Interdependence of a Mite, Siteroptes reniformis, and a Fungus, Nigrospora oryzae, in the Nigrospora Lint Rot of Cotton

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Accepted for publication 8 September 1972.

ABSTRACT

The mite, *Siteroptes reniformis*, was consistently associated with Nigrospora lint rot of cotton bolls in California. Infection of bolls in the field averaged 23% when the soil at the base of plants was artificially infested with both *Nigrospora oryzae* and *S. reniformis*; 4%, when only *N. oryzae* was added to the soil; and 1%, when no inoculum of either was applied in the checks. When tubes containing *N. oryzae* and the mite, or *N. oryzae* alone, were attached to individual bolls, 99.2% and 15.1%, respectively, became infected. *S. reniformis* fed on *N. oryzae* mycelium, and young females had one to two spores of *N. oryzae* within their hysterosoma soon after exposure to the fungus. Infections developed in 91.6% of the locks inoculated with one-three mites carrying one spore each, whereas all locks were infected when inoculated with one-three mites carrying two spores each. *S. reniformis* required *N. oryzae* for normal growth and reproduction. The mite aids the fungus in dissemination, inoculation, and early growth. Temperature optima of *N. oryzae* (21-27 C) and *S. reniformis* (27 C) were similar. *S. reniformis* overwintered in diseased cotton locks. These data and observations indicate that *S. reniformis* serves as a vector of *N. oryzae* in the initiation of Nigrospora lint rot disease in California, and that a mutualistic form of symbiosis exists between them.

Phytopathology 63:308-315

Additional key word: symbiosis.

Hansford (7) first reported *Nigrospora oryzae* (Berk. & Br.) Petch on cotton in 1929 in Uganda. He isolated the fungus from the interior of cotton seeds. Jaczewski (9) reported *N. gossypii* Jac. causing a lint rot of cotton in southern Russia that same year. Houston & Garber (8) observed *N. oryzae* as a causal agent of the lint rot disease of cotton bolls in the United States in 1959. Lint in affected locks (carpel contents) failed to fluff as the boll opened and infected fibers were weakened. The presence of dark brown to black conidia on the surface and in the lumen of the fibers darkened the lint. Healthy portions of affected bolls, however, fluffed normally at maturity since *N. oryzae* attacked only the lint and not the carpel wall. One lock could be completely destroyed while adjacent ones remained healthy.

Bondarovich (4) reported that *N. gossypii* affected maturing fibers and dying parts of cotton bolls in Russia, and that conidia of *N. gossypii* are carried into the bolls on the bodies of the mite *Siteroptes graminum* (Reut.), that feeds on the fungus. Several workers (1, 5, 15, 16) noted the association of *Pediculopsis (Siteroptes) graminum* (Reut.) or *Pediculopsis* sp. with *N. oryzae* or *Fusarium poae* (Pk.) Wr. & Reinking. Three of them (5, 15, 16) also suggested that these associations were a type of symbiosis. *N. oryzae* conidia are not deciduous; this fact, together with the preceding reports of fungus transmission by mites, suggested that an investigation into the possibility of a mite vector for *Nigrospora* lint rot might prove fruitful. This paper presents the evidence for mite transmission of *N. oryzae* and the nature of the relationship between the vector and the pathogen.

MATERIALS AND METHODS.—Field experiments were carried out during 1968 and 1969 at the San Joaquin Valley Agricultural Research and Extension Center of the University of California, Fresno County, and at the University of California, Davis.

We isolated *N. oryzae* from cotton bolls (*Gossypium hirsutum* L.), Bermudagrass [*Cynodon dactylon* (L.) Pers.], and sorghum stalks (*Sorghum vulgare* Pers.) by placing infected plant tissues on water agar and making hyphal tip subcultures. Also, potato dextrose agar (PDA) was seeded with *N. oryzae* conidia from infected plant tissues, and later the germinated spores were transferred to petri dishes or culture tube slants. All cultures were grown on PDA and, for extended storage, kept in tubes at 1 C.

We started colonies of *S. reniformis* by transferring individual young females from *Nigrospora*-infected locks that had been incubated in a moist chamber at room temperature to petri dish and tube cultures of *N. oryzae*. After birth, some young mites crawled onto the cotton plugs in the tubes. We then started new colonies by exchanging these mite-infested cotton plugs with the plugs in new tube cultures of *N. oryzae*. Mite colonies were maintained and increased in this manner throughout the study. Mites were mounted in Hoyer's (14) solution on a glass slide and identified. Preliminary identification of mites made by the authors was confirmed by G. W. Krantz, Oregon State University, Corvallis.

Mature cotton bolls of Acala 4-42 and SJ-1 were produced in the greenhouse for use in all the greenhouse and laboratory experiments. Laboratory experiments were conducted at 22-24 C, unless otherwise indicated.

N. oryzae-infected cotton bolls from several cotton-growing areas in the San Joaquin Valley of

California were collected during Fall of 1967 and checked for the presence of mites. Infected locks from these bolls were placed in sterile vials containing a strip of wet blotter paper. The mouth of each vial, covered with a test tube cap, was ringed with petroleum jelly to prevent mites from entering or escaping.

We prepared inoculum for field trials by growing N. oryzae on a 1:1 mixture of wheat and barley seeds in a 1-quart Mason jar for 17-21 days at 22-24 C. Plastic petri dishes and culture tubes containing N. oryzae on a PDA and barley straw medium were infested with S. reniformis. These cultures were incubated for 6-8 days at 22-24 C before use.

Observations on the behavior of the mites were facilitated by use of Plexiglas microscope slides with a 1.5-cm hole cut in the center. A square cover glass was glued over the hole on one side of the slide with epoxy-resin adhesive. The slide and cover glass were sterilized in 95% ethyl alcohol and placed in a sterile petri dish containing 2-4 ml of sterile water. A 10-mm disc of PDA was placed in the well of the slide, inoculated with N. oryzae, and covered with a sterile No. 1, 22 X 40 mm cover glass. Mites to be observed were then placed on the developing fungus colony after 24-48 hr.

Mites were killed and fixed in alcohol and prepared for sectioning by passage through a 25 to 100% alcohol dehydration series, with 10% increments. Specimens were kept in each concentration for 24 hr, except for 48 hr in 100% alcohol. The mites were finally placed in one change of an epoxy resin (17) for 24 hr, after which they were collected by centrifugation and embedded in the same medium. Sections (7-μ) cut with a glass knife on a Porter-Blum ultramicrotome were mounted in epoxy resin (Wilhold brand) and examined with a phase contrast microscope.

Prior to examination with the Cambridge Stereo Scan Mark IIa scanning electron microscope (SEM), mites were glued to the aluminum specimen stub with an adhesive made from Scotch brand transparent tape and benzene. Mites were transferred directly to the stub and killed by freezing or fixed in Carnoy's (10) solution for 48 hr, before being placed on the stub. All specimens were coated with gold prior to observation.

RESULTS.—*Frequency of association of mites with diseased bolls.*—B. R. Houston (*personal communication*) stated that during a study of *Nigrospora* lint rot of cotton in California from 1955 to 1959, mites were occasionally found in the infected cotton bolls in the field and also in cultures of N. oryzae isolated from the center of diseased locks. Diseased cotton bolls collected from the field were frequently infested with several species of mites. The following experiment was conducted to identify the mites present and to determine whether any species was consistently associated with Nigrospora lint rot.

Field-collected diseased locks were incubated as described and examined for mites every other day. The first mites were seen 9 days after the start of

incubation, and were found in 261 of 265 diseased locks examined after 25 or less days of incubation. These observations indicated that S. reniformis was consistently associated with locks infected with N. oryzae. Therefore, experiments were initiated to demonstrate Leach's rules for proof for "insect" transmission of a fungus (12).

The relationship of S. reniformis to N. oryzae transmission.—The following experiments were performed to determine whether N. oryzae was transmitted to healthy bolls by S. reniformis. Approximately 1 acre of cotton, Acala 4-42 variety in 1968 and SJ-1 variety in 1969, was planted in two half-acre blocks. Standard cultural practices were followed, except that no pesticides were used in 1968 and one application of Kelthane (Rohm and Haas) and Azodrin (Shell) was applied in 1969 for spider mite and lygus bug control. The test blocks were irrigated differently in the 1968 trial to determine if sprinkler or furrow irrigation affected the incidence of *Nigrospora* lint rot. The method of irrigation apparently made no difference; therefore only furrow irrigation was used in 1969. Each treatment was replicated 15 times. Each plot consisted of six rows, 83 ft long. The treatments were: (i) untreated controls; (ii) N. oryzae alone; (iii) N. oryzae with S. reniformis; and (iv) S. reniformis alone, placed on the soil surface at the base of the plants in the inner four rows of each plot. Inoculum prepared as described was applied 3 times at monthly intervals beginning in mid-August. The incidence of disease was determined by microscopic examination of bolls harvested in mid-November from the inner two rows of each plot.

S. reniformis could not be maintained in the absence of the fungus. Consequently, the treatment with mites alone was not attempted in 1969.

Incidence of boll rot was not significantly different in plots treated with N. oryzae alone from the untreated control in either the 1968 or 1969 experiments (Table 1). The marked increase of infected bolls when both N. oryzae and S. reniformis were added indicated that the mites were serving as a vector of the fungus.

The presence of the mite was confirmed by examination of N. oryzae-infected locks from 85 bolls, representing all treatments from the field and incubated as described earlier. Mites appeared 8 days after the start of incubation, and by the 15th day,

TABLE 1. Incidence of boll rot when *Nigrospora oryzae* alone and N. oryzae with *Siteroptes reniformis* were placed on the soil surface at the base of cotton plants

Treatment	Infected bolls/total[a]	
	1968	1969
Noninoculated control	34/1,695 a	7/1,409 a
N. oryzae alone	133/1,829 a	8/1,403 a
N. oryzae with mites	599/2,052 b	232/1,325 b

[a] Duncan's multiple range test used. Values differing ($P = .01$) are followed by different letters.

205 of 206 diseased locks examined yielded *S. reniformis*.

Further substantiation of data from the field plot was obtained in tests using individual cotton bolls. Tube cultures of *N. oryzae*, and *N. oryzae* with *S. reniformis*, were prepared. When the female mites were about to produce young (6-8 days after infestation of the fungus cultures), the tubes were fitted with a paper funnel and attached to unopened cotton bolls with a rubber band. Before the tubes were put in place, the tip of each boll was cut off to expose the lint. Controls consisted of empty tubes or tubes containing *N. oryzae* alone attached to the bolls. The tubes were removed after 2 weeks, and the bolls were harvested and examined 1 week later. Thirty bolls were used in each treatment, and the tests were repeated twice each in 1968 and 1969. Of the 30 bolls to which tubes containing only *N. oryzae* were attached, only four and six in 1968, and one and seven in 1969 became diseased. Those bolls to which tubes containing *N. oryzae* and *S. reniformis* were attached were 100% infected in both tests in 1968, and 29 and 30 bolls became diseased in 1969. The bolls with empty tubes had five and nine diseased in 1968 and one and one diseased in the 1969 tests, which was equivalent to the number of infections in bolls exposed to *N. oryzae* alone. All the *N. oryzae*-infected bolls from the empty tube and fungus alone controls were examined, and most were infested with *S. reniformis*, indicating that the naturally occurring population of *S. reniformis* was responsible for most if not all of these infections. The low incidence of lint rot where either empty tubes or *N. oryzae* alone were applied, and the marked increase in infected bolls in the fungus-mite treatment, indicated that *S. reniformis* played a major role in the increased incidence of *Nigrospora* lint rot.

Observations of S. reniformis and the association of the pathogen with the mite.—It was noted in the preceding experiment that *S. reniformis* could not be maintained in the absence of *N. oryzae*. Consequently, critical observations of mites were made in an effort to explain this phenomenon.

When young female mites emerged from their mother, they were hyaline with a dark inverted "T" (body contents) in the center of the hysterosoma (Fig. 1-a). After a short exposure to cultures of *N. oryzae*, young females had one to two black bodies on their hysterosoma just inside and behind the fourth pair of legs (Fig. 1-b). These same bodies were also present after young females were exposed to diseased locks of cotton. When dissected from the mite and plated on PDA, these bodies germinated and produced *N. oryzae* cultures.

Transmission of N. oryzae to cotton locks by S. reniformis under controlled conditions in the greenhouse.—Small cages were made from 2-cm lengths of 3/16-inch glass tubing, and one end was sealed with cigarette paper. Several cotton fibers were placed in each cage to impede mite movement. The cages with cotton fibers inside were sterilized in propylene oxide for 36-48 hr before use. Three mites, each carrying 0, 1, or 2 spores, were placed in each cage just prior to attachment to a locule on a fully developed boll. Three mites were used to ensure the presence of at least one mite/cage because some escaped between the time the mites were placed in the cages and attachment to the locules. Controls consisted of empty cages. Before the cages were attached, the bolls were surface-disinfected with a 2.62% sodium hypochlorite solution, and a 3/16-inch hole was cut into the locule with a sterile cork borer. The cages remained in place for 10-14 days, after which the bolls were harvested and examined for boll rot. Each type of inoculation was repeated 22 to 27 times. All locules exposed to mites carrying two spores each became infected. When each mite was carrying one spore, 22 of 24 locules became infected with *N. oryzae*. Three locules of 23, after exposure to mites without spores, and two of 22 locules used in the controls were infected. These results showed that one to three mites carrying one or two spores each were able to successfully inoculate the locule to which they were exposed.

Infections that occurred in the locules not exposed to mites, or to mites without spores, were probably due to escapes while the cages were being attached. In addition, infections that occurred later in the same glasshouse probably came from mite escapes from earlier experiments.

Nutrition of S. reniformis.—Fungi other than *N. oryzae* were obtained from rotting bolls. Experiments were conducted to determine if these and other fungi would support the growth and reproduction of *S. reniformis*. Ten tube cultures were made of each of 50 species of fungi in 34 genera. When the mycelium covered the agar surface, mites were introduced by the cotton plug transfer technique. The infested fungus cultures were incubated 14 to 21 days and examined periodically for evidence of gravid females. *S. reniformis* survived for several days on an *Epicoccum* sp., *Rhizoctonia bataticola* Taub, *R. solani* Kuehn, and *Stemphylium botryosum* Wallr.; whereas *Helminthosporium carbonum* Ullstrup, *H. carbonum* (albino strain), and *H. pedicellatum* Henry supported limited reproduction. Often while feeding on the latter fungi, the body of the female began to enlarge, but collapsed before eggs were mature. Also,

Fig. 1. a) *Siteroptes reniformis* female soon after emergence from the mother. Note dark inverted "T" on the hysterosoma (× 360). b) *S. reniformis* female with two spores of *Nigrospora oryzae* in the pouch and one adhering to the posterior surface (× 360). c) Longitudinal section of *S. reniformis*. Note the spore (S) of *N. oryzae* in the pouch with the slit (sl) extending to the midpoint of the opisthosoma (× 920). d) Scanning electronmicrograph of the ventral surface of the hysterosoma of a female *S. reniformis*. Note the opening of the pouch (a) and the bulges of two spores (S) of *N. oryzae* inside (× 1,200).
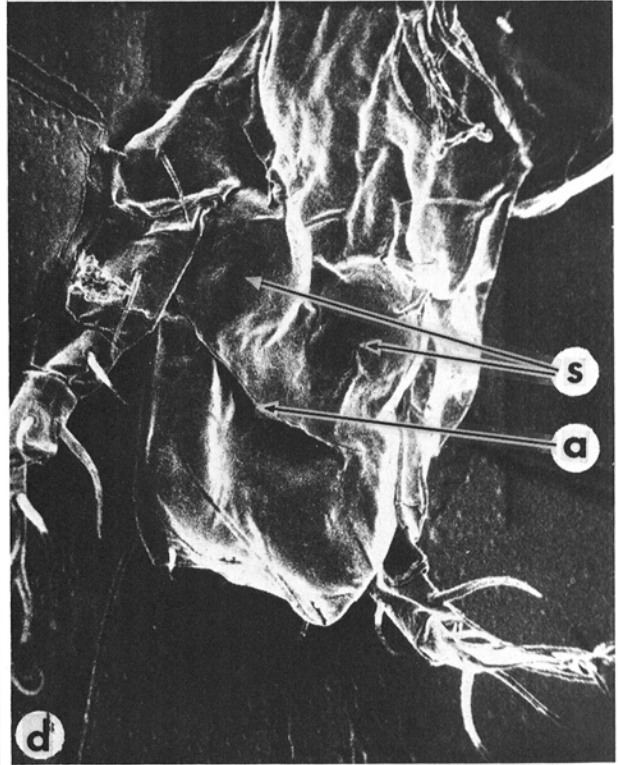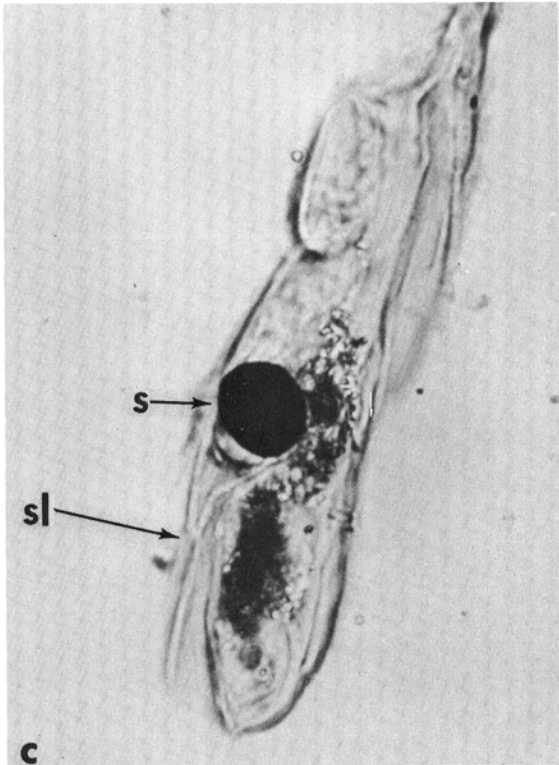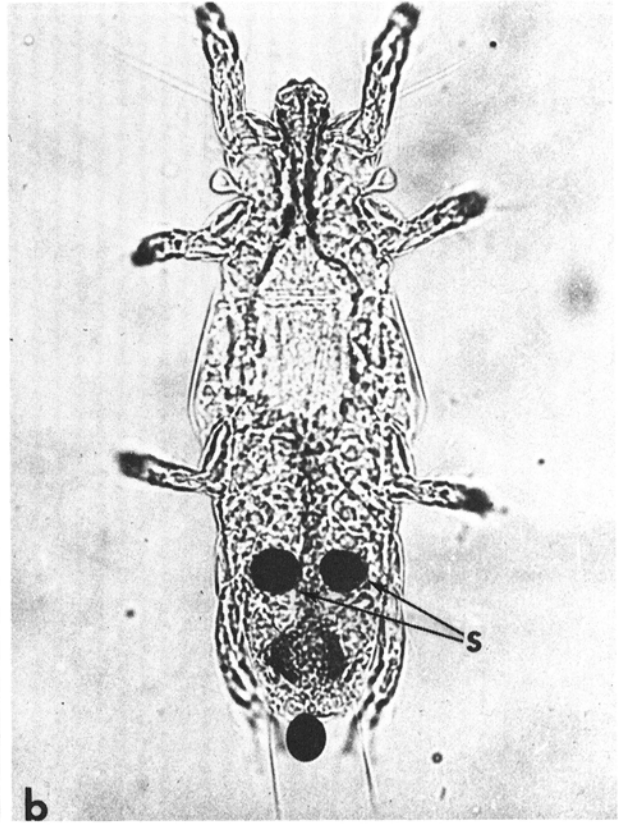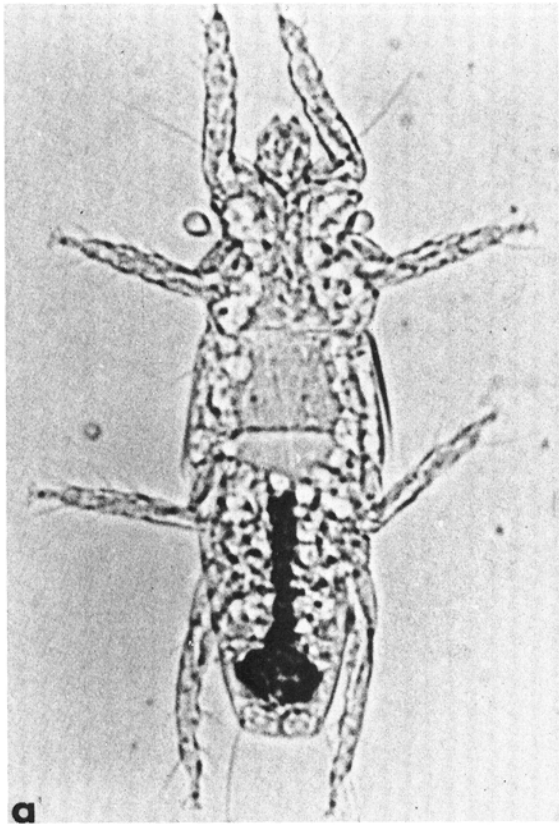
the second generation females failed to produce young, indicating the lack of some factor(s) needed for reproduction. Of the fungi in this test, only *N. oryzae* supported normal mite growth and reproduction.

Stimulation of N. oryzae growth by S. reniformis.—When young *S. reniformis* females settled and began to feed on *N. oryzae*, a small mycelial tuft formed in less than 24 hr around the head of the mite, suggesting that the mite stimulated fungus growth; hence the following experiment.

Petri dish cultures of *N. oryzae* were infested with *S. reniformis*. After 18-24 hr, mites that had settled and had begun to feed were transferred with a small disc of medium to one sector of a three-sectored petri dish containing PDA. The remaining two sectors of the dish received either a disc with fungus containing a mite that had been killed by crushing at the time of transfer, or a disc containing only the fungus. Incubation was for 24 hr at room temperature, after which the diameter of each colony was measured. Each treatment was replicated 40 times.

Mycelial mats averaged 22.0, 22.1, and 18.4 mm when grown in the presence of a live mite, dead mite, and no mite, respectively. Thus, *S. reniformis*, living or dead, increased ($P = .01$) the linear growth of *N. oryzae*. It was initially thought that the increased rate of growth was caused by the physical wounding of the fungus by mite feeding. However, stimulation of fungus growth by dead mites suggests that a chemical substance(s) was responsible.

Optimum temperature for fungus and mite growth.—The optimum temperature for the growth of *N. oryzae* was determined using the technique of Halisky et al. (6). To determine the optimum temperature for mite growth, females of *S. reniformis* were placed on *N. oryzae* cultures and allowed to settle. Individual females then were transferred with a small disc of medium to another dish of *N. oryzae* and incubated at constant temperatures ranging from 9-36 C at 3° intervals. The plates were examined at regular intervals, so that the start and completion of mite emergence could be determined. When emergence was completed, the plates were removed from the incubators and the numbers of female young produced in 10 replicates at each temperature were determined by counting the mites after they had settled and enlarged sufficiently to make them easily visible (Fig. 2). The number of offspring plus the number of days required to attain maturity were the criteria used to determine the most favorable temperature for growth and reproduction of the mite.

Halisky et al. (6) found the optimum temperature for the growth of *N. oryzae* to be 24 C. The present study confirmed this finding. However, the rate of fungus growth was greater at all temperatures (with the exception of 12 C) than as reported by Halisky et al. For the mite, the number of days necessary to reach maturity was shortest at 33 C, but the average number of offspring per female was only 74, whereas the average number of young produced per female at 27 C was 443 and only 1 more day was required to attain maturity. Consequently, 27 C is considered the
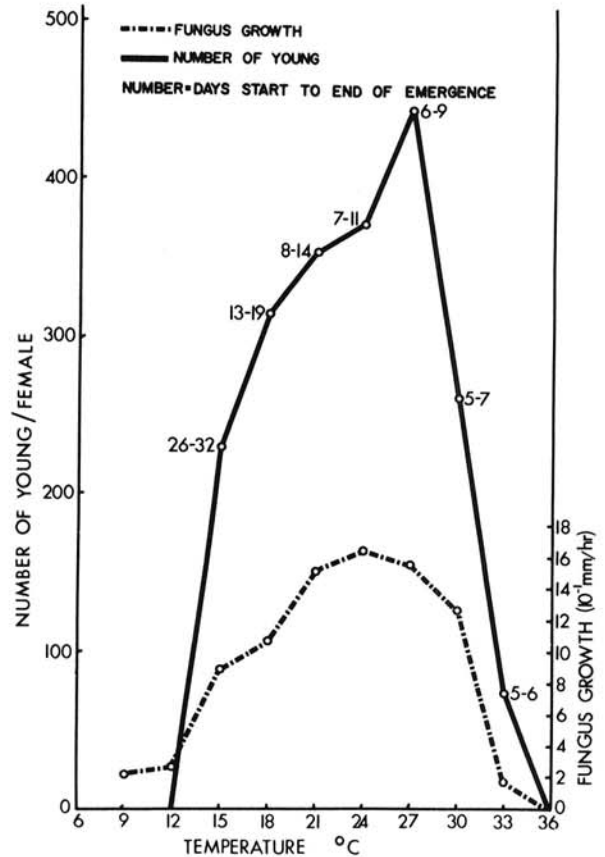

Fig. 2. Growth curves for *Siteroptes reniformis* and *Nigrospora oryzae*. Average number of young per female (o—o), together with the days from start to completion of emergence. *N. oryzae* growth rate on potato dextrose agar (o-·-o).

optimum for the mite which is within the optimum range (21-27 C) for the fungus.

At 15 C, a number of the young females produced, settled, began to enlarge, and then collapsed. At 12 C the development of the mites that were used to start the test was arrested shortly after eggs became visible within the body cavity. The development of the test mites at 9 C was arrested very early, and most of them collapsed and died. Thus, temperatures of 15 C and below are detrimental to mite survival. The similarity of the temperature-growth curves for *N. oryzae* and the number of young per female (Fig. 2) suggest that the reproductive capacity of the mite is closely correlated with the growth rate of the fungus.

Survival of the mite.—Winter temperatures in the San Joaquin Valley of California frequently fall below 15 C; hence an experiment was initiated to determine some of the environmental conditions under which the mite could survive.

Cotton bolls infected with *N. oryzae* were stored for 15 and 27 months at room temperature in air-dry conditions, and 4 months at 0.5 to 1 C at 80-90%

relative humidity. A third set of bolls was left on the cotton plants in the field until 13 February 1969. Eleven to 54 infected locks from each treatment were incubated in moist chambers and examined as described earlier. *S. reniformis* emerged after incubation for 8-38 days from all but two of the locks, from the air-dry at room temperature treatment. Thus, these results show that the mite survived in cotton locks under the extreme conditions used in the laboratory as well as under the variety of climatic factors present in the field.

The cold-tolerant stage of *S. reniformis* was not determined, but previous research has shown that some members of the family Pymotidae can survive as eggs or as first-stage females (3). The incubation period in this experiment was 8-38 days. Eight days is about the same period of time required to produce one generation of mites at room temperature (22 C). This suggests that the mites survived as quiescent females, but further research must be done to verify this hypothesis.

Effect of S. reniformis on germination of N. oryzae spores.—*S. reniformis* requires *N. oryzae* for survival, but does the fungus require the mite? Large unopened cotton bolls together with 15-20 cm of the stem were harvested. Bracts of the involucre were removed; the bolls were washed in tap water and surface-sterilized with 50% ethyl alcohol. The stems were placed in water in large shell vials. Holes were cut into the locules on opposite sides of the boll with a sterile cork borer to expose the lint. The lint was inoculated in one of the following ways: (i) one mite carrying zero, one, or two spores; and (ii) one or two spores of *N. oryzae*. Controls were not inoculated. The hole in the locule was covered with a microbeaker (0.5 ml) immediately after each inoculation. The experiment was repeated, except that we exposed the lint by cutting slits in the carpel wall near the apex, and after inoculation the slits were left exposed to simulate natural boll opening. The bolls were incubated 14 days and checked for *N. oryzae* infection.

TABLE 2. *Nigrospora oryzae* infections in covered[a] and exposed cotton locks resulting from inoculation with *Siteroptes reniformis* carrying *N. oryzae* spores, and with spores alone

Treatment	Infected locks/total	
	Covered	Exposed
Mite with two spores	55/62	20/20
Mite with one spore	44/62	20/20
Mite with zero spores	3/64	0/24
Control (untreated)	0/52	0/14
No mite, one spore	43/50	11/28
No mite, two spores	32/50	16/30

[a] Lock was covered with a microbeaker immediately after inoculation.

One mite carrying one or two spores of *N. oryzae* inoculated a locule. When the locules were left

exposed, there were 54% more infections when the mite was present.

It therefore appears that the mite serves to transport and place *N. oryzae* spores in an environment favorable for germination and growth. Partially opened cotton bolls appear to provide these conditions, and their selection by mites is most likely accidental. However fortuitous the choice may be, this selection results in propagation of the fungus, which provides a food supply for the mite.

S. reniformis and its relationship to N. oryzae.—Most young female mites were observed carrying one or two spores of *N. oryzae* on their hysterosoma within a short time after exposure to the fungus. Critical observations were made on the development and behavior of *S. reniformis*, especially with regard to its relationship to *N. oryzae*.

The gravid female when it began to feed became immobile. Even though the hysterosoma of the female enlarged several hundred times before birth of the young, the front part of the body (gnathosoma and propodosoma) remained movable, allowing the mite several microns of lateral mobility which enabled her to feed on the nearby hyphae. The mouth parts of female mites move horizontally and are very pointed distally. Apparently *S. reniformis* punctured the hyphae and fed on the liquid contents, since no hyphal fragments were ever observed around the head. The feeding activity caused the fungus to branch profusely in the vicinity of the mites' head.

S. reniformis is viviparous; the eggs develop inside the body of the mother, and each young mite is enclosed in a membrane until it emerges as a young adult.

The length of time required to produce young was dependent on temperature. At 22-24 C from 7-10 days were required (Fig. 2). The number of offspring produced appeared to be dependent on the availability of food and space (under crowded conditions fewer young were produced). As the embryos enlarged, so did the body of the mother, and since the young mites are nearly full size at birth, the mother's body enlarged approximately one time for each mite produced. Initially, one mite at a time emerged, but as more mites matured, the body of the mother collapsed and became a mass of emerging young. Males were first to emerge. When females emerged, they mated one to several times, then left the vicinity of the mother. The females wandered for a short time, crawled over a cluster of spores, hesitated momentarily, and rapidly moved their fourth pair of legs. A dislodged spore was placed in a "sac" (1) on the ventral surface of the hysterosoma. If not successful, the process was repeated, often several times. If the instinct to acquire spores was not satisfied within a short period, the mites apparently gave up and wandered about without spores or with only one attached.

After spores had been attached or the stimulus to attach spores was lost or satisfied, the crawling pace of the mites quickened and they began to disperse, often leaving the *N. oryzae* colony. Females placed on a new fungus colony at this time crawled about

slowly until a suitable spot was found to begin feeding.

Spore deposition.—It was noted that partially enlarged female mites no longer carried spores. Whether the spores were expelled due to the increase in size of the hysterosoma or voluntarily discharged by the mite before it settled was not observed directly. Attempts to watch the discharge of spores by observation of mites carrying spores after being placed on agar in petri dishes were not successful. Indirect evidence that spores were released soon after the mite reached a suitable habitat was obtained in the following experiment. Locks from a large unopened cotton boll were aseptically removed and placed in sterile petri dishes, and water agar was added until only a small portion of cotton lint remained exposed. Six mites, each carrying two spores, were placed on the exposed lint in each of five petri dishes and covered with a microbeaker for 24 hr. During this time and periodically afterward, the surface of the lint was examined for mites and deposited spores. The locks were dissected and examined for developing mites and *N. oryzae* infections 3 days later.

Two pairs of spores were found on the surfaces of two locks within 15 min after placement of mites. They were spaced about the same distance apart as they had been on the mite. Also, a mite was recovered that was no longer carrying spores. When the locks were dissected, two mites were recovered, both without spores. Both mites were attached to a small clump of lint which had an incipient *N. oryzae* infection. These limited observations suggest that the mites deposited their spores soon after being placed on the locks and then fed on the mycelium that developed.

Spore-carrying structures of S. reniformis.—The "abdominal sacs" described by Alfaro (1) could not be resolved in observations of intact mites with the light microscope. Consequently, specimens of *S. reniformis* were prepared for sectioning and examination with the scanning electron microscope (SEM).

The micrograph (Fig. 1-c) shows that the spores enclosed in a ventral pouch that opens by a slit at the midpoint of the opisthosoma and shows the narrow slit of Fig. 1-c, is a pouch that opens (Fig. 1-d) on the ventral surface of the opisthosoma with spores inside. Thus, *S. reniformis* is well adapted morphologically for carrying *N. oryzae* spores. The structure of the pouch is such that it could accommodate spores varying several microns in size and shape, but only spores of *N. oryzae* were observed in it.

DISCUSSION.—When *Nigrospora* lint rot disease of cotton bolls was first reported and described by Houston & Garber (8), the mode of dispersal of the pathogen was not mentioned. It was reported, however, that the fungus could not penetrate the carpel wall, and that infections occurred only when the pathogen made direct contact with the immature lint in the locule. Their studies showed that inoculations had to be made into a locule while the fibers were still moist and under conditions of high ambient humidity for successful fungus colonization. This evidence suggested that infection occurred at an early stage of boll opening (8) or, in cases where the locules in the boll are not completely independent (2), that the pathogen could be introduced to the lint before the boll opened by means of the natural opening at the "blossom end". Studies on the *Nigrospora* lint rot disease in California during the last 3 years have shown a consistent association between *Siteroptes reniformis*- and *Nigrospora oryzae*-diseased cotton bolls. With evidence for the presence of the conidia of *N. oryzae* on this mite, plus successful transmission of the pathogen by the mite in the field, and in the greenhouse under controlled conditions, Leach's four rules for "insect" transmission (12) have been fulfilled. The results and observations derived from this study suggest that the present concepts of the epidemiology and control of diseases caused by *N. oryzae* in California should be altered because of the probability of a mite vector being necessary for spore dispersal.

Krantz (11) in 1957 synonymized the genus *Pediculopsis* Reuter 1907 with the genus *Siteroptes* Amerling 1861. Thus, *Siteroptes* mites, according to this classification, have been associated with fungus species of the genera *Fusarium* and *Nigrospora* for some time and are essentially worldwide in distribution (1, 4, 5, 13, 15). Several suggestions that a form of symbiosis exists between the mites and fungi were based entirely on studies of them in vector-fungus-host relationships (5, 15, 16). None, however, has reported investigations of the mite-fungus relationship separated from the host plant as in our present study, where the host plant was used only as a medium to facilitate the clarification of the mite-fungus interaction. We showed that *S. reniformis* is dependent on *N. oryzae* for normal growth and reproduction, and that *N. oryzae* is disseminated and its growth is increased by *S. reniformis. N. oryzae* and *S. reniformis* are also similar in their temperature requirements, and the mite can survive winter temperatures in diseased cotton bolls. Thus, these results, and the demonstration that *S. reniformis* is morphologically adapted to carry *N. oryzae* conidia, support the conclusion that a mutualistic type of symbiosis exists between *Siteroptes reniformis* and *Nigrospora oryzae.* They also indicate that Nigrospora lint rot in cotton and possibly other diseases caused by *N. oryzae* in California, such as Nigrospora stalk rot of sorghum and corn, occur primarily on host plants that provide wandering females of *S. reniformis* with environmental conditions required for their fungus culturing and reproduction. The incidence of diseases caused by *N. oryzae* in the field therefore may be more dependent on *S. reniformis* activity than on the presence of *N. oryzae* alone.

LITERATURE CITED

1. ALFARO, A. 1946. El Acaro Pediculopsis graminum Reut. y. el hongo Nigrospora oryzae (Berk. et B.) Petch, en asociacion parasitaria sobre Tigos aragoneses.

Bol. Pat. Veg. Entomol Agri., Madr. 16:321-334.

2. ASHWORTH, L. J., JR., D. C. HILDEBRAND, & M. N. SCHROTH. 1969. Erwinia-induced internal necrosis of immature cotton bolls. Phytopathology 59:1016 (Abstr.).

3. BAKER, E. W., & G. W. WHARTON. 1952. An introduction to acarology. Macmillan Co., New York. 465 p.

4. BONDAROVICH, M. Y. 1961. Nigrosporasis of cotton and its control. Zashch. Rast. Vrez. Bolez. Moskva. 6:27-28. (Abstr. Rev. Appl. Mycol. 41:306).

5. COOPER, K. W. 1940. Relation of Pediculopsis graminum and Fusarium poae to central bud rot of carnations. Phytopathology 30:853-859.

6. HALISKY, P. M., W. C. SCHNATHORST, & M. A. SHAGRUN. 1961. Severity and distribution of cotton boll rots as related to temperature. Phytopathology 51:501-505.

7. HANSFORD, C. G. 1929. Cotton diseases in Uganda 1926-1929. Empire Cotton Growing Rev. 1:10-26, 160-167, 240-245.

8. HOUSTON, B. R., & R. H. GARBER. 1959. A lint-rot of cotton in California caused by Nigrospora oryzae. Plant Dis. Reptr. Suppl. 259:233-235.

9. JACZEWSKI, A. A. 1929. Diseases of cotton fibres. J.

Microbiol. Inst. Bact. Pasteur (Leningr.) 9:159-167. (Abstr. Rev. Appl. Mycol. 9:307).

10. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., N.Y., London. 523 p.

11. KRANTZ, G. W. 1957. Dolichocybe keiferi, a new genus and new species of pyemotid mite with a description of a new species of Siteroptes (Acarina: Pyemotidae). Ann. Entomol. Soc. Amer. 50:259-264.

12. LEACH, J. G. 1940. Insect transmission of plant diseases. McGraw-Hill. New York. 615 p.

13. PETCH, T. 1924. Monotospora oryzae B. et Br. J. Indian Bot. Soc. 4:21-22.

14. PETERSON, A. 1959. Entomological techniques. How to work with insects. Edwards Bros., Inc., Ann Arbor, Mich. 435 p.

15. PETRI, L. 1927. Rassegna dei casi fitopatologici piu notevoli osservati nel 1926. Boll. R. Staz. Pat. Veg., VII, 1, p. 1-45.(Abstr. Rev. Appl. Mycol. 6:394-395).

16. PEYRONEL, B. 1950. Associazione muluatistica fra acari del genere Pediculopsis e taluni funghi parassiti delle piante. Atti. Accad. Torino, 84, 9 p. (Abstr. Rev. Appl. Mycol. 30:101).

17. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.