Role of Insect Injury and Powdery Mildew in the Epidemiology of the Gummy Stem Blight Disease of Cucurbits

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ABSTRACT

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Observation of recent epidemics of gummy stem blight, caused by *Mycosphaerella melonis*, on cucurbits indicated a relationship with high incidence of either cucumber beetle feeding or powdery mildew. In two field experiments, gummy stem blight was reduced significantly in plots that were caged to exclude insects. In addition to predisposing plants to infection, the striped cucumber beetle (*Acalymma vittatum*) served as a vector of the causal fungus. In laboratory experiments, both powdery mildew and melon aphids markedly predisposed cucurbit leaves to *M. melonis*. Filtered, aqueous washings of mildewed leaves and aphid-infested leaves provided the same level of disease stimulation as the organisms themselves. Uninjured cucurbit leaves were virtually immune to unamended *M. melonis*, but they were susceptible when inoculum was amended with 0.1% sucrose and 0.05% hydrolyzed casein. Late-summar foliar necrosis of cucurbits in Kentucky may be caused by the interaction of powdery mildew, insects, and *M. melonis*.

Additional key words: *Aphis gossypii, Didymella bryoniae*

Gummy stem blight, caused by the fungus *Mycosphaerella melonis* (Pass.) Chiu & Walker, is one of the most serious diseases of cucurbits around the world (2,5,6,9–11,15–17). Symptoms may develop on any plant part including stems, fruits, and foliage (5,6,9–11,15–17). Between 1978 and 1980, we observed epidemics of this disease on cucumber and muskmelon in areas where cucurbits had been grown continuously for a number of years. The appearance of symptoms was not random; gummy stem blight was most severe in areas where there was also extensive injury from cucumber beetles (*Diabrotica undecimpunctata howardii* Barber and *Acalymma vittatum* Fabricius) or severe infection with powdery mildew (*Erysiphe cichoracearum* DC. or *Sphaerotheca fuliginea* (Schlect.) Poll.). It is a characteristic of this disease that uninjured, young cucurbit tissues are resistant to infection by *M. melonis* but that mechanically injured tissues rapidly develop a watery rot following inoculation (5,11,15,16).

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The present study was conducted to determine whether the specific types of injury provided by insect feeding and powdery mildew infection predispose cucumber and muskmelon to gummy stem blight in the field.

MATERIALS AND METHODS

Culture of pathogen and hosts. An isolate of *M. melonis* was obtained from muskmelon leaves showing symptoms of gummy stem blight and was maintained on green bean agar at 24 C in the dark. To ensure minimal change in virulence, the fungus was periodically inoculated onto cucumber and reisolated. Conidial suspensions were prepared by scraping spores from the surface of 5- to 9-day-old cultures, suspending them in distilled water, and filtering them through four layers of cheesecloth to remove mycelial fragments. Spore concentrations were determined with a hemacytometer, and inoculum densities were adjusted with water to give suspensions of 10^6 conidia per milliliter.

Susceptible cucumbers (*Cucumis sativus* L. 'Straight-8' and 'Wisconsin SMR-58') and muskmelons (*C. melo* L. 'Iroquois' and 'Mainstream') were grown in 10-cm plastic pots containing Pro-Mix Bx (Premier Peat Moss Co., New York, NY 10036) in a greenhouse at 23–31 C under daylight supplemented with fluorescent light. Plants were fertilized twice weekly with 250 ml of a 4.6 g/L solution of 23-19-17 fertilizer (Ra-Pid-Gro, Dansville, NY 14437).

Beetle predisposition experiments. Iroquois and Mainstream muskmelons as well as Straight-8 and SMR-58 cucumbers were set out in the field in areas infested with cucumber beetles, and beetles were allowed to feed on them for 2–4 hr. Control plants were set out in insect-proof cages or were left in the greenhouse. Plants were brought back to the greenhouse and were immediately sprayed with *M. melonis* conidial suspensions until runoff. Plants were incubated in closed moisture chambers for 24 hr, followed by 24 hr in partially open chambers. A few plants in each group were inoculated with spore suspensions that were amended to give final concentrations of 0.1% sucrose and 0.05% hydrolyzed casein (Sigma Chemical Co., St. Louis, MO 63178), a modification of the procedure of Svedelius and Unestam (15).

Field experiments. Two experiments in which cucumber beetles were excluded from cucumber plants by cages placed over the plots were done in 1980 at the University of Kentucky South Farm at Lexington. Wooden frame cages (85 × 85 × 170 cm) were covered with aluminum window screening stapled onto the wood. Edges were sealed with enamel paint, and soil was shoveled around the base of each cage to prevent entrance of insects. Soil was fumigated with methyl bromide and covered with plastic 1 mo prior to experimentation in order to kill overwintered adult beetles.

Table 1. Effect of insect exclusion on the development of gummy stem blight on Straight-8 cucumber in the field (1980)

	Severity of gummy stem blight[y]		
Treatment[x]	15 Aug.	4 Sept.	18 Sept.
Uncaged	1.3 a	5.0 a	5.0 a
Caged	0.2 b	1.7 b	5.0 a

[x]Wooden frames (85 × 85 × 170 cm) were covered with aluminum window screening (caged plots) or with wide nylon mesh (uncaged plots). Plants were inoculated with *Mycosphaerella melonis* on 4 August.

[y]0 = no infection, 5 = completely necrotic. Means were based on six plots with 10 plants per plot. Means in the same column followed by the same letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

In the first experiment (Table 1), Straight-8 cucumber plants were transplanted to the field at the three-leaf stage on 21 July. There were six screened plots and six unscreened control (frames covered with wide mesh to simulate shading effect) plots in a completely randomized design with 10 plants per plot. All plants were sprayed with *M. melonis* conidial suspension on 4 August. Very little disease was evident the week following inoculation. Plants were evaluated on 15 August and 4 and 18 September on a 0–5 scale (0 = no infection, 5 = completely necrotic plants) for severity of gummy stem blight.

In a second experiment (Table 2), SMR-58 cucumbers were transplanted on 24 September, inoculated on 1 October, and rated on 15 October. There were three uncaged and six caged plots. Plants in three of the caged plots were inoculated with spores amended with nutrients. After inoculation, plants were covered with plastic for 24 hr to maintain high relative humidity. After the final rating, pieces of stem were cut from each of the plants in the uncaged plots. Stem pieces were surface sterilized in 0.5% sodium hypochlorite for 5 min, rinsed in sterile distilled water, and plated on nutrient agar plus 1% dextrose to check for the presence of *Erwinia tracheiphila* (E. F. Smith) Bergey et al. Suspect colonies were inoculated into cucumber plants to check for bacterial wilt development.

Tests of beetle transmission. Striped cucumber beetles were collected from the field and maintained on cucumbers in the laboratory. The ability of striped beetles to transmit *M. melonis* was tested on three different occasions. Beetles were allowed to feed overnight on plants showing gummy stem blight symptoms. Twenty beetles were then transferred to a small open vial that was placed inside of a closed plastic humidity chamber containing six healthy SMR-58 cucumber plants that had been misted with water. A similar chamber of plants without beetles was used as a control.

Table 2. Effect of insect exclusion on the development of gummy stem blight on SMR-58 cucumbers in the field (1980)

Treatment[x]	Severity of gummy stem blight[y]
Uncaged	4.7 a
Caged + nutrients[z]	2.0 b
Caged	1.3 b

[x]Wooden frames (85 × 85 × 170 cm) were covered with aluminum window screening (caged plots) or with wide nylon mesh (uncaged plots).

[y]0 = no infection, 5 = completely necrotic. Means were based on three plots with 10 plants per treatment. Plants were inoculated with *Mycosphaerella melonis* conidia on 1 October and rated on 15 October 1980. Means followed by the same letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

[z]0.1% sucrose and 0.05% hydrolyzed casein added to *M. melonis* inoculum.

Leaf disk inoculation. Leaf disks 14 mm in diameter were cut with a cork borer from fully expanded leaf three of SMR-58 cucumber and Mainstream muskmelon plants. Ten disks (one from each of 10 plants) were placed with the adaxial surface up on plates of 2% water agar. Each disk was inoculated with a 10-μl droplet of *M. melonis* conidial suspension. In addition to disks from healthy leaves, some disks were cut from leaves that had colonies of melon aphids (*Aphis gossypii* Glover) or that were infected with powdery mildew. Aphid colonies were maintained on cucumber. Powdery mildew inoculations were made by dusting leaves with conidia from cucumber (sexual stage not identified). Some disks were then inoculated with unamended *M. melonis* spores, whereas others were inoculated with spores amended with 0.1% sucrose and 0.05% hydrolyzed casein.

In some treatments, 10 μl of washings from SMR-58 cucumber leaves that were either healthy, infested with aphids, or infected with powdery mildew were placed on the disks and allowed to dry to a film before inoculation. To prepare washings, eight fully expanded leaves were washed with 80 ml of distilled water plus one drop of Tween 20. Washes were filtered through 0.22-μm pore size filters (Millipore Corporation, Bedford, MA 01730) and were concentrated to 20 ml on a rotary evaporator. Tween 20 (one drop per 100 ml) was added to inoculum placed on mildewed leaf disks to enhance wetting of the leaf surface. In a separate experiment, Tween 20 was found to have no effect on disease expression.

After inoculation, plates of disks were placed in clear plastic boxes lined with moist germination paper and were incubated for 4 days at 24 C under 10,000 lux fluorescent light (14-hr photoperiod beginning with dark). Gummy stem blight developed as a necrotic soft rot in leaf disks, and the diameters of the necrotic lesions were measured in millimeters.

RESULTS

Beetle predisposition experiments. When plants injured by beetles were inoculated with *M. melonis*, they developed typical gummy stem blight symptoms within 3 days. Furthermore, lesions developed exclusively around sites of injury (Fig. 1D). Uninjured control plants did not develop disease except in a few plants where guttation fluids accumulated at the leaf margins. When uninjured cucurbits were inoculated with *M. melonis* spores amended with nutrients, they developed disease similar to the injured plants inoculated with unamended spores. SMR-58 cucumber plants infested with melon aphids were also susceptible to unamended *M. melonis* inocula, as were plants infected with powdery mildew.

Field experiments. In the first experiment, gummy stem blight developed much slower in the caged as compared with the uncaged plants (Table 1). By 4 September, all the uncaged plants were killed, whereas the caged plots were green and vigorous although infected with powdery mildew (Fig. 1E). The caged plants were dead by 18 September.

In a second experiment conducted with young cucumber plants, caged plants again developed significantly less gummy stem blight than did uncaged plants (Table 2). However, addition of nutrients to the inoculum did not significantly increase disease in the caged plots (Table 2). Necrosis was caused by *M. melonis* rather than by *Erwinia tracheiphila*. Pycnidia and spores of *M. melonis* were found on all plants from uncaged plots, and *E. tracheiphila* was not isolated from any plants.

Tests of beetle transmission. In three experiments, striped cucumber beetles transmitted *M. melonis* from infected cucumber plants to two, three, and five of six healthy cucumbers, respectively, as judged by symptom development 3 days after exposure to beetles. Symptoms subsequently appeared on all test plants within a week after introduction of beetles. No transmission was observed when beetles were fed for 3 hr on healthy leaves between transfer from infected leaves and enclosure with test plants. Transmission, therefore, appeared to be in a nonpersistent manner.

Leaf disk inoculations. Injury and nutrient predisposition to *M. melonis* seen in cucurbit plants were also expressed in cucurbit leaf disks (Table 3). Cucumber and muskmelon leaf disks were virtually immune to unamended *M. melonis*. However, when disks were taken from leaves infested with aphids or infected with powdery mildew, inoculation with unamended *M. melonis* resulted in rapid development of necrosis and soft rot. Exogenous nutrients (sucrose and hydrolyzed casein) stimulated disease in uninjured disks (Table 3). Washings from mildewed leaves and honeydew washed from aphid-infested leaves stimulated gummy stem blight development to the same extent as powdery mildew and aphids did. Washings from healthy leaves did not stimulate the disease.

DISCUSSION

Uninjured, young cucurbit leaves are very resistant to *M. melonis* spores, but infection occurs when cucurbit tissues are mechanically injured (5,11,15,16). Insects and other pathogens may injure cucurbits in such a way as to predispose them to *M. melonis*. For example, Grossenbacher (10) reported that outbreaks of gummy stem blight in 1907 on greenhouse-grown cucurbits were directly preceded by attacks of red spider mites (*Tetranychus telarius* L.). We have consistently isolated the pycnidial and perithecial stages of *M. melonis* from lesions on cucurbit leaves and stems in close association with either cucumber beetle feeding wounds or with powdery mildew infections. It has yet to be determined whether insect injury on cucumber fruits (Fig. 1F) plays an important role in predisposition of fruits to black rot caused by *M. melonis*. Exclusion of beetles from cucumbers in the field reduced the severity of gummy stem blight until after powdery mildew appeared (Tables 1 and 2). We found that cucumber beetles, melon aphids, and powdery mildew all effectively predisposed cucumber and muskmelon to *M. melonis*.

The role of injury in predisposition goes beyond providing an infection court for the fungus because uninjured tissues are infected when exogenous nutrients are present (5,15; Table 3). Chiu and Walker (5) found that spore germination was greatly stimulated by plant extracts (orange and cucurbit). Svedelius and Unestam (15) found little effect on spore germination but great stimulation of hyphal growth by casein hydrolysate and sucrose. When nutrients were sprayed onto leaves and allowed to dry prior to inoculation with *M. melonis*, there was great stimulation of disease (15). Wounding may enhance disease by releasing nutrients to the fungus. Aphid honeydew, for instance, is rich in sugars and other phloem constituents (7). Even in uninjured tissues, *M. melonis* often infects the leaf margins where guttation fluids collect (15). Guttation is known to enhance infection by other fungi (18).

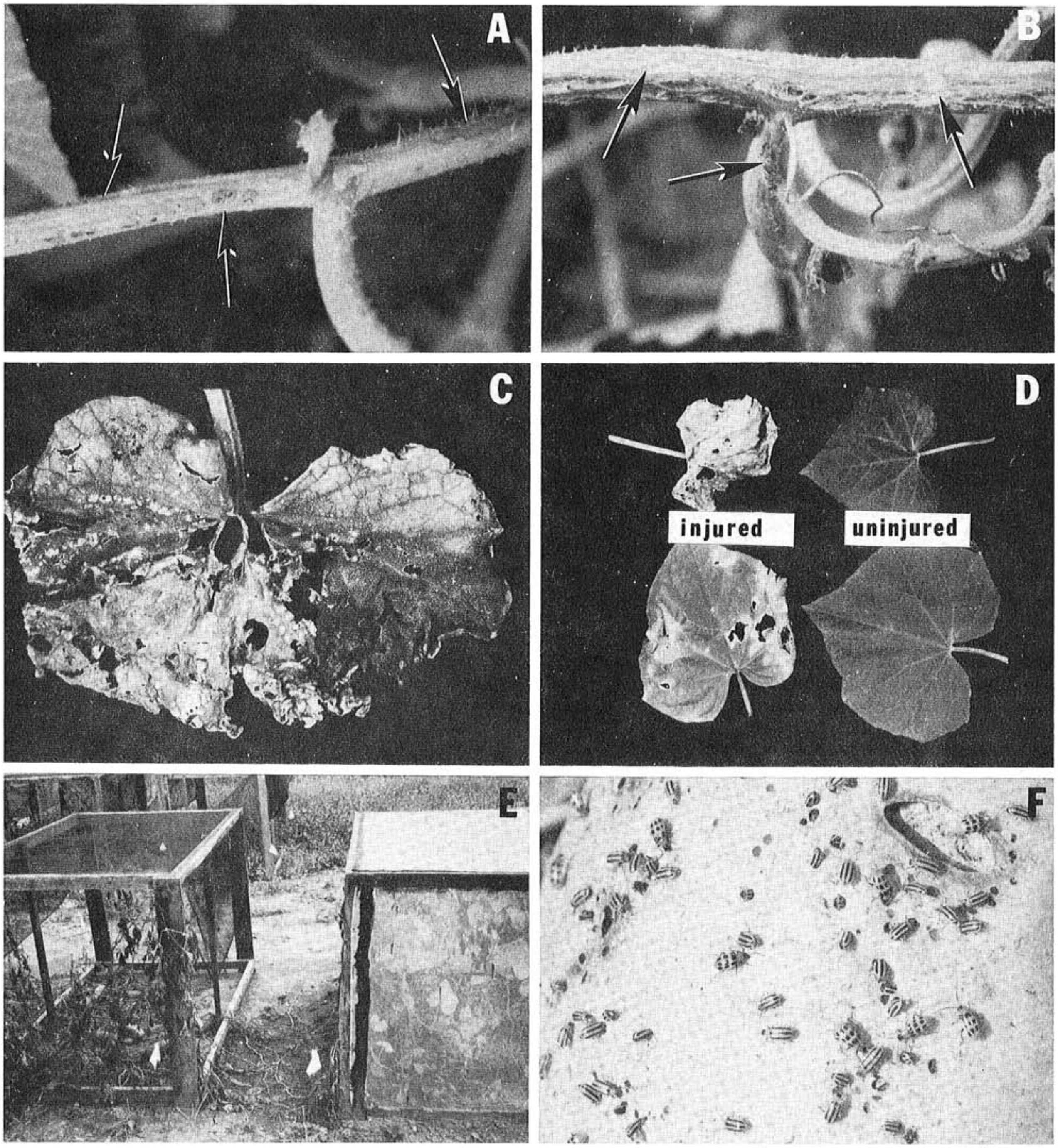

Fig. 1. Association of cucumber beetles with gummy stem blight of cucurbits: **(A)** Muskmelon stem showing feeding wounds (arrows) from cucumber beetles. **(B)** Gummy stem blight symptoms (stem-cracking, gummosis, soft rot) in association with beetle injuries (arrows) on stem and branch of muskmelon. **(C)** Cucumber leaf naturally infected with *Mycosphaerella melonis* in association with beetle feeding wounds. **(D)** Beetle-injured and uninjured cucumber leaves 4 days after inoculation with *M. melonis*. **(E)** Caged and uncaged cucumbers 1 mo after inoculation with *M. melonis* (data in Table 1). **(F)** Striped and spotted cucumber beetles feeding on the rind of a pumpkin fruit.

Table 3. Effects of aphids, powdery mildew, and nutrients on development of gummy stem blight in cucumber (SMR-58) and muskmelon (Mainstream) leaf disks 4 days after inoculation with *Mycosphaerella melonis* conidia

	Diameter of necrotic lesions (mm)	
Factors on leaf disks	**Cucumber**	**Muskmelon**
Nutrients alone	0 ± 0[a]	0 ± 0
Spores alone	0 ± 0	0 ± 0
Spores + nutrients[b]	12.0 ± 0.8	11.6 ± 0
Aphids alone	0 ± 0	0 ± 0
Spores + aphids	14.0 ± 0	14.0 ± 0
Powdery mildew alone	0 ± 0	...[c]
Spores + mildew	14.0 ± 0	...[c]
Spores + healthy leaf wash[d]	1.6 ± 0.8	0.5 ± 0.5
Spores + aphid-leaf wash[d]	13.7 ± 0.3	14.0 ± 0
Spores + mildew-leaf wash[d]	13.8 ± 0.2	13.4 ± 0.6

[a] Mean + standard error based on three separate experiments with one leaf disk from each of 10 plants in each experiment.
[b] Nutrients were 0.1% sucrose and 0.05% hydrolyzed casein.
[c] Mainstream muskmelon is resistant to powdery mildew.
[d] Eight fully expanded SMR-58 cucumber leaves that were either healthy, aphid infested, or powdery mildew infected were washed with 80 ml of distilled water (plus one drop of Tween 20). Washes were filtered through 0.22-μm Millipore filters and concentrated to 20 ml.

Nutrients may serve directly as substrates for metabolism as in the case of sooty blotch fungi (1,4), or they may modify the effects of inhibitory compounds on the host surface. Induced susceptibility by powdery mildews is known in muskmelon and other plants (13).

Chopping of cucurbit cotyledons also stimulated disease by releasing volatile plant compounds that enhanced the growth of *M. melonis* (V. L. Pharis, T. R. Kemp, and D. E. Knavel, *personal communication*). Insect injury and powdery mildew presumably also induce release of such volatiles from cucurbit tissues.

In addition to predisposing plants to infection, the striped cucumber beetle can be a vector of *M. melonis*. The actual importance of this is yet to be determined, but cucumber beetles may play a predominant role in the epidemiology of several cucurbit diseases. Cucumber beetles have previously been shown to transmit *Colletotrichum lagenarium* (Pass.) Ell. & Halst. (12), *Erwinia tracheiphila* (14), *Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner (3), and *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow (8).

Sudden development of foliar necrosis is often observed on cucurbits in central Kentucky in late summer prior to plant senescence. *M. melonis* and powdery mildew as well as extensive insect injury are consistently found in conjunction with this necrosis. We suggest that late-summar foliar necrosis in Kentucky is caused by the interaction of insects, powdery mildew, and *M. melonis*. Because injury and powdery mildew play an important role in the development of gummy stem blight in the field, these factors need to be taken into consideration in the selection of resistant varieties of cucurbits in breeding programs. Furthermore, incorporation of insecticides and powdery mildewcides into control strategies for gummy stem blight may be more successful than the use of protectant fungicides alone.

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