methods. Upon drying of the unwashed spheres, 85% of their weight is lost as gaseous ammonia, carbon dioxide, carbon monoxide, and water vapor. The evolution of these gases occurs in large amounts in specific temperature regions. During drying, the microspheres crack due to excessive gas release. Two quasi-isothermal drying systems were developed to control the rate at which gas is evolved. The first system uses a DuPont thermogravimetric analyzer interfaced with a Macsym III microcomputer. This system monitors total weight loss and controls the rate of heating through a feedback loop. The second system uses a CVC time-of-flight mass spectrometer to monitor the rate at which specific components are released from the sample. The microcomputer similarly controls this system.

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FIRST OCCURRENCES OF *CYRNEA COLINI* AND *DISPHARYNX* SP. IN BOBWHITES IN TENNESSEE

W. ALAN MCRAE[1] AND RALPH W. DIMMICK

University of Tennessee
Knoxville, TN 37901

ABSTRACT

Occurrences of helminth parasites *Cyrnea colini* and *Dispharynx* sp. are reported in the bobwhite (*Colinus virginianus*) in Tennessee. Two of 76 birds examined contained *C. colini* while 4 of 76 were infected with *Dispharynx* sp.

INTRODUCTION

A review of available literature by Kellogg and Doster (1972) indicated that helminth parasites *Cyrnea colini* and *Dispharynx* sp. have occurred in bobwhites from Georgia, Florida, North Carolina, Ohio and New Jersey. *Cyrnea* have also been recovered from bobwhites in all other southeastern states (except Tennessee) as well as in Texas, Wisconsin, Montana, northeastern U.S., and Cuba (Wehr 1972). In addition, *D. nasuta* has infected quail in Louisiana, Illinois, New Jersey and New York (Kellogg and Doster 1972).

Previous studies on helminth fauna of bobwhites from Tennessee (Cram 1931; Blakeney and Dimmick 1971; Dabney and Dimmick 1977) have not reported *C. colini* or *Dispharynx* sp. The purpose of this paper is to report the presence of both nematodes from bobwhites in Tennessee.

METHODS

Gastrointestinal tracts (posterior to the crop) of 76 bobwhites collected by live trapping or shooting on Ames Plantation, Fayette County, TN, were examined for helminth parasites following procedures outlined by Blakeney and Dimmick (1971). A minimum of 10 birds was collected each month from December 1977 to May 1978.

RESULTS AND DISCUSSION

Two birds (2.6%) contained nematodes identified as *C. colini* in the proventriculus or at the proventricular-gizzard junction. The numbers of *C. colini* were one and three individuals per bird. Cram (1931:266) reported finding a high incidence of this nematode in every southeastern state except Tennessee. In that study, mean infection rate was over 63% with a mean of 3.3 *C. colini* per infected bird (range = 1-31). Cram (1931) and Wehr (1972) attributed little or no pathogenicity to this nematode.

Four birds examined (5.3%) harbored *Dispharynx* sp. in the wall of the proventriculus. One infected bird contained two of the nematodes; the other three birds had one each. Positive species identification was not possible because no male *Dispharynx* were recovered. However, Goble and Kutz (1945) concluded that all forms of this genus in the Western Hemisphere were actually *D. nasuta.* Considering that no other species of the genus have been reported in bobwhites (Kellogg and Doster 1972), our specimens are most likely *D. nasuta.*

Bendell (1955) considered *D. nasuta* a major limiting factor on blue grouse (*Dendragapus obscurus fuliginosus*) populations, and Bump (1935) called this nematode the most important parasite of wild game birds in New York. Heavy infections of *D. nasuta* resulted in high mortality of pigeons in the southern United States (Cram 1928; Hwang et al. 1961). Kellogg and Prestwood (1968) reported that crop worm (*Capillaria contorta*) and *D. nasuta* infections were associated with severe ulceration of the proventriculus and mortality of 4000 bobwhites on a Georgia game farm. Madsen (1941) noted that infected birds usually die as a result of *D. nasuta* infection, but Kellogg and Doster (1972) felt that *D. nasuta* alone seldom caused death. The light infections encountered in this study did not cause any apparent pathologic changes in the proventriculus. Most problems result from heavy infections leading to thickening and maceration of the proventricular wall, epithelial desquamation, hypersecretion of mucus, congestion and secondary bacterial invasion (Hwang et al. 1961).

Even though the incidence of infection and numbers per infected bird were low in this study, all birds examined had reached somatic maturity, and incidence in young birds is usually significantly greater than in adults (Goble and Kutz 1945). *Dispharynx* sp. also requires an intermediate host which might make incidence in winter lower than in summer and fall.

There are differences between the helminth fauna of bobwhites from our study area and those from the Ames field trial course; these sites are approximately 10 km apart. All studies using birds from the field trial course have reported cestode infection in low incidence and numbers (Cram 1931; Blakeney et al. 1973; Dabney and Dimmick 1977). Cestodes were

[1] Present address: International Paper Co., Silver Lake Station, Bainbridge, GA 31717

completely absent from bobwhites in our sample. This absence may be a seasonal phenomenon since all cestodes previously reported at Ames require an insect as an intermediate host. However, it is now generally believed that denser bobwhite populations have a greater variety of parasites (Kellogg and Prestwood 1968). This could account for the presence of several species of cestodes in the denser population on the field trial course. The field trial course has also been subject to heavier stocking of pen-reared birds that might have harbored these cestodes, particularly *Raillietina cesticillus*. The absence of *C. colini* and *Dispharynx* sp. in samples from the field trial course is difficult to explain considering the high incidence of these nematodes in bobwhites from other southeastern states.

ACKNOWLEDGEMENTS

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SEASONAL PREY CAPTURE BY THE SCREECH OWL IN TENNESSEE

LINDA J. TURNER[1] AND RALPH W. DIMMICK

University of Tennessee

Knoxville, TN 37901

ABSTRACT

Prey utilization by Screech Owls (*Otus asio*) was determined by examining stomach contents of 90 dead Screech Owls collected from roads in Tennessee from November 1976 to June 1978, and by examining food items cached by Screech Owls in 42 nesting structures in East Tennessee. Mammals were most important in Screech Owl stomachs in late fall and winter, and insects were important items consumed in all seasons. Birds were predominant in food caches in spring and winter.

INTRODUCTION

Little information is available on prey utilization by Screech Owls (*Otus asio*) in the southern United States, and most of these accounts are based on small samples or are anecdotal. Fisher (1893) described the stomach contents of 255 Screech Owls, but only 17 stomachs were from southern areas (only one from Tennessee). Ijams (1931:3) reported that the Screech Owl in Tennessee, " . . . eats lots of mice and beetles, but is not averse to songbirds." Laskey (1933:21) examined a nest box previously occupied by a Screech Owl in Tennessee that contained crayfish parts and feathers ". . . of at least two Bluebirds, two Cardinals and one Robin." Stupka (1953) identified stomach contents of 39 Screech Owls collected over a 15 year period in the Great Smoky Mountains National Park, Tennessee. He found insects to be of major importance in the owl's diet although mammals, birds, crayfish, and amphibians were also identified.

In addition to the paucity of regional data on prey utilization by the Screech Owl, different data collection techniques may have resulted in discrepancies in reports of its food habits throughout the species' range. Studies based on stomach contents may show significant deviations from studies based on examination of food caches in nest boxes, or regurgitated pellents.

This study was undertaken to delineate seasonal patterns of prey utilization by Screech Owls in Tennessee by identifying stomach contents of dead-on-road (DOR) birds and prey items cached by owls in structures used for winter roosting and nesting.

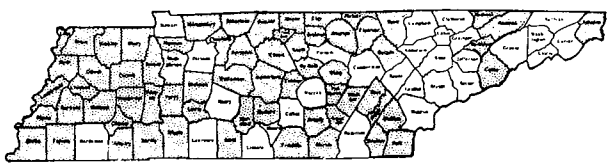

FIG. 1: *Counties (unshaded) in Tennessee where dead on road Screech Owls were collected from November 1976 to June 1978.*

[1] Present Address: Tennessee Valley Authority, Norris, TN 37828

Methods

Stomachs were removed from 90 DOR Screech Owls collected in 28 Tennessee counties from November 1976 to June 1978 (Figure 1). Various ornithologists, biologists, and other interested parties participated in the collection of these owls. Fourteen stomachs were empty and excluded from further analysis. Stomach contents were sorted and identified to the lowest taxon possible. After identification, specimens were blotted dry and their volume was measured to the nearest 0.1 ml using 10 and 25 ml graduated cylinders. Percentage volume (the volume of each food item divided by the volume of the entire stomach contents) and percentage occurrence (the number of stomachs in which a food item occurred divided by the total number of stomachs examined) were determined.

Food caches were collected from nest boxes located along the Holston River, Hawkins Co. and in various sections of Knox Co. from March 1977 to June 1978. Nest boxes were checked at least once during the winter months (December to February) and two or more times from March to June for intact carcasses as well as other prey remains. Caches were removed from the nest box only when immediate identification was not possible. Food items were counted, but no attempt was made to weigh or to measure the items. Data were compiled by seasons: spring (March-May), summer (June-August), fall (September-November), and winter (December-February). Since nest boxes were not checked during the fall or after the young birds fledged (late June), food cache data were not available for these time periods.

Results and Discussion

Spring

Identification of stomach contents of six Screech Owls collected during the spring months revealed heavy utilization of insects (Table 1). Insects (mostly beetles, moths, caterpillars, and crickets) occurred in 83.3 percent of the stomachs examined and composed 85.0 percent of the total stomach content volume. The remaining stomachs contained crayfish, spiders and centipedes. No vertebrate remains were found.

TABLE 1. *Stomach contents of 76 DOR Screech Owls collected from November 1976 to June 1978 in Tennessee.*[1]

	Spring			Summer			Fall			Winter			Total		
Prey Items	n	%occ	%vol	n	%occ	%vol	n	%occ	%vol	n	%occ	%vol	n	%occ	%vol
Mammals (predominately *Peromyscus* sp.)	0	0	0	0	0	0	6	24.0	52.6	15	36.6	60.8	21	27.6	54.4
Birds	0	0	0	1	25.0	11.8	2	8.0	2.5	2	4.9	.2	5	6.6	1.4
Fish	0	0	0	0	0	0	0	0	0	3	7.3	2.6	3	3.9	1.4
Amphibians	0	0	0	0	0	0	0	0	0	1	2.4	.5	1	1.3	.3
Crayfish	2	33.3	8.3	0	0	0	1	4.0	2.2	4	7.3	7.8	7	7.9	5.4
Spiders	1	16.7	3.3	0	0	0	12	32.0	2.9	23	24.4	2.8	36	25.0	2.8
Insects (mostly Lepidopterans, Coleopterans, and Orthopterans)	38	83.3	85.0	43	100.0	86.3	117	92.0	39.0	149	46.3	22.2	347	67.1	32.2
All Other Invertebrates	1	16.7	1.7	0	0	0	6	12.0	.7	4	9.8	1.2	11	10.5	1.0
Unidentified	1	16.7	1.7	1	25.0	2.0	0	0	0	7	17.1	1.9	9	11.8	1.2
TOTAL	43			45			144			208			440		

[1] *A complete list of all prey items can be found in Duley (1979).*

TABLE 2. *Food items cached in 48 nest boxes in east Tennessee during spring (March-May) 1977 and 1978 and winter (December-February) 1977-1978.*[1]

Prey Item	Number of Items	
	Spring	Winter
Mammals		
Meadow Mice (*Microtus* sp.)	4	0
Eastern Harvest Mouse (*Rheithrodontomys humulis*)	2	0
Others	5	2
Subtotal (percent occurrence)	11(12.0%)	2(4.2%)
Birds		
Blue Jay	8	11
Mockingbird	4	9
Brown Thrasher (*Toxostoma rufum*)	2	4
Cardinal	10	1
Song Sparrow	5	0
Others	29	10
Subtotal (percent occurrence)	58(63.7%)	35(72.9%)
Amphibians		
Pickerel Frog (*Rana palustris*)	1	0
Bullfrog (*R. catesbeiana*)	2	1
Subtotal (percent occurrence)	3(3.3%)	1(2.1%)
Fish		
Bluegill (*Lepomis macrochirus*)	3	0
Whitetail Shiner (*Notropis galacturus*)	2	0
GizzardShad (*Dorosoma cepidianum*)	0	6
Others	1	1
Subtotal (percent occurrence)	6(6.6%)	7(14.6%)
Invertebrates	9(9.9%)	3(6.2%)

[1] *A complete list of all prey items can be found in Duley (1979).*

A total of 91 food items was cached by Screech Owls in 27 boxes during the 1977 and 1978 nesting seasons (7 March-7 June, Table 2). Most of the food was stored in the nests during the first two weeks after the young owls had hatched (late April and early May) as was also reported by VanCamp and Henny (1975). Fifty-eight birds of 19 species were identified, comprising 63.7 percent of all food items found during the nesting season. The most frequently occurring birds were Cardinals (*Cardinalis cardinalis*), Blue Jays (*Cyanocitta cristata*), and Song Sparrows (*Melospiza melodia*), all permanent residents of Tennessee. Mammals, mostly voles (*Microtus* sp.) and house mice (*Mus musculus*), comprised 16.6 percent of the cached food items. Amphibians (3.3 percent), fish (6.6 percent), and various invertebrates (9.7 percent) made up the remaining food items cached. A red bat (*Lasiurus borealis*) found on 23 April 1978 was of special interest. There is no other record of the Screech Owl feeding on this species, although Cahn and Kemp (1930) found two other species of bats in the diet of the Screech Owl in Illinois.

Several northern studies have supplied most of the information on the food habits of the Screech Owl during the spring season. Allen (1924) identified 77 birds of 18 species brought to nestling owls in New York. Out of 40 days of observation, remains of birds were found on 35 days, insects on 28 days, crayfish on 24 days, amphibians on 15 days, mammals on 12 days, fish on 6 days, and spiders, snails, and reptiles on 1

day each. VanCamp and Henny (1975) identified 477 items in food caches during the nesting season (26 March-7 June) in northern Ohio. Fifty-three species of birds were recorded, amounting to 64.8 percent of the food items found during the nesting season. VanCamp and Henny concluded that Screech Owls take advantage of the spring migration of birds to feed their young. In a Michigan study, Craighead and Craighead (1956) found mammals and birds to be important in the diet of the Screech Owl from analysis of regurgitated pellets. Neither VanCamp and Henny (1975) nor Craighead and Craighead (1956) reported utilization of insects during the spring season.

Summer

All four stomachs collected in the summer months contained insects (beetles, grasshoppers, crickets, moths, and wasps). Vertebrate remains (a bird) were present in only one stomach (Table 1). No studies were available which discussed the summer diet of the Screech Owl.

Fall

Mammals and insects appeared to be the most important prey items consumed during the fall from analysis of the stomach contents of 25 Screech Owls (Table 1). Insects were present in 92 percent of the fall stomachs and accounted for 33.9 percent of the total volume. The most commonly occurring insect order, Orthoptera, (mostly Tettigoniidae and Gryllidae) was found in 60 percent of the fall stomachs. Thirty-two percent of the stomachs examined contained mammals (mostly *Peromyscus*), which comprised 24 percent of the total stomach content volume. Also, surprisingly important were spiders, which were identified in 32 percent of the stomachs. Birds, crayfish, millipedes, and centipedes were also encountered, but less frequently.

In Illinois, Cahn and Kemp (1930) collected 143 Screech Owl pellets in the late fall and winter and found that 92 percent of the items identifed were mammals. Wilson (1938) examined 1408 pellets collected in Ann Arbor, Michigan, primarily in the fall and winter. He identified 1,549 skulls primarily of mammals, three-fourths of which were meadow mice (*Microtus* sp.) and deer mice (*Peromyscus* sp.). Birds, crayfish, insects, and fish were present in low numbers.

Winter

Forty-one stomachs examined during the winter months were composed primarily of mammals (60.8 percent) and insects (22.2 percent) by volume. Insects (46.3 percent, predominantly coleopterans of the family Carabidae) ranked first in frequency of occurrence, followed by mammals (36.6 percent), spiders (24.4 percent), crayfish (7.3 percent), and fish (7.3 percent).

A total of 48 food items was cached in 15 nest boxes during the winters of 1977 and 1978 (Table 2). Thirty-five birds of nine species were identified, amounting to 72.9 percent of the total food items cached during the winter. Two permanent residents, the Blue Jay and the Mockingbird (*Mimus polyglottos*), accounted for over half of the avian food items cached. The remaining 27.4 percent was composed of fish (14.6 percent), crayfish (6.2 percent), mammals (4.2 percent), and amphibians (2.1 percent).

Craighead and Craighead (1956) examined Screech Owl pellets collected in the winters of 1942 and 1948 in Superior Township, Michigan. Meadow mice and white-footed mice (95.3 percent in 1942 and 87.2 percent in 1948) comprised the majority of the winter diet. In late winter, the consumption of small birds increased (1.2 percent and 11.4 percent) in both years. VanCamp and Henry (1975) examined 121 food items cached by Screech Owls over a twenty year period during the fall and winter in northern Ohio. They reported that the diet of the Screech Owl consisted of 60.3 percent mammals, 26.4 percent birds, 5.8 percent fish, 5.0 percent frogs, and 2.5 percent crayfish by percent occurrence. Nonmigratory House Sparrows (*Passer domesticus*) and Cardinals comprised over half of the avian food items, while meadow mice and deer mice made up more than half of the mammals eaten.

CONCLUSION

A diversity of food items was consumed by Screech Owls during all seasons. Stomach content analysis showed heavy dependence on insects in spring (83.3 percent occurrence), summer (100 percent), fall (92 percent), and winter (46.3 percent). A total of 347 insects of seven orders was identified. Lepidopterans, orthopterans, and coleopterans were the most common of these. Mammals appeared to be the most important prey items consumed by volume (54.4 percent). Insects occurred more frequently than mammals (67.1 percent compared to 27.6 percent) but occupied only 32.2 percent of the total volume.

The two different methods of studying food habits (stomach analysis and food cached identification) indicated different prey utilization by the Screech Owl. Food caches defined a diet consisting primarily of birds in spring and winter, while the identification of stomach contents detected significant consumption of mammals and insects. The preponderance of birds in food caches was probably due to the fact that " . . . it was impossible for them (Screech Owls, in general) to eat a bird without dropping some of the feathers. . . (Allen 1924: 7)." The absence of invertebrates in food caches was probably due to the fact that invertebrates are usually consumed in their entirety when captured, and are rarely cached as suggested by VanCamp and Henny (1975). However, the scarcity of mammals in the food caches (12.0 percent in spring, 4.2 percent in winter) was possibly due to less need for prey handling compared to birds (no plucking, etc.). Mammals are small enough to be swallowed entire and contain few inedible parts (such as feathers and feet of birds).

In general, researchers (Cahn and Kemp 1930, Craighead and Craighead 1956, and VanCamp and Henny 1975) have underrated the importance of invertebrates in the diet of the Screech Owl because their study methods (pellet analysis and food cached identification) failed to detect the significance of this group of organisms. Admittedly, most food habits studies of the Screech Owl were conducted in northern areas of the United States in predominantly fall and

winter seasons. It is possible that insects were neither as abundant nor as available in these northerly areas. However, Fisher (1893) analyzed 254 Screech Owl stomachs, many of which were collected in northern areas, and consistently found insects in fall and winter birds. In the Great Smoky Mountains National Park, Tennessee, where a comparatively "northerly" climate exists, Stupka (1953) found that 85 percent of the 39 Screech Owl stomachs examined over a 15 year period contained at least one insect. A variety of insects were encountered during the fall and winter months and formed a significant portion of the birds' diet. In Missouri, Korschgen and Stewart (1972) examined 419 pellets during a 6 year period (no reference to season was given) and found that mammals comprised 95 percent of the total food content volume. The only insect mentioned in this study, the June bug, was recorded in .2 percent of the 419 pellets. In summary, researchers should be aware of the inadequacies associated with each study method to avoid misrepresentation of the diet of the Screech Owl.

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OCCURRENCE OF LAKE STURGEON, *ACIPENSER FULVESCENS*, IN THE CUMBERLAND RIVER OF TENNESSEE

Craig N. Harned and Peter A. Hackney
Tennessee Valley Authority
Norris, Tennessee 37828

Abstract

The lake sturgeon, *Acipenser fulvescens,* once plentiful in the Tennessee and Cumberland River systems of Tennessee is now rare and endangered. The scarcity of reports or collections of lake sturgeon in these river systems, since the advent of reservoir formation, makes the recent capture of two specimens noteworthy.

Introduction

The last previously known occurrence of lake sturgeon, *Acipenser fulvescens,* in the Cumberland River of Tennessee and Kentucky was recorded by the Tennessee Wildlife Resources Agency in a fish kill investigation (Tennessee Game and Fish Commission, unpublished data) at Cumberland River Mile (CuRM) 188.3 on 2 August 1968. This species was once abundant in the Tennessee River (Tennessee Valley Authority 1957) and Cumberland River (R.M. Little of Little Fish and Oyster Company, Nashville, Tennessee, personal communication). Despite this reported former abundance, other records of lake sturgeon in the Cumberland River system are unknown to us. The rarity and concern for this species in Tennessee is indicated by a status of endangered on the Tennessee Wildlife Resources Agency's list of endangered and threatened fishes, effective 12 August 1975.

Results

During a study of paddlefish, *Polyodon spathula* (Walbaum), in Old Hickory Reservoir, Tennessee (Pasch et. al. 1978), two lake sturgeon were captured below Cordell Hull Dam. Both fish were netted in a 12.7 cm (five inch) mesh gill net set on the river bottom in water approximately 20 feet deep. The first specimen, taken on 24 May 1977 immediately below the dam (CuRM 313.5), appeared to be in good physical condition. Sex and age were not determined. Total length was 145 cm, and weight, which could only be approximated due to a 22 kg limit of available scales, was about 23 kg. A scar tissue mass was present on the right anterior of the snout. The fish was released unharmed; this deformity should permit future identification if this individual is recaptured. A second specimen was captured on 12 April 1978, at CuRM 311.5. Its large size prevented boating. Total length and weight were estimated at 2 m and 50 kg, respectively.

Origin of these lake sturgeon is unknown. R. M. Little (personal communication) stated that Cumberland River lake sturgeon were occasionally marketed in his fish house prior to impoundment with few seen thereafter. Pflieger (1975) noted a reduction of elimination of populations in Missouri following the construction of dams. Whether these individuals were

present in the Cumberland River prior to impoundment of Old Hickory Reservoir by the Corps of Engineers in 1957 or migrated into this lake through navigation locks, possibly from the Ohio River, is unknown. Small individuals have not been collected in recent years, and reproduction may be inadequate or absent.

This "rediscovery" of a species thought to be extirpated has particular significance in this case because of its endangered status in Tennessee. It is probable that the Cumberland River population is the last stock of lake sturgeon in Tennessee, except for the Mississippi River. Future developmental projects on the Cumberland River will need to address the presence of this fish and any potential for adverse impacts.

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THE EFFECT OF TEMPERATURE AND SUBSTRATE ON GROWTH AND SPORULATION OF *CERCOSPORA ZEAE-MAYDIS* ON AGAR MEDIA.

F. TUPPER GARDEN AND J. W. HILTY
University of Tennessee
Knoxville, Tennessee USA 37919

ABSTRACT

Potato dextrose agar proved best of five media tested for growth and sporulation of a *Cercospora zeae-maydis* isolate. Optimum sporulation in total darkness occurred at 28 C, and conidial lengths varied with temperature. Conidia in culture were longest when produced at 16 C. Conidia from naturally infected leaf tissue generally were longer than those produced in culture, reaching lengths of 155 μm. Conidiophores had as many as six geniscars, and conidia were observed with up to 13 septa.

Artificial inoculation of maize with *C. zeae-maydis* resulted in infection when conidial suspensions were atomized onto leaf surfaces and when fresh, ground diseased leaf material was dusted into whorls under conditions of high humidity.

INTRODUCTION

Cercospora zeae-maydis (Tehon and Daniels) causes gray leaf spot, a foliar disease of corn (*Zea-maydis L.*). Since first described in 1925, (Tehon and Daniels, 1925) the disease has been mentioned only briefly in the literature, or primarily has been regarded as a disease of senescent corn and of little economic value (Hyre, 1943; Miller and Wood, 1947; Roane, 1950). The disease has increased in severity and distribution in the Southeast since 1970, a condition that has been linked to increased adoption of minimum-tillage corn production (Roane, *et. al.*, 1974).

No information has been published on growth and sporulation of *C. zeae-maydis* in culture, or on procedures for artificial inoculation of plants with the fungus. However, references to cultural and inoculation studies with other *Cercospora* species abound. Isolation methods for *Cercospora* spp. (Berger and Hanson, 1963; Goode and Brown, 1970; Marlatt, 1970; Nagel, 1934; Nagel, 1938) and techniques for increasing and transferring *Cercospora* inoculum (Berger *et al.*, Calpouzos *et al.*, Goode *et al.*, 1970; Jones, 1958; Marlatt, 1970; Marlatt, 1972; Ruppel, 1972; Stavely *et al.*, 1969) have been reported.

The objectives of this study were to (1) develop techniques for isolation and culture, (2) determine effects of temperature and substrate on growth and sporulation and (3) achieve artificial inoculation of corn with *C. zeae-maydis.*

MATERIALS AND METHODS

Isolation of the fungus from fresh, diseased corn leaf material was accomplished as suggested by Latterell (1977). Leaves with gray leaf spot lesions were placed in moist chambers at room temperature overnight to induce sporulation. A 2 mm^2 sliver of sterile agar was cut with a flattened dissecting needle. Under a dissecting microscope, the agar sliver was brought into contact with the conidia without touching the leaf surface. The sliver with conidia was transferred to a petri dish containing V-8 juice agar. Individual colonies developed and variation in radial growth was observed. The most actively growing and sporulating isolate was selected for laboratory and greenhouse experiments.

All inoculum for cultural experiments was prepared by transferring conidia from stock tubes containing the fungus to V-8 juice agar plates with a sterile cotton swab. The swab was streaked across the agar surface to evenly distribute conidia. Uniform growth occurred over the entire surface within 7 days. Cultures were used as a source of conidia two weeks after inoculation of plates.

Growth on agar media. In a preliminary test with corn leaf decoction agar, a temperature of 25 $\pm$ 2 C was determined best for radial growth of *C. zeae-maydis*, and this temperature was employed in determining radial growth on various agar media.

Radial growth of *C. zeae-maydis* was observed on five agar media: potato dextrose (PDA), Czapek-Dox (CDA), V-8 juice (VJA), corn meal (CMA), and corn leaf decoction agar (CLDA). PDA and CDA were prepared as described in Tuite's manual (1969). CLDA was prepared by modifying Kilpatrick's carrot leaf decoction agar recipe with 300 g corn leaves instead of carrot leaves (Kilpatrick and Johnson, 1956; Tuite, 1969). CMA was prepared as described by Johnson and Curl (1972). VJA was prepared as described by Stevens (1974).

A 7-mm-diam. cork borer was used to cut discs from 2 week-old *C. zeae-maydis* cultures for transfer to the center of 9-cm-diam. petri dishes containing 15 ml agar medium. Five repli-

cations of each medium were used. The plates were kept in complete darkness, and two perpendicular colony diameters were averaged for each plate after 14 days.

Sporulation on agar media. The same media were used to observe relative sporulation at 25 C in total darkness. A conidial suspension was prepared by flooding a 14-day-old culture with 10 ml of a 5% sucrose solution containing 0.2% Tween 20 (polyethylenesorbitan monolaurate), and scraping the colony surface with a rubber policeman. The conidial suspension was filtered through cheesecloth and diluted to 100 ml with sterile distilled water. Conidial concentration was determined with the aid of a hemacytometer. One-half ml of suspension was transferred to each plate of test medium. In the first trial this was approximately 4 x 10^5 conidia. In the second trial approximately 2 x 10^5 conidia were transferred to each plate. Conidia were distributed over the agar surface with a sterile cotton swab and incubated 14 days. Conidia were suspended by flooding each plate with 10 ml of sterile H_2O, and dislodging spores with a rubber policeman. Conidial suspensions were filtered through cheesecloth, diluted 1:1000 and counted with the aid of a hemacytometer. Four counts per plate were averaged to determine sporulation for each plate. Conidial concentrations for five replicate plates of each medium were averaged.

Effect of temperature or sporulation. The effect of temperature on production and length of conidia in culture was evaluated at 12, 16, 20, 24, 28 and 32 C on PDA. Five replicates were observed at each temperature. Inoculum was prepared as in the previous experiment, and 0.4 ml (approximately 10^3 conidia) was transferred to each plate. Sterile cotton swabs were used to distribute the conidia over the agar. Cultures were placed in incubators adjusted to the six temperatures and kept in constant darkness. Conidial counts were conducted after 7 days (trial 1) and 9 days (trial 2) as previously described. An average of the counts for each of the five replications at each temperature represented relative sporulation at those temperatures.

Effect of temperature on conidial length. Lengths of conidia produced at each temperature were measured using an ocular scale calibrated with a stage micrometer. Fifty conidia produced at each temperature were selected at random and measured at 400X. Length measurements of conidia from corn leaves naturally infected with *C. zeae-maydis* also were made. Seventy-five of these conidia were randomly selected and measured.

A completely randomized design was used to analyze data from the growth, sporulation, and conidial length tests. All data were analyzed at the 5% level of significance and Duncan's new multiple range test was used for mean separation.

Artificial inoculation. Six methods of artificial inoculation of corn with *C. zeae-maydis* were tested in the greenhouse. Corn was planted in 60, 15-cm-diam. pots. Plants were inoculated by: (1) planting seeed in the soil from a field with a history of heavy gray leaf spot disease; (2) planting seed in steam-sterilized soil amended with 15 g of diseased leaf material; (3) planting seed in steam-sterilized soil amended with 25 g of diseased leaf material; (4) planting seed in steam sterilized soil amended with 50 g of diseased leaf material; (5) dusting seedlings with milled, fresh, diseased leaf material; and (6) atomizing a conidial suspension of *C. zea-maydis* onto seedlings. All plants were enclosed in wire-supported transparent bags to maintain high humidity. Plants were placed on a greenhouse bench in a randomized block design with 10 replications per treatment. Seedlings were assessed for gray leaf spot symptoms 25 days after planting, and fungi were isolated from lesions and identified.

TABLE 1. *Effect of selected media on radial growth of single spore isolates Cercospora zeae-maydis at 24 C.*

Medium	Mean radial growth	Radial growth range
CMA	32a[2]	30-34
PDA	31a	28-36
CLDA	23b	22-25
VJA	22bc	20-25
CDA	22c	20-24

[1] Corn meal agar (CMA), potato dextrose agar (PDA), corn leaf decoction agar (CLDA), V-8 juice agar (VJA), Czapek-Dox agar (CDA).

[2] Values are means of five replications; means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

RESULTS

Radial growth on agar media. The fungus grew on all media tested with the greatest radial growth occurring on CMA and PDA (Table 1). The least radial growth occurred on CDA and VJA. Type of medium affected color topography, and general appearance of the colonies. In general, colonies were slightly raised, feltlike, had no radial folds, and were olive gray to gray. Colony diameters ranged from 22mm to 36 mm. Mycelial variants were observed on CMA and PDA. The variants were white, sterile, and transfers of the variants resulted in sterile cononies. Transfer of wild type cultures every 2 weeks prevented contamination of cultures with the sterile variant.

TABLE 2. *Effect of selected media on sporulation of a single spore isolate of Cercospora zeae-maydis at 24 C.*

Medium [1]	Mean Sporulation (conidia/ml)
PDA	2.27 x 10^6a[2]
VJA	2.02 x 10^6b
CLDA	6.30 x 10^5c
CMA	5.28 x 10^5c
CDA	3.00 x 10^5d

[1] Potato dextrose agar (PDA), V-8 juice agar (VJA), corn leaf decoction agar (CLDA), corn meal agar (CMA), Czapek-Dox agar (CDA).

[2] Values are means of five replications; means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Sporulation on agar media. The fungus sporulated on all substrates, but spore production varied significantly among substrates (Table 2). Most abundant sporulation occurred on PDA. Fewest conidia were produced on CDA. Conidial counts ranged from 3.0 x

TABLE 3. *Effect of temperature on sporulation of a single spore isolate of Cercospora zeae-maydis on potato dextrose agar.*

Sporulation (conidia/ml)			
Trial I		Trial II	
Temperature (°C)	Mean	Temperature (°C)	Mean
28	5.37 x 10^5a[1]	28	7.45 x 10^5a
24	3.59 x 10^5b	24	4.95 x 10^5b
20	1.45 x 10^5c	20	2.46 x 10^5c
16	1.22 x 10^4d	16	1.20 x 10^4d
12	3.40 x 10^3d	12	4.00 x 10^3d
32	—	32	—

[1] Values are means of five replications; means followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

10^5 conidia/ml to 2.27 x 10^6 conidia/ml. Conidia were formed uniformly across the surface of colonies on PDA and VJA. CLDA and CMA, but some conidiophores arose from older mycelia. CDA colonies produced spores almost exclusively around the periphery of colonies.

Effect of temperature on sporulation. Significant differences in sporulation due to temperature occurred in two trials (Table 3). Sporulation was greatest at 28 C. No growth or sporulation occurred at 32 C, and very little sporulation was observed at 16 or 12 C. Conidial counts from cultures grown at different temperatures ranged from 3.4 v -10^3 conidia/ml to 5.37 x 10^5 conidia/ml.

Effect of temperature on conidial lengths. Lengths of conidia produced on PDA varied significantly with temperature (Table 4). Conidial lengths ranged from 7.5 μm to 135 μm and averaged 30 μm. Conidia formed at 16 C were significantly longer than those formed at other temperatures. Shortest conidia were produced at 23 C, the temperature of greatest sporulation.

TABLE 4. *Effect of temperature on length of Cercospora zeae-maydis conidia grown on potato dextrose agar compared with conidia produced or naturally-infected corn leaves.*

Source	Mean conidial length (μm)	Conidial length range (μm)
Naturally Infected	75.8a[1]	44 -155
16 C	36.6b	10 - 85
20 C	33.6c	15 - 93
24 C	34.2c	12.5-135
12 C	23.9d	10 - 85
28 C	22.6d	7.5- 45

[1] Values for conidia from naturally infected leaves and for conidia produced at different temperatures based on 75 and 50 measurements, respectively. Means followed by the same letter are not significantly different according to the Duncan's multiple range test (P=0.05).

Conidia from naturally infected corn leaves were significantly longer than those produced in culture. Lengths of these conidia ranged from 45 μm to 155 μm with a mean of 76 μm (Table 4).

Artificial inoculation. Dusting corn seedling whorls with milled diseased corn tissue, and atomizing a conidial suspension onto the foliage resulted in infection with *C. zeae-maydis*. The pathogen was isolated from lesions that developed in both cases. Only an unidentified *Helminthosporium* species was isolated from lesions that developed from the other inoculation treatments.

DISCUSSION

Mass conidial transfers over agar surfaces often have been necessary to achieve maximum growth and sporulation in culture (Berger and Hanson, 1963; Calpouzos and Stallknecht, 1966; Stavely and Nimmo, 1969), and periodic transfers of cultures often are necessary to maintain sporulating colonies (Goode and Brown, 1970; Nagel, 1938).

Diachun and Valleau (1941) reported significant differences in sporulation, growth, and pathogenicity among isolates of *C. nicotianae* Ell. and Ev. Other workers reported similar variations within other species of *Cercospora* (Jones, 1958; Kilpatrick and Johnson, 1956; Miller, 1949; Ruppel, 1972).

Growth and sporulation of *Cercospora* spp. are influenced by mutations and environmental factors, and effects of various media on growth and sporulation have been reported (Berger and Hanson, 1963; Kilpatrick and Johnson, 1956; Marlattt, 1970; Nagel, 1934; Ruppel, 1972). Temperature effects on growth ond sporulation have been reported for *C. zebrina* (Berger and Hanson, 1963), *C. nicotianae* (Stavely and Nimmo, 1969), and an unnamed *Cercospora* species pathogenic to *Fiscus elastica* (Marlatt, 1970). The influence of temperature on conidial germination in *Cercospora* spp. also has been investigated (Berger and Hanson, 1963; Judd and Peterson, 1972; Oso, 1972).

In our studies, temperature and substrate significantly influenced growth and sporulation of *C. zeae-maydis* in culture. The best general agar medium investigated for growth and sporulation of the fungus was PDA. PDA and VJA were good media for sporulation, and should be suitable for maintaining cultures and producing inoculum. Cultures on fresh plates of these media yielded abundant conidia when large numbers of conidia were initially distributed over the agar surfaces and allowed to germinate and grow for 7-10 days. Sporulation in darkness was greatest at 28 C, and longest conidia were formed at 16 C. Conidial length is a secondary taxonomic character of *Cercospora* species, although the validity of this character has been questioned (Johnson and Valleau, 1949; Welles, 1924). The range in conidial length observed in this study further indicated the difficulties of using this character.

C. zeae-maydis conidia collected from infected corn in Tennessee measured up to 155 μm, which is much longer than previously reported (Chupp, 1957; Kingsland, 1963; Tehon and Daniels, 1925). Also, conidia were observed with up to 13 septa, and with as many as six geniscars on a single conidiophore. The isolate observed in this study clearly differed from the original description of *C. zeae-maydis* by Tehon and Daniels (1925), and the subsequent description by Chupp (1953) and Kingsland (1963). These previous descriptions indicate a mean length of 70.3 μm, with up to 10 septa.

Successful artificial inoculation of plants with *Cercospora* spp. has been reported on a number of hosts, employing a variety of inoculation techniques (Georgopoulos and Dovas, 1973; Judd and Peterson, 1972; Marlattt, 1972; McKay and Pool, 1918; Miller, 1949; Ruppel, 1972). In all cases, high relative humidity (90-95%), and temperatures of 20-30 C were necessary for infection. Excellent infection was obtained in this study by atomizing a suspension of virulent conidia onto corn seedlings. Isolates varied in pathogenicity and growth rates. Fresh diseased material must be used when inoculating plants with milled *Cerco-*

spora-infected leaves. Numerous trials with 4-8 month-old disease material yielded no infection by *C. zeae-maydis*.

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AMINO ACIDS IN NON-DIAPAUSING PUPAE OF *HELIOTHIS ZEA* (LEPIDOPTERA: NOCTUIDAE)

Baldev S. Mangat
Alabama A. and M. University
Normal, Alabama 35762

Abstract

Seventeen amino acids were positively identified in hydrolysates of non-diapausing pupae of *Heliothis zea* (Boddie); namely, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phyenylalanine, proline, serine, threonine, tyrosine and valine. No values for tryptophan are reported because it is destroyed during acid hydrolysis.

Introduction

Protein is a major constituent of every living organism. Proteins are composed of smaller units arranged like beads on a chain. In proteins, however, the beads are amino acids, which are composed of carbon, hydrogen, oxygen, nitrogen and sometimes sulfur. Proteins play many important roles: as enzymes, as structural components of the cells, and as regulators of a variety of body functions. Enzymes speed up otherwise sluggish chemical reactions in the body. Protein is considered to be of high quality if it contains those indispensable or essential amino acids needed to maintain health. Protein malnutrition exerts its most devastating effects on the young. It is the most common disorder affecting small children in underdeveloped countries.

Nutritionists agree that protein might become a major limiting factor in our ability to feed a projected population of seven billion by the year 2000. If American agriculture will in fact be called upon to meet

at our long-term options. The set of interlocking constraints centered on resources and environment does not look promising. The present study was undertaken to determine the nutritional quality, i.e., kind and amount of amino acids of non-diapausing pupae of *H. zea*. Any public suggestion at this time that insects be used directly as food for human beings would be met with antagonism. The main immediate potential of insects therefore appears to be in their recycling of waste materials into proteinrich feeds for such animals as poultry, fish and livestock.

MATERIALS AND METHODS

The study was carried out in the biology laboratory at Alabama A & M University during 1972-73. *Heliothis zea* (Boddie) used in this study were from a restricted natural population occurring in the Tennessee Valley area. Moths were attracted from the area with a fluorescent black light (3600°A) trap and deflected into a cage during August 1972. Ten field-collected moths were confined in an oviposition cage. The oviposition cage(28x28-38cm) was made of steel and had metallic screens on all sides except the bottom and the door. A muslin sleeve was attached to the hinged door that allowed access to the interior. A strip of muslin was suspended through the middle top part of the cage to provide a resting place for moths and a surface for oviposition.

TABLE 1. *Composition and Nutrient Analyses of Modified Wheat Germ Synthetic Diet Used in Present Study to Rear H. zea Larvae (Vanderzant et. al. 1962).*

Component	Amount	Digestible Energy (cal/g)	Protein (%)	Fat (%)	Fiber (%)
Whole wheat germ	24.0 g	4712.4	29.1	12.1	3.3
Casein, Vitamin Free	28.0 g	3830.5	9.0	0.9	0.2
Sucrose	28.0 g	4361.2	0.0	0.0	0.0
Agar (Bacte)	20.0 g	0.0	0.0	0.0	0.0
Salt Mixture (Wesson's)	8.0 g	0.0	0.0	0.0	0.0
Sorbic acid	1.6 g				
Methyl parahydroxy-benzoate	1.6 g				
Potassium Hydroxide (10%)	16.0 ml				
Ethyl alcohol (95%)	15.0 ml				
Vitamin Solution	26.4 ml				
Water	650.0 ml				

The cage with moths was held in a constant temperature incubator, maintained at 24±1°C, and 85-90% relative humidity. High temperature and humidity are essential for egg laying. Two 14-Wattt Fluorescent day-light lamps were clock-controlled inside the incubator to provide a photoperiod of 14 hours of light and 10 hours of darkness.

Adults were fed 10% sugar solution and eggs were obtained from moths by exposing the muslin cloth for 24 hours. Eggs were surface sterilized in a freshly prepared 0.15% sodium hypochlorite solution. These eggs were then washed with sterile distilled water, and then transferred to a sterile test tube. The newly emerged larvae were reared on synthetic wheat germ diet, a slight modification of Vanderzant et. al. (1962). The ingredients and nutrient analyses of this diet are characterized in Table 1.

The vitamin mixture used in this diet is a modification of Vanderzant's (1959) B-vitamin mixture and is listed in Table 2. A plug of diet about 2.5cm long and 2.0cm diameter was cut and transferred to 95x25cm cotton-plugged sterile shell vials. One newly hatched larva was put in each rearing vial with the help of a fine camel's hair brush. The brush was sterilized by dipping in 70% alcohol before making each transfer. The vial was plugged and exposed to a constant-temperature cabinet operating at 24±1°C within 16 hours light and eight hours darkness.

TABLE 2. *The Ingredients Required to Make the Vitamin* Fortification Used for The H. zea (Boddie) Diet.

Ingredient	Weight (Gram)
Niacinamide	1.00
Calcium Pantothenate	1.00
Thiamine hydrochloride	0.25
Riboflavin	0.50
Pyridoxin hydrochloride	0.25
Vitamin B_{12} in Mannitol	2.00
Inositol	20.00
Folic Acid	0.25
Biotin	0.02
Choline Chloride	50.00
Alpha Tocopherol Powder	8.00
Ascorbic acid	270.00
Water (Make up to 1,000 ml in a Volumetric Flask)	

Diapause in this species was not induced when the larvae and the pupae were held at this condition (Mangat and Apple, 1966). These larvae were examined frequently and were transferred to fresh food when necessary. Five days after pupation, pupae were checked for non-diapause. The retention of the larvae eye spots or stemmata was found to be a valid characteristic of diapausing pupae of this species (Phillips and Newsom, 1966).

Amino acid determinations were made in triplicate of non-diapausing pupae. For each sample, 60 pupae were taken and dried in an oven operating at 80°C for 48 hours. One dried pupae were homogenized with a mortar and pestle. Each sample was hydrolyzed with 2 ml of 6N Hydrochloric Acid under nitrogen atmosphere at approximately 110°C for 24 hours. The hydrolysates were diluted at approximately 110°C for 24 hours. The hydrolysates were diluted with deionized water, filtered, and an aliquot was dried in a vacuum rotary evaporator. The dried sample was dissolved in an appropriate volume of 0.2N Sodium Citrate, pH 2.2. Twenty-five micrograms of protein hydrolysate were used for analysis in a Durrum D-500 Amino Acid Analyzer. This allowed the quantitative determination of 17 amino acids. Bcause tryptophan is destroyed during acid hydrolysis, no values for tryptophan are reported.

TABLE 3. *Amino Acid Composition of Dried Ground Non-diapausing Pupae of H. zea Raised on Synthetic Diet Containing Vitamins.*

Amino Acids	Dry Weight (Mg/Gram)
Alanine	29.8
Arginine	29.8
Aspartic Acid	41.6
Cysteine	2.7
Glutamic Acid	52.7
Glycine	25.8
Histidine	15.6
Isoleucine	19.4
Leucine	33.3
Lysine	29.7
Methionine	10.3
Phenylalanine	22.3
Proline	27.5
Serine	19.3
Threonine	20.2
Tyrosine	26.8
Valine	27.0

Results

The amino acid composition of non-diapausing pupae of *H. zea* is presented in Table 3 and is expressed in mg/gram dry weight. Positive identifications were made of alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine in non-diapausing pupae of this species. The amino acids present in highest quantitative concentrations were glutamic acid, aspartic acid and leucine, respectively. Cysteine was present but its concentration was very low.

Higher vertebrates are unable to synthesize ten of the amino acids and these are termed the essential amino acids; namely, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine (Young 1976). With the exception of trytophane all the essestial amino acids are present in *H. zea* pupae. Calvert et. al. (1969) reported from dried ground housefly pupae amino acids, namely, alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine.

Preliminary studies conducted by Calvert et. al. (1969) demonstrated that dried housefly pupae meal provided enough protein of sufficient quality to support normal growth of chicks during the first two weeks of life. Dried grasshopper meal on the other hand proved unsatisfactory for rapid growth of weanling rats in a ten day feeding trial as a result of insufficient methionine content or low digestibility of protein in grasshopper meal (Ueckert, Yang and Albin, 1972).

For optimum growth and development, the essential amino acid requirements for poultry are quite critical. The National Academy of Sciences (National Research Council) revises the estimates for the amino acid requirements as more research data becomes available. Their recent (1972) requirements of amino acids for 0-6 weeks old poultry chickens are arginine, histidine, isoleucine, leucine, methionine, phyenylaline, threonine, tryptophan, valine, lysine, glysine and serine. Loooking at the amino acids present in non-diapause *H. zea pupae* meal and comparing it with the established amino acid requirements for poultry by the National Research Council it is quite clear that this insect meal contains all the amino acids required for the growth and development of poultry with the exception of tryptophan which is destroyed during acid hydrolysis.

No feeding trials were conducted with *H. zea* meal. However, future studies are planned in which this meal will be fed to young poultry, rats and other monogastric animals without modification. The objective of these tests will be to see if this meal is of sufficient nutritional quality to support typical growth and development when compared to control groups. This work was supported in part by a research grant from the U.S. Department of Agriculture under CSRS Grant No. 216-15-02.

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