

Occurrence of Yellow Vine, A New Disease of Squash and Pumpkin, in Relation to Insect Pests, Mulches, and Soil Fumigation

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ABSTRACT

In 1988, a distinctly different disease from those previously described was observed in squash and pumpkin grown in Oklahoma. The disease has been designated as yellow vine, however, the etiology of this disease is as yet unidentified. Symptoms include wilting, plant yellowing, phloem discoloration in the primary root and crown, and plant death. Preliminary observations suggested that squash bug (*Anasa tristis*) populations, melon aphid (*Aphis gossypii*), and use of mulches were associated with the occurrence of yellow vine. A series of field studies were conducted to determine if there was a relationship between insect populations, mulches, soil-borne pathogens and yellow vine and to document the nature of this relationship. Yellow vine incidence was significantly greater ($P \leq 0.05$) in plants from plots with aluminum and black plastic mulch as compared to squash plants from plots with white paint mulch and on bare ground. In squash grown under insecticide-sprayed and no-insecticide regimes, the occurrence of yellow vine symptoms was significantly reduced ($P \leq 0.05$) when an insecticide application program was followed, regardless of mulch. Use of soil fumigation provided strong evidence that yellow vine was not soil-borne. Evidence suggests that insects are associated with yellow vine, perhaps acting as a vector of the causal organism.

RESUMEN

En 1988, se observó en plantas de calabacita y de calabaza cultivadas en Oklahoma una enfermedad diferente a otras previamente descritas. La enfermedad ha sido llamada enredadera amarilla (yellow vine), sin embargo, la etiología de la enfermedad permanece aun sin identificar. Los síntomas incluyen marchitez, amarillamiento de la planta, pigmentación del floema en la raíz primaria y la corona y finalmente la muerte de la planta. Observaciones preliminares indicaron que las poblaciones de la chinche de la calabaza (*Anasa tristis*), el pulgón del melón (*Aphis gossypii*) y el uso de acolchado, estuvieron asociados con la presencia de esta enfermedad. Se condujeron una serie de estudios de campo para determinar si existía relación entre la enfermedad enredadera amarilla y las poblaciones del insecto, el acolchado y los patógenos del suelo, así como para determinar la naturaleza de esta relación. La incidencia de la enfermedad enredadera amarilla fue significativamente mayor ($P \leq 0.05$) en las plantas de las parcelas con acolchado de aluminio y acolchado de plástico negro, en comparación con las plantas de calabacita creciendo en las parcelas con acolchado de pintura blanca y sobre suelo expuesto. En las plantas de calabacita cultivadas bajo regímenes con aspersión de insecticidas o sin aplicación de insecticidas, la presencia de la enfermedad se redujo significativamente ($P \leq 0.05$) cuando se siguió un programa de aplicación de insecticidas, indiferentemente del acolchado. El empleo de fumigación de suelo brindó una fuerte evidencia de que la enfermedad no proviene del suelo. Las evidencias sugieren que los insectos están asociados con la enfermedad enredadera amarilla, quizá actuando como vectores del organismo causal.

In 1988, a disease of unknown etiology was first observed on squash (*Cucurbita pepo* var. *melepepo*) and pumpkin (*Cucurbita pepo* var. *pepo*) in Oklahoma. Symptoms include a general and rapid yellowing of the leaves over a 3-4 day period followed by a gradual decline of the vine. Some affected plants, exhibiting no yellowing, may wilt and completely collapse in one day. Terminal leaves tend to stand in a vertical position and do not expand properly. However, there are no viral symptoms associated with the leaves, fruit, or flowers. The older leaves develop a blighted appearance

whereas the terminal leaves often remain alive but somewhat yellow. Root rot is not present in the early stages of disease development, although the phloem area in the primary root and crown is discolored exhibiting a golden to honey brown appearance. The xylem can exhibit some degree of general discoloration, especially in advanced stages of the disease.

There are a number of disease causing organisms capable of causing leaf yellowing, wilt, and vine declines in cucurbits (Bruton et al., 1988a). *Fusarium oxysporum*, *Fusarium solani*, and *Macrophomina phaseolina* are the predominant

fungi, isolated from diseased plants, that were considered capable of causing disease symptoms similar to those previously described (Bruton, unpublished). Martyn and McLaughlin (1983) demonstrated that *Fusarium oxysporum* f. sp. *niveum* race 1 can be a severe pathogen of certain cultivars of summer squash under greenhouse conditions. The highly aggressive race 2 has been reported in Texas, Oklahoma, and Florida (Martyn and Bruton, 1989). Numerous cross-inoculations with race 0, 1, and 2 of *F. o. f. sp. niveum* has established that the wilt fungus is not involved in yellow vine of squash and pumpkin (Bruton, unpublished data).

Field observations of yellow vine disease when it was first observed in 1988 suggested that the onset of symptoms was related to the occurrence of one or more of the primary cucurbit insect pests, particularly squash bug, *Anasa tristis* (DeGreer). Later studies demonstrated that squash and pumpkin were hosts of the yellow vine disease but not watermelon, muskmelon, and cucumber (Cartwright and Bruton, 1993). Subsequently, a series of studies in 1989 and 1990 were conducted to: 1) determine if populations of primary insect pests were related to the occurrence of the disease; 2) determine if soil-borne organisms were causative agents; and 3) gather additional data on the disease host range within agronomically important *Cucurbita* spp.

MATERIALS AND METHODS

Mulch Experiments. Initial observations in 1988 suggested that the disease was more pronounced in squash grown on plastic mulch than on bare soil. Therefore, we compared squash grown on black plastic, reflective mulch, white paint, and bare ground to determine if insect populations and ground cover, alone or in combination, were factors related to the occurrence of yellow vine. In 1989, an experiment was conducted at the Lane Research Center, Lane, OK to test the effects of insecticide treatment and mulches on the development of yellow vine in squash. A split-plot design, replicated 4 times, was used to compare two levels of insecticide (insecticide and no insecticide) as main plots and ground cover as sub-plots. Yellow straightneck squash *Cucurbita pepo* var. *meloepo* (cv. 'Goldbar') was transplanted into plots on 30 June 1989. Four ground cover treatments were compared as sub-plots; 1) aluminum mulch (paper-backed); 2) black plastic; 3) white paint liquid spray mulch; and 4) bare ground. Fourteen plants per plot were spaced 0.5 m apart on raised beds that were spaced 2 m apart. Plants were grown using trickle irrigation and production practices recommended by the Oklahoma State University Cooperative Extension Service. Fungicides were applied to all plots as needed. Three applications of mancozeb (2.8 kg/ha) and one application of triadimefon (0.42 kg/ha) were made during the season. Plots designated for maintaining insect free squash plants were sprayed weekly with either cypermethrin (0.11 kg a.i./ha) or esfenvalerate (0.06 kg a.i./ha) by ground application, using 54 liters of water/ha. Insecticide applications were begun 3 days after transplanting and continued through the harvest period.

Squash bug populations were assessed weekly by counting the number of adults, eggs, and nymphs on three randomly selected plants per plot commencing 7 July. Similarly, aphid populations were estimated by counting the number present on 20 randomly selected leaves from each plot. Symptoms of yellow vine, including plant yellowing or collapse, were recorded weekly by inspecting all plants in each plot. Isolations were routinely made on plants when yellow vine symptoms first became apparent. Isolations were made from the crown and roots, surface disinfested for 1-3 minutes in 0.5% NaOCl, and placed on potato dextrose agar (PDA) and/or water agar plus streptomycin sulfate (WA+S). As individual fungal colonies grew out, they were transferred to PDA and SNA (Nirenberg, 1981) and allowed to sporulate. Non-sporulating cultures were kept for about 30 days before being discarded.

Soil Fumigation Experiment. An experiment was conducted to investigate whether the disease might be induced by a soil-borne pathogen acting alone or in combination with insect attack. Elimination of microorganisms from the soil was accomplished by using methyl bromide as a soil sterilant. Soil in fumigation plots was covered with black plastic mulch and

sealed, then injected with methyl bromide at the rate of 67 g/m³ soil to a depth of 30 cm. Concurrently, we controlled the presence of key insect pests through the selective use of insecticides. A split-plot design, replicated 4 times, was used to test the effects of two levels of fumigation (fumigated and non-fumigated) and insecticide (sprayed and un-sprayed) on yellow vine occurrence. The soil type was a Bernow sandy loam located at the Lane Research Center. Soil samples were collected to 15 cm deep just prior to fumigation for each of the respective treatments to assay for potential soil-borne pathogens. Post-treatment assays were also conducted on soil samples after seven days for the presence of *Macrophomina phaseolina* (Papavizas and Klag, 1975) and *Fusarium* spp., (Nash and Snyder, 1962) as an index of soil sterilant efficacy. Yellow straightneck squash *Cucurbita pepo* var. *meloepo* (cv. 'Goldbar') was transplanted into plots on 11 August 1989. Plants were grown using production practices as previously described. Foliar fungicides were applied to all plots as needed. Two applications of mancozeb (2.8 kg/ha) and two applications of triadimefon (0.42 kg/ha) were made during the season primarily for powdery mildew. Plots designated for insecticide treatment, were sprayed weekly with cypermethrin (0.11 kg a.i./ha) by ground application, using 54 liters of water/ha. Insecticide applications were begun 4 days after transplanting and continued through the harvest period.

Squash bug populations were assessed weekly by examining three randomly selected plants per plot beginning 28 August and continuing for six weeks. Symptoms of yellow vine were recorded weekly beginning 28 August by inspecting all plants and recording those which exhibited at least one of the symptoms of yellow vine. Isolations were made from affected plants as previously described.

Squash/Pumpkin Cultivar Study. Because our initial observations and research indicated that symptoms of the dis-

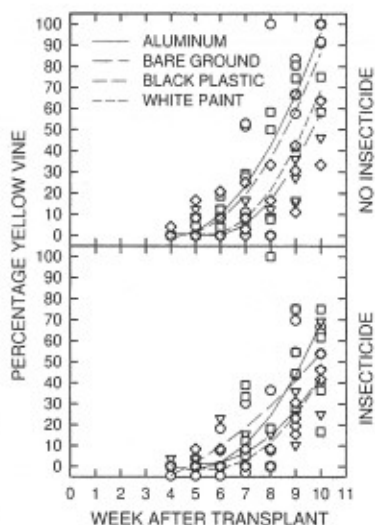


Fig. 1. Incidence of yellow vine disease in insecticide treated and untreated squash transplanted on different mulch types, 1989.

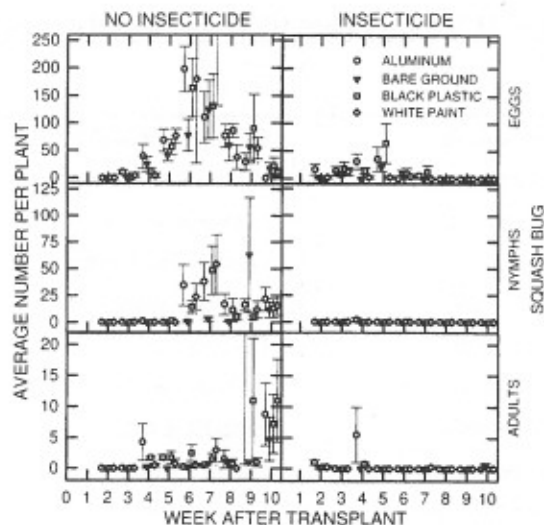


Fig. 2. Effect of mulch type and insecticidal treatment on squash bug populations in transplanted squash, 1989.

ease appeared to be restricted to squash and pumpkin, an expanded *Cucurbita* spp. study was conducted in 1990. A list of entries is presented in Table 1. Plots (4 m long) were established on the Lane Research Station and randomly arranged in 3 blocks. Beds were covered with black plastic and spaced on 3-m centers. Each cultivar was hand seeded on 25 May 1990 through a hole cut in the plastic to attain a plant spacing within rows of 0.5 m. Plants were grown under recommended production practices using trickle irrigation. Fungicides were applied to control foliar diseases in all plots as needed basis. Three applications of mancozeb (2.8 kg/ha) and 2 applications of triadimefon (0.42 kg/ha) were made during the season. Isolations were made from affected plants as previously described.

Insect populations were surveyed using the same methods as with previous cucurbit studies, with weekly counts beginning on 27 June 1990 and continuing until plants matured. Plants were checked weekly for symptoms of yellow vine after the first symptoms were observed and those displaying at least one of the symptoms were considered positive for disease.

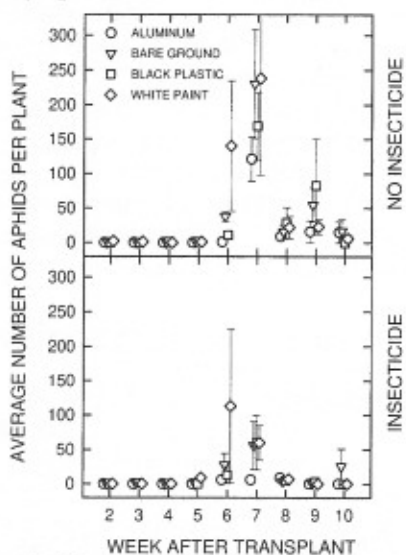


Fig. 3. Effect of mulch type and insecticidal treatment on melon aphid populations in transplanted squash, 1989.

RESULTS

Mulch Experiments. The most prevalent fungi isolated and identified are listed in order: *F. solani*, *F. equisiti*, *F. semi-tectum*, *Macrophomina phaseolina*, *F. oxysporum*, and *Rhizoctonia solani*. However, there was no consistent association with any fungus and symptomatic plants with the exception of *F. solani*. *F. solani* and the other fungi were isolated with approximately the same frequency on healthy appearing plants as well. Isolations on cucurbits typically yield *F. solani* almost 100% of the time and *F. oxysporum* about 10% of the time.

Yellow vine incidence was significantly ($P \leq 0.05$) greater on plants from plots with aluminum and black plastic mulch as compared to squash plants from plots with white paint mulch and on bare ground (Fig. 1). Furthermore, there was a significant ($P \leq 0.05$) reduction in yellow vine occurrence in plots receiving weekly insecticide treatments, irrespective of mulch (Fig. 1). When polynomial regression was used to relate the incidence of yellow vine to week in the field, it was

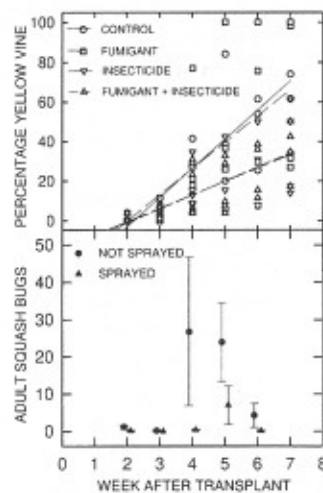


Fig. 4. Effects of fumigation and insecticidal treatment on the incidence of yellow vine disease of transplanted squash grown on black plastic mulch (upper); relationship of squash bug populations with respect to insecticidal treatment (lower), 1989.

Table 1. Percentage yellow vine in squash and pumpkin types.^a

Type	CV	Company	Yellow Vine (%)	SE
Winter Squash	Connecticut Field	Baxter	96	3.70
Summer Squash	Early Prolific	Gurdy	96	4.16
Winter Squash	Ghostrider	Stokes	94	5.55
Winter Squash	Golden Hubbard ^b	Stokes	92	8.33
Winter Squash	Jack-O-Lantern	Twilley	92	8.33
Summer Squash	Gold Slice	Peto	91	5.25
Summer Squash	Yellow Crook	Baxter	89	0.83
Winter Squash	Jack-Be-Little	Twiley	88	12.50
Winter Squash	Autumn Gold	Twilley	87	2.65
Summer Squash	Grey 5965	Gurney	87	1.66
Summer Squash	Sundance B	Baxter	82	13.63
Summer Squash	Sundance P	Peto	82	10.50
Summer Squash	Black 5966	Gurney	79	20.83
Winter Squash	Cushaw Green Stripe ^c	Baxter	77	9.70
Summer Squash	Goldbar	Twilley	75	8.08
Summer Squash	Hyrific	Baxter	74	13.24
Summer Squash	Gold Rush	Twilley	70	14.9

^aCultivars are *C. pepo* var. *meloepo* and var. *pepo* unless indicated otherwise.

^b*Cucurbita maxima*.

^c*Cucurbita argyrosperma*.

found that linear and quadratic effects were significant but cubic effects were not. Results of covariance analysis of the quadratic functions indicated that there was a significant ($P \leq 0.05$) difference between aluminum and black plastic mulch, no significant ($P \leq 0.05$) difference between bare ground and white paint, but there was a significant ($P \leq 0.05$) difference between the group aluminum - black plastic and the group bare ground - white paint for both insecticide and no insecticide treatments. It should be noted that the white paint mulch lasted only about one week under field conditions. Therefore, it should be considered as essentially bare ground. There was a significant ($P \leq 0.05$) insecticide by week interaction in that the rate of increase in yellow vine was greater in the insecticide treatments.

Numbers of squash bug eggs and nymphs (Fig. 2) and melon aphids (Fig. 3) exhibited significant ($P \leq 0.01$) effects from insecticide, week, and the week by insecticide interaction.

Squash bug populations were highly variable over week although the incidence of yellow vine did not follow a similar pattern. Melon aphid populations were highest in the unsprayed plots with bare ground and white paint mulch. In contrast, plants on aluminum and black-plastic mulch had lower aphid populations, yet had the highest incidence of yellow vine. These results provide substantial evidence that aphids may not be involved in yellow vine disease.

Soil Fumigation Experiment. Pre-treatment population means over all plots for *Fusarium* spp. and *M. phaseolina*

were 6781 (range:3149-11458) and 29 (range:15-41) CFU/g of soil, respectively. Post-treatment mean populations in the nonfumigated plots were 10,115 (range:5120-15423) and 18 (range:11-42) CFU/g of soil for *Fusarium* spp. and *M. phaseolina*, respectively. In the fumigated plots, the mean CFU/g of soil were reduced to 4 (range:0-16) and 0 (range:0-10) for *Fusarium* spp. and *M. phaseolina*, respectively. Fungal isolations on affected and healthy appearing plants, from fumigated plots, followed a similar pattern as in the mulch study with *F. solani*, *F. oxysporum*, and *M. phaseolina* being isolated 93, 12, and 16% of the time, respectively.

Fumigation had no effect on disease incidence or squash bug populations. However, insecticide did have a significant ($P \leq 0.05$) effect on the incidence of yellow vine, regardless of fumigation (Fig. 4). Plants from plots receiving insecticide averaged 36% yellow vine, whereas, those receiving no insecticide averaged 65% yellow vine. Squash bug numbers were

reduced in sprayed compared to unsprayed plots but the differences were not significant ($P=0.05$).

Squash/Pumpkin Cultivar Study. Yellow vine incidence by the end of the study ranged from 70% in Gold Rush summer squash to 96% in Connecticut Field Pumpkin (Table 1). Analysis of variance showed no significant ($P \geq 0.05$) difference between the squash and pumpkin cultivars for incidence of yellow vine. When all squash bug life stages were averaged over the entire time of the experiment, there was no significant

(PÚ.05) affinity for a particular cultivar.

DISCUSSION

Results of this study suggest that the yellow vine disease may be insect related. The strongest evidence for the implication of an insect association resulted when significant reductions in yellow vine incidence were observed when insecticides were used. Also, either the associated insect appears to prefer squash and pumpkin as a host, or other cucurbits (Cartwright and Bruton, 1993) are either resistant, non-hosts, or escape the yellow vine disease agent. Studies of insect fauna on cucurbits in Illinois indicate that aphids, squash bugs, cucumber beetles, beet leafhoppers, and thrips prefer squash or pumpkin over other cucurbit hosts (Howe and Rhodes, 1976). However, in no choice situations, all of the above mentioned insects may feed, or attempt to feed, on most cucurbit species.

Mulches are known to repel insect pests capable of transmitting disease agents (Jones and Chapman, 1968; Toscano et al., 1979). For example, incidence of watermelon mosaic virus and populations of leafhoppers, aphids, thrips, cucumber beetles, and whiteflies are greatly reduced in squash, but not altogether eliminated, when reflective mulches are used (Daiber and Donaldson, 1976; Chalfant et al., 1977; Necibi et al., 1992). Also, the use of insecticides, even systemic insecticides, usually serve only to check the spread of some types of aphid and leafhopper-borne diseases. Mulches do tend to increase populations of the squash bug in squash (Cartwright et al., 1990). In this study, the squash bug populations and the incidence of yellow vine also increased significantly where mulches were used. While there may appear to be a relationship between the incidence of yellow vine and squash bug occurrence, evidence collected in insecticide treated vs. non-treated plots suggested that disease incidence and squash bug populations were independent. Squash bugs are not known to transmit any known disease causing organism, although, squash bug feeding can result in a sudden wilting of the plant known as Anasa wilt (Robinson and Richards, 1931). While some researchers have suggested that squash bug injected toxins may be responsible for the wilt, no toxin has been conclusively identified to date. However, squash feeding does adversely affect xylem tissue which can result in plant wilt (Neal, 1993).

Root rot is not associated with yellow vine of squash and pumpkin in the early stages of symptom development. However, the roots deteriorate rapidly as yellow vine progresses. Consequently, the disease was initially suspected to be caused by the root rot pathogen *Fusarium solani* f. sp. *cucurbitae* or the wilt pathogen *F. oxysporum*. They were two of the more prominent species isolated and the symptoms were similar in some respects to both diseases.

Martyn and McLaughlin (1983) had demonstrated that *F. o. f. sp. niveum* race 1 was capable of causing a wilt of summer squash in the greenhouse. *Fusarium* wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* race 2 had recently been reported in Oklahoma (Bruton et al., 1988b) and there is no information available as to how squash and pump-

kin react to race 2. Numerous squash isolates of both fungi (*F. solani* and *F. oxysporum*) were compared to known pathogenic races representing each fungus. Cross-inoculations demonstrated that the *F. oxysporum* from squash was rarely pathogenic to watermelon, cantaloupe, cucumber, or squash. Conversely, the known races of 0, 1, and 2 of *F. o. f. sp. niveum* were highly virulent only to watermelon and occasionally to a small percentage of the other cucurbits tested (data not shown). Some *F. solani* isolates from squash induced root browning and decay on watermelon, cantaloupe, and squash, however, there was no similarity to the highly virulent *F. solani* f. sp. *cucurbitae* as described by Tousson and Synder (1961) (data not shown).

It should be noted that in this study *F. oxysporum* from squash was only occasionally isolated from the xylem of yellow vine affected plants. Furthermore, Scanning Electron Microscopy has rarely revealed the presence of a fungus in the crown xylem of affected plants (Bruton, unpublished data). Therefore, based on symptomology, fungal isolations, physical observations, and fumigation, *Fusarium* spp. were eliminated as a possible causal agent of squash yellow vine.

Soil fumigation provided strong evidence that yellow vine was not soil-borne, although, it did not rule out the possibility of the disease being seed-borne. However, communications with seed industry representatives as well as research and extension personnel indicated that yellow vine was not known to exist in surrounding states. The disease has never been recorded in the greenhouse on squash from the same seed lots as planted in the field studies, further suggesting that this disease is not seed-borne.

Evidence suggests that the yellow vine disease of squash and pumpkin is not soil-borne or seed-borne but rather is related, directly or indirectly, to insect pests. In the latter case, insect feeding may accentuate the onset of yellow vine symptoms. However, we were unable to definitively establish whether squash bug, aphids, or other insects were involved in the occurrence of yellow vine.

Symptoms of yellow vine normally began to appear within 3-5 weeks after being transplanted into the field. No causal agent has been determined in the syndrome known as yellow vine. Clearly, the true relationship between insects, the causal agent, and yellow vine has not been established.

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