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EFFECT OF TEMPERATURE AND SOIL MOISTURE CONTENT ON PERSISTENCE OF INFECTIVITY OF *PHYTOPHTHORA FRAGARIAE* IN NATURALLY INFESTED FIELD SOIL

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Soil baiting using the highly susceptible *Fragaria vesca* clone VS1 was used to assess the persistence of infectivity of *Phytophthora fragariae* (cause of strawberry red core root disease) in naturally infested soil held under a variety of storage conditions and in drainpipe columns exposed in the field.

The fungus was detected on all sampling dates during three years in soil samples held at −1 and +3 °C, most consistently in those that were wet and moist, and less consistently in those stored dry.

At 15° infectivity declined markedly in wet and dry samples but increased in moist samples during the second, before declining markedly in the third year. At 30° infectivity declined rapidly in all samples and it was not regained after a period of storage at 3°.

Infectivity was detected on all sampling occasions in soil from the drainpipes where it fluctuated markedly, declining in summer then rising in autumn.

Phytophthora fragariae Hickman, the cause of red core root disease of strawberry, may survive in soil for many years in the absence of a host. Alcock & Howells (1936) surmised that it 'certainly remains in an affected soil for eight years' and stated that it was neither easily killed by dry weather nor affected by severe frost. Montgomerie (1951) reported a possible case of survival for 15 years in a garden soil: certified strawberry runners developed symptoms of the disease when they were planted into an area where 15 years earlier there had been an outbreak of the disease, and where, in the intervening period, it had been a grass lawn. Fulton (1959) noted the disease in a recently planted strawberry field where an outbreak had been recorded 13 years earlier. As the new planting stock had come from a runner bed which did not show any symptoms of disease, and as in the intervening years the field had supported crops of wheat, oats, rye and sudan grass, he concluded that the fungus had survived from the previous outbreak.

Hickman (1940) and Bain & Demaree (1945) were unable to infect a wide range of plant species with this fungus although other workers found in the field (McKeen, 1958) and in inoculation studies (Converse & Moore, 1966; Pepin, 1967) that it could infect species in genera closely related to strawberry. In the two examples cited above, no known potential alternative hosts were grown between the old and new outbreaks. However, it is possible that the replant stocks, although apparently free from red core, could have been lightly infected (Duncan, 1979).

Apart from the work of Bain & Demaree (1945), who used bait plants to show that *P. fragariae* survived for two and a half years in a site which they had artificially infested with diseased roots, the longevity of this fungus in soil and the factors affecting it have not been studied experimentally.

Duncan (1976, 1977*a*) developed a quantitative baiting technique for estimating the infectivity of soil samples infested with *P. fragariae* and this paper reports the results of using this technique to examine the infectivity of an infested field soil both *in situ* and when stored under various conditions.

MATERIALS AND METHODS

Naturally infested field soil was collected from site II at the Scottish Horticultural Research Institute, Mylnefield (Duncan, 1976), on 30 Oct. 1975. Samples were taken at the intersections of a 1·82 m square grid in the middle of the bare alleyways between plants of cv. Cambridge Favourite which had been planted in the previous April. Each sample, of *c.* 1 l, was dug with a cleaned spade to a depth of *c.* 100 mm and the 306 samples obtained were pooled, stored at 4° for 4 days, then screened through a 6·3 mm mesh sieve to remove stones, break up clods of earth, and

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assist mixing. The screened soil was spread on a clean concrete floor and mixed thoroughly using clean shovels before being divided into three lots for 'wet', 'moist' and 'dry' treatments:

'wet' – the soil was placed in 300 mm diam plastic plant pots which were slowly lowered into tap water at 4° until the soil was thoroughly soaked and the pots left to drain for four days at 4°;

'moist' – the soil was kept at its original moisture content in polythene bags at 4°;

'dry' – the soil was spread on polythene sheeting in an unheated glasshouse, in which the temperature ranged from 5° to 15°, and allowed to air dry for 5 days.

Soil moisture contents, expressed as percentages of the weight of soil after drying to constant weight at 105° and as percentages of the moisture-holding capacity were: wet, 29·9 % (103 % MHC); moist, 18·6 % (64 % MHC); dry, 3·7 % (13 % MHC).

Portions of each soil type, equivalent to *c.* 900 g of oven-dried soil, were placed in clean polythene bags that were sealed with twist ties. One bag of each type was placed in larger polythene bags which were also sealed with twist ties and these were loaded into plastic dustbins which were placed in cabinets held at −1°, +3°, +15° and +30°. The cabinet at −1° had an 8 h defrost cycle lasting *c.* 30 min during which the temperature rose to *c.* +1°; no samples stored in this cabinet were ever visibly frozen.

Moist soil was also returned to the field in sections of plastic drainpipes 100 mm diam by 160 mm long. Their lower ends were capped with nylon netting (TYGAN T103), with a pore width of *c.* 1 mm, on to which was laid a 15 mm layer of autoclaved soil followed by another piece of netting. The columns were packed with moist soil (*c.* 900 ml/column) to within *c.* 30 mm of their tops. They were buried upright in the sampled field so that the level of the surface in the columns was the same as that of the surrounding soil. The layer of autoclaved soil acted as a barrier to prevent direct interchange of inoculum between the infested field soil and the soil in the columns. Soil which had been collected from an adjacent uninfested area, was autoclaved at 121° for 30 min and used to fill check columns.

Other samples of the wet, moist, dry and autoclaved check were used to prepare three different dilutions of soil in University of California soil-less compost (U-C). The dilutions were dispensed in 100 ml portions into 63 mm diam plastic plant pots and baited with individual rooted runners of *Fragaria vesca* L. clone VS1 for quantitative estimates of infectivity as described by Duncan (1976).

The soil for the three dilutions was weighed rather than measured by volume to avoid the problems associated with differential compaction of the dry and wet samples. For each sample, the first dilution consisted of 24 pots each containing the equivalent of 25 g oven-dried soil; the second – 15 pots each containing the equivalent of 12·5 g oven-dried soil, the third – 9 pots each containing the equivalent of 6·25 g oven-dried soil. The dilution series so obtained were almost identical to those which would have obtained by preparing a series from the unamended moist soil on a volume basis (the first was equivalent to a 1/4 dilution, the second a 1/8 dilution, and the third a 1/16 dilution of the soil). Three blanks, consisting of baits grown in compost alone, were prepared with each dilution series and the pots placed at random among the test plants to check for cross-contamination between pots on the wet benches (Duncan, 1976) used in the bait test.

During the 3 years 1975–8 samples of soil were removed from store and from the drainpipe columns and, after soil moisture determinations, baited in the manner described above.

The numbers of infected baits in each dilution series after 5 weeks on the wet benches were used to estimate the concentration of infective units of inoculum in the original soil sample using the GLIM (Generalised Linear Interactive Modelling) statistical package (Baker & Nelder, 1978). The estimates so obtained are identical to those calculated with the Rothamsted Maximum Likelihood Program used by Duncan (1977*a*).

The GLIM package was also used to fit models to describe the variation over time of concentration of infective units. The adequacy of the models was assessed by partitioning the deviance in a manner analogous to the fitting of multiple regression models. In the results section, statements marked (†) are supported either by analysis of deviance or by non-parametric tests with a significance level not greater than 0·05. The numbers enclosed thus [,] behind estimates of infectivity refer to the 95 % confidence limits of those estimates.

RESULTS

The temperature of the 30° cabinet was inadvertently raised to 37° from the 118th to 274th day of storage. The soils stored therein lost water, the moisture contents of the dry, moist, and wet samples falling steadily throughout the experiment to stand at 2 %, 6 % and 16 % respectively after 1028 days in store. In samples stored at the other temperatures, water loss was detectable only in the wet samples, the moisture contents of which ranged between 21 % and 25 % after 1028 days storage.

The concentration of infective units of the

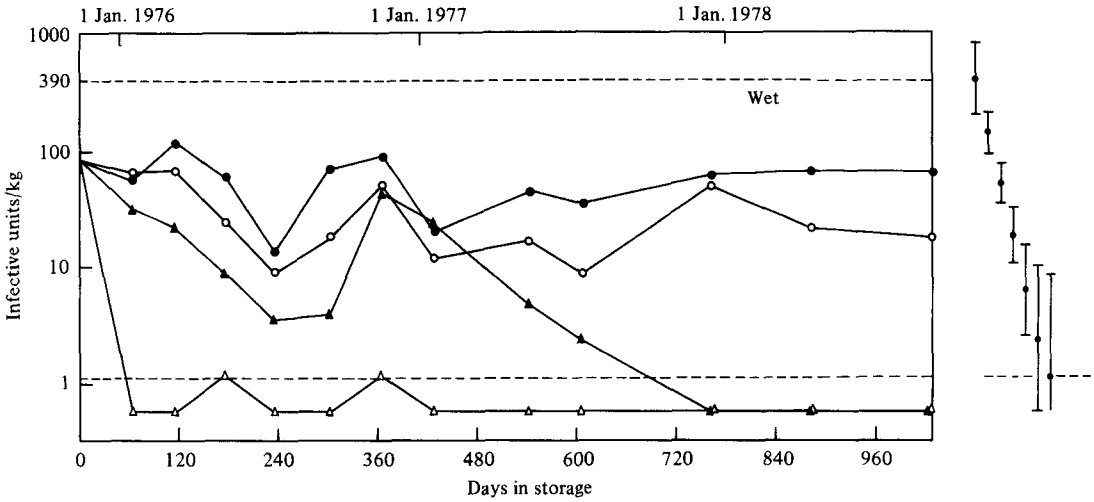

Fig. 1. Infectivity estimates (infective units/kg of oven dried weight of soil) for wet soil samples plotted against the time the samples were held in store. Storage temperatures were −1 °C (●——●), +3° (○——○), 15° (▲——▲), +30° (△——△). The bars at the side of the figure indicate 95 % confidence intervals for a representative selection of estimates. The upper and lower broken lines refer to the maximum estimable (MEC) and minimum detectable concentrations (MDC) respectively. Samples producing all positives were arbitrarily plotted above the MEC and those producing all negatives were plotted below the MDC. These arbitrary values were the MEC and MDC for a dilution series with twice the numbers of bait plants than actually used.

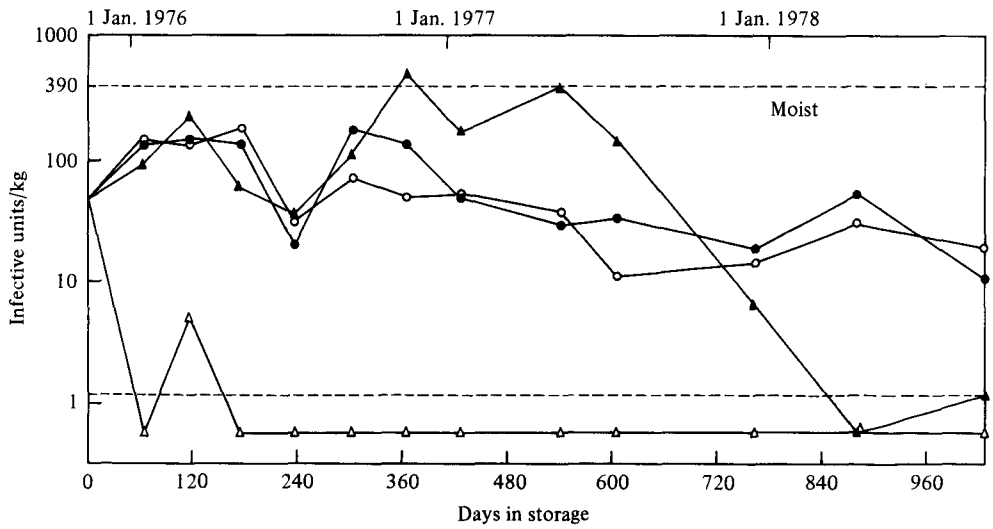

Fig. 2. Infectivity estimates (infective units/kg of oven-dried weight of soil) for moist soil samples plotted against the time the samples were held in store. Storage temperatures were −1 °C (●——●), +3° (○——○), +15° (▲——▲), +30° (△——△).

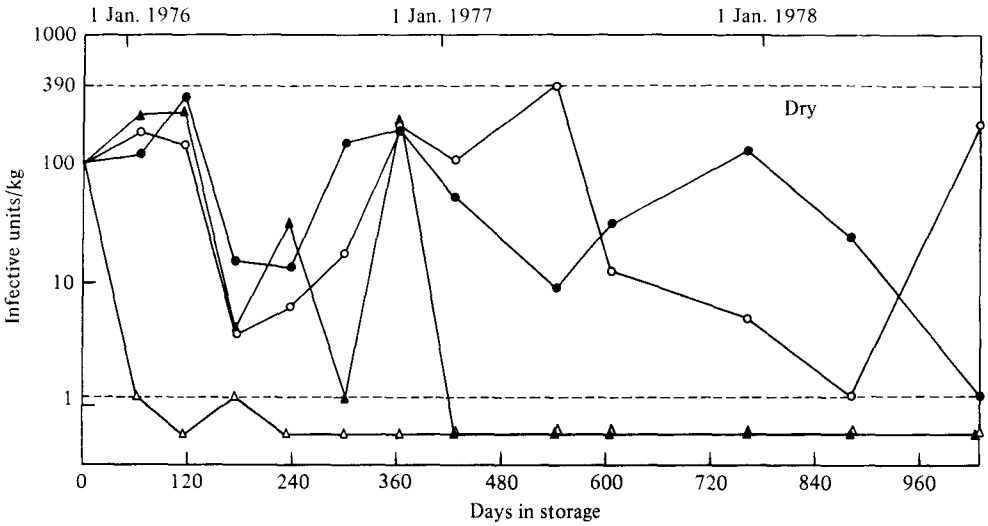

Fig. 3. Infectivity estimates (infective units/kg of oven-dried weight of soil) for dry soil samples plotted against the time the samples were held in store. Storage temperatures were −1 °C (●——●), +3° (○——○), +15° (▲——▲), +30° (△——△).

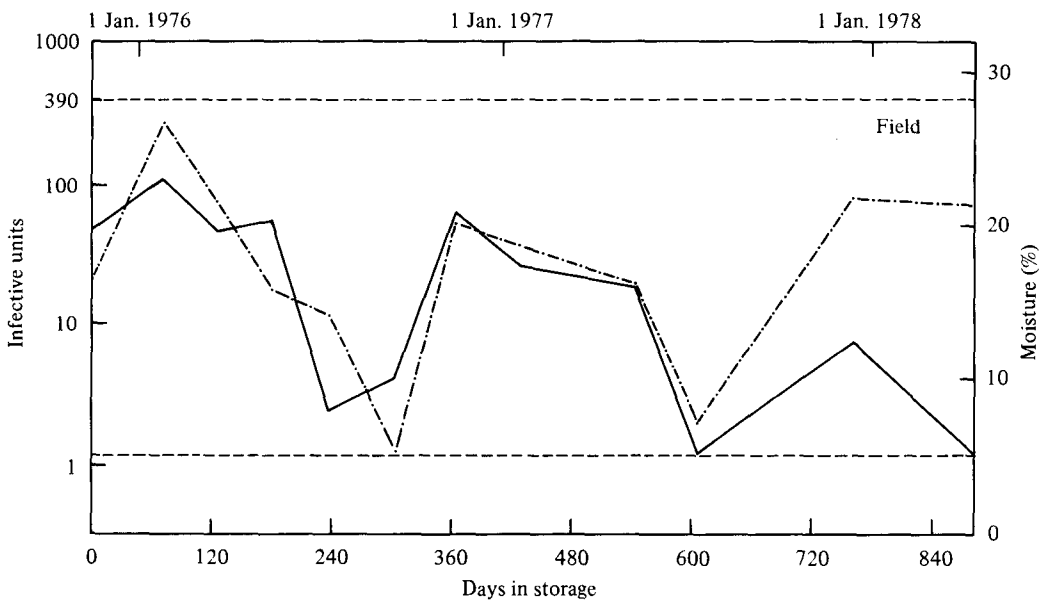

Fig. 4. Infectivity estimates (infective units/kg of oven-dried weight of soil) for soil samples held in drainpipe sections in the field (——), and the percentage moisture contents of the samples on removal from the field (–·–) plotted against the time the samples were held in the field.

fungus in the cabinet and field samples is plotted semi-logarithmically against time in Figs 1–4.

The simplest model for the change in concentration over time would be exponential decay with perhaps different rates for each treatment, which would correspond to straight-line plots. Apart from the wet samples at -1° a declining trend was detectable in all treatments (†). However, simple exponential decay was not sufficient to describe the complex variation in concentration over time for any of the treatments (†).

Both the drying and the wetting treatments had elevated initial concentrations compared with the unamended soil: wet 83 units/kg [57, 120]; moist 47 [31, 70]; dry 96 [66, 140] (†). The results from the three moisture treatments are described separately below.

In the wet samples, parallelism (a tendency to increase or decrease in step) was observed between the -1° and $+3^\circ$ treatments (Fig. 1) (†). The estimates from the samples at -1° were displaced above those at $+3^\circ$ by a multiplicative factor of 2·0 [1·7, 2·5].

The estimates for wet samples at 15° were generally lower than for samples at the lower two temperatures (†) and no infectivity was detected in them after 607 days in store. At 30° there was a very rapid decline in infectivity estimates although a single infection was recorded in a sample which had been stored for 364 days.

In the moist samples (Fig. 2) there was no detectable displacement between the -1° and $+3^\circ$ treatments. Successive estimates deviated about a slow exponential decay (half-life 290 days). By contrast the infectivity of the samples at 15° showed a definite rise and fall. Over the five successive sampling dates between the 364th and 607th days the average concentration of infective units (geometric mean) was greater than the initial concentration by a factor of 3·6 [2·3, 5·7] (†). The concentration then declined rapidly to about or below the Minimum Detectable Level of *c.* 1 unit/kg by the 882 day of storage. At 30° there was a very rapid fall in infectivity, none being detectable after 175 days.

In the dry soil the fungus was detected on all sampling dates in samples held at -1° and $+3^\circ$ (Fig. 3), but it was not detected after 364 days at 15° and 175 days at 30°. Excluding the 30° treatment, the fluctuations in infectivity of the dry soil treatments from one sampling date to the next was greater than that shown by the moist and wet soil samples (†).

All the samples that had been stored at 30° and were apparently no longer infective were transferred to the 3° cabinet after 452 days to see if their infectivity could be restored. All remained non-infective for the remaining 576 days of the experiment.

The fungus was detected on all sampling dates in infested soil taken from the columns in the field. Overall infectivity steadily declined except in July 1976 and 1977, when there were sharp decreases followed by sharp increases. The decreases coincided with decreases in their moisture content (see Fig. 4). No infectivity was detected in any of the samples taken from the check columns of autoclaved soil. Likewise, disease was not detected in any of the plants growing in compost alone and placed among all the dilution series to check for cross-contamination on the wet benches.

DISCUSSION

The results show that soil naturally infested with *Phytophthora fragariae* can remain infective for almost 3 years in the field and in certain storage conditions in the laboratory, thus confirming and extending the observations of Bain & Demaree (1945).

In store, infectivity persisted longest and usually at higher levels in wet and moist samples held at lower temperatures, as observed in the other homothallic species *P. megasperma* (Pratt & Mitchell, 1975) and *P. lateralis* (Ostrofsky, Pratt & Roth, 1977). By contrast, infectivity disappeared rapidly from samples held at 30°, and as transferring them to 3° had no effect it is assumed that the inoculum had died. Although death may have been caused or accelerated by the accidental period of exposure to 37°, the results of an earlier experiment at 30° (Duncan, unpubl.) gave an almost identical pattern supporting the conclusion that prolonged storage in soil at 30° is alone sufficient to kill the fungus.

The results of storage at 15° are difficult to interpret; in general it eventually resulted in very low levels or the complete loss of infectivity. However, in the moist samples infectivity tended to increase during the middle period of the experiment. It is improbable that apparent increases in infectivity were the result of the fungus multiplying in soil because *Phytophthora* species are generally poor saprophytes (Pratt & Mitchell, 1975; Ofstrofsky, Pratt & Roth, 1977). It is more likely that the increases resulted from increased germination rates of some previously dormant propagule, probably oospores.

Various pre-treatments, including passage through snails (Shaw, 1967), incubation in snail enzyme extracts (Salvatore, Gray & Hine, 1973) and soil extracts (Ayers & Lumsden, 1975; Banihashemi & Mitchell, 1976), have been used

to stimulate germination of oospores of *Phytophthora* and *Pythium* spp. Duncan (1977*b*) showed that short periods of incubation at 25° sometimes increased the subsequent germination rate of oospores of *P. fragariae*. It is possible that prolonged exposure to a combination of physical and microbiological factors in the moist samples at 15° tended to break the dormancy of oospores.

Lumsden & Ayers (1975) showed that the oospores of *Pythium ultimum* Trow became thin-walled when dormancy was breaking and they were then more easily killed by drying than when dormant and thick-walled. Their observations may be related to the findings that the enhanced levels of infectivity in the 15° moist samples were not maintained. Perhaps the oospores, which were capable of germinating rapidly in response to environmental changes accompanying the bait test, were like those of *P. ultimum*, more susceptible to drying in the original soil samples or to invasion by mycoparasites as described by Sneh, Humble & Lockwood (1977).

There was a marked decline in the infectivity of the field samples by the end of the experiment. The columns were only 160 mm high and the depth of infested soil within them was *c.* 115 mm. Duncan (1978) found in this site that the highest levels of inoculum were between 100 and 200 mm below the soil surface, where temperature and moisture fluctuations are much lower than in the uppermost 100 mm of soil. Thus if the columns had been longer and the infested soil immersed at greater depths, its infectivity might have persisted at higher levels for a longer time.

The most notable feature of the field results was the sharp decline in infectivity recorded in July each year. The resurgence of infectivity during the autumn cannot be explained by fortuitous introduction of new inoculum from the surrounding field soil since the uninfested checks remained non-infective. The summer decline may have been due to the death of potential inoculum followed by activation of previously dormant oospores in the autumn, or they could be attributed to cyclical changes in the levels of dormancy of the inoculum, but there is no evidence to favour either hypothesis.

It is also possible that the summer decline could partly be due to a loss of sensitivity of the baiting technique caused by high temperatures in the glasshouse during the summer. Such a hypothesis would require an annual decline in the assays for all treatments whether stored in field or in cabinets. Although there was a fall in the infectivity of the cabinet stored samples in 1976 there was none in 1977.

Inspection of Figs 1–3 would suggest that the early patterns of the results were similar for samples of the same initial moisture content, regardless of whether the samples had been stored at −1°, +3° or 15°, and only with time did temperature modify the patterns.

The greater variability in the results of dry compared to wet and moist samples may be explained in terms of the relationship between moisture tension and content in dry soil. Thus very small variations in the moisture content could induce large changes in moisture tension of the dry but not the wet or moist samples and such changes in tension could affect the survival of the inoculum leading to more variable results. Again there is no evidence to support this hypothesis but it is noteworthy that although there was variation in the results from one sampling date to the next in all treatments, their hierarchy changed only slowly. Where there were large changes in infectivity, subsequent samples tended to confirm the results.

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REFERENCES

ALCOCK, N. L. & HOWELLS, D. V. (1936). The *Phytophthora* disease of strawberry. *Scientific Horticulture* **4**, 52–58.

AYERS, W. A. & LUMSDEN, R. D. (1975). Factors affecting production and germination of oospores of three *Pythium* species. *Phytopathology* **65**, 1094–1100.

BAIN, H. F. & DEMAREE, J. B. (1945). Red stele root disease of the strawberry caused by *Phytophthora fragariae*. *Journal of Agricultural Research* **70**, 11–30.

BAKER, R. J. & NELDER, J. A. (1978). GLIM Release 3 Manual, 97 pp. Numerical Algorithms Group, Oxford.

BANIHASHEMI, Z. & MITCHELL, J. E. (1976). Factors affecting oospore germination in *Phytophthora cactorum*, the incitant of apple collar rot. *Phytopathology* **66**, 443–448.

CONVERSE, R. H. & MOORE, J. N. (1966). Susceptibility of certain *Potentilla* and *Geum* species to infection by various races of *Phytophthora fragariae*. *Phytopathology* **56**, 637–639.

DUNCAN, J. M. (1976). The use of bait plants to detect *Phytophthora fragariae* in soil. *Transactions of the British Mycological Society* **66**, 85–89.

DUNCAN, J. M. (1977*a*). On the use of maximum likelihood analyses in strawberry red core detection. *Transactions of the British Mycological Society* **68**, 127.

DUNCAN, J. M. (1977*b*). Germination *in vitro* of *Phytophthora fragariae* oospores from infected root tissue. *Transactions of the British Mycological Society* **69**, 391–395.

DUNCAN, J. M. (1978). Autecology of the strawberry red core fungus (*Phytophthora fragariae*). *Report, Scottish Horticultural Research Institute, 1977* pp. 70–71.

DUNCAN, J. M. (1979). Autecology of the strawberry red core fungus (*Phytophthora fragariae*). *Report, Scottish Horticultural Research Institute, 1978* (in press).

FULTON, R. H. (1959). Spread of strawberry red stele root rot, *Phytophthora fragariae*, by resistant varieties and the survival period of the organism. *Plant Disease Reporter* **43**, 270–271.

HICKMAN, C. J. (1940). The red core root disease of the strawberry caused by *Phytophthora fragariae* n.sp. *Journal of Pomology and Horticultural Science* **18**, 89–118.

LUMSDEN, R. D. & AYERS, W. A. (1975). Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum*. *Phytopathology* **65**, 1101–1107.

MCKEEN, W. E. (1958). Red stele root disease of the loganberry and strawberry caused by *Phytophthora fragariae*. *Phytopathology* **48**, 129–132.

MONTGOMERIE, I. G. (1951). Studies of red core root disease of the strawberry caused by *Phytophthora fragariae* Hickman. Ph.D. Thesis, University of Edinburgh, UK.

OSTROFSKY, W. D., PRATT, R. G. & ROTH, L. F. (1977). Detection of *Phytophthora lateralis* in soil organic matter and factors that affect its survival. *Phytopathology* **67**, 79–84.

PEPIN, H. S. (1967). Susceptibility of members of the Rosaceae to races of *Phytophthora fragariae*. *Phytopathology* **57**, 782–784.

PRATT, R. G. & MITCHELL, J. E. (1975). The survival and activity of *Phytophthora megasperma* in naturally infested soils. *Phytopathology* **65**, 1267–1272.

SALVATORE, M. A., GRAY, F. A. & HINE, R. B. (1973). Enzymatically induced germination of oospores of *Phytophthora megasperma*. *Phytopathology* **63**, 1083–1084.

SHAW, D. S. (1967). A method of obtaining single-oospore cultures of *Phytophthora cactorum* using live water snails. *Phytopathology* **57**, 454.

SNEH, B., HUMBLE, S. J. & LOCKWOOD, J. L. (1977). Parasitism of oospores of *Phytophthora megasperma* var. *sojae*, *P. cactorum*, *Pythium* sp. and *Aphanomyces euteiches* in soil by oomycetes, chytridiomycetes, hyphomycetes, actinomycetes, and bacteria. *Phytopathology* **67**, 622–628.

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