Pugh, G.J.F., and M.D. Evans. 1970. Keratinophilic fungi associated with birds: 1. Fungi isolated from the

- feathers, nests and soils. Trans. Brit. Mycol. Soc. 54(2):233-240.
- Pugh, G.J.F. 1972. The contamination of birds' feathers by fungi. Ibis 114(2):172-177.
- Reitsma, J., and J.H. Van Emden. 1949. De bladpokkenziekte van de Thee. (The blister-blight of Tea). Bergcultures. 12:218-231, and 18:370-377. (In Rev. Appl. Mycol. 29:58).
- Rowe, R.C., S.A. Johnston, and M.K. Beute. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1297.
- Schlichting, H.E. 1960. The role of waterfowl in the dispersal of algae. Trans. Am. Microsc. Soc. 79:160-166.
- Shaw, D.E. 1965. Condition resembling blister-blight of tea on tea seedlings in quarantine in New Guinea. F.A.O. Plant Protect. Bull. 13:56-64.
- Sheridan, J.E. 1977. Drachslera spp. and other seed-borne pathogenic fungi in New Zealand cereals. New Zealand J. Agric. Res. 20(1):91-94.
- Sigurdsson, H., and S. Fridriksson. 1969. Birds and seed dispersal over long distances. Plants and Gard. 25:54.
- Sladen, W.J.L., and P.K.C. Austwick. 1955. The mycoflora of wild Pink Footed Geese sampled in Iceland and Scotland, 1953. pp. 133-138. In the Wildfowl Trust 1953-1954. P. Scott and H. Boyd (eds.). Country Life, Ltd. London. 235 pp.
- Sturkie, P.D. 1976. Avian physiology. Springer-Verlag New York, Inc. 400 pp.
- Tiffany, L.H., J.C. Gilman, and D.R. Murphy. 1955. Fungi from birds associated with wilted oaks in Iowa. Iowa State Coll. J. Sci. 29:659-706.
- Tiunina, E.V. 1931. (Life cycle of yeast). Tr. Sev. Kav. Inst. Spetsial. Nyk. Tekhnicheskikh Kultur. 1(1):101-127. (In Biol. Abstr. 8(1):163).

- Trykoz, H.O. 1975. Spores of fungi in nests of Muridae rodents, on gamasoids, fleas and lice. *Ukranian Bot. Zh.* 32(5):603-611. (In *Biol. Abstr.* 64(4):2236).
- Warner, G.M., and D.W. French. 1970. Dissemination of fungi by migratory birds: survival and recovery of fungi from birds. *Can. J. Bot.* 48:907-910.
- Zilka, P.J., and R.O. Tinnin. 1976. Potential avian influence in the distribution of dwarf mistletoe. *Northwest Science* 50(1):8-16. (In *Forestry Abstr.* 38(3):146).

APPENDIX

Appendix Table I. Procedure for making Hunter's medium
(Hunter 1970).

1. K_2HPO_4	1g
2. $MgSO_4 \cdot 7H_2O$	0.5g
3. KCl	0.5g

Stock Micronutrients

$0.44g Fe_2(SO_4)_3 \cdot 9H_2O = 0.1mg Fe^{+3}$ per ml

$0.15g MnSO_4 \cdot H_2O = 0.5mg Mn^{+2}$ per ml

$0.88g ZnSO_4 \cdot 7H_2O = 0.2mg Zn^{+2}$ per ml

Add above 3 chemicals to 990ml of distilled water, and add 10ml concentrated HCl to prevent Fe precipitation.

OR:

To replace 1ml of stock micronutrient, add per liter:

Mn^{+2}	0.05mg
Zn^{+2}	0.2mg
Fe^{+3}	0.1mg

4. Dextrose	100g
5. Casein Hydrolysate	0.05g

Procedure

Take 1., 2., and 3. above and add to 1 liter distilled H₂O.

Take 1ml of stock micronutrient and add per liter. ²

Adjust pH to 6.5 with 1 N KOH.

Add 4. and 5.

Pour approximately 100ml into small flasks.

Autoclave for 15 minutes.

Appendix Table II. Procedure for making Sucrose - TBZ plates
(Krigsvold and Griffin 1975).

1. Sucrose	10g
2. Peptone	15g
3. KH_2PO_4	1g
4. $\text{MgSO}_4 \times 7\text{H}_2\text{O}$	0.5g
5. Agar	20g
6. Streptomycin, in 14ml sterile H_2O	0.210g
7. Chlortetracycline HCl, in 14ml sterile H_2O	0.210g
8. Pentachloronitrobenzene (PCNB)	0.1g
9. Oxgall	2g
10. Thiabendazole (TBZ), in 14ml Acetone	0.017g

Procedure:

Add 1. through 5. above per liter of water to large flasks.

Autoclave 20 minutes, cool to handling in water bath.

Add 4ml from solutions 6. and 7. for each liter of medium.

Add 8. and 9. per liter of medium to be made.

Add 3ml of 10. for each liter.

Adjust to pH 4 with 10 N HCl.

Pour plates under hood.

Cool plates, store in refrigerator.

Appendix Table III. (continued)

Species	05/20/77									
	12	13	14	15	16	17	18	19	20	
Canada Goose										
Pigeon Hawk										
Bobwhite										
Killdeer										
Upland Sandpiper										
Ring-Billed Gull										
Rock Dove										
Mourning Dove	2									
Common Flicker		1								
Red-Bellied Woodpecker										
Red-Headed Woodpecker										
Horned Lark							1			
Barn Swallow										
Purple Martin										
Blue Jay										
Common Crow									1	
Mockingbird										
Brown Thrasher										1
Robin										
Eastern Bluebird										
Starling									10	
House Sparrow										
Eastern Meadowlark										
Red-Winged Blackbird										
Common Grackle										1
Brown-Headed Cowbird									10	
Cardinal										
Indigo Bunting										
Savannah Sparrow										

Appendix Table III. (continued)

Species	07/23/77						
	12	13	14	15	16	17	18
Canada Goose							
Pigeon Hawk							
Bobwhite							
Killdeer							
Upland Sandpiper							
Ring-Billed Gull							
Rock Dove							
Mourning Dove							
Common Flicker							
Red-Bellied Woodpecker							
Red-Headed Woodpecker							
Horned Lark							
Barn Swallow							
Purple Martin							
Blue Jay							
Common Crow							
Mockingbird							
Brown Thrasher							
Robin							
Eastern Bluebird							
Starling							
House Sparrow							
Eastern Meadowlark							
Red-Winged Blackbird							
Common Grackle							
Brown-Headed Cowbird							
Cardinal							
Indigo Bunting							
Savannah Sparrow							

Appendix Table III. (continued)

10/22/77

Species	Fields in Suffolk									
	12	13	14	15	16	17	18	19	20	
Canada Goose									1	
Pigeon Hawk									1	
Bobwhite										
Killdeer	8						18			
Upland Sandpiper										
Ring-Billed Gull										
Rock Dove										
Mourning Dove	40		3				50			
Common Flicker	6								1	
Red-Bellied Woodpecker										
Red-Headed Woodpecker										
Horned Lark										
Barn Swallow										
Purple Martin										
Blue Jay										
Common Crow						200	1		2	
Mockingbird										
Brown Thrasher										
Robin										
Eastern Bluebird										
Starling	25			5	3	5	40			
House Sparrow										
Eastern Meadowlark						10				
Red-Winged Blackbird									8	
Common Grackle										
Brown-Headed Cowbird	3									
Cardinal										
Indigo Bunting										
Savannah Sparrow										

Appendix Table III. (continued)

Species	Fields in Smithfield											
	1	2	3	4	5	6	7	8	9	10	11	
	10/23/77											
Canada Goose												
Pigeon Hawk												
Bobwhite												
Killdeer												
Upland Sandpiper												
Ring-Billed Gull												
Rock Dove									50			
Mourning Dove												
Common Flicker												
Red-Bellied Woodpecker												
Red-Headed Woodpecker												
Horned Lark												
Barn Swallow												
Purple Martin												
Blue Jay				2								
Common Crow												
Mockingbird												
Brown Thrasher												
Robin												
Eastern Bluebird												
Starling									10			
House Sparrow												
Eastern Meadowlark												
Red-Winged Blackbird									10			
Common Grackle												
Brown-Headed Cowbird												
Cardinal												
Indigo Bunting												
Savannah Sparrow												
Blackbirds (Mixed)											1000's	

Appendix Table IV. List of bird species whose banding records were studied, total number of records reviewed, and the number of birds recaptured or recovered within the boundaries of the study area that were originally banded to the south of the study area.

	Total recoveries and recaptures in study area	Records where original banding site was south of study area
Canada Goose	936	97
Killdeer	1	0
Ring-Billed Gull	202	0
Mourning Dove	2113	9
Horned Lark	0	0
Blue Jay	246	2
Common Crow	4	0
Fish Crow	2	0
Robin	81	3
Starling	186	1
Eastern Meadowlark	1	0
Red-Winged Blackbird	67	0
Common Grackle	368	0
Brown-Headed Cowbird	155	1
Cardinal	460	0
Indigo Bunting	9	0
Savannah Sparrow	2	0

Appendix Table V. List of original banding sites of six bird species subsequently recovered within the study area boundary (36.0 to 39.0 North and 76.0 to 79.0 West).

Species	Original banding site ¹		Number
	Latitude	Longitude	
Blue Jay	34.0	79.4	1
	34.2	82.2	1
Robin	25.5	81.0	1
	27.3	80.2	2
Brown-Headed Cowbird	32.2	86.2	1
Starling	32.4	85.2	1
Mourning Dove	31.5	85.0	1
	32.5	80.0	1
	33.3	80.2	1
	34.3	79.5	1
	35.0	80.0	1
	35.4	76.3	1
	35.4	78.3	2
35.5	77.0	1	

Table V. (continued)

Species	Original banding site ¹		Number
	Latitude	Longitude	
<hr/>			
Canada Goose	26.3	80.2	3
	28.4	82.3	5
	30.0	84.1	6
	30.0	84.2	1
	30.2	84.1	7
	30.3	84.1	4
	33.3	79.0	1
	33.3	80.2	16
	34.3	86.5	14
	35.0	80.0	2
	35.2	76.0	3
	35.2	76.1	9
	35.2	84.5	5
	35.3	76.0	1
	35.4	75.3	8
	35.4	76.3	5
	35.4	84.2	1
35.5	87.5	6	

¹ Confined to banding sites South of the study area.

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THE ROLE OF BIRDS IN SPREADING THE
CYLINDROCLADIUM BLACK ROT OF PEANUTS

by

Richard Berton Hiller

(ABSTRACT)

Bird utilization of twenty fields was observed at monthly intervals for one year, from May 1977 to April 1978. The 20 fields were located in 2 areas of southeastern Virginia. A total of 30 bird species were observed using the fields. The migratory and movement patterns of some of the species were determined from bird banding data. A small proportion of 6 species recovered in the study area were originally banded to the south of the study area. Fecal material, soil from feet and crop contents were taken from several bird species collected in the field and analysed for Cylindrocladium crotalariae. Soil samples from a blackbird roost in the area were also analysed for C. crotalariae. None of the samples taken showed presence of the fungus. Microsclerotia of the fungus were force fed by different methods to 3 species of captive birds (Canada Geese, Japanese Quail, Bobwhite Quail) to determine viability after

passage through the gastrointestinal tract. No viable microsclerotia were detected in fecal material taken from birds force fed loose microsclerotia. A very small number (a total of 5 of 10,000 microsclerotia from 2 geese) proved to be viable after passage through Canadian Geese after being force fed microsclerotia in porous bags.