

1992

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Recommended Citation

Yang, X. B.; Tebeest, D. O.; and Moore, E. L. (1992) "Biological Vectors for the Dispersal of *Colletotrichum Gloeosporioides*," *Journal of the Arkansas Academy of Science*: Vol. 46 , Article 18.

Available at: <http://scholarworks.uark.edu/jaas/vol46/iss1/18>

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Journal of the Arkansas Academy of Science, Vol. 46 [1992], Aug. 18

BIOLOGICAL VECTORS FOR DISPERSAL OF *COLLETOTRICHUM GLOEOSPORIOIDES*

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ABSTRACT

Green treefrogs (*Hyla cinerea*) and grasshoppers (*Melanoplus differentialis* and *Conocephalus fasciatus*) commonly observed in Arkansas rice fields, are dispersal vectors for *Colletotrichum gloeosporioides* f. sp. *aeschynomens*, a causal agent of anthracnose of northern jointvetch. Treefrogs and grasshoppers captured from rice or soybean fields with diseased northern jointvetch were placed in containers in contact with healthy northern jointvetch plants. An average of 90% of northern jointvetch plants was infected by the pathogen with up to 10 lesions per plant using treefrog vectors. Experiments were done in the greenhouse on frog dispersal by monitoring disease development from a point source in closed rice-weed patches. Treefrogs dispersed the pathogen from the source plant to healthy plants resulting in 95% infection. In the field, grasshoppers were frequently observed feeding on anthracnose lesions. In six separate experiments, approximately 20% of grasshoppers collected from fields with diseased northern jointvetch transferred the disease after feeding or contacting healthy plants. By feeding pathogen-free grasshoppers on anthracnose lesions, we found that 66% of these grasshoppers transferred the disease to healthy plants. The grasshopper may be important in spreading the inoculum among weed patches. Green treefrogs appear to be efficient vectors of the disease because they preferred northern jointvetch plants as shelters.

INTRODUCTION

Colletotrichum gloeosporioides f. sp. *aeschynomene* (Penz) (CGA) incites an anthracnose of northern jointvetch (*Aeschynomene virainica* B.S.P.), a tall leguminous weed of rice and soybean fields in the Mississippi River Delta region. Collego, a commercially used mycoherbicide, was developed using this fungal pathogen. The ecology of this pathogen has been extensively studied as a model system for *Colletotrichum* species. Information on dispersal mechanisms for this pathogen is limited to physical vectors such as rain-splash studies (Templeton *et al*, 1979; Yang and TeBeest, 1991). Because species of *Colletotrichum* are important agents in weed biocontrol (TeBeest, 1990), understanding the dispersal of this fungus is important for development of mycoherbicides. Field observations suggest that the dispersal complex of CGA in rice fields consists of both physical and biological components. Grasshoppers have been observed feeding on anthracnose lesions of diseased northern jointvetch plants. A hypothesis that grasshoppers may be a vector of the pathogen was made as early as the 1970's (Templeton *et al*, 1979) but has not been tested experimentally. Green treefrogs (*Hyla cinerea* Schneider) are commonly observed in rice and soybean fields in the south, however, little is known about the role of amphibians spreading fungal pathogens, and no studies of pathogen dispersal by frogs were found. The objectives of this study were to determine if grasshoppers and green treefrogs can act as dispersal vectors of CGA and to determine the importance of these vectors to the development of disease epidemics in northern jointvetch.

MATERIALS AND METHODS

TREEFROG TRANSMISSION.

Frogs were captured near Stuttgart, Arkansas on four separate occasions during the 1991 rice growing season from 10 different rice fields infested with diseased northern jointvetch plants. In the first sampling, 15 and 18 frogs were caught from two patches of northern jointvetch. Twelve frogs per patch were caught for the second and third samplings. The fourth sampling was taken during the harvesting season from two rice fields. One of the two sampled fields did not contain northern jointvetch but was adjacent to a field infested with diseased northern jointvetch plants. In the field without northern jointvetch, the green treefrogs were captured from rice plants. Treefrogs from each sampling were returned to the laboratory in Fayetteville, AR in plastic

bags or plastic bottles on the same day. Each patch was considered as a sampling unit and all treefrogs from one patch were bulked as one sample.

In the laboratory, treefrogs from each patch were placed in glass containers 45 cm high x 22 cm diameter or plastic containers 45 cm high x 35 cm diameter for 24 hr. Each container had three or four pots of healthy northern jointvetch plants approximately 40 cm tall. The number of plants per container varied for different experiments (Table 1). After

Table 1. Infection of northern jointvetch plants after contact with green treefrogs (*Hyla cinerea*) from rice fields infested with northern jointvetch plants infected by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*.

| Experiment (mon/day) | Field* | Patch | Number of frogs | Number of plants | Infected plants (%) | Lesions/plant |
|----------------------|--------|-------|-----------------|------------------|---------------------|---------------|
| 1 (06/08) | Com 1 | 1 | 15 | 14 | 100 | 6.4 |
| | | 2 | 18 | 14 | 100 | 10.1 |
| 2 (08/29) | Exp 1 | 1 | 12 | 13 | 85 | 1.7 |
| | | 2 | 12 | 8 | 25 | 1.0 |
| | | 3 | 12 | 10 | 100 | 3.8 |
| 3 (09/06) | Com 1 | 1 | 12 | 10 | 100 | 5.7 |
| | | 2 | 12 | 12 | 62 | 1.6 |
| | | 3 | 12 | 11 | 91 | 2.5 |
| | Exp 1 | 1 | 12 | 15 | 100 | 9.7 |
| | | 2 | 12 | 15 | 100 | 5.1 |
| | | 3 | 12 | 15 | 100 | 2.1 |
| | | 4 | 12 | 15 | 100 | 3.5 |
| 4 (10/06) | Exp 4* | 1 | 10 | 7 | 57 | 1.0 |
| | | 2 | 10 | 9 | 78 | 1.8 |
| | | 3 | 10 | 9 | 78 | 1.8 |

* Com = commercial fields, Exp = experimental fields.

† This field was adjacent to a field with diseased northern jointvetch plants.

‡ Sample was a mixture from two fields.

24 hr, frogs were removed and plants were placed in a dark dew chamber at 28 C for 24 hr. A control treatment in which plants were not placed in contact with treefrogs was included for each test. After incubating inoculated plants in growth chambers at 28 C for 5 days, the number of infected plants and number of lesions/plant were determined for each sampling unit.

To determine if frogs vectored the inoculum of *C. gloeosporioides* f. sp. *aeschynomene* from plant to plant, simulated rice-weed patches were assembled in a greenhouse. Each patch was enclosed with screen in a frame 122 x 81 x 100 cm with the bottom of each frame containing a water reservoir 2 cm deep with a surface area of 76 x 115 cm. Twenty-

four rice plants at heading stage were transplanted into each frame. The average number of tillers per rice plant was 14 and there were 336 rice tillers per patch. Ten healthy northern jointvetch plants taller than the rice plants were evenly distributed in each rice patch and a diseased northern jointvetch plant with 5 to 7 anthracnose lesions was placed at the center. Three treatments, each with 2 replicates, consisting of 10 frogs/patch, 2 frogs/patch, and 0 frog/patch were established. Test frogs were placed in a dew chamber at 28 C for 24 hr prior to their use, to rid them of residual spores. Temperature in the greenhouse was maintained at 25 C and free moisture was provided every two days using humidifiers and by covering the frame with plastic sheeting. Treefrogs were fed commercial crickets every two days. The number of diseased plants, killed plants, and lesions/plant was counted twice for each patch during the test.

To quantify green treefrog movement and shelter selection, the number of frogs sitting on the 336 rice tillers, on the 10 northern jointvetch plants, or on the screen of the frame were counted two to four times per day from 8 AM to 8 PM only in the 10 frog/patch treatment. A total of 56 observations was recorded. These observations were then averaged by counting the number of treefrogs on rice or on northern jointvetch plants and plotting these against time.

GRASSHOPPER TRANSMISSION.

Two different experiments were performed to determine grasshopper transmission. The first experiment was to determine if the pathogen was carried by grasshoppers in rice or soybean fields infested with diseased northern jointvetch plants. From the previously mentioned commercial rice or soybean fields, shorthorn (*Melanoplus differentialis*) and longhorn (*Neoconocephalus crepitans* and *Conocephalus fasciatus*) grasshoppers were captured with an insect net during the growing season. Grasshoppers were returned to the lab the same day and each grasshopper was placed for 24 hr in a test chamber constructed of a transparent plastic bottle (10 cm in diameter and 22 cm in height) which contained a healthy northern jointvetch plant approximately 3 weeks old. Chambers were then placed under a light bench or in a growth chamber for 24 hr. Five insect-free test chambers were used as controls. The plants were next moved into a dew chamber at 28 C for 24 hr to induce infection and then kept in a growth chamber at 28 C for four days. Lesions on each test plant were counted and grasshopper feeding marks were also noted. The experiment was repeated seven times during the growing season. Grasshopper sampling size at each replication varied, depending on the moisture condition on the sampling day. In the second experiment, grasshoppers were caught from the Fayetteville area of northwestern Arkansas where northern jointvetch, and the disease has not been reported to occur. Grasshoppers were fed wheat seedlings for one to two days before each test. Each grasshopper was put into a glass tube (4 cm in diameter and 30 cm in height) containing a 2 cm stem segment of northern jointvetch bearing a lesion caused by CGA. After insects were exposed to the lesion for 24 hr, each grasshopper was moved into a test chamber as described above. Healthy plants then received the same treatments as the first experiment. Numbers of infected plants and lesions per plant were recorded. Two control treatments were also set up for each replicate. In the first control treatment, ten (10) healthy northern jointvetch plants were treated only with 24 hr dew. In the second control treatment, ten healthy plants were placed in contact with insects which had been fed on healthy stem segments, the plants were then provided 24 hr dew at 28 C. The experiment was repeated two times.

RESULT

TREEFROG TRANSMISSION.

In four separate experiments, healthy northern jointvetch plants were infected by *C. gloeosporioides f. sp. aeshynomene* after coming in contact with frogs collected from 16 diseased northern jointvetch patches in 10 different rice fields (Table 1). Percentages of plants infected after contact with frogs ranged from 25 to 100%, with greater than 90% infection for most patches. No infection of control plants was observed in the four tests. The number of lesions/plant ranged from 1 to 10. Plants placed in contact with frogs sampled from a field without northern jointvetch plants in October were also infected.

C. gloeosporioides f. sp. aeshynomene was dispersed among healthy plants by treefrogs in the simulated rice-weed patch experiment after the introduction of diseased northern jointvetch (Table 2). New disease lesions were observed during the first experiment six days after diseased plants were introduced. In the second experiment, infected plants were observed eight days after the introduction of source plants. An average of 95% plants were infected within sixteen days of the introduction of source plants. An average of 4.5 and 5.5 northern jointvetch plants were killed in the first and second experiments for the 10-frog treatment. There were noticeable differences in lesions/plant between 2-frog and 10-frog treatments. No infected plants were observed in the treatment without frogs.

Table 2. Results of dispersal experiments of *Collectotrichum gloeosporioides f. sp. aeshynomene* by green treefrogs (*Hyla cinerea*) in simulated rice-weed patches, as indicated by the number of diseased northern jointvetch plants.

| Frogs/ patch | 1st observation* | | | 2nd observation | | |
|---------------------|------------------------|------------------|-------------------|--------------------|------------------|-------------------|
| | Plants infected | Plants killed | Lesions/ Plant | Plants infected | Plants killed | Lesions/ plant |
| Experiment 1 | | | | | | |
| 0 frog | 0.0 ± 0.0 ^b | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 2 frogs | 2.0 ± 1.4 | 0.0 ± 0.0 | 1.0 ± 0.0 | 7.0 ± 0.0 | 3.0 ± 0.0 | 1.7 ± 0.0 |
| 10 frogs | 2.0 ± 0.0 | 0.0 ± 0.0 | 4.0 ± 0.2 | 9.5 ± 0.7 | 4.5 ± 0.7 | 4.5 ± 2.1 |
| Experiment 2 | | | | | | |
| 0 frog | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 2 frogs | 2.0 ± 0.0 | 0.0 ± 0.0 | 1.3 ± 0.4 | 6.0 ± 0.7 | 2.5 ± 0.7 | 1.7 ± 0.0 |
| 10 frogs | 5.0 ± 1.4 | 1.0 ± 0.0 | 1.5 ± 0.1 | 9.5 ± 0.7 | 5.5 ± 0.7 | 5.2 ± 1.6 |

* Time of first and second observations was 8 and 16 days after introduction of source inoculum for experiment 1, and 10 and 18 days for experiment 2.
^b Mean values and standard error.

Green treefrogs were observed in rice fields in May when rice was planted. In the middle of the growing season, after the rice flowered and northern jointvetch plants were taller than rice, large numbers of treefrogs were observed. Treefrogs were often observed on the upper parts of northern jointvetch on clear days; however, during the early morning or on windy days, treefrogs were more frequently observed on lower parts of northern jointvetch plants beneath the rice canopy. Treefrogs were frequently observed sitting on anthracnose lesions on northern jointvetch plants above or inside the rice canopy (Fig. 1), especially later in the growing season when disease incidence was high.



Figure 1. A green treefrog (*Hyla cinerea*) sitting on an anthracnose lesion caused by *Collectotrichum gloeosporioides f. sp. aeshynomene* on a stem of northern jointvetch in a rice field.

The behavior of treefrogs in simulated rice-weed patches in a greenhouse appeared consistent with field observations. Frogs usually sat motionless on upper parts of northern jointvetch plants with their abdomens firmly in contact with the plant stem and appeared to prefer northern jointvetch plants as shelters with more than 80% of frogs observed on the northern jointvetch plants as compared to rice (Fig. 2). Most disease lesions were found on the upper portions of the stems.

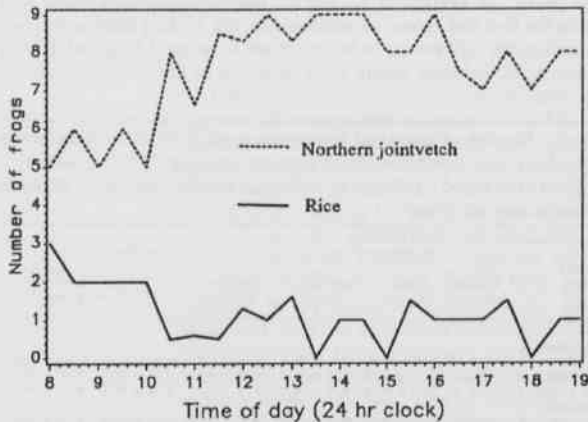


Figure 2. Results of shelter selection by green treefrogs (*Hyla cinerea*) in simulated rice-weed patches. Numbers of green treefrogs (*Hyla cinerea*) sitting on 336 rice tillers or on 10 northern jointvetch plants during day hours in simulated rice-weed patches.

GRASSHOPPER TRANSMISSION.

In rice fields, wounds caused by grasshoppers were frequently observed on northern jointvetch plants around the anthracnose lesions. In the first two experiments, approximately 10 *Neoconocephalus crepitans*, the longhorn grasshopper, were tested, but the insects did not feed on northern jointvetch stems. This grasshopper species was not used in later experiments. Grasshoppers transmitting *C. gloeosporioides* in these experiments were the longhorn meadow grasshopper (*Conocephalus fasciatus*) and the differential grasshopper (*Melanoplus differentialis*) which fed on northern jointvetch. Lesions appeared within the wound area of a stem three to four days after insect wounding. Occasionally, lesions were observed on part of a stem where no insect feeding wounds were noted. Among the five experiments in which grasshoppers were collected from rice, there was an increasing trend of disease with an average incidence of 22%. For the last experiment, a high incidence of 40% was found using grasshoppers obtained from one soybean field. In the experiment where grasshoppers acquired the inoculum by feeding on lesions, the average incidence was 70%.

DISCUSSION

Our studies revealed that both grasshoppers and green treefrogs are potentially important dispersal vectors of *C. gloeosporioides f. sp. aeschynomene* in rice fields. These vectors transfer a considerable amount of inoculum based on infection results (Tables 1 and 3). Treefrogs moved the pathogen from plant to plant (Table 2) and preferred northern jointvetch plants to rice as shelters. This is the first report of frogs as a vector of plant fungal pathogens.

Insects have been found to be major vectors in some plant pathosystems. However, the significance of grasshoppers in the studied pathosystem is not clear. Several factors may influence the importance of grasshoppers. Grasshopper populations vary from year to year, resulting in the variation of vector numbers. Importance is also determined by preference of grasshoppers to feed on disease lesions compared to healthy plant areas. If there is no preference, the chance of grasshoppers acquiring the inoculum will be a linear function of disease incidence. On

Table 3. Infections of plants facilitated by either grasshoppers fed with diseased lesions or grasshoppers from rice fields infested with northern jointvetch infected by *Colletotrichum gloeosporioides f. sp. aeschynomene*.

| Test | Field | Grasshoppers tested | Infected plants (%) | Lesions/plant |
|-------------------|-----------|---------------------|---------------------|---------------|
| From field | | | | |
| 21/07 | Brace | 20 | 15 | 1 |
| 31/07 | Brace | 27 | 20 | 1 |
| 14/08 | Brace | 12 | 17 | 2 |
| 28/08 | Brace | 4 | 25 | 1 |
| 06/09 | Yang | 9 | 33 | 1.3 |
| 27/09 | Soybean 1 | 7 | 14 | 2 |
| | Soybean 2 | 9 | 11 | 1 |
| | Soybean 3 | 10 | 40 | 1 |
| Lab test | | | | |
| | test 1 | 20 | 65 | 1.6 |
| | test 2 | 16 | 75 | 3.8 |

the other hand, if grasshoppers actively search for disease lesions, potential significance of this vector would be much greater. A future study of feeding preferences of the various species of grasshoppers toward diseased and healthy tissue is needed.

Green treefrogs may be efficient vectors in this pathosystem because of their behavior. Ecological studies (Dulleman and Trueb, 1986; Mattison, 1987; Wright and Wright, 1942) as well as the present data indicate that treefrogs prefer tall plants as shelters. This behavior prevents attacks from snakes and fish (Garton and Brandon, 1975; Wright and Wright, 1942) and provides better vision for predation (Freed, 1980). Northern jointvetch is one of several taller weeds in rice, and an infested rice field can be dominated by this weed. Because green treefrogs selectively seek northern jointvetch as shelters, the density of the frogs/m² may be concentrated in the weed patch compared to other parts of a rice field. In the weed patch, the chance of moving the inoculum from a diseased plant to a neighboring healthy plant is very high and should result in target-specific horizontal movement of inocula. It has been observed that disease lesions on northern jointvetch plants in rice fields are in positions taller than rice. This may be because of frog movement and because the upper parts of plants are more susceptible. In the field, direct contact with lesions may not be necessary for treefrogs to obtain fungal inoculum. Because CGA produces large numbers of conidia (Templeton *et al.*, 1979), large areas of the lower stem can become contaminated when rain washes these spores down from upper lesions. As the treefrogs move up and down the plants, the chance of acquiring the inoculum increases. Furthermore, vertical movement of frogs observed in our study as well as others (Dulleman and Trueb, 1986) provides a means of vertical dispersal moving inocula from lower to upper plant parts if the initial infection by seedborne (TeBeest *et al.*, 1992) or rain-splash inoculum (Yang and TeBeest, 1991) occurs at the base of the plant.

The finding that grasshoppers and treefrogs transmit *C. gloeosporioides f. sp. aeschynomene* suggests a role of biological vectors in the formation of spatial patterns of plant diseases. Because northern jointvetch is distributed as patches in rice (Yang unpublished), rain dispersal is not as likely to move the pathogen from one patch to another because the maximum dispersal distance in rice is only 1.5 m in a single rain episode (Yang and TeBeest, 1991). However, grasshoppers are capable of flying from patch to patch, and it is known that frogs migrate as far as several hundred meters during reproduction or when food is scarce (Dulleman and Trueb, 1986). Such long distance movement of these vectors may provide a dispersal mechanism from patch to patch.

Grasshoppers and treefrogs may be important factors to consider in biological risk assessment of mycoherbicides. Many species of *Colletotrichum* have been studied as potential mycoherbicides and genetic-engineering techniques are being investigated to enhance their efficacy (TeBeest, 1990). Eventually these engineered organisms will be subjected to field tests, and the presence of these vectors in test plots may increase the chances of unwanted dispersal. The distance of grasshopper movement is far greater than other physical dispersal mechanisms, which increases the risk level. Furthermore, shelter selection by treefrogs can

direct the movement of pathogens, and may increase the chance of gene exchange between different fungi. For example, mating has been observed between strains of *C. gloeosporioides* f. sp. *aeschynomene* and *C. aloesporioides* f. sp. *jussiaeae*, two related fungi infecting northern jointvetch and winged water primrose, respectively (TeBeest *et al.* unpublished). These two plants are both tall weeds in rice fields and are favorite shelters for treefrogs. The fact that these two weeds are frog shelters may greatly increase the chance of contact and gene exchange between the above pathogens.

ACKNOWLEDGMENT

We thank Dr. R.J. Smith Jr. for assistance and Drs. R. Cartwright and C. Cisar for reviewing the manuscript.

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