# Parasitism of *Uncinula necator* Cleistothecia by the Mycoparasite *Ampelomyces quisqualis*

S. P. Falk, D. M. Gadoury, P. Cortesi, R. C. Pearson, and R. C. Seem

Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456-0462.
Current address of P. Cortesi: Istituto di Patologia Vegetale, Università degli Studi di Milano, 20133 Milano, Italy.
This research was supported by the USDA Special Research Grants/Northeastern IPM Program and the New York State Integrated Pest Management Program. P. Cortesi was supported by a fellowship from the National Research Council of Italy.
We thank R. Alexander, D. George, D. Riegel, and J. Robinson for technical assistance and the Taylor Wine Co. for use of their vineyard.
Accepted for publication 3 April 1995.

## ABSTRACT

Falk, S. P., Gadoury, D. M., Cortesi, P., Pearson, R. C., and Seem, R. C. 1995. Parasitism of *Uncinula necator* cleistothecia by the mycoparasite *Ampelomyces quisqualis*. Phytopathology 85:794-800.

Parasitism of *Uncinula necator* cleistothecia by the mycoparasite *Ampelomyces quisqualis* was widespread in the Vitaceae around New York State. Although *A. quisqualis* did not survive in naturally parasitized *U. necator* cleistothecia on grape leaves, it did overwinter in parasitized cleistothecia on the bark of grapevines. Although only 1% of the total population of cleistothecia on bark was parasitized, the bark may still be an important site for overwintering of *A. quisqualis* since the mycoparasite is located adjacent to developing powdery mildew colonies on leaves, analogous to that of healthy cleistothecia, which also overwinter on bark and release primary inoculum to infect emerging grape leaves. In vitro studies of parasitism of *U. necator* cleistothecia showed that infection occurs only during early stages of development prior to or at the earliest stages of the formation of appendages but before darkening of the cleistothecial wall. When *A. quisqualis* was applied to grapevines from colonized cotton-wick cultures suspended above vines, parasitism of cleistothecia on leaves increased compared to naturally occurring parasitism, although during a season with high rainfall the level of parasitism was similar by the end of the season. The impact of increased parasitism was a reduction in the number of cleistothecia dispersed from leaves to bark and a reduction (50 to 60%) in the number of cleistothecia overwintering on bark of grapevines. Thus, biological control of grape powdery mildew with *A. quisqualis* may be further enhanced by a reduction in the level of overwintering inoculum for the next season.

*Ampelomyces quisqualis* Ces. is a naturally occurring mycoparasite on both the anamorph and teleomorph of many species of powdery mildews. The mycoparasite infects and forms pycnidia within powdery mildew hyphae, conidiophores, conidia, and cleistothecia (2,3,11,23,27,28,30,32). This parasitism reduces sporulation and production of cleistothecia and may eventually kill the mildew colony (3,12,18,24). The mycoparasite has been recorded on more than 64 species in the genera *Brasiliomyces*, *Erysiphe*, *Leveillula*, *Microsphaera*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca*, *Uncinula* and in the anamorphic genera *Oidium* and *Oidiopsis*. These reports have come from 256 species of host plants representing 172 genera in 59 families and from collections from 28 countries (S. P. Falk, *unpublished data*). This wide host range and tolerance to a number of fungicides used in the control of powdery mildews (16,19,21,26,28) makes *A. quisqualis* a highly desirable candidate for biological control.

Much research has focused on foliar application of *A. quisqualis* to control powdery mildews (13,18,19,24,25,28), while only a little has focused on the ecology of the mycoparasite in the field. In particular, no studies have addressed the impact of parasitism of cleistothecia on the host powdery mildew or the fate of these parasitized cleistothecia, despite reports of parasitism of cleistothecia in many species of powdery mildew, including *Uncinula necator* (Schwein.) Burrill (7).

*U. necator* cleistothecia are dispersed from infected leaves to bark by rainfall, where they become the only source of primary inoculum for grape powdery mildew in New York vineyards (1,7,8,9,15) and an additional source of inoculum in some California (29) and Australian vineyards (14). A reduction in this overwintering inoculum has substantially delayed powdery mildew epidemics in New York vineyards (10). Therefore, an added benefit of biological control with *A. quisqualis* may be a reduction of overwintering inoculum due to parasitism by the mycoparasite.

In this study we examined the development and fate of parasitized cleistothecia of *U. necator* under natural conditions and when an exogenous source of inoculum of the mycoparasite was applied to grapevines. Our specific objectives were to determine: i) whether different hosts of *U. necator* within the Vitaceae in New York State bore cleistothecia parasitized by *A. quisqualis*; ii) the location of overwintering cleistothecia parasitized with *A. quisqualis*; iii) the effect of maturation of cleistothecia on susceptibility to parasitism; iv) the impact of an exogenous source of *A. quisqualis* on development of cleistothecia; v) the effect of increased parasitism of *U. necator* on the number of cleistothecia dispersed from leaves to bark prior to overwintering; and vi) whether increased parasitism of *U. necator* impacts the number of cleistothecia surviving on bark. Preliminary reports of this research have been published (4,5).

## MATERIALS AND METHODS

**Occurrence of *A. quisqualis* in *U. necator* cleistothecia within the Vitaceae.** Plant parts infected with *U. necator* and bearing cleistothecia were collected from several *Vitis* interspecific hybrid cultivars, *V. labrusca* L. 'Concord,' *V. riparia* Michx., and *Parthenocissus quinquefolia* (L.) Planch. around New York during late summer and early fall in 1991, 1992, and 1993. Colonies of *U. necator* from 5 to 10 leaves per collection were examined

Corresponding author: S. P. Falk; E-mail address: stuart_falk@cornell.edu

under a dissecting microscope for the presence of parasitized cleistothecia shortly after collection or after 24 h of incubation in humidity chambers. Parasitism by *A. quisqualis* was confirmed by the presence of conidia in cleistothecia or by isolating the mycoparasite on agar media from conidial droplets exuded from parasitized cleistothecia.

**Overwintering of *A. quisqualis* in naturally parasitized cleistothecia on leaves.** Leaves were collected on 29 October 1991 from a 10-year-old planting of the *Vitis* interspecific hybrid cultivar Chancellor at Geneva, NY. Leaves were uniformly and heavily infected with *U. necator* and had begun senescing. Cleistothecia were counted in 20 5-mm-diameter fields of view separately on the upper and lower surfaces of each of 10 leaves under a dissecting microscope at 50× magnification. Parasitized cleistothecia were recognized by their flaccid and dull fawn-colored appearance or by the presence of conidial droplets of the mycoparasite on the surface of the ascocarp. Mature cleistothecia were dark brown with fully developed appendages. The viability of *A. quisqualis* was determined by observing the germination of conidia from 100 parasitized cleistothecia squashed on the surface of water agar (WA) in petri plates after 24 h of incubation at room temperature. Germination was scored when the germ tube exceeded the length of the conidium. Additional leaves (approximately 20) were placed in each of several pouches (about 30 cm by 30 cm) made from fiberglass screening to examine the effect of overwintering on the number and viability of parasitized cleistothecia on leaves. Pouches containing leaves were either hung in the trellis or placed on the ground and secured with tent pegs. On 15 January, 26 February 1992, and 6 April 1992, pouches were retrieved from the field, and leaves were examined for cleistothecia. The upper and lower surfaces of 10 leaves hung in the trellis and of 20 leaves from the ground were examined; more leaves from the ground were examined because there were fewer cleistothecia. The observations were repeated on 25 March 1994 by sampling the leaves that remained in the trellis and on the ground in the same vineyard after the winter. Ten leaves were examined from both the trellis and the ground. To determine whether leaf litter remained in the vineyard until bud break, the density of leaf litter on the vineyard floor was determined by a point-intercept method (6) on 26 March and 4 May 1992 and on 25 March and 15 May 1994.

**Overwintering of *A. quisqualis* in naturally parasitized cleistothecia on bark.** Bark samples were collected from the cordons of a 10-year-old planting of Chancellor at Geneva, NY, at 3-week intervals from March to June 1992 and were repeated at monthly intervals between September 1992 and May 1993. A bulk sample of bark was collected from the vines in two entire rows and divided into four subsamples. Cleistothecia were collected by a wet-sieving technique (1,15). Each sample of 20 g of air-dried bark was added to 1 liter of cold water and shaken vigorously for 3 min; the washings were poured through nested 50 (300 μm), 120 (125 μm), and 170 (90 μm) mesh sieves. Washings with 1 liter of water were repeated two more times for 1 min each. Cleistothecia retained on the 125- and 90-μm sieves were removed by backwashing, and the volume was adjusted to either 50 or 100 ml. Three or four aliquots of 4 or 5 ml were removed from each 125- and 90-μm sieve and applied to filter paper, where parasitized and nonparasitized cleistothecia were trapped and counted under a dissecting microscope. Counts from each sieve were combined and expressed as cleistothecia per g of bark. Putative parasitized cleistothecia were transferred and squashed onto the surface of WA in petri plates and examined under a compound microscope after 24 h of incubation at room temperature. The percent viability of *A. quisqualis* conidia from individual parasitized cleistothecia was determined with germination counts of 50 conidia.

**Effect of maturation of cleistothecia on development of parasitism.** Leaves, bearing cleistothecia at different stages of development, were collected from a 20-year-old planting of the *Vitis* interspecific hybrid cultivar Delaware at Geneva, NY, on 22 September 1993 and from a 10-year-old planting of Chancellor at Geneva, on 26 July and 3 August 1994. Four colonies on each of 15 leaves were examined at 50× and determined to be free of natural parasitism by *A. quisqualis*, and 5-mm-diameter areas in these colonies were marked by pinpricks. Ten leaves were sprayed with an aqueous suspension of *A. quisqualis* conidia, and five leaves were sprayed with distilled water using a nonchlorinated fluorocarbon aerosol sprayer (Preval, Precision Valve Corporation, Yonkers, NY).

Conidial suspensions were prepared by first growing the fungus, originally obtained from parasitized *U. necator* colonies on leaves of the *Vitis* interspecific hybrid cultivar Rosette in Geneva, during the autumn of 1989, on wheat bran malt agar (WBMA) (strained extract from 100 g of wheat bran, 20 g of malt extract, 2 g of DL-asparagine, 15 g of agar, 1 liter of distilled water, pH 6.5). After 2 to 3 weeks in the dark at 24°C, conidia were harvested by adding distilled water to the petri dishes and rubbing the lawn of pycnidia with a glass rod. Conidia in the suspensions were counted with a hemacytometer and adjusted to $2 \times 10^6$ conidia per ml in 0.02% Tween 20 (germinability of 100 conidia, 81 ± 2.8%, $n = 5$). Leaves were incubated in moist chambers at room temperature (~25°C) for 24 h and then in partially covered plastic boxes for an additional 5 days. Numbers of cleistothecia at different stages of development were counted in each colony in a 50× field of view under a dissecting microscope at the beginning of the experiment and under the same field of view at the end of the experiment. Cleistothecia were recognized as occurring in one of six stages of development, which in order of development are: hyaline (<40 μm in diameter); pale yellow (40 to 80 μm in diameter) without appendages; yellow (80 to 100 μm in diameter) with appendages; orange (≥100 μm in diameter) with appendages; brown (≥100 μm in diameter) with appendages; and black (≥100 μm in diameter) with appendages. Parasitized cleistothecia were recognized by their appearance and confirmed to be parasitized by examining squashed cleistothecia under a compound microscope for the presence of conidia of *A. quisqualis*.

Two relationships were examined. First, the relationship between the stage of development 6 days after treatment of cleistothecia with the mycoparasite and percent parasitism was examined. Cleistothecia diameter (in micrometers), a continuous variable calculated from the midpoint of cleistothecia diameter in each of the six stages of development, was regressed against percent parasitism at the end of the experiment. Second, the failure of individual cohorts to advance to a more mature stage of development due to parasitism was examined. This was determined by assuming that the number of healthy cleistothecia of a particular maturity stage present at $t = 6$ days included those already at that stage of development at $t = 0$ days plus some or all of those from successively less mature stages of development until all the cleistothecia in the present maturity stage at $t = 6$ days were accounted for. By beginning these calculations with the most mature cleistothecia and continuing with each successively less mature stage of development, it was possible to determine the cleistothecia that failed to mature due to parasitism.

**Deployment of *A. quisqualis* to grapevines in the field.** *A. quisqualis* was applied to grapevines from cotton-wick cultures in 1992 and 1993. Cotton-wick cultures of *A. quisqualis* were prepared by growing the fungus on cotton twine (30-ply cotton/polyester blend) (King Cotton, John H. Graham & Co., Oradell, NJ). Cotton twine was washed in distilled water for 2 h and wrung out to remove most of the water, and single 1-m lengths of twine were placed in 113-g baby-food jars and sterilized. Jars was inoculated with 8 ml of a suspension of $1 \times 10^7$ conidia per ml in cooled (40°C) modified WBMA (0.1% agar) and incubated at 24°C in the dark. After 3 weeks, the twine cultures were dried overnight and stored at 4°C until needed. Wick cultures were suspended about 30 cm above vines by attaching to nylon twine

suspended between spikes nailed into the tops of vineyard posts. Two 1-m lengths of wick were suspended above each vine. In 1992 cotton-wick cultures were deployed above vines in a 10-year-old planting of Rosette at Geneva, NY, and in a 20-year-old commercial vineyard of *Vitis* interspecific hybrid cultivar Aurore at Dresden, NY. On Rosette, all vines in 4 vine plots were treated with *A. quisqualis* or were untreated in a randomized complete block with three replicates. Cotton wicks were deployed at the 3-cm shoot growth stage on 15 May, and additional wicks were deployed over those already present at prebloom on 4 June and at the 1-cm berry stage on 27 July. On Aurore, all vines in 36 vine plots (six rows by two panels) were treated with *A. quisqualis* or were untreated. Plots were completely random with three replicates. Treatments were deployed at 10 cm of shoot growth on 21 May and again at early bloom on 18 June and at the 1-cm berry stage on 22 July.

In 1993, *A. quisqualis* was applied to a different part of the Aurore vineyard used in 1992. Plots composed of 36 vines in six rows, with four replicates arranged in a randomized complete block were used. Colonized wicks were successively deployed on the following dates: 11 May at 3 cm of shoot growth, 14 June at bloom, and 13 July, at the 1-cm berry stage. Experimental plots were surrounded by vines that were sprayed with pesticides using a hooded boom sprayer as part of the normal vineyard maintenance. At Dresden, a minimum distance of approximately 15 m occurred between plot corners.

**Impact of parasitism by *A. quisqualis* on development of cleistothecia.** Leaves were collected from vines beneath cotton-wick cultures of *A. quisqualis* to compare the amount of parasitism of the powdery mildew and the impact of parasitism on the formation of cleistothecia. Ten leaves were collected from between the third and seventh nodes on randomly selected vines in each of the replicated plots of *A. quisqualis*-treated and untreated vines in the Rosette and Aurore vineyards described above. On 30 June 1992, 30 leaves were collected from each plot of Aurore vines. Leaves were collected from Rosette in Geneva on 7 July, 18 August, and 7 October and from Aurore in Dresden on 30 June and 25 August in 1992. In 1993, leaves were collected from Aurore in Dresden on 27 July, 17 August, and 20 September. Powdery mildew colonies on both the upper and lower surfaces of each leaf were examined under a dissecting microscope. The number of parasitized and nonparasitized cleistothecia (including both immature and mature ascocarps), was counted in 10 5-mm-diameter areas of powdery mildew colony per leaf surface. Cleistothecia were confirmed to be parasitized by the presence of *A. quisqualis* conidia when squashed and examined under a compound microscope. Colonies were considered parasitized when either parasitized cleistothecia or pycnidia of *A. quisqualis* were observed.

**Impact of *A. quisqualis* parasitism on the dispersal of cleistothecia to bark.** Cleistothecia were collected in filter-paper funnels attached to the bark of grapevines as described by Gadoury and Pearson (7) to determine whether parasitized cleistothecia were dispersed from leaves to grapevine bark by rainfall during the fall. Funnels were attached to Aurore vines at Dresden that were untreated or treated with *A. quisqualis*-colonized wicks in 1993 as described previously. Two funnels were attached to the upper trunks of each of five vines in each of the replicated *A. quisqualis*-treated and untreated plots. The funnels were collected after rain events and examined under a dissecting microscope for parasitized cleistothecia as previously described. Funnels were in place 5 days prior to collection on 29 September 1993 and 6 days prior to collection on 5 October 1993.

**Impact of *A. quisqualis* parasitism on overwintering of cleistothecia on grapevine bark.** Bark was collected from Aurore vines at Dresden during the spring of 1993 and during the winter and spring of 1994 to determine whether the mycoparasite affected the number of cleistothecia overwintering on bark. Samples of bark (3 g) were collected from each of 10 vines in each plot of 36 vines untreated or treated in 1992 and 1993 with *A. quisqualis* on cotton twine. Three replicate samplings were conducted in 1993 and four replicates in 1994 prior to bud break. The 30 g of air-dried bark was wet-sieved as previously described, and putative parasitized cleistothecia were examined under a compound microscope. Viability of the *A. quisqualis* conidia was assessed by germination on WA.

**Statistical analyses.** The software package Systat 5.2 (Systat Inc., Evanston, IL) was used to perform analyses of variance,

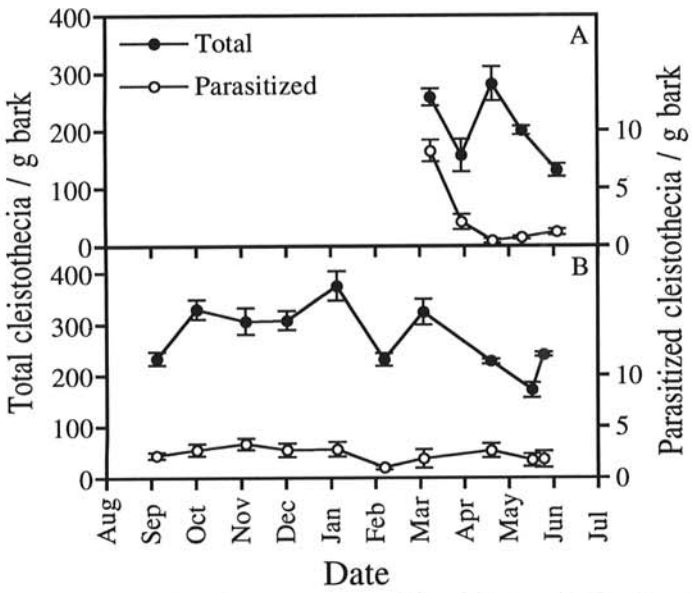


**Fig. 1.** Total number of *Uncinula necator* cleistothecia (● and left axis) and cleistothecia naturally parasitized by the mycoparasite *Ampelomyces quisqualis* (○ and right axis) per g of air-dried bark from Chancellor grapevines, **A,** during the winter and spring of 1992 after the 1991 growing season and **B,** during the fall of 1992 and winter and spring of 1993 after the 1992 growing season at Geneva, NY. Bars are one standard error of the mean from four replicates.

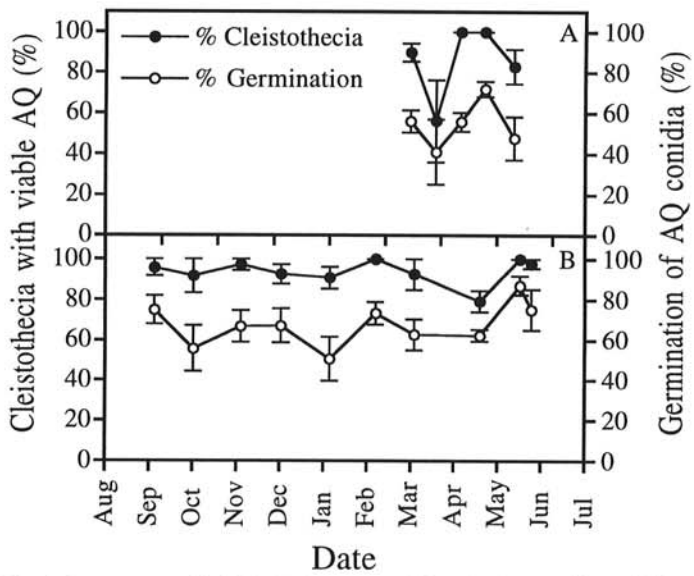


**Fig. 2.** Percent parasitized *Uncinula necator* cleistothecia containing viable *Ampelomyces quisqualis* (AQ) conidia (●) and percent germination of *A. quisqualis* conidia from parasitized cleistothecia (○) collected from the bark of Chancellor grapevines naturally parasitized by the mycoparasite **A,** during the winter and spring of 1992 after the 1991 growing season and **B,** during the fall of 1992 and winter and spring of 1993 after the 1992 growing season at Geneva, NY. Bars are one standard error of the mean from four replicates.

regressions, and $t$ tests with unequal variances. Values followed by plus/minus refer to the mean and the standard error of the mean.

## RESULTS

**Occurrence of *A. quisqualis* in *U. necator* cleistothecia within the Vitaceae.** *U. necator* cleistothecia naturally parasitized by *A. quisqualis* were observed on leaves throughout New York from cultivars Aurore and Seyval at Dresden and Chancellor, Delaware, and Rosette at Geneva; *V. labrusca* at Dresden and Geneva; *V. riparia* at Feura Bush and Albany; and *P. quinquefolia* at Feura Bush and Dundee. Parasitized cleistothecia were typically dull, fawn colored, flaccid, and ranged in size from 64 to 130 μm in diameter ($n$ = 95). Appendages were sometimes evident in the more developed cleistothecia that were parasitized, but fully mature cleistothecia, which were concave-convex with uncinate-tipped appendages and contained asci and ascospores, were never observed to be parasitized. Parasitized cleistothecia usually occurred in areas of powdery mildew colonies devoid of sporulation and mature cleistothecia. They were readily found in powdery mildew colonies on leaves but also could be found in powdery mildew colonies on canes, rachises, pedicels, and berries collected during the fall. Parasitized cleistothecia observed under the compound microscope were devoid of asci. Instead they contained conidia of the mycoparasite that were 7.5 to 9 × 2.5 to 3.5 μm, cylindrical to fusiform, occasionally curved, and biguttulate. *A. quisqualis* could be readily grown on various agar media from these conidia that were often exuded from parasitized cleistothecia placed under high humidity.

**Overwintering of *A. quisqualis* in naturally parasitized cleistothecia on leaves.** Cleistothecia occurred in high numbers on leaves of Chancellor during the fall of 1991, with a level of 22.3% natural parasitism by *A. quisqualis*. Eighty-four percent of parasitized cleistothecia contained viable conidia of *A. quisqualis*. However, no viable conidia were found during the spring of 1992 or 1994 in samples overwintered in pouches. In addition, no leaf litter was present on the vineyard floor prior to bud break.

**Overwintering of *A. quisqualis* in naturally parasitized cleistothecia on bark.** Naturally parasitized cleistothecia were isolated from the bark of Chancellor grapevines during the winter and spring of 1992 and from the fall of 1992 into the spring of 1993 (Fig. 1). The number of parasitized cleistothecia detected averaged 0.95 ± 0.18 ($n$ = 15) of the total number of cleistothecia. The total number of cleistothecia varied somewhat across the sampling dates, but reached a plateau during October 1992 of about 300 cleistothecia per g of bark and then declined during early spring to about 200 cleistothecia per g of bark at bud break during May 1993 (Fig. 1B). Sampling after the 1991 season (Fig. 1A) was conducted only during this declining phase. A high percentage of parasitized cleistothecia, averaging 91.44 ± 2.39% ($n$ = 15), contained viable *A. quisqualis* conidia across both periods (Fig. 2). An average of 63.15 ± 2.67% ($n$ = 15) of these conidia germinated on WA after 24 h (Fig. 2).

**Effect of maturation of cleistothecia on development of parasitism.** Parasitism was present in all but black cleistothecia 6 days after treatment of *U. necator* cleistothecia on leaves with a suspension of *A. quisqualis* conidia (Fig. 3A). When maturity classes were converted to a continuous variable by calculating the midpoint of cleistothecia diameter in each class, a significant inverse relationship was found between the size of cleistothecia and the likelihood of parasitism: percent parasitized = 129.53 − 0.9113 diameter (micrometers); $R$ = 0.839, $P$ = 0.037, $n$ = 6. When individual cohorts were examined, greater than 90% of the cleistothecia that were hyaline or pale yellow at $t$ = 0 did not mature due to parasitism by *A. quisqualis* (Fig. 3B). Significantly fewer ($P \leq 0.001$, $n$ = 3) yellow cleistothecia with appendages (14.6 ± 7.8%) failed to mature due to parasitism; no cleistothecia that were orange, brown, or black at $t$ = 0 failed to mature (Fig. 3B). In the absence of *A. quisqualis*, all cleistothecia had matured to a more advanced stage of development after 6 days, except for 32.5 ± 6.1% of hyaline cleistothecia.

**Impact of *A. quisqualis* applied to vines from cotton-wick cultures on the development of cleistothecia on leaves.** The impact of parasitism by *A. quisqualis* on the production of cleistothecia on leaves is shown in Table 1 for Rosette at Geneva in 1992 and for Aurore at Dresden in 1992 and 1993. On Rosette, parasitism was first detected in *U. necator* colonies on *A. quisqualis*-treated vines on 7 July. By 18 August, parasitism of cleistothecia occurred in both *A. quisqualis*-treated and untreated plots, with proportionally more cleistothecia (67.4%) parasitized under *A. quisqualis* treatment than under natural parasitism (25.1%). This increased parasitism significantly ($P$ = 0.037) reduced the number of mature cleistothecia on leaves from 12,500 cleistothecia per m$^2$ leaf area to 310 cleistothecia per m$^2$ leaf area. By 7 October, the proportion of cleistothecia parasitized was similarly high in both *A. quisqualis*-treated (80%) and untreated plots (69.4%), but the number of cleistothecia per m$^2$ had decreased from the previous sampling in the untreated plots. On Aurore in 1992 parasitized colonies were detected on 30 June. By 25 August, the proportion of cleistothecia parasitized was similar and high, and very few cleistothecia were present on leaves. At Dresden in 1993, parasitized cleistothecia were not found until mid-August, when a few occurred in the *A. quisqualis*-treated plots. At that time, 2,400 mature cleistothecia per m$^2$ leaf area

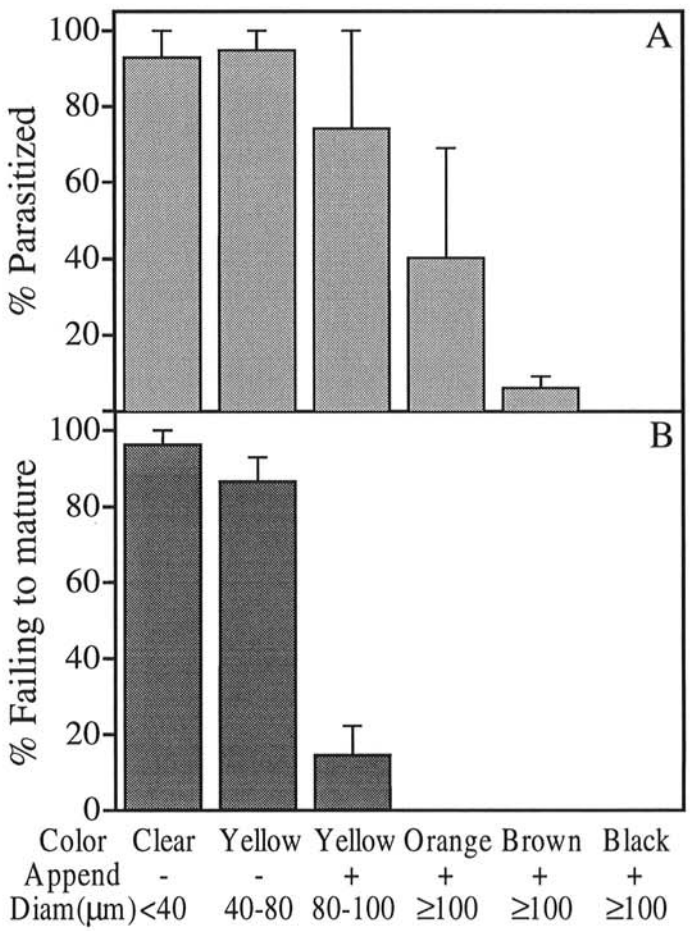


**Fig. 3.** Parasitism of *Uncinula necator* cleistothecia at different stages of maturity 6 days after treatment with a suspension of *Ampelomyces quisqualis* conidia applied to detached leaves of Delaware and Chancellor grapevines; **A,** percent cleistothecia parasitized after 6 days and **B,** percent cleistothecia failing to mature in each maturity class considering their stage of maturity at $t$ = 0. Bars are one standard error of the mean from three replicated experiments.

occurred in *A. quisqualis*-treated plots and 970 mature cleistothecia per $m^2$ leaf area occurred in untreated plots ($P = 0.070$). Although more mature cleistothecia occurred in *A. quisqualis*-treated than untreated plots at that time, the number of mature cleistothecia in the treated plots was the same (2,300 mature cleistothecia per $m^2$ leaf area) at the next sampling on 20 September, and the level of parasitism was high (66.8%). Between sampling periods the number of mature cleistothecia formed on leaves in untreated plots significantly increased ($P = 0.02$, separate variance $t$ test; $n = 4$) to 5,300 mature cleistothecia per $m^2$ leaf area. The level of natural parasitism was significantly less (1.6%) than in the *A. quisqualis*-treated plots.

**Impact of *A. quisqualis* applied to vines from colonized wicks on the dispersal of cleistothecia to bark.** Parasitized cleistothecia were collected in filter-paper funnels attached to Aurore bark at Dresden in 1993 (Table 2). Increased parasitism after treatment with *A. quisqualis* resulted in the removal of fewer mature cleistothecia from leaves to bark during fall rain events. This was significant at $P = 0.095$ on 29 September and at $P = 0.033$ on 5 October. Significantly more parasitized cleistothecia ($P = 0.009$ and 0.011, respectively) were removed from leaves in *A. quisqualis*-treated plots than from leaves in untreated plots. Compared to the total number of cleistothecia dispersed from leaves to bark, 52.7 and 68.7% were parasitized on 29 September and 5 October, respectively.

**Impact of *A. quisqualis* applied to vines from colonized wicks on the overwintering of cleistothecia on bark.** Bark examined on 20 April 1993 after the 1992 season showed an average of 2.5 cleistothecia per g of bark on *A. quisqualis*-treated vines compared to 7.2 cleistothecia per g of bark on untreated vines ($P = 0.176$) (Table 3). Parasitized cleistothecia (8.3%) were found only on bark from vines that had been treated with *A. quisqualis* (Table 3). Bark collections after the 1993 growing season contained higher numbers of cleistothecia than collections taken during the 1992 growing season. Significantly ($P \leq 0.01$) fewer cleistothecia occurred on bark from *A. quisqualis*-treated vines than untreated vines on all the sampling dates, and from 3.5 to 5.4% of the cleistothecia on *A. quisqualis*-treated vines were parasitized versus 0 to 0.2% on untreated vines (Table 3).

## DISCUSSION

*A. quisqualis* naturally parasitized colonies and cleistothecia of *U. necator* on several hosts throughout New York State. Parasitized cleistothecia could be found in mildew colonies on any plant part where cleistothecia were found. Parasitism of cleistothecia of *U. necator* is probably widespread wherever *A. quisqualis* occurs.

Previous research on overwintering of *A. quisqualis* suggested that morphologically distinct pycnidia that were darker, more spherical, and that had a definite circular ostiole were formed saprophytically in powdery mildew colonies on leaves during the fall to allow the mycoparasite to overwinter (22,31). Other studies have suggested that the mycoparasite overwintered by the formation of perithecia inside leaf tissue beneath powdery mildew colonies (3), although a formal description of this teleomorph was never published. No such structures were observed in *U. necator*-infected grape leaves overwintered on the ground or in the canopy. Overwintering of *A. quisqualis* in parasitized cleistothecia in leaf litter has been suggested (22), but our study showed that the mycoparasite did not survive in leaves on the ground. The ground also was an unsuitable environment for the overwintering of healthy cleistothecia in New York, since earth-

TABLE 1. Occurrence of *Ampelomyces quisqualis*, applied to grapevines from multiple deployed colonized cotton-wick cultures, infecting cleistothecia and colonies of *Uncinula necator* on grape leaves in 1992 and 1993

| Location Cultivar | Date | Treatment[a] | Cleistothecia/$m^2$ of leaf area | | Cleistothecia parasitized (%)[b] | Colonies parasitized (%)[c] |
|---|---|---|---|---|---|---|
| | | | Parasitized | Mature | | |
| Geneva Rosette | 7 Jul. 1992 | Untreated | 0 | 0 | ... | 0 |
| | | *A. quisqualis* | 0 | 0 | ... | 7.5 |
| | 18 Aug. 1992 | Untreated | 6,400 | 12,500 | 25.1 | 62.5 |
| | | *A. quisqualis* | 1,100 | 310 | 67.4 | 98.3 |
| | | (*P*)[d] | 0.208 | 0.037 | 0.031 | 0.153 |
| | 7 Oct. 1992 | Untreated | 2,600 | 1,100 | 69.4 | 38.7 |
| | | *A. quisqualis* | 900 | 670 | 80.0 | 37.4 |
| | | (*P*) | 0.215 | 0.558 | 0.589 | 0.906 |
| Dresden Aurore | 30 Jun. 1992 | Untreated | 0 | 0 | ... | 0.1 |
| | | *A. quisqualis* | 0 | 0 | ... | 1.1 |
| | | (*P*) | ... | ... | ... | 0.295 |
| | 25 Aug. 1992 | Untreated | 860 | 30 | 78.5 | 66.8 |
| | | *A. quisqualis* | 190 | 0 | 83.3 | 71.8 |
| | | (*P*) | 0.192 | ... | 0.808 | 0.310 |
| Dresden Aurore | 27 Jul. 1993 | Untreated | 0 | 0 | | 0 |
| | | *A. quisqualis* | 0 | 0 | | 0 |
| | 17 Aug. 1993 | Untreated | 0 | 970 | 0 | 0 |
| | | *A. quisqualis* | 30 | 2,400 | 0.1 | 0.2 |
| | | (*P*) | ... | 0.070 | ... | ... |
| | 20 Sep. 1993 | Untreated | 530 | 5,300 | 1.6 | 3.2 |
| | | *A. quisqualis* | 8,000 | 2,300 | 66.8 | 59.1 |
| | | (*P*) | 0.001 | 0.064 | <0.001 | <0.001 |

[a] *A. quisqualis* treatment consisted of colonized cotton-wick cultures deployed over *Vitis* interspecific hybrid cultivar Rosette vines during 1992 on 15 May, 4 June, and 27 July; over *Vitis* interspecific hybrid cultivar Aurore vines during 1992 on 21 May, 18 June, and 22 July; and over Aurore vines during 1993 on 11 May, 14 June, and 13 July.

[b] Percentage of total cleistothecia parasitized; total cleistothecia = parasitized + mature + immature cleistothecia.

[c] Percentage of colonies with either parasitized cleistothecia or *A. quisqualis* pycnidia inside conidiophores.

[d] Probability values within dates compare untreated and *A. quisqualis*-treated plots by separate variance $t$ tests ($n = 3$ in 1992 and 4 in 1993).

worms bury many leaves (7). However, we found naturally parasitized cleistothecia on the bark of grapevines over two successive winter seasons. On average, only about 1% of the total cleistothecia on bark were naturally parasitized, but these were highly viable (80 to 100%), containing conidia with a germinability of 50 to 80%. This level of parasitism is much reduced compared to the 22% natural parasitism of cleistothecia on leaves that occurred in the same vineyard during the fall of 1991 just prior to bark sampling. Rainfall that dispersed mature cleistothecia from leaves to bark (7) also dispersed some parasitized cleistothecia. The occurrence of parasitized cleistothecia on bark adjacent to developing powdery mildew colonies may place *A. quisqualis* in an ideal location to initiate parasitism, analogous to that of healthy cleistothecia and emergent grape leaves. The bark may be an important overwintering site for *A. quisqualis* residing within parasitized cleistothecia.

Our in vitro studies on parasitism of developing *U. necator* cleistothecia by *A. quisqualis* indicate that infection occurs only during early stages of development. This occurs while the cleistothecia are hyaline or pale yellow, less than 80 µm in diameter, and prior to or at the earliest stages of the formation of appendages but before darkening of the cleistothecial wall. Yellowing of cleistothecia is due to intracellular accumulation of lipids (7) and occurs simultaneously with a thickening of the walls of cells of the outer-most layer (7). Mature cleistothecia were never parasitized in vitro. We also have never observed parasitism in mature cleistothecia (e.g., dark brown to black with uncinate-tipped appendages) collected from leaves or bark from untreated or *A. quisqualis*-treated plots. Instead, parasitism by *A. quisqualis* was observed only in hyaline, yellow, orange, or brown cleistothecia collected from the field, and the frequency of parasitism was inversely proportional to stage of development.

Based on our in vitro studies, cleistothecia were likely parasitized while hyaline or pale yellow and matured slightly before they were killed by the mycoparasite. Parasitism may occur through the parent hyphae and anchorage hyphae that connect developing cleistothecia to the powdery mildew colony. Emmons (3) believed that infection of cleistothecia occurred this way in *Erysiphe cichoracearum*, since direct penetration of a cleistothecium by an *A. quisqualis* conidium was never observed but the mycoparasite was observed in the thin-walled hyphae of the powdery mildew colony and in the pseudo-antheridial and pseudo-oogonial hyphae from which the cleistothecia form. Infection of cleistothecia of *Microsphaera viburni* by *A. quisqualis* also occurred through parent hyphae, as observed by Speer (23). Infection of developing cleistothecia could have occurred similarly in our in vitro studies. Even though parent hyphal connections are intact until the final stages of maturation of a cleistothecium (7), we did not see parasitism of mature cleistothecia, so hyphae might only function as routes of infection during early maturation. Direct penetration of cleistothecia also might be possible during early maturation of cleistothecia, until changes in the cleistothecium wall (7) prevent penetration. Speer (23) illustrated *A. quisqualis* inside the cleistothecium wall of an immature cleistothecium of *M. viburni*. This mode of entry also would be consistent with our observations.

When *A. quisqualis* was applied to grapevines as wick cultures, parasitism was increased, and production of cleistothecia on leaves was reduced at some sampling dates in two of three site years. In 1992, this effect was only evident during early and mid-season where parasitism of powdery mildew on *A. quisqualis*-treated vines occurred earlier and at higher levels than natural parasitism. However, rainfall during June through August of 1992 was above

TABLE 2. Effect of *Ampelomyces quisqualis* cotton-wick cultures applied to *Vitis* interspecific hybrid cultivar Aurore vines during the 1993 season at Dresden, NY, on the number of *Uncinula necator* cleistothecia removed from leaves to grapevine bark

| Date | Treatment[a] | Cleistothecia/funnel | | Cleistothecia parasitized (%)[b] |
|---|---|---|---|---|
| | | Parasitized | Mature | |
| 29 Sep. 1993 | Untreated | 0.2 | 21.2 | 0.6 |
| | *A. quisqualis* | 14.6 | 6.9 | 52.7 |
| | (*P*)[c] | 0.009 | 0.095 | 0.010 |
| 5 Oct. 1993 | Untreated | 0.5 | 15.1 | 2.4 |
| | *A. quisqualis* | 18.9 | 3.8 | 68.7 |
| | (*P*) | 0.011 | 0.033 | <0.001 |

[a] *A. quisqualis* treatment consisted of colonized cotton-wick cultures deployed on 11 May, 14 June, and 13 July.
[b] Percentage of total cleistothecia parasitized; total cleistothecia = parasitized + mature + immature cleistothecia.
[c] Probability values within dates compare untreated and *A. quisqualis*-treated plots by separate variance *t* tests ($n = 4$).

TABLE 3. Effect of *Ampelomyces quisqualis* cotton-wick cultures applied to *Vitis* interspecific hybrid cultivar Aurore vines during the 1992 and 1993 growing season on the number of *Uncinula necator* cleistothecia overwintering on bark

| Date | Treatment[a] | Cleistothecia/g of bark | | Cleistothecia parasitized (%)[c] | Cleistothecia with viable *A. quisqualis* (%) | Germination of *A. quisqualis* conidia (%) |
|---|---|---|---|---|---|---|
| | | Parasitized | Healthy[b] | | | |
| 20 Apr. 1993 | Untreated | 0 | 7.2 | 0 | ... | ... |
| | *A. quisqualis* | 0.1 | 2.5 | 8.3 | 100 | 60.1 |
| | (*P*)[d] | ... | 0.176 | ... | | |
| 1 Dec. 1993 | Untreated | 0.1 | 52.1 | 0.2 | 100 | 64.0 |
| | *A. quisqualis* | 1.4 | 28.6 | 5.4 | 100 | 79.2 |
| | (*P*) | 0.015 | 0.004 | 0.063 | ... | 0.641 |
| 17 Jan. 1994 | Untreated | 0 | 47.7 | 0 | ... | ... |
| | *A. quisqualis* | 0.8 | 20.4 | 4.3 | 100 | 54.3 |
| | (*P*) | ... | 0.010 | ... | | |
| 31 Mar. 1994 | Untreated | 0.1 | 40.0 | 0.2 | 100 | 70.0 |
| | *A. quisqualis* | 0.7 | 19.0 | 3.5 | 87.5 | 64.9 |
| | (*P*) | 0.021 | 0.004 | 0.104 | ... | ... |
| 28 Apr. 1994 | Untreated | 0 | 35.6 | 0 | ... | ... |
| | *A. quisqualis* | 0.5 | 10.9 | 3.8 | 100 | 71.8 |
| | (*P*) | ... | <0.001 | ... | | |

[a] *A. quisqualis* treatment consisted of colonized cotton-wick cultures deployed over vines during 1992 on 21 May, 18 June, and 22 July and over vines during 1993 on 11 May, 14 June, and 13 July.
[b] All cleistothecia not parasitized by *A. quisqualis*.
[c] Percentage of total cleistothecia parasitized; total cleistothecia = parasitized + healthy cleistothecia.
[d] Probability values within dates compare untreated and *A. quisqualis*-treated plots by separate variance *t* tests ($n = 3$ in 1992 and 4 in 1993).

average (D. G. Gadoury, *unpublished data*), which may have promoted naturally occurring parasitism (12,13,17,18,19,20), allowing it to increase to the high levels of parasitism that had already been reached in the *A. quisqualis*-treated vines. By contrast, during the relatively dry 1993 season, fewer opportunities for parasitism may have occurred due to reduced rainfall. However, under the wick cultures, parasitism may have been greater due to the availability of *A. quisqualis* inoculum at times when rain did occur.

It was possible to observe the impact of the increased parasitism by *A. quisqualis* on the number of cleistothecia dispersed from leaves to bark and on the number of cleistothecia overwintering on bark of Aurore grapevines at Dresden. The eventual outcome of increased parasitism under *A. quisqualis* treatments was a significant reduction in the number of mature cleistothecia overwintering on grapevine bark in 1994 after the 1993 season. A similar trend was observed in 1993 after the 1992 season, but the total numbers of cleistothecia was very low in both *A. quisqualis*-treated and untreated vines, presumably due to very high natural parasitism. Even though reduced numbers of cleistothecia overwintered on bark of *A. quisqualis*-treated vines in 1994, this represents a reduction of inoculum of only 50 to 60% compared to untreated vines. The number of cleistothecia overwintering on *A. quisqualis*-treated vines in 1994 was still greater than the number overwintering in either treatment in 1993 after the 1992 season when natural parasitism was very high.

Interestingly, powdery mildew development during the 1993 growing season was delayed and never reached the high levels experienced during the 1992 growing season (S. P. Falk, *unpublished data*). The low level of overwintering inoculum available in spring 1993 may be partially responsible for the delayed powdery mildew development. However, we have no data on the amount of inoculum available during the spring of 1992 when the level of powdery mildew development was greater. Reducing the level of overwintering inoculum does affect subsequent powdery mildew development. A reduction of 90% of overwintering inoculum was estimated to substantially delay development of powdery mildew caused by *U. necator* in a study in which over-the-trellis lime-sulfur treatments were applied to dormant grapevines to eradicate cleistothecia on the bark of the vine (10).

Based on these results, we believe that season-to-season variation in natural parasitism may have a greater impact on numbers of overwintering cleistothecia, and possibly subsequent powdery mildew development, than the present method of deploying *A. quisqualis,* which relies on rainfall to disperse the mycoparasite. However, it does illustrate the potential for the mycoparasite to substantially affect levels of overwintering inoculum. Indeed, an added benefit of using the mycoparasite *A. quisqualis* to reduce disease development during the growing season may be a reduction in the level of overwintering inoculum that is available for the next season.

## LITERATURE CITED


1. Cortesi, P., Gadoury, D. M., Seem, R. C., and Pearson, R. C. 1995. Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevines. Plant Dis. 79:15-19.
2. de Bary, A. 1870. *Eurotium*, *Erysiphe*, *Cicinnobolus*: Nebst Bemerkungen über die Geschechtsorgane der Ascomyceten. Abh. Senckenb. Naturforsch. Ges. 7:361-455.
3. Emmons, C. W. 1930. *Cicinnobolus Cesatii*, a case study in host-parasite relationships. Bull. Torrey Bot. Club 57:421-441.
4. Falk, S. P., Cortesi, P., Gadoury, D. M., and Pearson, R. C. 1992. Survival of *Ampelomyces quisqualis* in parasitized cleistothecia of *Uncinula necator*. (Abstr.) Phytopathology 82:1119.
5. Falk, S. P., and Gadoury, D. M. 1994. Reduced production of cleistothecia by *Uncinula necator* due to the mycoparasite *Ampelomyces quisqualis*. (Abstr.) Phytopathology 84:543.
6. Gadoury, D. M., and MacHardy, W. E. 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. Phytopathology 76:112-118.
7. Gadoury, D. M., and Pearson, R. C. 1988. Initiation, development, dispersal, and survival of cleistothecia of *Uncinula necator* in New York vineyards. Phytopathology 78:1413-1421.
8. Gadoury, D. M., and Pearson, R. C. 1990. Ascocarp dehiscence and ascospore discharge in *Uncinula necator*. Phytopathology 80:393-401.
9. Gadoury, D. M., and Pearson, R. C. 1990. Germination of ascospores and infection of *Vitis* by *Uncinula necator*. Phytopathology 80:1198-1203.
10. Gadoury, D. M., Pearson, R. C., Riegel, D. G., Seem, R. C., Becker, C. M., and Pscheidt, J. W. 1994. Reduction of powdery mildew and other diseases by over-the-trellis applications of lime sulfur to dormant grapevines. Plant Dis. 78:83-87.
11. Hashioka, Y., and Nakai, Y. 1980. Ultrastructure of pycnidial development and mycoparasitism of *Ampelomyces quisqualis* parasitic on Erysiphales. Trans. Mycol. Soc. Jpn. 21:329-338.
12. Hijwegen, T. 1988. Effect of seventeen fungicolous fungi on sporulation of cucumber powdery mildew. Neth. J. Plant Pathol. 94:185-190.
13. Jarvis, W. R., and Slingsby, K. 1977. The control of powdery mildew of greenhouse cucumber by water sprays and *Ampelomyces quisqualis*. Plant Dis. Rep. 61:728-730.
14. Magarey, P. A., Emmett, R. W., Gadoury, D. M., Biggins, L. T., Clarke, K., Magarey, R. D., Wachtel, M. F., Masters, J., and Wilkins, B. J. 1993. Cleistothecia as primary inoculum sources of grapevine powdery mildew (*Uncinula necator*) in Australia. Aust. N.Z. Wine Ind. J. 8:239-241.
15. Pearson, R. C., and Gadoury, D. M. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology 77:1509-1514.
16. Philipp, W.-D., Beuther, E., and Grossmann, F. 1982. Untersuchungen über den Einfluß von Fungiziden auf *Ampelomyces quisqualis* im Hinblick auf eine integrierte Bekämpfung von Gurkenmehltau unter Glas. Z. Pflanzenkr. Pflanzenschutz 89:575-581.
17. Philipp, W.-D., Beuther, E., Hermann, D., Klinkert, F., Oberwalder, C., Schmidtke, M., and Straub, B. 1990. Zur Formulierung des Mehltauhyperparasiten *Ampelomyces quisqualis* Ces. Z. Pflanzenkr. Pflanzenschutz 97:120-132.
18. Philipp, W.-D., and Crüger, G. 1979. Parasitismus von *Ampelomyces quisqualis* auf Echten Mehltaupilzen an Gurken und andern Gemüsearten. Z. Pflanzenkr. Pflanzenschutz 86:129-142.
19. Philipp, W.-D., Grauer, U., and Grossmann, F. 1984. Ergänzende Untersuchungen zur biologischen und integrierten Bekämpfung von Gurkenmehltau unter Glas durch *Ampelomyces quisqualis*. Z. Pflanzenkr. Pflanzenschutz 91:438-443.
20. Philipp, W.-D., and Hellstern, A. 1986. Biologische Mehltaubekämpfung mit *Ampelomyces quisqualis* bei reduzierter Luftfeuchtigkeit. Z. Pflanzenkr. Pflanzenschutz 93:384-391.
21. Philipp, W.-D., and Kirchhoff, J. 1983. Wechselwirkungen zwischen Triadimefon und dem Mehltauhyperparasiten *Ampelomyces quisqualis in vitro*. Z. Pflanzenkr. Pflanzenschutz 90:68-72.
22. Puzanova, L. A. 1989. Studies of the hyperparasite of the genus *Ampelomyces* Ces. ex Schlecht. in the south of Russia (Krasnodar Region). Pages 167-177 in: Frontiers in Applied Microbiology. Vol. 3. K. G. Mukerji, V. P. Singh, and K. L. Garg, eds. Rastogi and Co., Meerut, India.
23. Speer, E. O. 1978. Beitrag zur Morphologie von *Ampelomyces quisqualis* Ces. Sydowia Ann. Mycol. 31:242-246.
24. Sundheim, L. 1982. Control of cucumber powdery mildew by the hyperparasite *Ampelomyces quisqualis* and fungicides. Plant Pathol. 31:209-214.
25. Sundheim, L. 1984. *Ampelomyces quisqualis*, a hyperparasitic fungus in biological control of powdery mildews on greenhouse cucumber. Acta Hortic. 156:229-236.
26. Sundheim, L., and Amundsen, T. 1982. Fungicide tolerance in the hyperparasite *Ampelomyces quisqualis* and integrated control of cucumber powdery mildew. Acta Agric. Scand. 32:349-355.
27. Sundheim, L., and Krekling, T. 1982. Host-parasite relationships of the hyperparasite *Ampelomyces quisqualis* and its powdery mildew host *Sphaerotheca fuliginea*. I. Scanning electron microscopy. Phytopathol. Z. 104:202-210.
28. Sztejnberg, A., Galper, S., Mazar, S., and Lisker, N. 1989. *Ampelomyces quisqualis* for biological and integrated control of powdery mildews in Israel. J. Phytopathol. 124:285-295.
29. Thomas, C. S., Gubler, W. G., and Bettiga, L. 1991. *Uncinula necator* ascospore release, viability and infection in field conditions in California. (Abstr.) Phytopathology 81:1182-1183.
30. Tulasne, L. R., and Tulasne, C. 1931. Selecta Fungorum Carpologia. Vol. 1, Erysiphei. W. B. Grove, trans. A. H. Reginald Butler and C. L. Shear, eds. The Clarendon Press, Oxford.
31. Yarwood, C. E. 1939. An overwintering pycnidial stage of *Cicinnobolus*. Mycologia 31:420-422.
32. Yukawa, Y., Katumoto, K., and Takahashi, H. 1971. Studies on *Microsphaera euonymi-japonicae* Vienn.-Bourg. and its hyperparasite. I. Scanning electron microscopic observations on the mycoparasitism. Bull. Fac. Agric. Yamaguti Univ. 22:197-208.