Cleistothecia and Flag Shoots: Sources of Primary Inoculum for Grape Powdery Mildew in the Western Cape Province, South Africa

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Little is known about the mode of survival and sources of primary inoculum of *Uncinula necator,* the causal pathogen of grapevine powdery mildew, in vineyards in the Western Cape province. A study was therefore undertaken to determine whether cleistothecia and flag shoots are formed on vines in local vineyards. Flag shoots were found shortly after budbreak in September 1997 in a Carignane vineyard near Somerset West. Cleistothecia were first observed during April to May 1996 on severely infected leaves from three vineyards in the main grape-growing areas of Stellenbosch. This was the first report of cleistothecia and flag shoot formation in vineyards in the Western Cape province. Cleistothecia occurred in small numbers on leaves (1 – 10 per leaf) and all were immature. Cleistothecia were dispersed by late summer and autumn rains from leaves to bark of grapevines, where they overwinter. No conclusion could be made regarding the viability of cleistothecia. However, the characteristics of the first symptoms that developed on leaves, namely separate, individual lesions formed at random on first-formed leaves growing in close proximity to the bark, provided circumstantial evidence that cleistothecia are dispersed to the bark. Weather conditions suitable for release of ascospores from overwintered cleistothecia occurred frequently between budbreak and bloom in all the areas.

Grape powdery mildew, caused by *Uncinula necator* (Schw.) Burr., is a disease of major economic importance in cultivated grapevines worldwide (Pearson & Gadoury, 1992). There are two principal sources of primary inoculum, namely hyphae inside dormant buds and cleistothecia on the surface of the vine (Bulit & Lafon 1978). Shortly after budbreak, the fungus is reactivated in infected buds and the developing shoots, which are called flag shoots, become covered with white mycelium. Conidia produced on these flag shoots infect neighbouring shoots and vines (Pearson & Gadoury, 1987). Flag shoots are most easily detected 3–8 weeks after budbreak, before the canopy closes over (Magarey *et al.,* 1994). Flag shoots appear to be most prevalent on vines of more susceptible varieties (cultivars Carignane and Thompson Seedless) that were heavily diseased early in the previous season. Most flag shoots also appear on the same vines year after year (Bleyer *et al.,* 1998). Rain is generally considered to be deleterious to the development of epidemics by the anamorph stage of *U. necator* (Gadoury & Pearson, 1990a).

Initiation of cleistothecia requires hyphal contact between two mutually exclusive mating types (Gadoury & Pearson, 1991; Evans *et al.,* 1997; Délye & Corio-Costet, 1998). Environmental factors such as temperature, day length, humidity, leaf age and host resistance do not affect cleistothecium initiation and, once initiated, only temperature and host resistance affect their growth. Cleistothecia can form on all infected tissues from early summer to autumn (Pearson & Gadoury, 1987). Cleistothecia are washed by late summer and autumn rain to the bark of the vine where they overwinter (Pearson & Gadoury, 1987). Although disease incidence and severity may determine the potential population available for dispersal, rain events determine the actual efficiency of transfer from infected organs to the bark of the vine. Ascospores are released in spring between budbreak and bloom of grapevines only during or immediately following rains or over-vine irrigation (Emmett *et al.,* 1992) of more than 2.5 mm (Gadoury & Pearson, 1990a). Ascospores are capable of germinating in water as well as relative humidities as low as 54% (Pearson, 1990). Rainfall is therefore a critical event in the release of ascospores and the initiation of powdery mildew epidemics in areas where cleistothecia are sources of primary inoculum.

Although both cleistothecia and flag shoots produce primary inoculum beginning shortly after budbreak, they may result in different patterns of disease development. Flag shoots are intense point sources of inoculum and will cause disease foci centered on the location of the flag shoot. The incidence of flag shoots in vineyards is usually very low, ranging from 0 to 0.2% (Emmett *et al.,* 1990). If so, flag shoots will cause disease foci centered on the location of the flag shoot. If flag shoots are numerous, the pattern of disease development might be a more random or uniform distribution of disease throughout the vineyard (Pearson & Gadoury, 1987). Where cleistothecia are the principal source of primary inoculum, disease if often randomly distributed throughout the vineyard. Initial infections due to ascospores are most often found on the undersides of the first-formed leaves of shoots growing in close proximity to the bark of the vine. Ideal conditions for abundant primary infection will therefore depend on the type(s) of primary inoculum present. Spread from flag shoots can be expected to be favoured by optimal temperatures for sporulation and the absence of free water on susceptible tissues. Temperatures of 20–27°C (optimum 24–25°C) are favourable for conidial germination and disease development (Fessler & Kassemeyer, 1995; Willocquet *et al.,* 1996), although germination can occur between 6 to 33°C (Delp, 1954). Temperatures above 32°C (Fessler & Kassemeyer, 1995) or 35°C (Delp, 1954), inhibit germination of conidia and temperatures above 40°C will

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kill conidia. However, release of ascospores is most likely to be maximised by frequent rains during the period between budbreak and bloom. The optimal temperature for infection by ascospores is between 20°C and 25°C. Infection is significantly reduced at 15°C or below. No infection occurs at or below 5°C, nor does it occur at or above 31°C (Gadoury & Pearson, 1990b; Pearson, 1990; Jailloux *et al.,* 1998).

In New York (Pearson & Gadoury, 1987) and some Italian vineyards (Cortesi *et al.,* 1997) cleistothecia are the source of primary inoculum. In California (Gubler *et al,* 1988), Germany (Hill *et al.,* 1995), France, Romania (Bulit & Lafon, 1978), Russia, Peru (Pearson & Gadoury, 1992), Iran (Banihashemi & Parvin, 1995), Australia (Wicks *et al.,* 1985) and other Italian vineyards (Cortesi *et al.,* 1997) cleistothecia are an additional source of primary inoculum. Although powdery mildew has been present in the Western Cape province since 1880 (Du Plessis, 1948), the sexual stage of the fungus has never been reported, nor has the occurrence of flag shoots been documented in vineyards. Van der Spuy and Matthee (1977), however, demonstrated that the fungus overwinters in the buds of potted vines as dormant mycelia or conidia.

Little is known of the mode of survival and sources of primary inoculum of *U. necator* in the Western Cape province. More knowledge of this aspect could lead to more efficient control measures. This study was conducted to determine if flag shoots and the sexual stage of the pathogen are formed in the viticultural regions of the Western Cape province.

MATERIALS AND METHODS

Vineyards: A survey was conducted to identify vineyards with a history of high powdery mildew incidences. This entailed sending a detailed questionnaire to extension officers, consultants and producers in the viticultural regions. According to the information gathered by the survey, four Bukettraube vineyards (Nietvoorbij "disease garden", Lievland, Nooitgedacht and Simondium), two Cape Riesling vineyards (Nietvoorbij "disease gardens" F1 and F2), one Chenin blanc vineyard (Klapmuts) and a Carignane vineyard (Somerset West) were selected. The vineyards were used during 1996 to 1997 for studies on flag shoot and cleistothecia formation. Favourable periods for cleistothecium development and dispersal, ascospore release and ascospore infection in the different vineyards were determined from weather station data collected at Nietvoorbij, Klapmuts, Simondium and Faure. A 24h period with temperatures ranging between 4 and 32°C was considered as a favourable period for cleistothecium development (Gadoury & Pearson, 1988), whereas precipitation of ≥ 1mm was considered as a rain event favourable for cleistothecium dispersal (Cortesi *et al.,* 1995). Precipitation of ≥ 2.5mm and a temperature ≥10°C were considered as a favourable condition for ascospore release and ascospore infection (Gadoury & Pearson, 1990b).

Flag shoots: Thirty vines were selected at random in each vineyard. Starting after budbreak, the vineyards were monitored for seven consecutive weeks for the appearance of typical flag shoot symptoms.

Cleistothecia on leaves and bunches: During late summer and autumn (February to May) infected leaves were collected from vines in the different vineyards and placed in paper bags. In addition, during the 1996/1997 growing season, infected bunches (5–20 bunches per vineyard) were collected in mid- and in late summer (December to March) from both wine and table-grape vineyards in the main viticultural regions. Infected bunches were cut and wrapped in clean, healthy grapevine leaves and wrapped in newspaper. The material was taken to the laboratory and examined for cleistothecia at 20–30X.

Dispersal of cleistothecia: The dispersal of cleistothecia by late summer and autumn rain from the leaves to the bark of grapevines was determined on selected vines by the method of Cortesi *et al.* (1995). Five to six vines were selected at random in each of three Bukettraube vineyards (Nietvoorbij "disease garden", Lievland and Simondium) and three Cape Riesling vineyards (Faure, Nietvoorbij F1 and Nietvoorbij F2). Funnels prepared from folded 9-cm disks of No. 1 filter paper were attached to the cordons and trunks and were secured to the vines by push-pins inserted through the top edge of the funnel into the vine. A total of 12 funnels were attached to each vine, two funnels to each of the two cordons, four to the upper trunk and four the lower trunk. Funnels were first installed during the beginning of April 1996 and 1997, when the first immature (light brown) ascocarps were observed on the leaves of the Bukettraube vineyard at Nietvoorbij. The funnels were replaced after each rain event until June and examined for cleistothecia at 20–30X. In both years of the study 90% of the leaves had fallen from the vines at the last sampling, except at Simondium, where the farmer removed the vineyard at the end of May 1996, and Nietvoorbij (Bukettraube), where pruning started on 3 June 1996.

In July 1996 and August 1997 seven vines on which cleistothecia were positively identified during 1996 were selected in the Bukettraube vineyards at Nietvoorbij and the Chenin blanc vineyard at Klapmuts. Bark was collected form both the upper trunk and the cordons of the vines and cleistothecia were recovered from the bark using the method of Cortesi *et al.* (1995). The bark (10g) was placed in a 2L Erlenmeyer flask containing 500 mL of water. The flask was shaken vigorously for 3 min, and the resultant suspension was poured into a stack of nested Cobb sieves of 60, 120, 150 and 170 mesh, corresponding to pore sizes of 250, 125, 106 and 90 µm, respectively. Cleistothecia and bark debris collected on each sieve were resuspended in 25 mL of water, and four 5 mL aliquots of the suspension were transferred to four 9 cm filter paper disks. The filter paper disks were allowed to dry before investigation at 64X. The bark remaining in the Erlenmeyer flask was then resuspended in 500 mL of water and shaken an additional 60 seconds. The suspension was poured into the nested sieves and the process was repeated for a total of four rinses for each of the seven bark samples. Cleistothecia recovered were counted (Cortesi *et al.,* 1995); they were crushed on glass slides in water, stained (Widholm, 1972) with 1% fluorescein diacetate, viewed under fluorescence microscopy and ascospore viability asessed (Gadoury & Pearson, 1991).

RESULTS

Flag shoots: No flag shoots were found during the study period on any of the vines in the Bukettraube, Cape Riesling and Chenin blanc vineyards. In the Carignane vineyard flag shoots were observed shortly after budbreak in both seasons. The shoots were stunted, leaves were deformed and heavily colonised with mycelial growth. Most of the flag shoots occurred at the second node, although some occurred at the first node. Secondary infections were observed two weeks later.

tribution of ascospore release and the association of ascosporic infection with crop loss in local vineyards.

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