Growth of *Eutypa lata* in Grapevine Wood Extracts

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Nitrogen and carbohydrate contents in extracts from Cape Riesling canes/shoots and wood were determined. Nitrogen content from extracts of both canes/shoots and wood was the highest during June, July and August. Sugar content from canes/shoots was highest during July and August, while the sugar content in wood was the highest during July, August and September. Growth of *Eutypa lata* was determined in these extracts *in vitro*. *Eutypa lata* had the highest mycelium yield when grown in canes/shoot extracts collected during July and August. *Eutypa lata* showed the best growth in extracts from wood collected during July. It is suggested that the high nitrogen and sugar content in grapevine wood could play a role in the susceptibility of pruning wounds during the pruning season (i.e. June to August) to *Eutypa lata*.

Dieback caused by the fungus *Eutypa Lata* (Pers. Fr.) Tul. Syn. *Eutypa armeniacae* (Hansf. & Carter) is found in all vine growing areas of the world (Petzoldt, Moller & Sall, 1982). The fungus is a wound parasite (Carter, 1957). Ascospores are discharged during or shortly after rain and germinate on pruning wounds. The fungus firstly infects the xylem tissue and subsequently spreads to the cambial and phloem regions (Moller & Lehoczky, 1980).

Moller & Kasimatis (1980) found that pruning wounds made in late winter were highly susceptible to *E. lata.* Similarly vines in California were most susceptible to *E. lata* when pruned in December and least susceptible in March. Wounds on one year old wood were more resistant than those on older tissue. Trese, Ramsdell & Burton (1982), however, found that wood age had no influence on susceptibility when wounds were infected on the same day that they were inflicted. According to Petzoldt, Moller & Sall (1981) the season of the year, however, influences wound susceptibility. In addition, the nutrient status of a plant can also influence its susceptibility to pathogens. Beever (1970) proved a relationship between nutrients in xylem sap of stone fruits and their seasonal susceptibility to the silver leaf disease caused by *Stereum purpureum* (Pers.) Fr.

This study was undertaken to determine the nutrient status of vine wood at monthly intervals during the season and to determine difference in growth of *E. lata* in wood extracts from canes/shoots and those from wood of Cape Riesling vines.

MATERIALS AND METHODS

Collection of wood samples: Wood samples were collected monthly within three days from the first day of the month to determine the nutritional status of the wood and the extent to which it could support fungal growth. The term canes/shoots is taken for wood less than one year old, while the word "wood" is used for permanent wood older than one year.

Canes/shoots were cut at the second internode from the base of five year old Cape Riesling vines. One kilogram canes from four vines were pooled and shredded in a compost shredder (Lescha-Zak model 1802) prior to freeze-drying. One kilogram of wood from cordons on four vines was also collected and all the canes were removed. These wood pieces were cut into 5 mm thick planes with a bandsaw before shredding through the compost shredder. The shredded wood was then freeze-dried. All freeze-dried samples were subsequently milled in a Wiley Mill and passed through a 0,4 mm sieve.

Extraction procedure: Extracts from canes/shoots and wood were made using a modified method of Burton & Gardner (1963). Milled material was soaked in 2L of distilled water for 4 hours and stirred hourly for 5 min. with a glass rod. The extract was first filtered through cheesecloth followed by filtration through Whatman no. 54 filter paper.

Growth of *Eutypa lata* in extracts: The filtrates were poured into six 1L flasks (200 mL/flask) and sterilised by irradiation (7kGy). After irradiation each flask was inoculated with a 6 mm mycelium plug *E. lata* culture actively growing on potato dextrose agar (PDA), and incubated at 24°C for 14 days on a rotary shaker. After incubation, the mycelia were harvested by filtration and dry weights were determined after drying mycelia on filter paper at 60°C for 24 hours. The mass of the filter paper was determined prior to the procedure.

Determination of macro and micro elements: Two hundred millilitres, divided into two 100mL aliquots, from each of the two wood samples were used for determination of pH (Beckman 70 pH meter), carbohydrate (Schoorl method), and nitrogen content (Kjeldahl method). Analyses of macro and micro elements were done by the Soil Science Department, at the Winter Rainfall Region, Elsenburg by means of a direct current plasma emission spectrograph (Spectrspan 5 model).

Growth of *Eutypa lata* in glucose and ammoniumnitrate: Trials indicated that the good growth of *E. lata* during June, July and August in extracts from canes/shoots and wood could be due to a higher nitrogen and carbohydrate content during this period,

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the following three concentrations of glucose and ammoniumnitrate in 200 mL sterile distilled water were used: (pH was adjusted to 5,8):

1) glucose (100 mg/L) + ammoniumnitrate (100 mg/L)

2) glucose (4500 mg/L) + ammoniumnitrate (200 mg/L)

3) glucose (9000 mg/L) + ammoniumnitrate (400 mg/L)

Three 1L flasks, each containing 200 mL of a specific concentration, was inoculated with *E. lata* and incubated on a rotary shaker at 25°C for 14 days, whereafter the mycelium mass was determined for each concentration, respectively.

Statistical procedures: A complete randomized experiment was carried out in a Cape Riesling vineyard with two replications with the treatment design as a 2x12 factorial. The factors were two wood sources (canes/shoots and wood) and 12 extract times (Jan. to Dec.). The data were analyzed with standard factorial analyses of variance. Polynomial regressions were fitted over time and compared using, SAS (1990). Student's t-LSD was calculated to compare treatment means.

RESULTS AND DISCUSSION

The pH of extracts from wood showed a decline from January to December (Fig. 1). The lowest pH (4,82) of canes/shoots extracts was recorded from extracts of young canes during November. The highest pH occurred in the period May to June. Extracts from wood showed the highest pH during January (6,46)

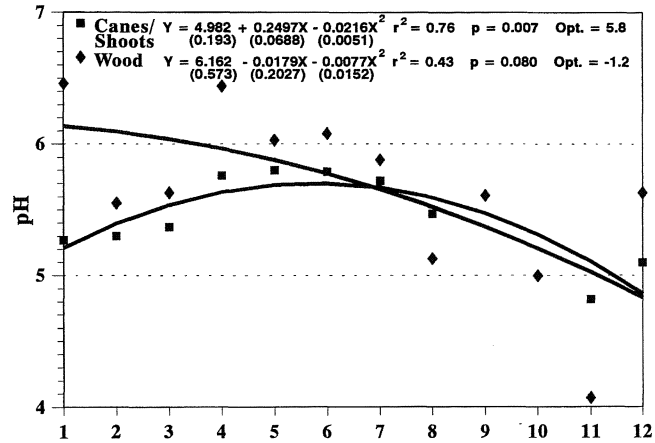

FIGURE 1
The pH of wood extracts from canes/shoots and older wood of Cape Riesling vines.

TABLE 1
Amounts of macro and micro elements (mg/L) in extracts from canes/shoots*

Element	Months									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Nov	Dec
P	70,0	75,0	104,5	30,5	23,0	36,0	31,5	47,6	94,0	44,5
K	941,0	837,0	1130,0	380,0	262,0	311,0	365,0	680,0	664,0	1019,0
Ca	204,0	185,0	222,0	175,0	115,0	117,0	109,0	119,0	173,0	182,0
Mg	58,0	44,0	72,0	37,5	32,0	52,5	37,5	41,7	62,0	37,0
Na	10,6	9,5	12,8	26,5	20,0	14,0	19,5	17,8	18,6	15,1
Cu	0,29	0,30	0,52	0,18	0,09	0,15	0,30	0,49	1,18	0,83
Zn	0,69	0,89	0,89	0,88	0,36	0,10	0,61	0,81	3,25	1,26
Mn	0,45	0,17	0,92	0,23	0,29	0,83	0,73	0,65	0,40	0,49
Fe	0,15	0,25	0,61	0,45	0,33	0,18	0,10	0,21	0,35	0,20
B	0,01	0,01	0,05	0,11	0,14	0,13	0,14	0,12	0,03	0,05

*No shoots were available during September and October.

TABLE 2
Amounts of macro and micro elements (mg/L) in extracts from wood.

Element	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
P	60,5	56,5	55,0	38,5	36,0	34,0	38,0	36,2	33,5	33,4	32,5	77,5
K	560,0	606,0	513,0	394,0	311,0	357,0	363,0	343,0	352,0	319,0	324,0	912,0
Ca	182,0	190,0	183,0	196,0	157,0	113,0	129,0	179,0	194,0	189,0	202,0	208,0
Mg	66,0	62,0	66,0	67,0	86,5	64,5	65,0	63,8	89,0	94,0	104,0	93,0
Na	11,2	8,4	11,2	15,0	30,2	13,0	7,0	29,7	49,3	42,5	132,0	15,2
Cu	0,43	0,21	0,68	0,40	0,27	0,29	0,45	0,39	0,44	0,48	0,47	0,53
Zn	4,36	3,03	2,25	1,67	1,65	2,12	1,94	1,20	0,93	1,10	0,47	3,87
Mn	0,50	0,25	0,81	0,48	0,79	0,83	0,58	0,49	0,43	0,37	0,33	0,64
Fe	0,17	0,23	0,54	0,10	0,51	0,12	0,06	0,73	0,52	0,59	1,00	0,73
B	0,01	0,01	0,13	0,12	0,15	0,11	0,13	0,13	0,02	0,01	0,01	0,02

with a sharp decline during the ripening period (Jan.-Febr.) and at harvest and increased during autumn. As for canes/shoots, the lowest pH of wood occurred at the beginning of the growing season. These pH values are very favourable for the growth of *E. lata*, since it can grow at pH values between 2,5 and 8 with an optimum at pH 5,8 (Ferreira, unpublished).

The macro and micro element content of extracts from wood and canes/shoots are given in Tables 1 and 2, respectively. The method of extraction of nutrients from canes/shoots and wood used in this study may not have resulted in complete extraction, but has the advantage that no heat or chemicals were used which could have influenced the chemical nature of the compounds in that extract.

Only carbohydrate and nitrogen content showed a peak during the pruning season (June, July and August). The carbohydrate content of extracts from canes/shoots and wood is shown in Fig. 2. The carbohydrate content in canes/shoots was relatively high during January compared to the carbohydrate content of the wood. In the extracts from canes/shoots the carbohydrate content was lowest in samples collected from February to May, with the highest carbohydrate content during July and August. The carbohydrate contents in the wood followed more or less the same pattern and was high during July, August and September. Significantly lower carbohydrate content of the wood exracts was observed in November compared to the carbohydrate content of canes/shoots. Uys (1981) also found an increase in carbohydrate content of vine rootstock material during the dormancy period.

The nitrogen content of the canes/shoots (Fig. 3) was relatively low during the period January to April whereafter it was high from May until November and peaked during June, July and August. The nitrogen content of the wood was low from January to May and relatively low from September to December with a peak during June, July and August. A higher nitrogen content in canes/shoots and permanent wood during leaf fall and the dormancy period was also observed by Uys (1981) and Conradie (1985).

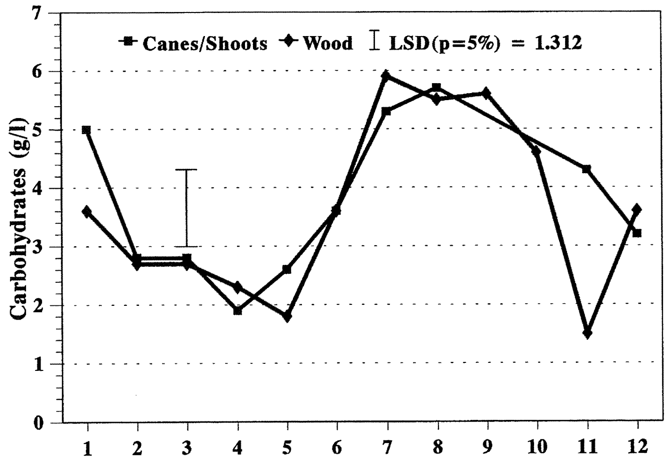

FIGURE 2
Carbohydrates content of extracts from canes/shoots and older wood from Cape Riesling vines.

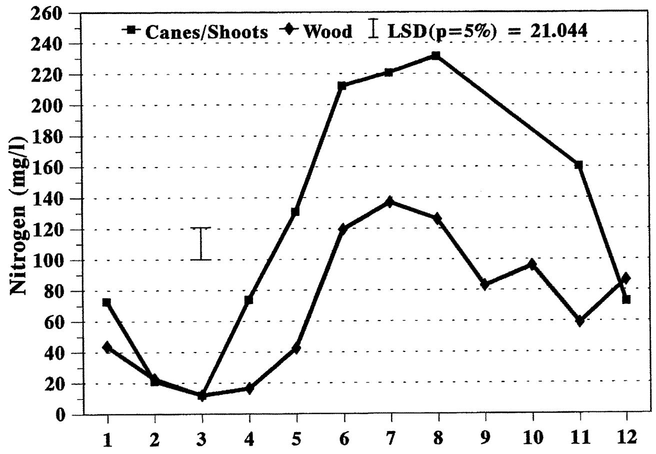

FIGURE 3
Nitrogen contents of canes/shoots and older wood from Cape Riesling vines.

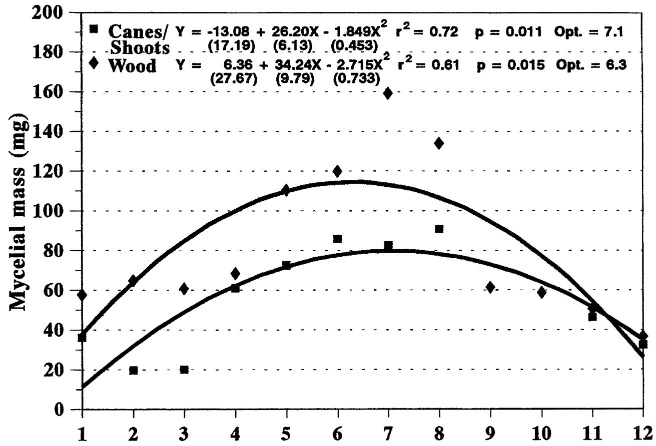

FIGURE 4
Mycelium growth of *Eutypa lata* in monthly extracts from Cape Riesling canes/shoots and older wood.

The highest mycelium yields of *E. lata* were obtained in extracts from canes/shoots and wood collected in May, June, July and August (Fig. 4). The higher concentration of carbohydrate and nitrogen during winter could be reponsible for the better growth of *E. lata* during this period.

Eutypa lata showed an optimum growth in extracts from canes/shoots collected during June and July. The optimum growth in wood extracts was during July and August. *Eutypa lata* grew relatively better in extracts from canes/shoots than those of wood. This could be due to the higher nitrogen content in canes/shoots during this period (Fig. 3).

The growth of the fungus, as determined by mycelium mass, increased with an increase in concentration of glucose and nitrogen (Fig. 5).

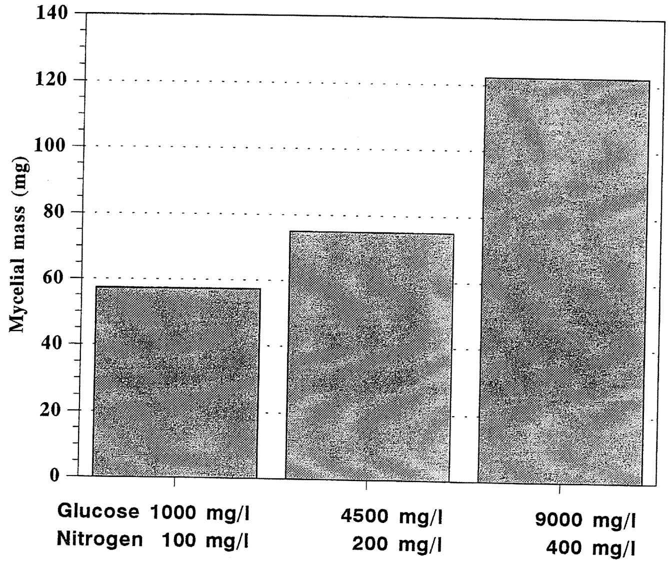

FIGURE 5
Mycelium growth of *Eutypa lata* in a synthetic medium at different levels of glucose and ammoniumnitrate.

Growth in the synthetic medium, however, was lower compared to the growth in the wood extracts at a comparable carbohydrate and nitrogen content. This is probably an indication that the other nutrients in the canes/shoots and the wood extracts also had an effect, but it is generally accepted that nitrogen and carbohydrate content will have the greatest influence on fungal growth. The more active growth of the fungus in extracts from wood during the winter months and the higher nutritional value of the extracts during this period indicate that pruning wounds made during this period could be more susceptible to infection by *E. lata.*

With xylem sap of peach trees, Beever (1970) also found a higher carbohydrate and nitrogen content during winter which also supported better growth of *Stereum purpureum* than extracts made during the other seasons of the year. He suggested that this could be the reason for seasonal susceptibility of stone fruits to this fungal disease.

The findings by several workers (Carter & Moller, 1970; Ramos, Moller & English, 1975; Carter & Price, 1975; Moller & Kasimatis, 1980; Petzoldt *et al.*, 1981; Petzoldt, *et al.*, 1982) that susceptibility of pruning wounds in apricot and vines decreased with increasing time between wounding and inoculation, could possibly be attributed to a depletion of the carbohydrate and nitrogen content in such wounds due to the growth of other non-pathogenic wound invading fungi. The physical aspect of drying of the wound and subsequent abstriction of cells can also play a role. Carter & Moller (1970) ascribed this decrease in susceptibility to fast colonisation of pruning wounds by other micro-organisms, which inhibit the growth of *E. lata* by competition for nutrients and/or possible formation of metabolites which may be toxic to this fungus.

Several workers found that pruning wounds made during late autumn are more susceptible to *E. lata* than pruning wounds made in early spring with an intermediate susceptibility during winter (Ramos *et al.*, 1975; Moller & Kasimatis, 1980; Petzoldt *et al.*, 1982, Trese *et al.*, 1982). This phenomenon may be attributed to a high spore dispersal of *E. lata* in autumn and early spring with an intermediate dispersal during winter (Ramos *et al.*, 1975; Pearson, 1980; Petzoldt *et al.*, 1982) rather than nutrient status of the wood.

The results of this study indicate that higher nitrogen and carbohydrate content of vine wood during the dormancy period may promote the susceptibility of pruning wounds to *E. lata.* Protection of wounds against *E. lata* during this period is essential.

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