Establishment of *Dicyma pulvinata* in *Cercosporidium personatum* Leaf Spot of Peanuts: Effect of Spray Formulation, Inoculation Time, and Hours of Leaf Wetness

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ABSTRACT

Mitchell, J. K., Taber, R. A., and Pettit, R. E. 1986. Establishment of *Dicyma pulvinata* in *Cercosporidium personatum* leaf spot of peanuts: Effect of spray formulation, inoculation time, and hours of leaf wetness. Phytopathology 76:1168-1171.

Visible signs of colonization by *Dicyma pulvinata* on lesions of *Cercosporidium personatum* appeared within 58–65 hr (21–31.5 hr of leaf wetness) after inoculation in growth chamber experiments at 26 C. Spores of *D. pulvinata* suspended in distilled H_2O, 0.3% methylcellulose (CMC), 0.2% citrus pectin, or 0.25% ghatti gum colonized more lesions of *C. personatum* and at a faster rate when plants sprayed with *D. pulvinata* were subjected to an initial dry-leaf compared with an initial wetted-leaf incubation period. Spores of *D. pulvinata* suspended in CMC exhibited the least amount of variability in colonization rates between these two initial incubation periods. The efficacy of several different isolates of *D. pulvinata* for colonization of *C. personatum* also was investigated. Mutants were selected for growth under low relative humidity (RH80), high temperature (TR16), and resistance to benomyl (BR30). The isolate RH80 of *D. pulvinata* colonized lesions of *C. personatum* more rapidly than either the wild-type or BR30 isolates at 26 C.

Additional key words: biological control, *Arachis*, mycoparasites.

The fungus *Dicyma pulvinata* (Berk. & Curt.) v. Arx (= *Hansfordia pulvinata* (Berk. & Curt.) Hughes) is a mycoparasite. It was found on late leaf spot lesions in south Texas in 1978 (9) and may be a useful agent for the control of this leaf spot in peanuts caused by *Cercosporidium personatum* (Bert. & Curt.) Deighton (= *Phaeoisariopsis personata* (Berk. & Curt.) v. Arx) (8).

Biological control of a foliar disease such as late leaf spot is a highly desirable method for use in conjunction with chemical methods of disease control. Only partial success of biological control has been shown in field tests with many biocontrol agents, which points to knowledge gaps that relate to the impact of the field environment and application technology. A step toward narrowing this gap would be the selection of a suitable carrier for the biocontrol agent that would aid in its survival and mycoparasitic activity. Presently, several different carrier systems are under investigation for possible use with biocontrol agents. For soilborne diseases, carrier systems under study include fluid drilling with gels (5), diatomaceous earth granules (1), wheat bran cultures (4), lignite-stillage (6), and pelletized sodium alginate (10). Reports concerning carrier systems for foliar biocontrol agents are sparse compared with soilborne systems, but a few carriers have been described. The postemergent mycoherbicide Collego (Upjohn Co., Kalamazoo, MI) is sold as a two-component product; component A consists of a water soluble rehydrating agent (carrier) that allows the spores of *Colletotrichum gloeosporioides* (Penzig) Penzig & Sacc. f.sp. *aeschynomene* (component B) to take up H_2O before germination. The use of 2% sucrose-0.1% yeast extract as a carrier system for phyllosphere yeasts of wheat was shown to enhance biological control of both *Septoria nodorum* Berk. and *Cochliobolus sativus* (Ito & Kuribay.) Drechsler & Dastur (3).

Our objectives were to find a carrier system that would optimize the mycoparasitic activity of *D. pulvinata* on lesions of *C. personatum* under field conditions and to compare the efficacy of several different isolates of *D. pulvinata* on lesion colonization once the best carrier system had been chosen.

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MATERIALS AND METHODS

Preparation of *Cercosporidium* infected plants. Thirty-day-old plants of the susceptible *Arachis hypogaea* L. cultivar Tamnut-74 grown in 11- × 14-cm plastic pots (three plants per pot) within the greenhouse were inoculated with 150–300 ml of a conidial suspension of *C. personatum* (1×10^4 spores per milliliter) using a hand sprayer. Plants were placed into a dew-deposition chamber (Percival model RFCS) adjusted for 13 hr dark (10.5 hr dewpoint) and 11 hr light (55% RH) at constant 26 C. After 5 days plants were transported back to the greenhouse and kept there until lesions attained 2–3 mm in diameter.

Effect of spray carrier and time of inoculation on the rate of colonization by *D. pulvinata*. Forty pots of peanut plants infected with *C. personatum* were placed into a dew-deposition chamber adjusted as described previously and left for 2 days to acclimate. The plants were divided into two groups and each group sprayed with 50 ml of spores of *D. pulvinata* (1×10^6 spores per milliliter) suspended in either distilled H_2O, 0.3% methylcellulose (CMC), 0.2% citrus pectin, or 0.25% ghatti gum 30 min before being placed into the chamber (five pots per treatment). Plants in group 1 were placed into the dew-deposition chamber at the beginning of the day cycle (initial period of dry leaves); plants in group 2 were placed into the chamber at the beginning of the night cycle (initial period of wetted leaves). Percentage of total lesions of *C. personatum* exhibiting visible signs of colonization by *D. pulvinata* was recorded at each day/night cycle change. A total of at least 200 individual lesions of *C. personatum* per carrier tested were used and the experiment was repeated three times.

Isolates of *D. pulvinata* tested. Six isolates of *D. pulvinata* were tested. Wild-type isolates included *D. pulvinata* collected from Atascosa County, TX (AWT); Potts farm, Brazos Co., TX (POTTS); and Poth, Wilson County, TX (POTH). Isolates TR16 (high-temperature tolerant), BR30 (benomyl tolerant, 10 ppm a.i.), and RHG80 (low-relative-humidity tolerant) mutants of *D. pulvinata* were obtained by mutation of the wild-type isolate AWT with ethyl methanesulfonate (EMS) by a modification of the procedure of Lindegren et al (7). For the isolation of isolate TR16, 0.1 ml of the appropriately diluted EMS-treated spore suspension was spread onto V-8 agar plates and incubated at 30 C. This isolate exhibited sporulation after 30 days at this temperature. Isolation of BR30

encompassed seeding V-8 agar plates containing benomyl (0.5 μg/ml a.i.) with EMS-treated spores and selecting fungal colonies formed at 25 C after 7 days. For the isolation of RH80, attached peanut leaves infected with *C. personatum* were sprayed with a suspension of EMS-treated spores of *D. pulvinata*. The leaves were sealed into 1-qt Mason jars at the petiole using sealing putty. Mason jars contained 250 ml of 51% aqueous glycerol (w/w), which maintained a relative humidity of approximately 80% at 26 C (2). Plants with jars were placed into a growth chamber adjusted for 12 hr dark/12 hr light at 26 C. RH80 was isolated from hyphal strands of *D. pulvinata* in a single colonized lesion after 9 days of incubation. All three mutant isolates maintained their selected traits during the period of 3 mo under which the experiment was performed.

Efficacy of different isolates of *Dicyma*. Thirty pots of peanut plants were infected with *C. personatum* as described previously and placed into a dew-deposition chamber, adjusted for 13 hr dark (10.5 hr of dew point) and 11 hr light (55% RH) at the beginning of the day cycle (initial dry-leaf period). Thirty minutes before being placed into the chamber, plants were divided into groups of five pots each and each group was sprayed with a 50-ml spore suspension (1×10^6 spores per milliliter) of an isolate of *D. pulvinata* in 0.3% CMC. Isolates tested included AWT, BR30, TR16, RH80, POTTS, and POTH. Percentage of total lesions of *C. personatum* exhibiting visible signs of colonization by *D. pulvinata* was recorded at each day/night cycle change. This experiment was repeated twice.

RESULTS

Interactions of spray formulation and initial leaf moisture. In growth chamber experiments at 26 C, visible signs of colonization by *D. pulvinata* of lesions of *C. personatum* appeared within 58–65 hr (21–31.5 hr of leaf wetness) after application. Spores of *D. pulvinata* suspended in either distilled H_2O, 0.3% CMC, 0.2% citrus pectin, or 0.25% ghatti gum colonized more lesions of *C. personatum* and at a faster rate when lesions of *C. personatum* on peanut leaves were subjected to an initial dry-leaf period as opposed to an initial wetted-leaf period; day vs. night inoculation, respectively (Fig. 1). Most rapid colonization of leaf tissue by *C. personatum* was observed with plants subjected to an initial dry-leaf period that were sprayed with spores of *D. pulvinata* suspended in distilled H_2O (Fig. 1, left). Spores of *D. pulvinata* suspended in CMC exhibited the least amount of variability between these two initial environmental conditions (Fig. 1, left). The differences in initial environmental conditions on the leaf surface and their effect on establishment of *D. pulvinata* became more apparent by plotting percentage of colonization vs. hours of leaf wetness (Fig. 1, right). As shown previously, lesions were colonized more rapidly with spores of *D. pulvinata* suspended in distilled H_2O, or 0.2% citrus pectin, or 0.25% ghatti gum when plants were subjected to an initial dry-leaf period (Fig. 1, right). However, there was no visible difference in the rates of lesion colonization by *C. personatum* between these two initial environmental conditions with spores of *D. pulvinata* suspended in 0.3% CMC (Fig. 1, right). The CMC formulation decreased the time lag in colonization by approximately 10 hr of leaf wetness on plants placed in the chamber with an initial dry-leaf vs. wetted-leaf period.

A comparison of the efficacy of the different spray formulations on rates of lesion colonization by *C. personatum* is shown in Figure 2. Regardless of the spray formulation used, all lesions of *C. personatum* exhibited visible signs of colonization by *D. pulvinata* after 133 hr in plants subjected to an initial dry-leaf incubation period (Fig. 2A). There was no major visible difference between rates of lesion colonization between the different spray formulations. Lesions were colonized more rapidly when peanut plants were subjected to an initial dry-leaf period (Fig. 2A)

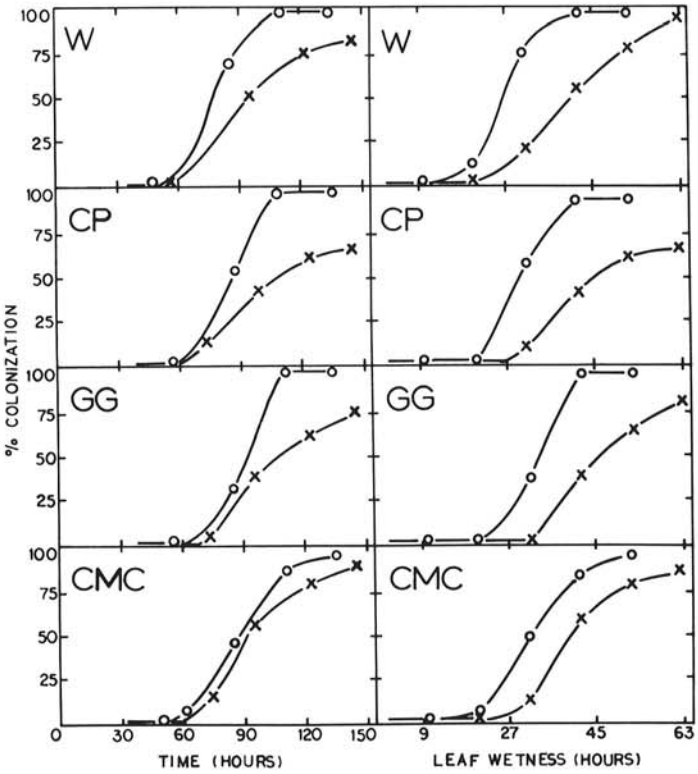

Fig. 1. Effect of spray carrier and initial environmental conditions on the rate of colonization by *Dicyma pulvinata*. **Left,** total hours after inoculation. **Right,** hours of leaf wetness after inoculation. Infected peanut plants were sprayed with spores of *D. pulvinata* suspended in either distilled H_2O (W), 0.2% citrus pectin (CP), 0.25% ghatti gum (GG), or 0.3% methylcellulose (CMC). Inoculated plants were subjected to either an initial dry-leaf (o) or an initial wet-leaf (×) incubation period.

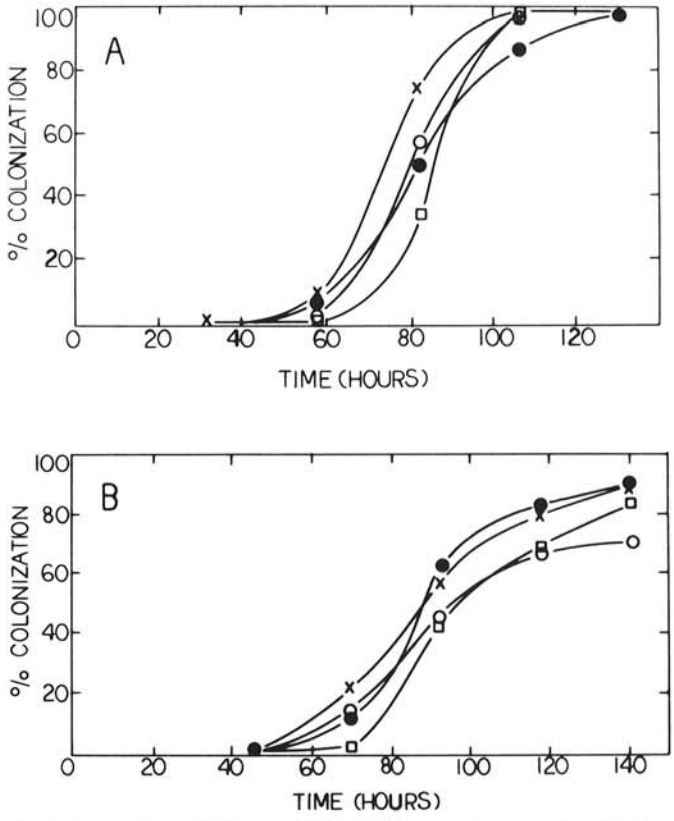

Fig. 2. Comparison of efficacy of different spray carriers on rates of lesion colonization by *Cercosporidium personatum*. **A,** Initial dry leaves; and **B,** Initial wetted leaves. Infected peanut plants were sprayed with spores of *Dicyma pulvinata* suspended in either distilled H_2O (×), 0.2% citrus pectin (o), 0.25% ghatti gum (□), or 0.3% methylcellulose (•).

compared with an initial wetted-leaf period (Fig. 2B). Furthermore, asymptotic levels of lesion colonization by *C. personatum* averaged only 81% (Fig. 2B) between different spray formulations after 133 hr with plants subjected to an initial wetted-leaf period (compared with 100% colonization observed at this time with plants subjected to an initial dry-leaf period).

Efficacy of different *Dicyma* isolates. Rates of lesion colonization of *C. personatum* with different isolates of *D. pulvinata* are shown in Fig. 3. Isolate RH80 colonized lesions just as effectively as the AWT isolate. Slowest colonization rates of lesions of *C. personatum* were observed with isolates TR16, POTTS, and POTH of *D. pulvinata*.

DISCUSSION

Our results support two conclusions: The composition of the spray formulation used is important in enabling the mycoparasite to colonize its host most efficiently, and the initial environmental conditions proceeding application of the mycoparasite also affect efficient establishment of the mycoparasite. Most rapid lesion colonization of *C. personatum* was observed with plants subjected to an initial dry-leaf period that were sprayed with spores of *D. pulvinata* suspended in distilled H_2O. However, as with citrus pectin and ghatti gum, the visible rates of lesion colonization by *C. personatum* with the H_2O carrier varied greatly between plants subjected to an initial dry- vs. an initial wetted-leaf environment. Light exhibited no effect on the rate of spore germination of *D. pulvinata* (J. K. Mitchell, *unpublished*). This would rule out the possibility of a light-induced decrease in the lag time of germination, which would result in the earlier appearance of colonization by *D. pulvinata* recorded with plants subjected to an initial dry-leaf period (day inoculated). It may be that the absence of moisture after application stimulates spore germination of *D. pulvinata*. The presence of free moisture during the initial incubation period (night-inoculated plants) may limit O_2 concentration in the leaf surface microclimate, which may hinder spore germination of *D. pulvinata*. These results present an advantage for the practical use of this mycoparasite. Spores of *D. pulvinata* would be applied to plants in early evening to take advantage of cooler temperatures and lack of leaf wetness compared with inoculation of plants during dew point, which most likely would not occur until early morning hours.

Spores of *D. pulvinata* treated with 0.3% CMC before inoculation of plants exhibited little variability in rates of lesion colonization of *C. personatum* between the two initial incubation environments. Therefore, the CMC formulation stimulated colonization not observed with other spray formulations found after an initial wetted leaf incubation period. Presently, the mechanism for this phenomenon is not fully understood. CMC appeared to swell and contract between wetted- and dry-leaf periods (night vs. day cycles) during the course of the experiment. This swelling during periods of leaf wetness may result in a more aerobic environment stimulating the mycoparasite to grow better. The effect of the CMC formulation on colonization by *C. personatum* becomes even more apparent by plotting the percentage of colonization vs. hours of leaf wetness (instead of total hours). Lesions of *C. personatum* were colonized at similar rates with plants subjected to either an initial dry-leaf or wetted-leaf period; colonization rates were parallel. Subjecting plants to an initial dry-leaf period after inoculation resulted in a decrease in the time lag of colonization by approximately 10 hr of accumulated leaf wetness, compared with plants subjected to an initial wet-leaf period.

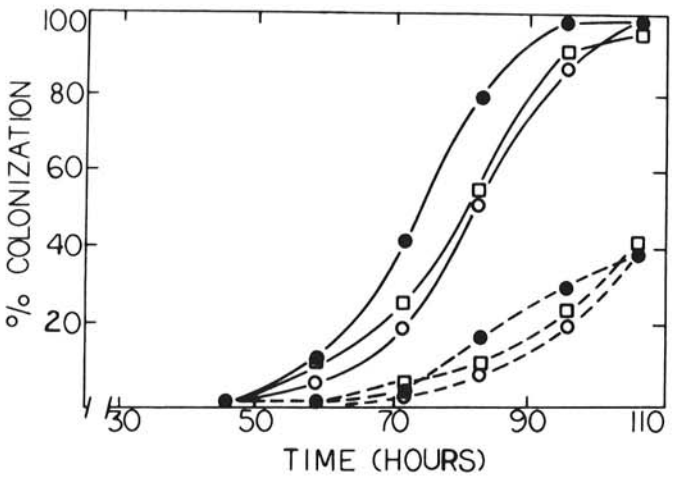

Fig. 3. Efficacy of different isolates of *Dicyma pulvinata* on rate of lesion colonization of *Cercosporidium personatum*. Isolates of *D. pulvinata* included: RH8O (•–•), AWT (□–□), BR30 (o–o), TR16 (o- -o), POTH (□- -□), and POTTS (•- -•).

There were no major visible differences in rates of lesion colonization between the different spray formulations when data were compared within either an initial dry-leaf incubation period or an initial wet-leaf incubation period. Rates of lesion colonization with different spray formulations were more rapid in plants subjected to an initial dry-leaf period versus wetted-leaf period. Not only did the initial environmental condition affect the rate of lesion colonization, but it also affected the asymptotic levels. Asymptotic levels of colonization averaged 100% after 133 hr for the different spray formulations in plants subjected to an initial dry-leaf period and 81% in plants subjected to an initial wetted-leaf period. It is important to realize that if only one type of initial environmental parameter had been investigated, the advantage of the CMC formulation over the other formulations would have been missed. If this had been the case, H_2O would have been chosen as the best formulation because lesions of *C. personatum* were colonized more rapidly by *D. pulvinata* suspended in this carrier. However, the effects of this latter formulation on rates of lesion colonization were shown to be affected by changes of leaf surface moisture after inoculation. The use of the CMC formulation would enable *D. pulvinata* to be more tolerant to sudden shifts in the leaf surface microclimate (as often occurs under field conditions) as pertaining to moisture. Optimal use of this mycoparasite would be the inoculation of plants 10–12 hr before expected dew point with a spray formulation consisting of 0.2–0.3% CMC.

Six different isolates of *D. pulvinata* were evaluated for their effectiveness for lesion colonization by *C. personatum*. It is interesting that of the three naturally occurring isolates (AWT, POTTS, and POTH), the AWT isolate colonized lesions much more effectively, especially because the POTH and AWT isolates were from a common geographical area of the state. The RH80 (low-relative-humidity tolerant) isolate colonized lesions of *C. personatum* more rapidly than any of the other isolates of *D. pulvinata*, including the AWT (prototroph of *D. pulvinata* mutants RH80, BR30, and TR16). It is interesting that the selection pressure used to obtain RH80 also carried along with it the selection for a more aggressive strain than the prototroph AWT. The BR30 (benomyl-tolerant) isolate colonized lesions just as well as the AWT isolate. The selection pressure for the BR30 isolate did not carry along with it any change in the aggressiveness of the prototroph. Isolates TR16 (high-temperature tolerant), POTTS, and POTH colonized lesions of *C. personatum* much more slowly than any of the other isolates.

Several factors affecting the biological control of Cercosporidium leaf spot of peanuts by *D. pulvinata* have been discussed. Information presented demonstrates that the initial microclimatic conditions (wet vs. dry leaves) following application of this mycoparasite not only affected the subsequent rate of lesion colonization, but also affected the asymptotic levels of colonization achieved. Furthermore, the use of a specific spray formulation (e.g., CMC) was shown to play a major role in overcoming this effect. These results exemplify the possibility of artificially manipulating the leaf surface microclimate with certain spray formulations to optimize conditions for mycoparasitic activity. The merits of these factors will become more apparent when enough experimental data have been collected and reviewed

over a period of time. Work in progress with this biocontrol agent will deal with obtaining more information that relates to the impact of the field environment and application technology.

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