Survival of *Didymella bryoniae* in Infested Muskmelon Crowns in South Carolina

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ABSTRACT

Keinath, A. P. 2008. Survival of *Didymella bryoniae* in infested muskmelon crowns in South Carolina. Plant Dis. 92:1223-1228.

The crowns of muskmelon (*Cucumis melo* subsp. *melo*) plants are susceptible to cankers caused by the fungal pathogen *Didymella bryoniae*. The objective of this study was to compare the length of time *D. bryoniae* survived in infested crowns that were buried or left on the soil surface. Dried crowns with cankers were buried 12.5 cm deep, placed on the soil surface, or placed on top of raised beds covered with white-on-black polyethylene mulch from July 2002 to June 2003, December 2003 to October 2004, July 2004 to November 2005, and November 2005 to November 2007. At regular intervals, crowns or crown debris were retrieved, washed, cut into pieces, and cultured on semiselective medium to recover *D. bryoniae*. *D. bryoniae* was not recovered from crowns buried 35 and 45 weeks in 2003 and 2004 but was recovered from 2.5% of crowns buried 66 weeks in 2005. In contrast, *D. bryoniae* was recovered after 48, 45, 66, and 103 weeks from 66, 6.3, 2.5, and 10% of crowns on the soil surface in 2003, 2004, 2005, and 2007, respectively. *D. bryoniae* also was recovered after 66 and 103 weeks from 12.5 and 8% of crowns on mulched beds in 2005 and 2007. In two additional experiments, the pathogen was recovered from 15.0 and 20.1% of infested muskmelon debris left in place for 42 and 38 weeks on polyethylene-mulched beds. To reduce the time *D. bryoniae* survives after a cucurbit crop, crop debris should be incorporated into soil promptly after harvest.

Additional keywords: gummy stem blight, plasticulture

The cucurbit diseases gummy stem blight and black rot are caused by the fungal pathogen *Didymella bryoniae* (Auerw.) Rehm (anamorph *Phoma cucurbitacearum* (Fr.:Fr.) Sacc.). Gummy stem blight occurs on all vegetative portions of plants, whereas black rot occurs on fruit (22,28). Several sources of inoculum have been identified in different studies. *D. bryoniae* was isolated from the seed coat, perisperm, and cotyledons of cucumber and pumpkin seed grown in several countries in Asia, Africa, and Europe (17). Reports of gummy stem blight on cucumber and watermelon seedlings grown in greenhouses also suggest that the pathogen can be seedborne (5,16). Airborne ascospores of the fungus have been trapped in watermelon fields in Florida and inside and outside cucumber greenhouses in The Netherlands (20,26). Infested cucurbit debris that remained from previous crops was a source of conidia that were able to infect seedlings in greenhouse assays (13). However, in previous studies, *D. bryoniae* survived for less than 9 months in infested debris (6,13,26). Pycnidia and pseudothecia present on infested debris at the beginning of the studies were devoid of spores

at the end of the studies (6,26). The naturally infected crop debris used in these studies was watermelon and squash stems, watermelon vines, and cucumber stems, respectively. It is probable that the stems used in the studies by Chiu and Walker (6) and van Steekelenburg (26) were vine sections.

The crown of a cucurbit plant includes a short stem that branches into two or more primary vines. Infection of the crown by *D. bryoniae* leads to crown cankers (3,6,19,20,22). Cankers on crowns of muskmelon (*Cucumis melo* subsp. *melo*) begin as water-soaked areas that become dry, tan or white in color, and covered with pycnidia and pseudothecia. They can extend several centimeters along all the primary vines, but on the stem they usually do not extend more than 2 cm below the point of attachment of the vines. Cankers have been observed on 40 to 99% of muskmelon plants in field experiments and in a heavily infested commercial field in South Carolina (A. P. Keinath, unpublished). The commercial field in Florence County, SC, belonged to a limited-resource grower who had used polyethylene-mulched raised beds for two consecutive years to grow muskmelon. When the field was observed in June 2002, all of the plants in the oldest of three plantings had crown cankers of gummy stem blight (14). The remains of muskmelon crowns from the previous year with empty fruiting bodies of *D. bryoniae* were found in the rows, but the fungus was no longer viable.

Cucurbit vines and stems exhibit secondary growth, including a perivascular cylinder partially composed of lignin (9). Therefore, vines would persist longer than nonlignified portions of cucurbit plants, such as leaves or tendrils, but not as long as crowns, which include the thickest parts of stems. The objective of this study was to compare the length of time *D. bryoniae* would survive in infested crowns that were buried or left on the soil surface. The goal of this research was to more precisely define the length of time a field must be rotated out of cucurbits to reduce or eliminate infested debris from previous crops as a source of inoculum of *D. bryoniae* in South Carolina.

MATERIALS AND METHODS

Infested muskmelon crowns and treatments. Crowns of muskmelon cv. Athena with symptoms of gummy stem blight and signs of *D. bryoniae* were collected from a commercial field in Florence County, SC, in July 2002 and from experimental plots at the Clemson University Coastal Research and Education Center, Charleston, SC, in November 2003, July 2004, and November 2005. The crowns of muskmelon grown in experimental plots were inoculated on 20 September 2003, 8 May 2004, and 23 September 2005 (ca. 2 months before collection) with 10^6 conidia of *D. bryoniae* per milliliter. At the end of the growing season, crowns with extensive cankers were collected, and healthy portions of vines were cut from crowns so that only diseased canker tissue remained. In 2002, arbitrarily selected crowns were measured to ensure that each crown contained enough tissue to cut it into 10 1-cm-long portions. In other years, crown sizes were estimated visually. All crowns in all years contained fruiting bodies of *D. bryoniae*, but not all of the canker surface had fruiting bodies. Crowns were air-dried (2002 and 2003) or dried in an operating fume hood (2004 and 2005) for 1 week. Each crown (ca. 4 cm wide $\times$ 6 cm long) was placed in a 9.5 $\times$ 11.5 cm polypropylene mesh sample bag with 3 $\times$ 2 mm openings (Agdia, Inc., Elkhart, IN). The solid nylon-polyethylene side was removed from the bags before use. Bags that were to be buried were filled with soil dug from the site the day before burial. Bags were sealed with a heat-sealer.

Bags with crowns were placed in a field at the Coastal Research and Education Center on 2 July 2002, 1 December 2003, 29 July 2004, and 7 November 2005 to coincide with the end of the spring and fall

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Accepted for publication 29 April 2008.

muskmelon cropping seasons. A different field was used each year. All soil types were Yonges loamy fine sand (fine-loamy, mixed, thermic, Typic Albaquults), pH 6.7, 6.2, 6.5, and 6.3 in the 2002, 2003, 2004, and 2005 experiments, respectively. Rainfall data were obtained from the National Weather Service station at the Charleston International Airport (32°54'N, 80°2'W), 9.9 km north of the experimental site (Table 1).

In the 2002, 2003, and 2004 experiments, crowns were either placed on the soil surface or buried 12.5 cm deep. In 2004, a third treatment was added, where bags were placed on top of raised beds (0.5 m wide × 12 cm high) covered with white-on-black polyethylene mulch. In 2005, two treatments, soil surface and mulched beds, were examined. There were five replicate sites (blocks) in the 2002 experiment and four replicate sites in the subsequent experiments. Per experimental unit, eight crowns were used in 2003 and 2005, and 10 crowns were used in 2002 and 2004. At the beginning of each experiment, baseline colonization of crowns by *D. bryoniae* was determined by culturing 40 crowns in the 2002 and 2003 experiments, 28 crowns in the 2004 experiment, and 32 crowns in the 2005 experiment as described below.

The number of samplings in each experiment was determined by the number of infested crowns available each year. There were four samplings in 2002, 2004, and 2005 and three samplings in 2003. In 2002, one bag was retrieved from each treatment in each block every 9 to 14 weeks. In the 2003 and 2004 experiments, bags were retrieved every 15 to 17 weeks. Because *D. bryoniae* was recovered from at least one treatment at the last samplings in the 2002, 2003, and 2004 experiments, the sampling interval was extended to 26 weeks in the 2005 experiment. The four experiments lasted 48, 45, 66, and 103 weeks in 2002 to 2003, 2003 to 2004, 2004 to 2005, and 2005 to 2007, respectively.

Recovery of *D. bryoniae*. Crowns were removed from bags and washed under running tap water 3 to 5 min to remove excess soil. Crowns were dried in an operating fume hood for 3 days (13). At the 0-time samplings in all experiments and at all samplings in the 2002 experiment except at the last sampling, each crown was cut into 10 1-cm sections, which were then cut in half. Thereafter, the procedure for handling crowns was modified so that

more crown tissue could be placed in direct contact with the agar and recovery still could be quantified after crowns had begun to decompose. At the last sampling in the 2002 experiment and all samplings other than the baseline samplings in 2003 to 2005, individual dried crowns were shredded by hand into small fragments 0.5 to 1 cm long. In all experiments, pieces of crowns were rehydrated for 10 min in deionized water, surface-disinfested in 0.5% sodium hypochlorite solution for 1 min, rinsed with sterile deionized water, and placed on CSR medium (one-quarter-strength potato dextrose agar [QPD] amended after autoclaving with [per liter] 100 mg chloramphenicol, 100 mg streptomycin, and 60.5 mg metaxyl [0.25 ml Ridomil 2E]) (13). When crowns were shredded, crown fragments were placed into 10 small heaps per petri plate as described by van Bruggen and Arneson for soil organic matter (25). Plates were incubated at ambient temperature (22 to 24°C) under a 16-h photoperiod.

Colonies growing from crown sections or fragments were examined at ×30 to ×50 magnification after 3 to 4 days and again after 1 or 2 additional days of incubation. *D. bryoniae* was identified by characteristic hyaline aerial mycelia, dark green substrate mycelia, and round, tan pycnidia with a dark ring of cells around the ostiole (15,19,27). Pycnidia from selected colonies were excised from the agar, and conidia were examined at ×200 to ×400 magnification to confirm identification as *D. bryoniae* (15). A few colonies produced only pseudothecia and ascospores. The total number of sections or heaps plated and the number of sections or heaps from which colonies of *D. bryoniae* grew were recorded for each treatment and replication.

Over-wintering of infested vine debris on polyethylene-mulched beds. Muskmelon plants with crown and vine lesions were produced at the Coastal Research and Education Center, Charleston, SC, in 2003 and 2005. Transplants of muskmelon cv. Athena were set 0.46 m apart into raised beds covered with white-on-black plastic mulch on 28 August 2003 and 3 August 2005. Plots were 9.7 m long and two rows wide in 2003 and one row wide in 2005. There were six replications in each experiment. Each plant was inoculated by spraying the crown with a suspension of 10^6 *D. bryoniae* conidia/ml on 20 Septem-

ber 2003 and 13 and 23 September 2005. Essentially all plants developed crown and vine cankers of gummy stem blight. Presence of the pathogen was verified by observing pycnidia on vines. Plants were allowed to senesce in place on top of polyethylene-mulched beds. On 18 May, 24 June, and 15 July 2004 and 7 April, 15 May, and 19 June 2006, crown and vine debris was collected from each replicate plot. Debris was dried and prepared as described above. Eight small heaps of debris were placed onto plates of CSR medium; six plates were used per plot. The total number of debris heaps from which colonies of *D. bryoniae* grew was recorded for each plot.

Statistical analysis. For each experiment, PROC MIXED of SAS version 9.0 (SAS Inc., Cary, NC) was used to determine the effects of treatments and time on percentage of crowns or percentage of heaps yielding *D. bryoniae* at recovery. A split-plot repeated measures model was used with autoregressive covariance structure. Burial treatments and time were considered fixed effects, and replicate sites (blocks) were considered a random effect in the analysis of variance. The Restricted Maximum Likelihood method was used to estimate sums of squares. Treatments and recovery times were compared with least-squares means *t* tests.

RESULTS

At the beginnings of the experiments, *D. bryoniae* was recovered from 100, 100, 73.3, and 100% of the crowns cultured in 2002, 2003, 2004, and 2005, respectively. *D. bryoniae* was recovered from 77.3, 84.6, 16.4, and 83.9% of the crown sections in 2002, 2003, 2004, and 2005, respectively. Recovery was lower in 2004 because the assay was interrupted between the rehydration step and placing tissue on semiselective medium. Crown tissue debris recovered from the soil surface or raised beds remained relatively intact for 1 year after placement in the field; by the end of 2 years (2005 to 2007 experiment) only fragments of debris remained. Crowns that were buried were partially decayed, and occasionally vascular bundles were the predominant tissue remnants.

In the 2002 to 2003 experiment, percent recovery of *D. bryoniae* decreased significantly ($P < 0.0001$) between 0 and 35 weeks after crowns were buried or left on the soil surface (Fig. 1A). Percent recovery was significantly ($P \leq 0.0001$) higher from crowns left on the surface than from buried crowns at 9, 23, and 48 weeks (Table 2). *D. bryoniae* was not recovered from any buried crowns at 35 and 48 weeks. However, at 35 weeks, $6.0 \pm 3.6\%$ (SE) of the crowns left on the soil surface yielded *D. bryoniae*, and percent recovery increased significantly ($P \leq 0.0001$) to $66 \pm 7.8\%$ at 48 weeks (Fig. 1A). This increase in recovery at the end of the season only in the

Table 1. Rainfall (mm) during summer (July, August, and September) and winter (December, January, and February) months in Charleston, SC, during four experiments from 2002 to 2007

Buried	Year	Summer 1	Winter	Summer 2
July 2002 to June 2003	2002-2003	580	218	na ^a
December 2003 to October 2004	2003-2004	na	199	468
July 2004 to November 2005	2004-2005	468	121	272
November 2005 to November 2007	2005-2006	na	241	353
November 2005 to November 2007	2006-2007	na	219	476

^a Not applicable; crowns not in the field in this season.

crowns on the surface was the likely reason for the large treatment-by-time interaction term in this experiment (Table 2).

In the 2003 to 2004 experiment, recovery decreased over time in a similar pattern for buried crowns and crowns left on the soil surface (Fig. 1B). There was a steep decrease in the percentage of crowns yielding *D. bryoniae* between March and July 2004. However, the two treatments differed at the last two sampling dates, which resulted in a treatment-by-time interaction (Table 2). At 30 weeks after placement in the field, percent recovery was higher from buried crowns, but at 45 weeks, percent recovery was higher from crowns on the surface ($P \leq 0.02$). Averaged across the last two sampling dates and depths, *D. bryoniae* was recovered from $3.9 \pm 1.5\%$ of the crowns at the end of the 2003 to 2004 experiment.

At the March 2004 sampling, colonies that originated from debris on the surface were more likely to produce pseudothecia (86% of 32 colonies examined) than colonies that originated from buried debris (50% of 16 colonies examined). This was the only sampling period when an association between depth of burial and type of fruiting body produced was observed among all 15 sampling periods across all four experiments.

The experiment in 2004 to 2005 included three treatments: crowns buried at 12.5 cm or placed on the soil surface as in the previous two experiments, and crowns placed on top of polyethylene-mulched raised beds. For all three treatments, percent recovery after 66 weeks was lower than percent recovery at 0 weeks (Fig. 2). Treatments did not differ from each other at the 66-week (last) sampling ($P \geq 0.37$), when *D. bryoniae* was recovered from $12.5 \pm 9.5\%$ of the crowns placed on mulched beds, but only from $2.5 \pm 2.5\%$ of the crowns, i.e., 1 of 40 crowns, in each of the other two treatments. However, the decline in recovery followed a different pattern for each treatment, as indicated by the treatment-by-time interaction (Table 2). For buried crowns, percent recovery declined rapidly and significantly ($P = 0.0008$) between 0 and 16 weeks but gradually during the next 50 weeks (Fig. 2). For crowns on the soil surface, percent recovery varied between 0 and 33 weeks, but then declined steadily and significantly ($P \leq 0.01$) over the last half of the experiment. Percent recovery from crowns left on polyethylene-mulched raised beds did not change during the first 50 weeks of the experiment (Fig. 2), but then declined rapidly and significantly ($P \leq 0.0001$) in the final 16 weeks. Percent recovery was lower from the buried crowns compared with crowns on mulch at 16, 33, and 50 weeks after burial and with crowns on the soil surface at 33 weeks ($P \leq 0.004$). *D. bryoniae* was recovered from a higher percentage of crowns from mulched beds

than from the soil surface at 50 weeks ($P = 0.0002$). At the end of the 2004 to 2005 experiment, *D. bryoniae* was recovered from a mean of $5.8 \pm 3.4\%$ of the crowns, averaged across the three treatments.

In the 2005 to 2007 experiment, there was no overall effect of treatment (Table 2); this was the only experiment in which there was no treatment-by-time interaction. Percent recovery at 26 weeks did not differ significantly from the 0-time recovery, but by 78 weeks, fewer crowns yielded *D. bryoniae* than at 0 weeks (Fig. 3). At 52

weeks, percent recovery was significantly lower than at 0 weeks for crowns on mulched beds ($P = 0.0017$), but not for crowns on the soil surface ($P = 0.17$). After nearly 2 years in the field, $9.1 \pm 4.8\%$ of the crowns still yielded colonies of *D. bryoniae*, averaged across both treatments. However, this mean was not significantly different from 0 (t test, $P = 0.23$).

As with infested crowns, *D. bryoniae* persisted in infested muskmelon crown and vine debris that were left in place on top of mulched raised beds, although re-

Fig. 1. Percentage recovery of *Didymella bryoniae* from cankers on muskmelon crowns left on the soil surface or buried 12.5 cm deep from **A**, July 2002 to June 2003 and **B**, December 2003 to October 2004.

covery of the fungus declined over time (Fig. 4). In May 2004 and April 2006, 95 and 86% of the debris heaps yielded colonies of *D. bryoniae*, respectively. The slopes of the regression lines plotted for all three sampling times in 2004 and the last two sampling times in 2006 were not significantly different from each other, 0.13 ± 0.02 and 0.15 ± 0.02 , respectively. At the end of the experiments, which were timed to coincide with typical muskmelon harvest, $15.0 \pm 4.8\%$ and $20.1 \pm 6.5\%$ of the debris heaps yielded *D. bryoniae* in July 2004 (9.7 months after inoculation) and June 2006 (8.8 months after inoculation), respectively.

DISCUSSION

On the basis of the results of these six field studies, it is clear that *D. bryoniae* will survive at least 8 months in buried crowns and at least 15 months in crowns left on the soil surface. The pathogen also may survive longer than 24 months in crowns left on the soil surface or on polyethylene-mulched raised beds. Therefore, the length of time necessary to eradicate *D. bryoniae* from a field is more than 24 months. This is a significantly longer survival time than that determined in three previous studies, in which *D. bryoniae* was recovered from infested vine debris for 6 months (6), 7.4 months (13), or less than 9 months (26). One likely reason for the longer survival in crown debris than in vine debris is that crowns were approximately 1 cm thick when buried, whereas vine debris was only 2 mm thick (13). Another *Didymella* species, *D. rabiei*, survived less than 9 months on chickpea stems overwintered on the soil surface (24). Survival of *D. bryoniae* for 24 months is in agreement with the results of a previous study in South Carolina that related development of gummy stem blight to crop rotation intervals. Cucumbers became infected by *D. bryoniae* when they were planted into soils that were infested 6, 12, 18, or 24 but not 30 months previously with diseased cucumber vines and fruit (21).

Length of survival of *D. bryoniae* in crowns depended on where they were placed in the field. In the 2002, 2003, and 2004 experiments, *D. bryoniae* was recovered longer or more frequently from crowns aboveground than from buried crowns. *D. bryoniae* was not recovered from any buried crowns at the end of two

Fig. 2. Percentage recovery of *Didymella bryoniae* from cankers on muskmelon crowns buried 12.5 cm deep, left on the soil surface, or left on raised beds covered with white-on-black polyethylene mulch from July 2004 to November 2005.

Fig. 3. Percentage recovery of *Didymella bryoniae* from cankers on muskmelon crowns left on the soil surface or on raised beds covered with white-on-black polyethylene mulch from November 2005 to November 2007.

Table 2. Split-plot repeated measures analysis of variance for arcsine-square root-transformed percentage recovery of *Didymella bryoniae* from infested crowns of muskmelon in four experiments from 2002 to 2007

Source	2002-2003			2003-2004			2004-2005			2005-2007		
	df ^a	F value	P value	df	F value	P value	df	F value	P value	df	F value	P value
Treatment	1, 18.1	73.12	0.0001	1, 7.3	0.57	0.47	2, 17	15.52	0.0001	1, 13.7	2.24	0.16
Time	4, 28.5	97.28	0.0001	3, 15.5	717.90	0.0001	4, 33.1	23.27	0.0001	4, 20.7	33.03	0.0001
Treatment × time	4, 28.5	11.06	0.0001	3, 15.5	7.23	0.003	8, 33.1	4.81	0.0005	4, 20.8	1.08	0.39

^a Degrees of freedom (numerator, denominator) were estimated with the Satterthwaite option of SAS PROC MIXED.

of the three experiments that included this treatment. The maximum length of time that *D. bryoniae* survived in buried crowns was 66 weeks or 15 months; the mean survival time was 49 ± 9 weeks or 11 months averaged across the three experiments. In The Netherlands, however, *D. bryoniae* survived longer in buried debris (9 to 10 months) than in debris left on the soil surface over winter (5 months), when temperatures reached -9°C , presumably because soil temperatures were slightly higher belowground (26). The increased survival of *D. bryoniae* on the soil surface in the present study is analogous to increased survival of fungal pathogens in cereal residue in no-till production systems (4). In another study, *Cercospora zeae-maydis* was recovered in May from infested maize debris placed on the soil surface the previous December but not from buried debris (7). Population density of *Phialophora gregata* in soybean debris also was greater on the soil surface than at 20-cm depth (1).

Fewer treatment differences were observed in the two experiments that began in late fall of 2003 and 2005, when percent recovery of *D. bryoniae* remained constant during the initial 15 to 26 weeks after placement in the field, compared with the two experiments that began in the summer of 2002 and 2004. The more rapid decline during the summer may have been due to greater total rainfall in the summer than in the winter. Rainfall during the summer months of June through August was 2.5 times greater than rainfall during the winter months of December through February averaged across all four experiments. Van Steekelenburg (26) also concluded that *D. bryoniae* survived better in infested cucumber debris kept dry than in moist debris. Similarly, *Alternaria dauci* was recovered from infested carrot debris for 1 year in dry or nonirrigated soil in California but only for 7 months in soil watered or irrigated weekly (18).

In one of two experiments that included a comparison between the soil surface and polyethylene mulch, *D. bryoniae* survived longer in crowns on top of mulch-covered raised beds than on the soil surface. The main difference between these two treatments was a period of 16 weeks in the middle of the experiment from March to July 2005 when *D. bryoniae* did not decline in crowns on mulched beds but was declining in crowns on the soil surface. In the other experiment, *D. bryoniae* survived longer than 104 weeks in both soil surface and mulch treatments. In addition, *D. bryoniae* survived for 9 to 10 months in debris left in place on top of polyethylene-mulched raised beds, which was longer than *D. bryoniae* survived in vine sections left on the soil surface in my previous study (13). This is long enough to account for the severe outbreak of gummy stem blight observed in 2002 on a commercial

crop planted in undisturbed polyethylene-mulched beds that also had muskmelon with gummy stem blight in 2001 (14). Leaving infested crop debris on polyethylene mulch after harvest may represent an additional risk beyond leaving crop debris in place on the soil surface. This study is the first to document increased pathogen survival in crop debris in plasticulture systems compared with bare-ground culture.

The recovery assay used in this study was used in my previous study on survival of *D. bryoniae* in watermelon vines (13). In that study, *D. bryoniae* was not detected in additional samplings conducted after the first sampling period in which *D. bryoniae* was not recovered. Thus, the assay is reliable and sensitive enough not to produce false negatives. The large increase in recovery observed in 2003 in the surface treatment may have been the result of two factors. The assay was changed at this sampling from cutting the crowns into sections to shredding the remaining crown debris. It is likely that shredding the debris increased recovery, because this technique increased the debris surface area in contact with the semiselective medium. It is also likely that *D. bryoniae* reproduced on the over-wintered debris. In two previous studies, *D. bryoniae* produced new pycnidia on over-wintered debris in the spring in 1 of 2 years (6,26). Chiu and Walker (6) concluded that because old fruiting bodies were empty of spores, the fungus survived as dormant mycelia. Although other au-

thors (19,26,27) do not mention dormant mycelia, de Neergaard (8) observed mycelia growing in the lignified portions of infected cucumber stems. Several other plant-pathogenic genera in the *Ascomycota* also survive as dormant mycelia in crop debris (2).

Athena, the muskmelon cultivar currently grown widely in the eastern United States (10), may be particularly susceptible to gummy stem blight crown cankers, based on the high percentage of plants inoculated for this study that developed crown cankers and the high incidence of crown infection in the commercial field used as the source of crowns in 2002. Although few plants died from crown cankers before producing fruit, fruit were smaller than normal (14). In a survey of eight watermelon and muskmelon fields in South Carolina in 1995, crown cankers were found in two of four watermelon fields and no muskmelon fields; vine cankers were found in one muskmelon field (23). Chui and Walker (6) reported that the cultivar of muskmelon they inoculated in the field was more susceptible to cankers than the watermelon or cucumber cultivars tested.

All three sources of inoculum, seed, airborne ascospores, and infested debris, are important in the epidemiology of gummy stem blight. Because this is a polycyclic disease, even small amounts of initial inoculum can result in extensive development of gummy stem blight by the end of the growing season when weather conditions are conducive (12). Outbreaks of

Fig. 4. Percentage recovery of *Didymella bryoniae* from infested muskmelon vine debris left on raised beds covered with white-on-black polyethylene mulch in May to July 2004 and April to June 2006.

gummy stem blight on watermelon transplants in California, an area assumed to have lower levels of indigenous inoculum than the humid southeastern United States, indicate that seedborne inoculum is present (16). The role of airborne ascospores has not been examined in modern plasticulture systems currently used for cucurbit production. If infested crop debris remains in a field, some airborne spread of the pathogen is expected, at least within the same field (20). Thus, reducing survival of the pathogen on debris would reduce spread of the pathogen via direct contact of debris with plants and dissemination of ascospores through the air.

In South Carolina, the main cucurbit growing season is spring to summer. On a 2-year rotation cycle, a maximum of 21 months would elapse between harvest of a muskmelon crop for the last time at the end of June and transplanting another crop in March two calendar years later. Although some growers crop cucurbits in particular fields every year or every other year because of wells and permanent irrigation equipment located there, such frequent cropping could lead to increased development of gummy stem blight (11,21). On the basis of this study, a simple way to reduce survival of *D. bryoniae* is to remove polyethylene mulch and incorporate cucurbit crop debris into soil with thorough tillage after harvest is completed. Depth of incorporation is less important than completely covering debris with soil (13). With this management technique, it may be possible to plant cucurbits susceptible to gummy stem blight and black rot (i.e., muskmelon, watermelon, cucumber, pumpkin, gourd, and winter squash) every other year, i.e., one growing season without cucurbits. However, a low level of gummy stem blight inoculum may still be present. If fields of muskmelon are abandoned after harvest and debris remains on polyethylene-mulched beds or the soil surface for up to 6 months until the field is prepared the following winter, then a 3-year rotation, i.e., 2 growing seasons out of cucurbits, is necessary to completely eliminate *D. bryoniae* inoculum in infested debris. A treatment in which crowns re-

mained on mulched beds for a period of time and then were buried was not examined. However, because *D. bryoniae* did not decline during the first 26 to 50 weeks on mulched beds, it likely would take an additional 11 to 15 months for the fungus to decline in crowns after they were incorporated into soil. The only cucurbits that could safely be grown the first or second year following a cucurbit crop affected with gummy stem blight are summer squash and zucchini (*Cucurbita pepo*), which are resistant to gummy stem blight and black rot (21).

ACKNOWLEDGMENTS

I thank V. B. DuBose, W. H. May III, and A. W. Lassiter for technical assistance. This material is based upon work supported by the CSREES/USDA under project numbers SC-1700161 and 2003-34287-13481. Technical contribution no. 5456 of the Clemson University Experiment Station.

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