FIELD OBSERVATIONS OF *SCLEROTINIA SCLEROTIORUM*: APOTHECIAL DEVELOPMENT AND SCLEROTIAL PRODUCTION.

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SUMMARY

Culture grown sclerotia of *Sclerotinia sclerotiorum* were buried beneath kiwifruit vines in June 1991 and monitored for apothecial development. The first stipes and apothecia were seen on 14 October and 30 October respectively. Apothecia numbers increased throughout November. A mean of 4.3 apothecia were produced per sclerotium. Sclerotia were found inside cavities within the ovary of infected fruitlets. Infected fruitlets that had fallen to the ground by 9 January had larger and heavier sclerotia than infected fruitlets remaining attached to the vine. Sclerotia produced from infection of fruit were larger and heavier than sclerotia from infected fruitlets. Sclerotial production and subsequent emergence of apothecia are important factors in the ecology of *S. sclerotiorum*.

Keywords: *S. sclerotiorum*, apothecia, sclerotia.

INTRODUCTION

Disease caused by infections of the fungus *Sclerotinia sclerotiorum* (Libert) de Bary can result in the loss of immature kiwifruit (*Actinidia deliciosa*) in New Zealand kiwifruit orchards (Pennycook 1985). Infections occur on male blossoms, fruitlets and fruit, and sclerotia are often produced. Sclerotia are the over-wintering survival structures that 'germinate' during spring and summer to produce apothecia, which are the primary source of inoculum (Pennycook 1985).

Laboratory and field studies overseas (Marukawa *et al* 1975; Wang and Tourneau 1971) indicated that sclerotia production occurred over a range of temperatures (15-27°C), pH (2.5-9) and light conditions and is influenced by osmotic potential and the available sources of carbon and nitrogen. Apothecia require adequate moisture, suitable temperatures and high humidity for development (Grogan and Abawi 1975), and emerge from late September onwards in New Zealand kiwifruit orchards (Manning 1991).

There is little published data on production of sclerotia or apothecia in New Zealand kiwifruit orchards. This paper presents data on these two aspects of *S. sclerotiorum* ecology in a commercial kiwifruit orchard 3 km north west of Oropi, Tauranga.

METHODS

Apothecial development

Slices of kiwifruit were autoclaved and inoculated with mycelium from 5 day old cultures of *S. sclerotiorum*. These were incubated in petri dishes at ambient temperatures (20-25°C). After 2 to 3 weeks sclerotia were removed and air dried in petri dishes for 4 to 6 weeks. Sclerotia were buried in 11 replicates of 20, 1-2 cm deep under T-bar kiwifruit vines, on 13 June 1991. Replicates were spaced 5 x 5 m and consisted of five rows of four sclerotia in a grid measuring 350 x 240 mm. Replicates were covered with a wire cage and shade cloth to avoid disturbance and reduce the impact of rain on the soil surface.

The number of stipes, precups (an intermediate stage) and apothecia that emerged within each grid, were recorded from 20 September 1991 to 16 January 1992 (Fig. 2). The physical state of apothecia was recorded as 'fresh', 'older' (pale, few blemishes), 'dried and shrivelled' and 'dead' (loose or blackened). Rainfall and canopy

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air temperatures were monitored hourly during the study with a 'Squirrel' datalogger (Grant Instruments Ltd). Development times were based on the assessment dates when the maximum number of apothecia per individual sclerotium was first observed.

Sclerotial production

Sclerotial production was estimated from fruitlets with a withered brown appearance typical of *Sclerotinia* infection (Pennycook 1985). On 9 January 1992, 250 fruitlets were collected from those remaining attached to the vines and 250 from those fallen to the ground. The fruitlets were air dried for 1 to 2 weeks and the sclerotia were extracted by hand and counted. Sclerotia from the vine and ground collected fruitlets were bulked separately and sorted into six size categories using 5.0, 3.5, 2.0, 1.0 and 0.85 mm sieves. Sclerotia were then individually weighed. Mean sclerotia sizes were calculated using the number of sclerotia per size category and the average of the respective adjacent sieve sizes.

Sclerotia were collected from the decayed remains of *Sclerotinia* infected fruit in a nearby orchard on 12 February 1992, and processed as above.

RESULTS

Apothecial development

The proportion of germinated sclerotia that remained buried was 99%, producing a total of 928 apothecia. The four sclerotia that became exposed, possibly due to rain erosion, showed no sign of germination. The number of apothecia per germinated sclerotium ranged from 1-13 (mean 4.3 ± 2.3 S.D.). The diameters of most apothecia was between 3-6 mm, while the largest were up to 13 mm in diameter.

Fig. 1: Daily rainfall at Oropi, Tauranga from 15 September 1991 to 16 January 1992.

The first stipes and precups were observed on 14 October, 123 days after burial. The rainfall totalled 158 mm (Fig. 1) and the mean daily air temperature was 10.8°C over the 4 weeks before this date. Only 7 mm rain was recorded from 14-28 October and the soil surface was observed to dry out. On 21 October the numbers of stipes and precups had not increased and the development of those present had not progressed. The first mature apothecia were observed on 30 October and numbers of apothecia increased until the end of November (Fig. 2). The overall development time from the median number of stipes to the median number of apothecia was 8.5 days. The mean air temperature from 1-28 November was 12.5°C . The initial germination date and development times for individual sclerotia were variable. The time from 10% apothecia to 90% apothecia was 20 days (5-25 November).

From 14-28 November there were 12 days without rain and considerable deterioration of the apothecia was observed over this period. On 28 November 97% of the apothecia were classed as 'older' or 'dry and shrivelled' (Fig. 2). Further aging of apothecia and relatively dry weather through until the end of December coincided with a decrease in overall numbers of apothecia. On 16 December 100% of apothecia

Fig. 2: Total numbers of apothecia and their physical state, produced from 220 cultured sclerotia buried beneath kiwifruit vines.

were classed as 'dry and shrivelled' and on 23 December 40% were classed as 'dead'. However there was a decrease in the number of 'dry and shrivelled' apothecia on 5 and 23 December. Over the 24 days following the 23 December, decay continued and the remains of most apothecia had disappeared by 16 January 1992. Spore release ('puffing') was observed during some assessments before and including 23 December.

Apothecia from natural sclerotia were observed in the grids from 30 October to 5 December. These were distinguishable by their appearance and locality within the grids. They reached a maximum number of 24 on 11 November. These apothecia were 2-4 mm in diameter and lighter in colour than apothecia from the cultured sclerotia. They were only seen as singles or pairs of apothecia.

Sclerotial production

Sclerotia were present in 93% of fruitlets with *Sclerotinia* infection symptoms collected from the vines and from the ground. Most sclerotia were found inside cavities within the dried ovaries. However the mean sclerotia weights from vine and ground collected fruitlets were significantly different ($P > 0.001$). Also the mean sclerotia weights from fruit were significantly heavier ($P > 0.001$) than the mean sclerotia weights from vine and ground collected fruitlets (Fig. 3). The mean size of sclerotia extracted from vine and ground collected fruitlets was 1.8 mm and 2.2 mm respectively. The mean size of sclerotia from fruit infections was 3.2 mm. Observations in May 1992 indicate that at least some *Sclerotinia* infected fruitlets still remained attached to the vines.

Fig. 3: Frequency distribution of sclerotia weights from *S. sclerotiorum* infected fruitlets and fruits.

DISCUSSION

Emergence of apothecia from buried sclerotia beneath kiwifruit vines was observed to occur from late October to late December. This corresponds with the emergence period reported by Pennycook (1985). A similar flush of apothecial production was reported under field conditions overseas (Mitchell and Wheeler 1990; Schwartz and Steadman 1978).

The development of stipes and precups was slow in October when rainfall was low. Grogan and Abawi (1975) reported that low water potential (< -6 bars) inhibits apothecia production. This suggests that the drying out of the soil, observed on 21 October, contributed to the slow development of stipes and precups present at this time. Schwartz and Steadman (1978) demonstrated that production of apothecia is promoted by conditions which provide and maintain adequate soil moisture, such as irrigation and the presence of a dense plant canopy. The increase in apothecia numbers during November indicates that at this site, moisture levels were adequate for sclerotia germination and apothecia development over this period. The decrease in numbers of 'dry and shrivelled' apothecia on 5 and 23 December is evidence that apothecia in this state are capable of recovering to a less deteriorated state of 'older' under field conditions. Partyka and Mai (1962) reported recovery of 'dry' apothecia at 100% RH under laboratory conditions.

Sclerotia size has been suggested to affect viability (Hoes and Huang 1975) and may affect apothecia production (Willets and Wong 1980). Also culture-grown sclerotia of *S. sclerotiorum* have been reported to have less rind breakdown than natural sclerotia, a higher survival rate and produce apothecia earlier and in greater numbers (Merriman 1976; Merriman *et al* 1979; Mitchell and Wheeler 1990). These aspects of sclerotial survival and apothecial production may be factors contributing to the observed high germination rate and level of apothecia produced per sclerotia.

Sclerotia were produced inside cavities within the ovaries of a high proportion of fruitlets showing symptoms of infection with *Sclerotinia* in this orchard. These sclerotia were smaller and lighter than sclerotia from fruit infections and they made contact with the soil over a greater time period. In addition, the nutrient composition, and the physical environment during sclerotia formation, is likely to be different for infections of fruitlets compared with infections of fruit. The above aspects have been shown to affect sclerotia production and viability and apothecia production, under annual cropping situations (Merriman *et al* 1979).

The preliminary observations and data presented in this paper highlight the variability of sclerotia and production of apothecia in kiwifruit orchards. The effects of this variability on inoculum production and disease incidence require further research.

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