THE ASSOCIATION OF *NEMATOSPORA CORYLI* PEGLION, THE CAUSATIVE ORGANISM OF YEAST-SPOT DISEASE OF SOYBEANS, AND THE GREEN STINK BUG, *ACROSTERNUM HILARE* (SAY): (HEMIPTERA: PENTATOMIDAE)

by

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INTRODUCTION

The Green Stink Bug

The green stink bug, Acrosternum hilare (Say), was described in 1831 and placed in the genus Pentatoma (Van Duzee, 1917). It has also been listed under the genus Nezara. Its distribution is widespread, ranging from eastern Canada and New England, west to the Pacific Coast and south to Florida, Texas, and Arizona (Underhill, 1934).

The insect overwinters as an adult which becomes active in the spring and lays its eggs on wild hosts. The eggs hatch and there are 5 nymphal instars which complete their development on wild hosts. New adults from the first generation migrate to near by cultivated crops and a second generation occurs (Plate I).

Studies in Ohio indicated there was one brood per year, with an egg laying period of eight to ten months for each generation (Whitmarch, 1917). In Virgina, a similar life history study also indicated there was one generation per year (Underhill, 1934). Other workers have found that A. hilare has two generations per year, one on wild hosts and one on cultivated crops such as soybeans (Miner, 1966).

The importance of wild hosts to A. hilare was noted by Schoene and Underhill (1933). They found that a succession of wild hosts appeared to be necessary for the insect to maintain itself in large numbers. Their list of preferred hosts in order of preference were: mulberry, blackberry, black locust, elder,

EXPLANATION OF PLATE I

Adult *Acrosternum hilare* (Say) and its nymphal instars. (From left to right--adult, fifth, fourth, third, second and first instars.)

PLATE I

honeylocust, linden, mimosa, redbud, dogwood, boxelder and holly. Injury to adjacent crops occurs when wild hosts mature or fail to supply a good source of food to the insect.

In addition to soybeans, A. hilare has been reported as a pest of cotton (Butler, 1960), lima beans (Wingard, 1922), pecans (Hill, 1938) and peaches (Rings, 1957).

Many reports of damage to farm crops have been made. However, serious damage is usually confined to isolated areas (Underhill, 1934). Miner (1966) noted that once an infestation occurs in an area or field, it could be expected to occur to some degree year after year, provided that no major environmental change such as a lack of a cultivated crop or removal of wild hosts takes place.

Damage to soybeans is caused by the puncturing of the bean by the insect's stylets, the injecting of a histolytic substance and the sucking up of the liquified plant tissue. Damaged soybeans have a chalky appearance and brown spots. The chalky appearance is due to air spaces left in the bean when the cell contents have been withdrawn by the insect (Miner, 1966).

Stink bug damaged soybeans have shown a decrease in oil content and an increase in the percent of protein when compared with undamaged ones (Miner, 1966).

Daugherty (1964) showed that large numbers of stink bugs on soybean plants delays maturity. There is also a reduction in the germination percentage when the seeds have been punctured.

Nematospora coryli

Nematospora coryli Peglion, the causative organism of yeast-spot disease of soybeans, is an ascosporogenous yeast, reproducing vegetatively by budding, as well as forming an ascus and ascospores.

Peglion created this genus in 1897 when he isolated the organism from diseased hazelnuts in Italy. Because of the characteristic shape of the spores, he called the genus Nematospora from the word "nema" meaning thread. There is only one species in this genus; N. coryli is the type species and type strain (Lodder, 1952).

The description of N. coryli according to Lodder (1952) is as follows. The shape is variable, round, oval, elongate or irregular. Ascospores are thread like or spindle shaped with one end rounded and the other provided with a non-motile flagellum. (Plate II)

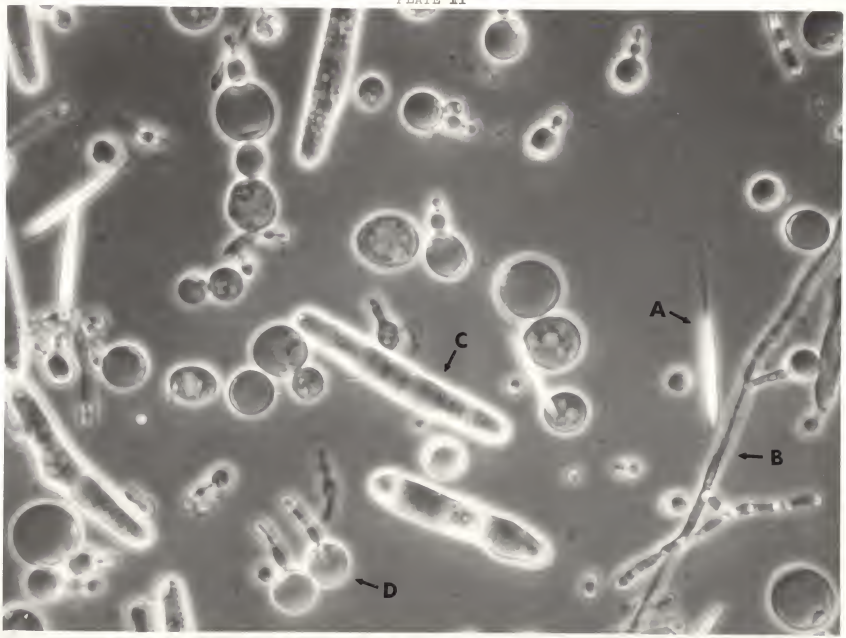
Spores are (2-3)x(38-40) excluding the flagellum and are produced in a cigar shaped ascus (6-8)x(65-70). Eight spores are normally produced in each ascus and are arranged in two groups of four. Vegetative cells are oval and round. A mycelium or pseudomycelium is produced when the yeast is cultured in a liquid medium. The yeast grows very well on solid media and poorly in liquid media.

Several cultures of N. coryli isolated from different hosts have been described as new species. However, they are

EXPLANATION OF PLATE II

Nematospora coryli, causative organism of yeast-spot disease of soybeans. A. Ascospore. B. Mycelium. C. Developing Ascus. D. Germinating vegetative cells.

PLATE II



probably just different strains of N. coryli. These include: N. lycopersici Schneider from tomatos, N. phaseoli Wingard from lima beans, and N. nagpuri Dastur from cotton (Lodder, 1952).

N. coryli has been reported many times in the literature in association with crop injury. It has been isolated from lima beans (Wingard, 1922), pecans (Weber, 1933a), sweet peppers and tomatos (Weber, 1933), pomegranates, tangerines (Fawcett, 1929) oranges, grapefruit (Weber, 1933; Fawcett, 1929), mungbeans (Preston and Ray, 1943) and soybeans (Lehman, 1943; Preston and Ray, 1943).

N. coryli infection in plants has been called by various names depending upon the host. Such common names as yeast-spot disease (soybeans), internal boll disease (cotton), coffee bean rot and kernel-spot (pecans) have been used.

Review of Literature

Ashby (1926) coined the word stigmatomycosis to cover the transmission of fungi to fruits by sucking insects. He defined stigmatomycosis as "a type of affection in which fruits externally sound are found to be internally infected as a result of punctures made by plant-feeding bugs of the suborder Heteroptera." Nematospora infections have a wide distribution including all of the West Indies, China, Japan, Philipines, Cuba, Mexico and the United States.

Nowell (1917) and his associates found that Nematospora infections in cotton bolls were associated with insect punctures of Dysdercus delauneyi, the cotton stainer bug.

Wingard (1925) found Nematospora phaseoli infections in lima beans entirely dependent upon the puncturing of the bean by Nezara hilaris, now Acrosternum hilare. He suggested that A. hilare was a carrier of the infective organism but was not able to isolate N. phaseoli from the insect.

Coffee bean rot in Kenya has been associated with the feeding damage of Antestia lineaticollis Stal. and A. facets Germ. (Carter, 1962).

According to Leach (1940), "the universal association between Nematospora and insects in such widespread regions of the world would suggest that the association is more than a casual one."

Leach and Clulo (1943) were unsuccessful in isolating the yeast from the body of A. hilare. They suggested that the transmission was merely mechanical and that the relationship was not a constant one.

Frazer (1944) did the first extensive study of the association of Nematospora with an insect, working with the cotton stainer bug and internal boll disease of cotton caused by Nematospora gossypii, now in the genus Ashbya. She succeeded in infesting insects by feeding them on Nematospora-infected seeds. Paraffin sections of the insects revealed spores to be present in the alimentary canal, mostly in the midgut. Spores, mycelium and germinating spores were present in the stylet pouches of the insect's head. The fungus was never detected in the living tissue of the host. The organism was lost during

the molting process. Frazer also speculated on how N. gossypii spores were deposited in the stylet pouches and how they could be injected into the plant tissue during the feeding process.

Frazer also successfully infested Dysdercus intermedius with N. coryli by feeding them on infected cotton-seed kernels. She again found germinating spores and mycelial buds in the stylet pouches and also spores in the alimentary canal. She successfully reisolated the yeast from the heads of two fourth instar nymphs.

Daugherty and his associates at the University of Missouri have done extensive work recently on the transmission of N. coryli to soybeans. He was the first researcher (1962) to isolate the yeast from soybeans which were fed on solely by the green stink bug (Daugherty, 1966).

Daugherty (1966) found the following Pentatomidae to be associated with the transmission of the yeast-spot disease organism to soybeans: Thyanta custator (F.), Acrosternum hilare (Say), Euschistus tristigmus (Say), E. servus, E. variolarius and E. euschistoides (Vollenhoven).

Daugherty and Foster (1966) isolated N. coryli from rice damaged by the rice stink bug, Oebalus pugnax (F.).

Foster (1966) did an extensive study of the association between N. coryli and the green stink bug, isolating the yeast organism from surface sterilized A. hilare. This was the first report of the isolation of the yeast from any field collected insect. He found the yeast to be harbored internally in all

three body regions. It was most often associated with the stylets, salivary receptacles, and the hindgut in that order.

Foster isolated the yeast from all instars except the third. He did not isolate the yeast from eggs, exuviae or fecal material of A. hilare. His failure to isolate from the exuviae contradicted the findings of Frazer. In September, at the end of the growing season for soybeans, 54.9% of field collected bugs harbored the yeast.

Objectives

Acreage of soybeans has increased tremendously in Kansas and other areas of the United States in the last decade. This is particularly true in those areas where abundant stink bug populations are present. While the association between N. coryli and A. hilare has been known for many years and demonstrated to be more than cursory, the exact nature of this association was not known. The purpose of this study was to investigate in detail the biological relationship between N. coryli and the green stink bug.

The basic objectives included:

1. Develop an efficient method of infesting A. hilare with N. coryli.
2. Determine how long the adults harbor the yeast.
3. Determine if the organism is lost during molting.
4. Determine the frequency of transmission to soybeans.
5. Determine seasonal history.
6. Determine if the yeast reproduces in the insect vector.
7. Determine if the yeast could be obtained from wild hosts if infested insects fed upon them.

MATERIALS AND METHODS

The Yeast Culture

The original culture of N. coryli was obtained in 1966, from Dr. D. M. Daugherty of the Department of Entomology, University of Missouri. It was cultured on nutrient agar plates (Plate III) prepared by the method to be described and was refrigerated at 6C until used.

The Insect Culture

A laboratory stock culture of A. hilare was started from insects collected in August 1967 from a soybean field in Cherokee County. A. hilare has been reared successfully through more than nine generations by the following method.

Insects were caged (Plate IV) in half-pint ice cream cartons with the lid replaced by saran screen. Food was green beans (Phaseolus vulgaris) purchased from a local supermarket. Distilled water was supplied by means of a small vial closed with a disposable 1½-inch cotton dental roll which served as a wick. A piece of 9-cm filter paper was placed on the floor of the cage. Every two days, fresh filter paper, green beans and distilled water were added. The rearing room was kept at 26C and the relative humidity ranged from 70-80%. Overhead fluorescent lighting provided 16-hour photoperiod.

EXPLANATION OF PLATE III

Nematospora coryli Peglion culture on a nutrient agar plate

PLATE III

EXPLANATION OF PLATE IV

A. hilare in rearing cage

PLATE IV

Soybeans

Clark 63 variety was used for all tests. Soybeans were planted in a greenhouse, 15 pots per planting, at 2-week intervals. The plants were sprayed regularly with Kelthane to control mites but some plants were discarded because of excess mite damage. Plants were kept at 26C and a 16-hour photoperiod in a growth chamber when insects were caged on them. After the insects were removed, the plants were returned to the greenhouse.

Sterilization Techniques

The Insects

After killing an insect in a cyanide bottle, its legs and antennae were cut off. It was placed in a 2.6% sodium hypochlorite (NaOCl) solution for 2 min, transferred with sterile forceps to sterile distilled water, and rinsed for 2 min. The forceps were sterilized by dipping them in 95% ethanol and passing them through a flame to burn off the alcohol. The procedure for mashing the insects varied in each of the experiments and will be discussed later.

Soybeans and Dogwood Berries

Soybeans and dogwood berries were surface sterilized by placing them individually or in small groups in 5.25% NaOCl solution for 1 min, transferring them to sterile distilled water

by means of sterile forceps and rinsing them for 1 min. After rinsing, they were transferred, again with sterile forceps, to filter paper and excess water was removed. They were then placed in individual sterile air tight containers and allowed to dry.

Microbiological Media

Two types of media were used in the isolation of N. coryli; a solid nutrient agar medium and a liguid nutrient broth overlay medium.

The nutrient agar was prepared by adding 8 g of dextrose, 4 g of yeast extract and 18 g of Difco nutrient agar powder to 700 ml of distilled water. The dissolved medium was autoclaved for 25 min at 16 psi of pressure. Immediately after removal from the autoclave, 10 ml of a 1% streptomycin solution was added. A series of petri dishes was then poured and covered. The streptomycin solution was prepared by adding 1 g of streptomycin sulfate base to 100 ml of sterile distilled water. The solution was covered and refrigerated until used. The streptomycin helped to inhibit the growth of some bacteria.

The nutrient broth overlay medium was prepared by adding 5.5 g of dextrose, 2.8 g of yeast extract and 4.4 g of Difco nutrient broth to 500 ml of distilled water. The solution was thoroughly mixed and 5-ml portions were pipetted into test tubes. The test tubes were closed with a cotton plug and autoclaved for 25 min at 16 psi of pressure (Foster, 1966).

Plates were incubated at room temperature (22C to 26C) for 3 to 4 days or until N. coryli was detectable.

Infesting the Insects

A. hilare was infested with N. coryli by allowing the insects to feed on a yeast suspension in sugar water. Five ml of a 10% sugar solution was pipetted into jelly cups. Yeast was added by means of a sterile bacteriological loop. The solution was mixed thoroughly and covered with parafilm stretched tight over the cup to prevent leakage. The insect was placed in a small sterile disposable petri dish covered with saran screen. The jelly cup was inverted and placed (parafilm down) on the saran screen. (Plate V) The cup was pierced with a hot insect pin to prevent CO_2 buildup from the fermenting sugar solution. The cup was removed after 24 hours and new yeast suspension was supplied if the insect had not fed.

It was found that if the parafilm was examined under a binocular microscope, the number of probes the insects had made could be detected. Miles (1959) found that some Hemiptera form a stylet sheath when feeding. A. hilare, when feeding through the parafilm membrane, left what was interpreted as a stylet sheath, on the inside of the parafilm. The criterium used to tell if an insect had fed on the yeast suspension was the finding of at least one stylet sheath, indicating one probe. The total number of probes each insect made was recorded in each of the following experiments.

EXPLANATION OF PLATE V

Method used to infest *A. hilare* with *Nematospora coryli*

PLATE V

Length of N. coryli retention by adult insects fed on yeast suspension

One to two-week old adult stink bugs were fed on the yeast suspension. Infested insects were placed in individual cages and fed on green beans which were changed daily. An insect was removed at 3-day intervals, 3 through 60 days, and isolation of N. coryli was attempted. The experiment was replicated three times.

Insects were surface sterilized by the procedure outlined previously. Each sterilized insect was cut with sterile scissors and divided into its head, thorax and abdomen. Each body region was placed, by means of sterile forceps, into a sterile test tube containing 5 ml of nutrient broth, and mashed with a sterile glass rod. The glass rod was sterilized by dipping it into 95% ethanol and passing it through a flame. The nutrient broth containing the mashed insect was agitated and poured on a nutrient agar plate. Non-infested insects served as checks. They were fed on green beans and removed at 3-day intervals, mashed and plated.

A similar experiment was conducted for 70-, 80-, and 90-day intervals. Five replications of each interval were used because of possible mortality from old age. Checks were treated in the same manner.

Length of N. coryli retention by adult insects fed on inoculated soybeans

To determine if results of retention times in the afore mentioned test were comparable with insects fed on inoculated soybeans, the following experiment was initiated.

Soybeans on the plants were inoculated with N. coryli. A yeast suspension in distilled water was prepared and a drop of suspension was placed on the soybean pods over each bean. Beans within the pods were pierced several times with a minuten insect pin through the drops on both sides of the pods. After 5 days, newly emerged adult stink bugs were caged individually over infected soybeans for 2 days. The cages were transparent plastic boxes 3 x 1¼ x 1¼ inches with 2 windows about 3/4 x 2½ inches covered with saran screen, on each side of the box. The infestation procedure was checked by caging the test insects over healthy soybeans for 2 days to see if they transmitted the yeast organism.

The infested insects were then caged separately and allowed to feed on green beans which were changed daily. Insects were removed from the cages at 15-, 30-, 45-, and 60-day intervals, eight insects at each interval and surface sterilized. The head, thorax and abdomen were then mashed and plated.

The non-infected soybeans were checked under a binocular microscope for feeding damage and were surface sterilized by the procedure described previously. The sterilized soybeans were placed individually in a sterilized mortar and pestle and ground

to powder. Five ml of sterile nutrient broth was added to each soybean and the mixture was plated.

Retention of N. coryli after a molt

To determine if N. coryli is retained by infested insects which have undergone a molt, fifth instar nymphs were fed on the yeast suspension. Insects which had probed several times were caged individually in ice cream cartons with a fresh green bean provided daily. After the insects had molted, the new adults were surface sterilized and their heads were plated separately from their thorax and abdomen. The same procedure was used for non-infested fifth instar nymphs which served as checks. In the case of the checks, the entire insect was plated and not divided.

Isolation of N. coryli from exuviae

To determine if N. coryli was present on insect exuviae, fifth instar nymphs were fed on the yeast suspension. The infested insects were placed individually in cages and fed on green beans which were changed daily.

When the end of their fifth stadium approached, as indicated by the size of the nymph, the insect was surface sterilized in 2.6% NaOCl. Because of the inability of the nymphs to withstand the standard period of time in the NaOCl solution and still live to molt, they were left in the sterilizing agent for only 20 to 30 sec. Nymphs were removed from the solution with

sterile forceps, rinsed in sterile distilled water and placed in individual sterile petri dishes until they molted. The new adults were surface sterilized, divided into head, thorax and abdomen and plated. The exuviae were collected with sterile forceps, placed separately in sterile test tubes containing 5 ml of nutrient broth, agitated and plated. The same procedure was used for non-infested fifth instars serving as checks.

Retention and Transmission of N. coryli by Infested fifth instar nymphs molting to adults

To determine if infested fifth instars are able to transmit the yeast after they had molted to adults, newly molted fifth instars were fed on the yeast suspension. The infested nymphs were caged individually on soybean pods on plants in a growth chamber. The insects were placed on a fresh soybean pod each day throughout their fifth stadium and for 5 to 6 days after they had molted to adults. The new adults were then surface sterilized, divided into head, thorax and abdomen and plated.

Each soybean pod was labeled and allowed to mature for several weeks. The soybeans were then harvested, removed from their pods and checked under a binocular microscope for feeding damage. The soybeans from each day's feeding were surface sterilized, ground to powder with a sterile mortar and pestle and plated in nutrient broth.

Isolation of N. coryli from fecal material

Isolation of N. coryli was attempted from fecal material by allowing adult green stink bugs to feed on a yeast suspension. The infested live insects were surface sterilized in NaOCl solution for 2 min and rinsed for 2 min in sterile distilled water. The surface sterilized insects were placed in sterile petri dishes and provided with a green bean which had been washed in distilled water. Each day, any fecal material deposited in the dishes was collected by means of a sterile bacteriological loop and smeared on agar plates. Five ml of sterile nutrient broth overlay medium was added to each plate. The insects were then resterilized for 1 min and placed in new sterile petri dishes and provided with a new freshly washed green bean. The same procedure was used for non-infested checks.

Isolation of N. coryli from salivary secretions.

Adult insects were infested by feeding on a yeast suspension and caged individually in ice cream cartons with a fresh green bean provided daily. After 7 days, the insects were surface sterilized while alive and their legs were cut off. It was found that if the antennae were removed the insects started to salivate freely. A salivating bug was held by forceps over a nutrient agar plate and the tip of its proboscis was drawn forward by means of a sterile probe and touched to the agar. Two to three ml of nutrient broth overlay medium was then added to the plate. Non-infested checks were treated in the same manner.

Transmission of N. coryli from insect to insect.

Two possible means by which non-infested adults could become infested in the field were investigated.

Because dogwood (Cornus drummondi Meyer) is one of the main wild hosts of A. hilare in Kansas, it was investigated as a possible site of adult infestation. A circular cage of fine wire screen 12 inches in length and 3¼ inches in diameter was used to confine ten infested adult bugs, infested by feeding on the yeast suspension, over a clump of 15 dogwood berries in the field. One end of the cage was provided with a cloth sleeve and the other was closed with saran screen. After 7 days, the insects were removed and 10 non-infested adults were caged over the same clump of berries. Seven days later, the surviving non-infested bugs were surface sterilized and their heads were mashed and plated.

In another test, 5 infested adults were caged singly over small clumps of 3 to 5 berries. After 7 days, 5 non-infested adults were caged over the same clumps. The 5 non-infested insects were removed after a week, surface sterilized, and their heads were mashed and plated.

Five non-infested adults were caged singly over small groups of berries and served as checks. They were also caged for 7 days and their heads were plated.

The dogwood berries from these two experiments were surface sterilized, mashed in sterile mortars and pestles and plated in nutrient broth.

Soybeans also were investigated as a possible site of adult infestation in the field. Adult stink bugs were infested with N. coryli by feeding on the yeast suspension. In the field, each infested adult was caged singly over a soybean pod for 2 days. Immediately after their removal from the plants, non-infested adults were caged over the same pods for 2 days. They were removed, surface sterilized and their heads were mashed and planted.

Concentration and Reproduction of N. coryli in the head of adult A. hilare

To determine if there is a difference in the yeast concentration in the heads of adults as related to the number of probes made and if N. coryli reproduces in the heads of the adults, adults were fed on the yeast suspension. The insects were grouped as to the number of probes each had made in the membrane, 1, 2, 3, 4, 5, 6 and 7 or more. Individuals from each group were surface sterilized and their heads were mashed in 5 ml of sterilized nutrient broth. Each test tube containing a mascerated head was aggitated and a 0.1 ml sample was pipetted from the homogeneous solution with a sterile pipette. The sample was deposited and uniformly smeared with a sterile bacteriological loop over a nutrient agar plate. The plates were incubated at room temperature and the number of colonies which started to grow were counted and recorded.

Several of the 1 probe group were separated and caged individually with a fresh green bean provided daily. After

10 days, these insects were surface sterilized and plated in the same manner.

Frequency of transmission of N. coryli by adults

To determine how often infested adults transmit the yeast organism to soybeans, twenty newly molted adults were infested by feeding on the yeast suspension. They were caged individually over soybean pods in a growth chamber. The plants were approximately 3 months old and pods were ¼ developed. The growth chamber was set at 26°C and a 16-hour photoperiod. The insects were caged over a new pod each day for 25 days. At the end of 25 days, the insects were surface sterilized and isolation of N. coryli was attempted.

Each soybean pod was labeled and the plants were moved to a portable greenhouse where they were allowed to mature for several weeks. The soybeans were then harvested, checked for feeding damage, surface sterilized, ground to powder in a sterile mortar and pestle and plated in nutrient broth.

The procedure was the same for 3 non-infested insects which served as checks.

Survey of Field Population

This study was designed to find what percent of field collected A. hilare are infested with N. coryli in Kansas. Three soybean fields, approximately three miles apart, located in Cherokee County near Columbus, Kansas were chosen for this study.

Adult green stink bugs were collected at 2 to 3 week intervals from late June through September. Ten adults were collected from each field, if possible, or as many as could be found during each sampling. The insects were surface sterilized and their heads were mashed and plated.

RESULTS AND DISCUSSION

Length of N. coryli retention by adult insects fed on yeast suspension

As seen in Table 1, adults of A. hilare were able to retain N. coryli for 60 days after feeding on the yeast suspension. The yeast was always isolated from mashed heads but less frequently from mashed thoraxes and abdomens, being found in 100, 29 and 35% of the mashed heads, thoraxes and abdomens, respectively. The number of probes made, each indicating one feeding, had no apparent effect upon retention time. Insects which had probed 1 or 6 times were able to retain N. coryli for 60 days. N. coryli was never isolated from non-infested checks (Table 2).

Table 3 shows the results of a similar experiment for 70-, 80-, and 90-day intervals. N. coryli was again isolated consistently from the heads of the insects. The yeast was also isolated from thoraxes and abdomens of some insects, most frequently from the abdomens. The yeast was not isolated from an 80-day check.

Bacterial growth was a problem in some cases, primarily on the plates containing the mashed abdomens. This undoubtedly interferred with the isolation of the yeast from the abdomen and thorax because the bacteria grew quite rapidly and could have inhibited the yeast's growth. For this reason, the frequency of N. coryli in the abdomen and thorax may have been higher than demonstrated in these experiments.

Table 1. Length of N. coryli retention by adult insects fed on yeast suspension.

INSECT	Number of Days	Number of Probes	HEAD	THORAX	ABDOMEN
1	3	4	+	-	+
2	3	2	+	-	+
3	3	5	+	-	+
4	6	2	+	+	-
5	6	1	+	-	+
6	6	2	+	-	-
7	9	1	+	-	-
8	9	1	+	-	-
9	9	1	+	-	-
10	12	6	+	+	+
11	12	1	+	-	-
12	12	1	+	+	+
13	15	2	+	-	+
14	15	2	+	-	+
15	15	3	+	-	+
16	18	3	+	-	-
17	18	2	+	+	-
18	18	1	+	-	-
19	21	2	+	-	-
20	21	2	+	-	+
21	21	3	+	-	-
22	24		o	o	o
23	24	3	+	-	-
24	24	1	+	-	-
25	27	3	+	-	-
26	27	2	+	-	-
27	27	1	+	+	+
28	30	4	+	+	+
29	30	2	+	-	+
30	30	1	+	-	-
31	33	6	+	-	-
32	33		o	o	o
33	33	3	+	-	-
34	36	5	+	-	-
35	36	1	+	-	-
36	36	1	+	-	-
37	39	4	+	-	-
38	39	3	+	-	-
39	39	2	+	-	-
40	42	2	+	+	+
41	42		o	o	o
42	42	1	+	-	-

Table 1. Continued

INSECT	Number of Days	Number of Probes	HEAD	THORAX	ABDOMEN
43	45	4	+	+	-
44	45	2	+	-	-
45	45	5	+	-	-
46	48		o	o	o
47	48		o	o	o
48	48		o	o	o
49	51	2	+	+	-
50	51	3	+	-	+
51	51		o	o	o
52	54	6	+	+	-
53	54	2	+	+	+
54	54	1	+	+	+
55	57	1	+	+	+
56	57	3	+	-	-
57	57		o	o	o
58	60	1	+	+	-
59	60	2	+	+	-
60	60	6	+	-	-

+ = yeast isolated
- = yeast not isolated
o = indicates that the insect died before the end of the interval.

Table 2. Checks on the length of *N. coryli* retention by adult insects fed on yeast suspension.

Number of the Insect	Number of Days	INSECT
1	3	-
2	6	-
3	9	-
4	12	-
5	15	-
6	18	-
7	21	-
8	24	-
9	27	-
10	30	-
11	33	-
12	36	-
13	39	-
14	42	-
15	45	-
16	48	-
17	51	o
18	54	-
19	57	o
20	60	-

- = yeast not isolated
o = insect died before the end of the interval.

Table 3. Length of N. coryli retention by adult insects fed on yeast suspension.

Number of Days	Number of Probes	HEAD	THORAX	ABDOMEN
70	5	+	-	+
70	4	o	o	o
70	2	o	o	o
70	3	-	-	+
70	2	o	o	o
80	4	+	-	+
80	4	+	-	+
80	4	+	-	-
80	3	+	+	-
80	5	+	-	+
90	2	+	-	-
90	4	+	+	+
90	5	o	o	o
90	2	o	o	o
90	1	o	o	o
		CHECKS		
70		o	o	o
80		-	-	-
90		o	o	o

+ = yeast isolated
- = yeast not isolated
o = insect died before the end of the interval.

Mortality was not a problem until the 70-, 80-, and 90-day intervals were used. Two insects, however, lived for 90 days after feeding on the yeast suspension and N. coryli was isolated from their heads.

The retention for 90 days is significant from the standpoint of the biological relationship between the yeast and the insect. The yeast appears to be well adapted for maintaining itself for long periods in the head of the host. The isolation of the yeast from the abdomen and thorax probably represented yeast material which had been recently ingested from the head during feeding.

The ability of the insect to harbor the yeast for at least 3 months may indicate a possible means by which N. coryli could overwinter. Adult insects, in the late fall, hibernate and overwinter and become active again in the spring. If an overwintering adult was infested in the fall, it could be possible for the yeast to remain viable in the insect's head and in the spring be transmitted to plant hosts by the feeding of the insect. All available evidence has indicated N. coryli to be associated with wild and cultivated hosts. The question as to how the yeast overwinters after the fruits mature and are harvested or drop to the ground and decompose has not been answered. Overwintering in an insect host such as A. hilare could be a possible means of overwintering for N. coryli.

Length of N. coryli Retention by Adult Insects Fed on Inoculated Soybeans

The method used to infest the insects in the previous experiment proved to be extremely efficient. However, the concentration of the yeast in the jelly cups was high. It was thought that this may account for the long retention time. This alternate infestation method was used to determine if comparable results could be obtained. Results of this experiment are found in Table 4.

Insects were shown to harbor the yeast 60 days using the inoculated bean method. Results were comparable with the yeast suspension method and reinforced the previous findings. By both methods, the adult insects were able to retain N. coryli 60 days.

This method, however, proved to be less reliable in that the percentage of insects infested was lower than those infested by the yeast suspension method. Seventy three percent of the insects fed on inoculated soybeans became infested compared with 100% of those fed on the yeast suspension.

The reason for the lower percentage was attributed to the fact that the insects may not have fed on the soybeans in the infected region. The infestation procedure was checked by having the insects feed on healthy soybeans and isolation of the yeast was attempted from these beans. The results showed that this was not always a reliable criteria for infestation because in some cases those which had not transmitted were

Table 4. Length of N. coryli retention by adult insects fed on inoculated soybeans.

Number of the Insect	Transmission to Soybean	HEAD	THORAX	ABDOMEN
		15 Days		
1	+	+	-	+
2	+	+	+	+
3	-	-	-	-
4	-	-	-	-
5	+	+	-	-
6	-	-	-	-
7	+	+	+	+
		30 Days		
8	-	+	-	-
9	+	+	+	+
10*	+	+	-	-
11	-	+	-	+
12	-	-	-	-
13	-	+	+	+
14	-	-	-	-
		45 Days		
15	-	-	-	-
16	+	+	-	-
17	+	o	o	o
18	+	o	o	o
19	-	+	+	+
20	-	+	+	+
21	+	+	-	-
22	-	+	-	+
		60 Days		
23	-	+	-	+
24	-	-	-	-
25	o	o	o	o
26**	+	+	-	-
27***	+	+	-	-
28	+	+	-	-
29	+	+	-	+
30	+	o	o	o

* this insect died after 28 days
** this insect died after 59 days
*** this insect died after 58 days
\+ = yeast isolated
\- = yeast not isolated

shown to harbor the yeast at the end of 30-, 45-, and 60-day intervals. For example, in the 30-day group, insects 8 and 11 did not transmit initially but N. coryli was isolated from their heads at the end of 30 days. This can be explained by the frequency of transmission of N. coryli by A. hilare to be discussed later. In most cases, those which did not transmit initially were found not to be harboring the yeast organism at the end of each interval.

These results, plus the results of the previous experiment, especially the indication that the number of probes appeared to be independent of the retention time, were used as the basis for adopting the yeast suspension method to infest bugs in all subsequent experiments.

Retention of N. coryli after a Molt

As seen in Table 5, N. coryli was lost from the heads of the infested fifth instars during molting. The yeast was isolated from the thorax and abdomen (mashed together) in 9 out of 10 insects tested. The yeast was never isolated from non-infested checks.

During molting the lining of the head capsule, the stylets, stylet pouches, foregut and hindgut are shed while the lining of the midgut is retained. Any yeast present at this point in the alimentary canal would not be affected by the molt. For this reason, the finding of N. coryli in the thorax-abdomen section of the body was not surprising.

Table 5. Retention of N. coryli after a molt.

Insect	Number of Probes	HEAD	THORAX AND ABDOMEN
1	1	-	-
2	5	-	+
3	6	-	+
4	5	-	+
5	5	-	+
6	3	-	+
7	3	-	+
8	3	-	+
9	12	-	+
10	4	-	+
		CHECKS	
1		-	-
2		-	-
3		-	-

Table 6. Isolation of N. coryli from exuviae.

Insect	Number of Probes	HEAD	THORAX	ABDOMEN (After Molting)	EXUVIAE
1	10	-	-	-	+
2	6	o	o	o	-
3	10	-	-	-	+
4	10	-	-	-	+
5	6	-	-	-	+
6	10	-	-	-	+
7	10	-	-	-	+
8	10	-	-	-	+
9	10	-	-	-	+
10	10	-	-	-	+
11	1	-	-	-	+
12	5	-	-	-	+
		CHECKS			
1		-	-	-	-
2		-	-	-	-

+ = yeast isolated
- = yeast not isolated
o = not mascerated

The loss of the yeast from the heads by A. hilare during molting is significant. After each molt, A. hilare nymphs must be re-infested in order for them to transmit the disease organism and adult insects must acquire the yeast after they become adults. The possibility of infection from the midgut will be discussed later. Frazer (1944) also found that infested cotton stainer bugs lost N. gossypii when they molted.

Isolation of N. coryli from Exuviae

Table 6 shows that N. coryli was isolated from 11 of 12 exuviae tested. These results contradict the findings of Foster (1966), who failed to isolate the yeast from the exuviae A. hilare. N. coryli was not isolated from the non-infested checks.

In the previous experiment, N. coryli was isolated from the thorax-abdomen section of the body of newly molted adults. During this experiment, N. coryli was not isolated from the thorax or the abdomen. The retention tests showed that the yeast is infrequently associated with the thorax (29%) and the abdomen (35%). The relationship does not appear to be a constant one.

Frazer (1944) found N. gossypii to be associated with the stylet pouches of the cotton stainer. The stylet pouches are shed during molting and consequently the insect would not be infested after molting. Isolation of N. coryli from the exuviae of infested A. hilare nymphs supports her findings.

Retention and Transmission of N. coryli by Infested Fifth Instar Nymphs Molting to Adults

The results of this experiment (Table 7) showed that infested fifth instars transmitted the yeast to soybeans during each feeding of their fifth stadium. When they molted, they no longer transmitted the yeast to soybeans. The yeast was not isolated from the head of these newly molted adults. These findings were consistent with the previous results. The yeast was not isolated from a non-infested check or from the beans it had fed upon.

Insect number 3 had N. coryli present in its abdomen but the yeast was not transmitted to the soybeans after this insect molted. The failure of this insect to transmit after molting showed that the yeast was not regurgitated from the gut or introduced into the soybeans by any other means during feeding in this case.

It is also of interest that 2 to 3 days prior to molting, the insects stopped feeding and in all cases started to feed the day after molting took place.

Isolation of N. coryli from Fecal Material

The yeast was isolated from the fecal material of 5 of 7 adults tested (Table 8). It was not isolated from insects 2 or 5. Insect number 1 passed the yeast 5 days in succession. The insects died in most cases after 10 days and on many days they failed to defecate. N. coryli was never isolated from non-

Table 7. Retention and transmission of N. coryli by infested fifth instar nymphs molting to adults.

Insect	Number of probes	HEAD (After Molting)	THORAX (After Molting)	ABDOMEN (After Molting)	SOYBEANS Number of days before and after molting 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	10	-	-	-	+	+	+	-	-	M -	-	-	-	-	-	-		
					f	f	f	n	n	n	f	f	f	f	f	n		
2	4	-	-	-	-	+	+	-	-	-	M -	-	-	-	-	-	-	-
					n	f	f	n	n	n	n	f	f	f	f	f	f	f
3	6	-	-	+	+	+	-	-	M -	-	-	-	-	-				
					f	f	n	n	n	f	f	f	f	f				
4	7	-	-	-	+	+	-	-	-	M -	-	-	-	-	-			
					f	f	n	n	n	n	f	f	f	f	f			
5	6	-	-	-	+	+	+	+	-	-	M -	-	-	-	-	-		
					f	f	f	f	n	n	n	f	f	f	f	f		
					CHECKS													
1		-	-	-	-	-	-	-	-	-	M -	-	-	-	-	-		
					f	f	f	f	n	n	n	f	f	f	f	f		

M = Molt took place
f = Soybean fed upon
n = Soybean not fed upon

+ = yeast isolated
- = yeast not isolated

Table 8. Isolation of N. coryli from fecal material.

Insect	Number of Probes	Number of days											
		1	2	3	4	5	6	7	8	9	10	11	12
1	2	+	+	+	+	+	-	nf	-	-	nf	nf	died
2	2	-	-	-	-	nf	nf	died					
3	1	-	-	-	-	-	nf	nf	+	died			
4	5	-	-	-	nf	-	nf	nf	nf	nf	-	nf	- died
5	1	-	+	-	nf	nf	nf	nf	nf	nf	died		
6	6	+	+	-	+	-	nf	nf	died				
7	4	-	-	+	-	-	died						
CHECKS													
1		-	-	-	-	nf	nf	-	-	nf	nf	-	-
2		-	-	-	-	nf	nf	-	-	-	nf	-	-

nf = no fecal material deposited
+ = yeast isolated
- = yeast not isolated

infested checks. The mortality of the insects was attributed to the repeated surface sterilization that was required to insure sterile conditions. The sterilization in NaOCl may also account for the lack of feeding as indicated by the lack of defecation on many days.

These results contradict the findings of Foster (1966) and Frazer (1944). Foster failed to isolate from fecal material. Frazer, in her paraffin sections, found N. gossypii in the alimentary canal but she thought that the yeast material was non-viable. These results showed that N. coryli did remain viable in the digestive tract of A. hilare. The yeast was isolated 30% of the time from the fecal material. The yeast in the alimentary canal probably represented yeast that was ingested from the head during feeding and was being passed through the insect.

The insects were never seen feeding upon their fecal material; yet this does appear to be a possible means of re-infesting themselves or other non-infested insects.

Isolation of N. coryli from Salivary Secretions of Adult Green Stink Bugs

As seen in Table 9, N. coryli was not isolated from the salivary secretions of the insects tested or from a non-infested check.

These results suggest that the yeast is not present in the salivary glands of the insects. To get to the salivary glands, the yeast must travel up the salivary channel of the

Table 9. Isolation of N. coryli from salivary secretions of adult green stink bugs.

Insect	Number of Probes	SALIVARY SECRETIONS
1	1	-
2	1	-
3	1	-
4	1	-
5	1	-
6	1	-
7	3	-
8	1	-
9	1	-
10	1	-
11	3	-
12	2	-
	CHECK	
1		-

- = yeast not isolated

probocis, up the efferent channel of the salivary duct into the salivary pump and then up the afferent salivary duct to the glands. To accomplish this, the yeast material must pass through two valves leading into and out of the salivary pump. These valves are one way valves and are not accessible in the reverse direction the yeast must travel. The other alternative is for the yeast to travel through living tissue. If the yeast was in living tissue of the host, it would not be lost during molting.

Frazer's results of finding N. gossypii in the stylet pouches seem quite plausible. She speculated that during feeding there is a leakage at the union of the pharynx and the maxillae (food channel) and in this way the yeast could have escaped from the food channel and into the pouches. Tower (1914) also thought that the air tight connection between the efferent channel of the salivary duct, the pharynx and the maxillae, would be loosened during probing and thus cause leakage to occur.

The question of how the yeast is introduced into the soybeans has not been answered. If there is leakage at the connection between the pharynx and the maxillae, especially when the stylets are moving back and forth, it could be possible for the yeast to move from the stylet pouches into the salivary channel. This would occur when the stylets were being moved back and forth and were being forced into the bean. The yeast could then be injected into the bean with the salivary secretions. During this particular study, the insects were not feeding and consequently there was no stylet movement. Also,

it is possible that the test insects were not salivating in the manner in which they normally do during the feeding process.

Another possible means of introducing the yeast organism into the soybeans is by regurgitation during feeding. Again, the yeast in the pouches, could re-enter the food channel during stylet movement. Since there are no valves in the pharynx, food could flow back into the bean if the pumping of the pharynx was interrupted.

These two means of introducing N. coryli into soybeans are speculative and no direct evidence is now available to support either. More critical work is needed in order to answer this question.

Transmission of N. coryli from Insect to Insect

In test 1 (Table 10), 5 of 7 non-infested adults caged as a group over a clump of 15 dogwood berries became infested with N. coryli. Isolation of N. coryli was attempted from 7 of the 15 berries and 6 of the 7 were found to be infected.

In test 2 (Table 10), 4 of 5 non-infested adults which were caged singly over groups of berries became infested. N. coryli was isolated from 4 of the 5 groups of berries. As also seen in Table 10, none of the non-infested checks became infested with N. coryli by feeding on berries in the field.

These results are important from the standpoint of the acquisition of N. coryli in the field. The results showed that it was possible for non-infested adults to become infested with

Table 10. Transmission of N. coryli from insect to insect.

DOGWOOD BERRIES

TEST # 1

Non-infested Insect	HEAD (After feeding on berries)
1	+
2	+
3	+
4	-
5	+
6	-
7	+

TEST # 2

Infested Insect	Number of Probes	Non-infested Insect	HEAD	BERRIES
1	3	1	+	+
2	2	2	+	+
3	1	3	+	+
4	1	4	+	+
5	1	5	-	-

CHECKS

Number of the Insect	HEAD
1	-
2	-
3	-
4	-
5	-

+ = yeast isolated
- = yeast not isolated

the yeast by feeding on berries which had been infected by the feeding of infested A. hilare. As mentioned previously, if the yeast is able to overwinter in the adults and is transmitted to dogwood berries in the spring, these berries could serve as a source of infestation for other non-infested insects.

Because of the gregarious habits of the nymphs of A. hilare, particularly the first, second, and third instars, the above method of acquiring the yeast in the field would be of particular significance. The early instars stay close to each other during development and at times 10 to 15 nymphs were found on a single group of berries. These nymphs could spread the yeast among themselves quite easily. After the nymphs molt, they could again acquire the yeast because they would often be on the same group of berries. Foster (1966) isolated N. coryli from field collected nymphs of all the instars except the third. This stage was not ruled out because the second and the fourth instars were found to harbor the yeast.

Table 11 gives the results of a similar study with soybeans. In this study, 2 of 9 non-infested adults became infested with the yeast in the field. This means of acquiring the yeast would be of importance later in the season when the insects are on soybeans. It could be possible for insects to become infested by feeding on infected soybeans infected by the feeding of infested adults.

The following is another point which could be of importance from the standpoint of the biological relationship be-

Table 11. Transmission of N. coryli from insect to insect

SOYBEANS

Infested insect	Number of probes	Non-infested insect	HEAD
1	4	1	-
2	4	2	-
3	3	3	-
4	1	4	+
5	2	5	-
6	1	6	-
7	6	7	-
8	2	8	-
9	2	9	+

+ = yeast isolated
- = yeast not isolated

tween the yeast and the insects. When working with the dogwood berries, it was noted that even though the berries were surface sterilized, several different types of fungi grew on the plates. These fungi undoubtedly came from within the berries. When insect heads were mashed and plated, however, only N. coryli, and infrequently bacteria or another very small microorganism were isolated. N. coryli appears to be retained within the head with greater regularity than any of the other forms.

Concentration and Reproduction of N. coryli in the Head of Adult A. hilare

There was a trend toward an increase in the yeast concentration after the 10-day interval (Table 12). Insects 7 and 9 produced more colonies than any of the other insects tested but the variability present when they are compared with insects 8 and 10 shed some doubt on these results.

There are several factors which could explain part of this variability. It was not possible to distinguish between probes. An insect may have probed the membrane but the length of actual feeding could not be determined. Some of the probes may have been only momentary or the insect may have fed for several minutes. In a previous experiment, it was noted that the number of probes did not appear to affect retention time. Insects which probed 1 or 6 times were able to retain the yeast 60 days. It could have been, however, that some of the insects which probed more than 1 or 2 times were not actually feeding for any great length of time and were not ingesting a large

Table 12. Concentration and reproduction of *N. coryli* in the head of adult insects.

Insect	Number of probes	Number of days	Number of colonies
1	1	1	0
2	1	1	2
3	1	1	0
4	1	1	4
5	1	1	0
6	1	1	1
7	1	10	25
8	1	10	7
9	1	10	50
10	1	10	1
11	2	1	0
12	2	1	4
13	2	1	12
14	2	1	0
15	2	1	1
16	2	1	17
17	3	1	1
18	3	1	1
19	3	1	0
20	4	1	5
21	4	1	0
22	5	1	7
23	5	1	3
24	5	1	10
25	5	1	22
26	5	1	2
27	6	1	3
28	6	1	5
29	7+	1	2
30	7+	1	9
31	7+	1	8
32	7+	1	24

amount of yeast. An insect which probed once may have ingested as much yeast as an insect which probed 5 times.

This experiment should be repeated and the factors affecting the standardization of the technique overcome. Large numbers of insects could be used and differences in concentration detected.

Frequency of transmission of N. coryli to Soybeans by Adult A. hilare

Results of this experiment are found in Tables 13 and 14. The 18 adult A. hilare were caged on a total of 409 soybean pods. Thirty eight of the pods were not accounted for because of various reasons such as being lost or mislabeled. A total of 371 pods were accounted for and examined for feeding damage. Of these, 268 pods had been fed upon and were classified as test pods and isolation of N. coryli was attempted from the beans. Of the test beans, 160 (60%) were found to be infested with N. coryli.

Table 13 gives the breakdown of each insect, including whether it was infested with the yeast, if determined, at the end of the 25 days or as long as the insect lived and how many probes the insect made through the parafilm. The check insects are included at the bottom of the table and it should be noted that the yeast was never isolated from a check bean that was fed upon by a non-infested adult A. hilare.

Table 14 summarizes the results of Table 13. A total of 72% of the beans were fed upon. The percent varied from

Table 13. Frequency of transmission of N. coryli by adult A. hilare.

Insect	No. of probes	HEAD	Isolation of N. coryli from each day's feeding on soybeans																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
242	5	+	+	+	-	+	-	+	-	N	N	-	N	-	N	N	+	+	-	+	+	+	+	o	N	+	+
244	1	+	N	-	+	-	+	o	o	o	+	-	+	+	-	+	+	+	+	+	+	N	N	N	N	Died	
246	1	+	-	o	+	-	o	o	N	N	+	+	-	o	-	o	N	+	-	+	+	+	+	-	+	-	Died
251	2	+	N	+	+	+	N	o	+	o	-	+	-	-	N	N	N	N	N	Died							
257	2	+	N	-	N	+	+	-	+	o	o	+	+	+	N	N	N	N	N	+	N	N	+	-	N	N	N
258	5	+	-	-	o	+	+	o	-	o	N	+	-	+	N	+	-	-	N	N	N	N	N	-	-	N	N
259	1	+	-	+	+	+	+	+	o	o	N	+	-	-	+	N	-	+	+	N	+	+	+	N	+	+	
260	8	+	-	-	-	-	+	+	+	o	-	N	-	+	N	N	-	+	+	N	N	N	N	Died			
261	3	+	-	-	-	-	-	+	N	o	N	N	N	-	N	N	N	Died									
263	6	+	N	+	-	+	+	-	+	N	+	-	-	o	+	-	+	N	+	o	+	-	+	o	+	+	+
264	4	+	-	+	N	-	o	o	+	o	+	N	+	N	N	N	+	+	-	+	+	+	+	+	-	+	-
265	3	o	o	-	N	-	o	+	+	o	o	-	-	-	-	N	+	N	N	Died							
267	4	+	-	+	-	-	o	+	-	-	+	-	-	N	N	+	N	+	+	+	+	-	+	+	+	+	N
271	4	+	+	+	-	+	-	o	+	+	+	-	+	-	+	+	N	-	+	N	-	+	+	+	N	+	o
272	3	o	-	-	+	-	-	-	o	-	+	N	o	-	-	+	N	N	N	N	Died						
274	5	+	-	-	-	+	+	+	o	-	+	-	o	+	-	+	+	+	+	+	+	+	+	+	N	+	o
289	1	+	-	-	-	N	+	-	o	+	-	+	+	-	N	N	+	+	-	+	+	+	+	N	N	+	+
291	1	+	N	-	N	-	-	+	o	N	+	-	+	-	N	-	N	N	-	N	+	N	-	N	N	N	N
CHECKS																											
2		-	o	o	o	-	-	-	-	-	-	-	o	N	N	N	N	Died									
3		-	o	N	-	o	o	-	-	-	o	-	-	N	N	-	-	N	N	N	-	-	-	N	-	N	-
4		-	-	-	-	-	-	-	-	-	N	-	-	N	-	-	-	N	-	-	-	-	-	-	-	N	-

\+ = Yeast isolated
\- = Yeast not isolated
N = Soybean not fed upon
o = Bean not accounted for

Table 14. Frequency of transmission of N. coryli to soybeans by adult A. hilare.

SUMMARY OF TABLE 14

INSECT	Total No. of Pods	No. Fed Upon	Percent Fed Upon	No. of Test Pods	N. coryli Isolated	Percent
242	24	18	75%	18	12	67%
244	20	15	80%	15	11	74%
246	19	16	84%	16	9	56%
251	15	8	53%	8	5	63%
257	23	11	48%	11	8	73%
258	22	13	59%	13	5	39%
259	23	19	83%	19	15	79%
260	20	13	65%	13	16	46%
261	14	7	50%	7	1	14%
263	22	19	86%	19	13	69%
264	22	17	77%	17	12	71%
265	13	9	69%	9	3	33%
267	24	20	83%	20	12	60%
271	23	20	87%	20	14	70%
272	16	11	69%	11	3	27%
274	23	21	91%	21	15	72%
289	24	19	79%	19	12	63%
291	24	12	50%	12	4	33%
			TOTALS			
18	371	268		268	160	
			AVERAGE			
1	21	15	72%	15	9	60%
			CHECKS			
2	11	7	64%	7	0	0%
3	21	13	62%	13	0	0%
4	25	21	84%	21	0	0%
			TOTALS			
3	57	41		41	0	
			AVERAGE			
1	19	14	72%	14	0	0%

48 (insect number 257) to 91% (insect number 274). In the check insects, 72% of the beans were also fed upon, ranging from 62 to 84%.

The frequency of transmission varied from 79 (insect number 274) to 14% (insect number 261) with an average of 60%. Eleven of the 18 insects had a percent higher than 60% and 6 insects had a percent higher than 70%. Seven of the 18 insects died before the end of the 25 days.

There appeared to be a relationship between the amount of feeding and the percent of transmission. The 6 insects which had the highest frequency of transmission (over 70%) also had a frequency of feeding higher than the average of 72%. An exception was number 257 which transmitted 73% of the time yet fed only 48% of the time, the lowest feeding percentage of any of the insects. On the other hand, those 6 insects which had the lowest frequencies of transmission (below 60%), also had the lowest feeding frequencies (below 72%). Four of these 6 insects died before the end of the 25 days.

Insects caged in the small plastic cages in the growth chamber in this test had a higher mortality rate than those reared in the ice cream cartons. The insects appeared to be under greater stress and it is possible that conditions were not conducive for maximum longevity and feeding.

The soybeans never reached maturity because of the feeding damage. The small size of the beans may have affected

the isolation technique. However, the results showed that N. coryli is transmitted a large percentage of the time during the feeding process by infested adult A. hilare under these simulated field conditions.

Survey of Field Population

The percentage of adult A. hilare (Table 15) infested with N. coryli in the field varied from 33% on July 16 to 10% on September 16. An average of 23% of all adults collected were infested. The insects were collected off dogwood bushes early in the summer and off dogwood and soybeans later in the year. The adult population never seemed to build up but flucuated between collection dates. The nymphal population appeared stable and nymphs were found continually from June 26 through October 2. These facts could account for the low yet steady percent of adults harboring the yeast. At the last collection date, many fifth instars were seen along with third and fourth instars. Most of the adults seen appeared to be newly molted individuals. These insects represent the overwintering population.

As seen in Table 16, there was little difference in the percentage from the three different fields: field 1 averaged 24%, field 2 averaged 23% and field 3 averaged 20% infested.

Some fifth instars of A. hilare were collected occasionally and were found to harbor the yeast. Two brown stink bugs

Table 15. Survey of Field Population

DATE	HOST	Number Collected	Number Infested	PERCENTAGE
June 26	Dogwood	25	6	25%
July 16	Dogwood	21	7	33%
July 30	Dogwood	33	9	27%
Aug. 12	Dogwood	25	5	20%
Aug. 29	Dogwood and Soybeans	30	8	27%
Sept. 16	Soybeans	30	3	10%
Oct. 2**	Dogwood	26	5	19%
TOTAL		190	43	23%

**Soybeans were harvested just prior to this collection date.

Table 16. Survey of Field Population

Breakdown by field

FIELD	Number (total) Collected	Number Infested	PERCENTAGE
1	68	16	24%
2	73	17	23%
3	49	10	20%

(Euschistus servus) were found to harbor the yeast. Five nymphs of the leaf-footed bug (Leptoglossus sp.) of the family Coreidae were collected and N. coryli was isolated from 2 of these. This is the first report of the isolation of the yeast from Hemiptera other than those belonging to the family Pentatomidae. Fawcett (1929) did find Leptoglossum zonatus (Dall.) to transmit N. coryli to citrus fruit, but he failed to isolate it from the insect.

SUMMARY

A method was found by which the green stink bug, Acrosternum hilare (Say), could be infested with Nematospora coryli Peglion, the causative organism of yeast-spot disease of soybeans.

Once infested, adult A. hilare were able to retain N. coryli in their head for as long as 90 days. Infested fifth instar nymphs lost the yeast from their heads when they molted and N. coryli was isolated from exuviae of newly molted adults. N. coryli was also isolated from the fecal material of infested adults, but it was not isolated from salivary secretions. The significance of these findings were discussed with respect to the location of the yeast in the body of the host and possible methods of transmission to soybeans.

Non-infested adults became infested in the field by feeding on dogwood berries (Cornus drummondi Meyer) and soybeans which had been previously infected with N. coryli by the feeding of infested adult A. hilare.

There was a trend toward an increase in N. coryli concentration in the head of the adults after a 10-day interval. Whether this represents reproduction of the yeast in the head of the insect is still to be determined.

Eighteen infested adults caged for 25 days on soybean pods were found to transmit N. coryli in 60% of their feedings. Six insects transmitted over 70% of the time and one adult transmitted in 79% of its feedings.

The caged insects had an average feeding frequency of 72%. Those which had fed most often had the highest frequencies of transmission.

Insects were collected from dogwood (Cornus drummondi Meyer) during most of the season before the soybeans podded and after the soybeans were harvested. Dogwood appeared to be their preferred wild host in Kansas. Twenty three percent of all adult A. hilare collected from the field were found to be infested with N. coryli. The percentage varied from 33% on July 16 to 10% on September 16.

Fifth instar nymphs collected from the field were found to harbor the yeast and the yeast was also isolated from field collected brown stink bugs (Euschistus servus). Two nymphs of the leaf-footed bug (Leptoglossus sp.) of the family Coreidae were found to harbor the yeast. This was the first report of N. coryli being isolated from Hemiptera other than members of the family Pentatomidae.

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THE ASSOCIATION OF NEMATOSPORA CORYLI PEGLION, THE CAUSATIVE ORGANISM OF YEAST-SPOT DISEASE OF SOYBEANS, AND THE GREEN STINK BUG, ACROSTERNUM HILARE (SAY): (HEMIPTERA: PENTATOMIDAE)

by

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AN ABSTRACT OF A MASTER'S THESIS

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A method was developed whereby the green stink bug, Acrosternum hilare (Say), could be infested in the laboratory with Nematospora coryli Peglion, the causative organism of yeast-spot disease of soybeans.

Adult A. hilare retained N. coryli in their heads up to 90 days after infestation. Infested fifth instar nymphs lost the yeast from their heads when they molted and N. coryli was isolated from the exuviae of newly molted adults. It was also isolated from the feces of infested adults but not from salivary secretions.

There were indications of increase in N. coryli concentration in the head after 10 days.

In the field, non-infested adults became infested by feeding upon dogwood berries and soybeans which had been previously infected with N. coryli by the feeding of laboratory infested adult A. hilare.

Eighteen infested adults transmitted N. coryli to soybeans in 60% of their feedings; one had a transmission frequency of 79%. Specimens which fed most often, produced the highest frequencies of transmission.

Twenty three percent of all field-collected adults were infested with N. coryli. It varied from 33% on July 16 to 10% on September 16.

Dogwood, Cornus drummondi Meyer, appeared to be the preferred wild host of A. hilare in southeastern Kansas. Fifth instar nymphs collected from the field harbored the yeast.

The brown stink bug, Euschistus servus, was also infested in the field.

Two field-collected nymphs of a leaf-footed bug, Leptoglossus sp. (Coreidae) were infested. This is the first report of the isolation of N. coryli from Hemiptera other than Pentatomids.