STUDIES ON SPECIES OF SEPTORIA CAUSING
LEAF-SPOTS OF PISTACHIO (PISTACIA VERA)

A Thesis submitted for the Degree of Doctor of Philosophy
in the University of London

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The pathogenic *Septoria pistaciae* and *B. vesicatoria*, causing leaf spot diseases of Pistachio (*Pistacia vera*) were studied. The morphology and ultrastructure of the two pathogens and also the ultrastructure of the host-pathogen interface were investigated. The conidia spores of both pathogens were narrowly elongate with several transverse septa and each cell contained one nucleus. Spore length and cell number were positively correlated. Spore germination was shown in detail in *S. pistaciae*. Spore germination, initially an increase in volume followed by an increase in length by cell division, and germination by the production of twin-pairs of secondary septate spores and/or hyphal germ tubes. A pattern of cell division, polarity and germination was established and an interactive model was described. The spore growth initially followed linear kinetics and then became exponential. The production of hyphal tips became continuous exponential at the same time and the hyphal growth unit stabilised at 40-60 μm. The germination pattern could be altered by manipulation of the nitrogen or phosphate source and levels in the medium. In the resting spore, the wall was composed of three layers: an outer layer (*o*) and two inner layers *I*₁ and *I*₂. Each primary septum was a double layer derived from *I*₁ and *I*₂ with an intermediate electron lucent layer. Perforation was typical with paired Vesamin nodules. During increase in length.
Abstract

Two pathogens, Septoria pistaciarum and S. pistacina, causing leaf spot diseases of Pistachio (Pistacia vera) were studied. The morphology and ultrastructure of the two pathogens and also the ultrastructure of the host-pathogen interface were investigated. The conidia spores of both pathogens were narrowly elongate with several transverse septae and each cell contained one nucleus. Spore length and cell number were positively correlated. Spore germination was shown in detail in S. pistaciarum. Spore germination, initially an increase in volume followed by an increase in length by cell division, and germination by the production of twin-pairs of secondary septate spores and/or hyphal germ tubes. A pattern of cell division, polarity and germination was established and an interactive model was described. The spore growth initially followed linear kinetics and then became exponential. The production of hyphal tips became continuous exponential at the same time and the hyphal growth unit stabilised at 40-60 μm. The germination pattern could be altered by manipulation of the nitrogen or phosphate source and levels in the medium. In the resting spore the wall was composed of three layers an outer layer (o) and two inner layers $I_1$ and $I_2$. Each primary septum was a duplex layer derived from $I_1$ and $I_2$ with an intermediate electron lucent layer. Perforation was simple with paired Woronin bodies. During increase in length
the development of new (secondary) septa showed the cross-wall material was derived from layer I₂ and development was centripetal. The germ-tube wall was formed from wall layer I₂ and wall layers 0 + I₁ were disrupted. Secondary spores were formed enteroblastically.

*S. pistaciarum* and *S. pistacina* were used in the ultrastructure of host-pathogen investigation, infection of the host by *S. pistaciarum* was by inoculation with conidia produced on culture whereas with *S. pistacina* inoculation was obtained from previously infected leaves. The spores germinated on the leaf surface and penetration was made by fine hyphae through the stomatal aperture in both species, growth of pathogen within the leaf was limited to the intercellular spaces. Penetration of the host cell wall did not occur. The hyphae were attached to mesophyll and palisade cells by an accumulation of extra-hyphal sheathing material. The initial host response to infection was shown by an accumulation of electron-dense material and disruption of the tonoplast, followed by disruption of chloroplasts and disorganisation of the host cell cytoplasm. Pycnidia were produced within the lesion. Pycnidial initials were formed in sub-stomatal spaces and spore tendrils were released on the leaf surface.
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<tr>
<td>P40</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 35 days.</td>
<td>160</td>
</tr>
<tr>
<td>P41</td>
<td>SEM. <em>S. pistaciarum</em> (freeze-fractured) infected leaves inoculated 20 days previously.</td>
<td>165</td>
</tr>
<tr>
<td>P42</td>
<td>SEM. <em>S. pistaciarum</em> (freeze-fractured) infected leaves inoculated 20 days previously.</td>
<td>166</td>
</tr>
<tr>
<td>P43</td>
<td>SEM. <em>S. pistaciarum</em> (freeze-fractured) infected leaves inoculated 20 days previously.</td>
<td>167</td>
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<tr>
<td>P44</td>
<td>SEM. <em>S. pistaciarum</em> freeze-fracture of infected leaf after 20 days.</td>
<td>168</td>
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<tr>
<td>P45</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 11 days.</td>
<td>169</td>
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<tr>
<td>P46</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 25 days.</td>
<td>174</td>
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<tr>
<td>P47</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 35 days.</td>
<td>171</td>
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<tr>
<td>P48</td>
<td>TEM. Section of leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 45 days.</td>
<td>172</td>
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<tr>
<td>P49</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 45 days.</td>
<td>173</td>
</tr>
<tr>
<td>P50</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 55 days.</td>
<td>174</td>
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<tr>
<td>P51</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistaciarum</em> after 55 days.</td>
<td>175</td>
</tr>
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</table>
### LIST OF PLATES (CONT'D)

<table>
<thead>
<tr>
<th>Plate</th>
<th>Description</th>
<th>Page</th>
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</thead>
<tbody>
<tr>
<td>Plate 52</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistacina</em> after 11 days.</td>
<td>183</td>
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<tr>
<td>Plate 53</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistacina</em> after 29 days.</td>
<td>184</td>
</tr>
<tr>
<td>Plate 54</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistacina</em> after 29 days.</td>
<td>185</td>
</tr>
<tr>
<td>Plate 55</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistacina</em> after 35-55 days.</td>
<td>186</td>
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<tr>
<td>Plate 56</td>
<td>TEM. Section through leaf of <em>P. vera</em> infected with <em>S. pistacina</em> after 45 days.</td>
<td>187</td>
</tr>
</tbody>
</table>

There are 20 species of *Pistacia* (tree or shrubs) wild or cultivated, distributed from the Mediterranean region to Asia, in Mexico and Texas. Only one species "Pistacia vera L." is grown as a commercial crop and the nut of "Pistacia" is the only commercially acceptable edible nut of the 20 species. Several species like *P. atlantica* Raf., *P. terebinthus* L., *P. chinensis* Bunge, *P. notica* Fisch. & Mey., *P. chinijik*, *P. tangutica* L., *P. integerrima* Steward have been introduced as root stocks upon which to bud and graft as ornamental.

In comparison with the other species of *Pistacia*, *P. vera* L. is known for the large size and spontaneous dehiscence of its fruits (Whitcombe 1957). *P. vera* is both deciduous and dioecious. Shoot extension begins at the end of March and terminates in late April to mid May, a panicked compound leaf at each node subtends one axillary bud.

A *Pistacia* tree after 3-7 years (unpublished report) ultimately produces a heavy crop one year, followed by little or none the next. The mechanism involved is unique in comparison with other biennial fruit and nut bearing trees.
1.0 Introduction

Pistacia is a genus of Anacardiaceus plants comprised of trees and shrubs which exude turpentine or mastic.

"Pista-cia" is derived indirectly from "Pesta" which is an old Persian (Iranian) name for Pistachio (Bailey 1925).

There are 20 species of Pistacia (trees or shrubs) wild or cultivated, distributed from the Mediterranean region to Asia, in Mexico and Texas. Only one species "Pistacia vera L" is grown as a commercial crop and the nut of "Pistachio" is the only commercially acceptable edible nut of the 20 species. Several species like P. atlantica Desf, P. terebinthus L., P. chinensis Bunge, P. mutica Fish & Mey, P. khinjik, P. lentiscus L., P. integerrima Steward have been introduced as root stocks upon which to bud and graft as ornamentals.

In comparison with the other species of Pistacia, P. vera L. is known for the large size and spontaneous dehiscence of its fruits (Whitehouse 1957). P. vera is both deciduous and dioecious. Shoot extension begins at the end of March and terminates in late April to mid May, a pinnately compound leaf at each node subtends one axillary bud.

A Pistachio tree after 3-7 years (unpublished report) ultimately produces a heavy crop one year, followed by little or none the next. The mechanism involved is unique in comparison with other biennial fruit and nut bearing trees.
Biennial bearing in other species is usually the result of greatly reduced flower bud formation during the year of a heavy crop. In contrast, in Pistachio it is by inflorescence bud abscission in the summer during which a heavy crop of nuts is produced (Woodroof 1979). Bud abscission generally begins in late June and is most intense during July and August when seed growth and development are most rapid. The degree of bud abscission (in some instances almost 100%) increases as the number of nuts per branch increases (Crane 1973-1975).

1.1 World Areas of Distribution and Cultivation.

Pistachio is a very xerophilous plant able to withstand great dryness of soil and air but humidity is unfavourable to its development. Pistachio can tolerate cold winter temperatures of 17.8°C (Kuska, U.S.S.R.) and hot summer temperatures of 42.2°C (in Iran). The best orchards in Iran are grown at altitudes of 1,000 m. with rainfall averages of 25-38 cm/annum and a summer temperature of 37.5°C. The Pistachio tree thrives best in areas having a cool winter for breaking bud dormancy and a hot summer for maturing the nuts.

In Central Asia, P. vera L. is not grown as a commercial plantation crop but occurs as a sporadically distributed species. It is widespread over Southern Central Asia in the low mountains and foothills from the Caspian Sea (Kopetdaph mountains) up to Fergana, the region of Tashkent and mountains of Karatan.
The wild Pistachio groves extend into Northern Afghanistan, beyond the river Amu-Daria in the low mountains of the Pamir-Alai (Whitehouse 1957). Historical records show Pistachios growing in many places where none exist today. The age of the trees ranged from 6 to 240 years (Popov 1929).

The commercial species of Pistachio P. vera L. has been planted commercially for hundreds of years in Iran, Turkey, Greece and Syria, and it has been introduced into Egypt from Syria along the Mediterranean coast between Alexandria and the Libyan frontier; and into Austria, Hungary, Italy, France, U.S.A. and Australia. In the United States of America, cultivation testing began in 1902 by the U.S. Department of Agriculture at Chicago, California and Texas, but most of the Pistachio nuts consumed in the U.S.A. were imported from other countries (Table 1).

In Iran, the Pistachio tree has been planted commercially for hundreds of years but only in the last sixty or seventy years has there been recognition of the value of the nut as an agricultural crop for export with the rate of new plantings keeping pace with the rapid increase in American consumption. The principal producing area in Iran is Kerman, which is located also in the vicinity of Rafsenjan, Sirjan and Ghazuin (Woodroof) and Damghan in approximately 120 villages scattered through this large fertile valley about 100 miles long and 50 miles wide (Whitehouse 1957).

Turkey is today one of the world's leading producers of Pistachio nuts. Pistachio culture in Turkey is centered in the dry, barren foothills and lower ranges of Southeast and
PISTACHIO NUTS

### Table 1

**IMPORTS OF PISTACHIO NUTS INTO UNITED STATES**

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<thead>
<tr>
<th></th>
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<tr>
<td>Italy</td>
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<td>88</td>
<td>28</td>
<td>1</td>
<td>28</td>
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<tr>
<td>Other</td>
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<td>2</td>
<td>133</td>
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<tr>
<td>Total</td>
<td>114</td>
<td>90</td>
<td>61</td>
<td>1</td>
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<tr>
<td><strong>Other Countries</strong></td>
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</tr>
<tr>
<td>Afghanistan</td>
<td>661</td>
<td>832</td>
<td>590</td>
<td>150</td>
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<td>India</td>
<td>177</td>
<td>96</td>
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<td>Iran</td>
<td>274</td>
<td>2,235</td>
<td>3,092</td>
<td>1,618</td>
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<td>Lebanon</td>
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<td>Syria</td>
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<tr>
<td>Turkey</td>
<td>86</td>
<td>2,459</td>
<td>4,226</td>
<td>3,632</td>
<td>3,163</td>
<td>3,868</td>
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<tr>
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<tr>
<td>Total</td>
<td>1,156</td>
<td>5,530</td>
<td>8,183</td>
<td>5,872</td>
<td>5,844</td>
<td>8,089</td>
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<tr>
<td>Grand total</td>
<td>1,270</td>
<td>5,620</td>
<td>8,183</td>
<td>5,933</td>
<td>5,845</td>
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### Table 1 (continued)

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<td>7</td>
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<td>14</td>
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<td>Iran</td>
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<tr>
<td>Turkey</td>
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<td>6</td>
<td>11</td>
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<td>Other</td>
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<td>...</td>
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<td>...</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>280</td>
<td>343</td>
<td>203</td>
<td>45</td>
<td>312</td>
</tr>
<tr>
<td>Grand total</td>
<td>230</td>
<td>291</td>
<td>360</td>
<td>209</td>
<td>52</td>
<td>317</td>
</tr>
</tbody>
</table>

*Three year average, 1937-1939.
*Eleven months, September-July.
*Includes 3 tons from Spain and 2 tons from Italy.
*Includes 113 tons from Syria and Lebanon.

Source: U.S. Foreign Service.
Western Turkey. The principal producers are the valleys of Gaziantep and Urfa, but there is some cultivation of the crop in eastern Seyhan, Southern Maras, Malatya, Diyarbakir, Northern Mardin and Siirt.

In Italy the nut is grown in the Calania and Girgenti Province, Carini (20 miles from Palermo).

Sicilian nuts are reported to be larger and greener and are particularly esteemed by consumers. Bronte exports more nuts than other Sicilian provinces. There are orchards near the mouth of the Rhone valley in France and in Australia (Bembower 1956).

1.2 Commercial Varieties of Pistachio Nuts.

Pistachio nuts are classified in the trade according to their origin, e.g. Afghan, Iranian, Sicilian, Syrian or Turkish.

Importers purchase their nuts in the "natural state", grading, processing and selling them under their own brand names. Iranian 8 star Colossal, Iranian 7 star Giants, Italian 5 star Extra Jumbo, Turkish 4 star Jumbo and Afghan 3 star Buds are the brand names of one New York firm. Iranian Giants (Monarch), Iranian Midgets (Tulip), Sicilian (Royal) and Syrian (Crown) are the brands of another.

Iranian growers in Rafsenjam area have been selecting and naming some of the best of their seedlings. The following are some of the better cultivars grown in Iran today: Ibrahimi Momtaz, Owhadi, Safeed Agah, Kalehghouchi. Some other local names
from Iranian and other countries are mentioned in Table 2.

A new variety (Sirora) is being developed in the U.S.A., it originated as a seedling from an open-pollinated Red Aleppo parent and the name is derived from the name Cisirio and acronym of Red Aleppo (Maggs 1981).
### Local names, shape and origin of Pistachio nuts

#### Table 2

<table>
<thead>
<tr>
<th>Local name</th>
<th>Shape</th>
<th>Origin</th>
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<tbody>
<tr>
<td>Badami</td>
<td>almond</td>
<td>Rafsenjan</td>
</tr>
<tr>
<td>Ohadi</td>
<td>hazelnut</td>
<td>&quot;</td>
</tr>
<tr>
<td>Montaz</td>
<td>almond</td>
<td>&quot;</td>
</tr>
<tr>
<td>Badami-riz</td>
<td>almond</td>
<td>&quot;</td>
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<tr>
<td>Shah-Passand</td>
<td>almond</td>
<td>Damghan</td>
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<tr>
<td>Jowzi</td>
<td>hazelnut</td>
<td>&quot;</td>
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<tr>
<td>Khanjari</td>
<td>almond</td>
<td>&quot;</td>
</tr>
<tr>
<td>Noghli</td>
<td>hazelnut</td>
<td>&quot;</td>
</tr>
<tr>
<td>Kallehpazi</td>
<td>almond</td>
<td>Ghazvin</td>
</tr>
<tr>
<td>Uzum</td>
<td>long and plump</td>
<td></td>
</tr>
<tr>
<td>Kirmizi</td>
<td>red hulled</td>
<td></td>
</tr>
<tr>
<td>Red Aleppo</td>
<td>red hulled</td>
<td></td>
</tr>
<tr>
<td>Alemi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obiad</td>
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<td></td>
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<tr>
<td>Mirhary</td>
<td></td>
<td></td>
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<tr>
<td>Achoury</td>
<td></td>
<td></td>
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<tr>
<td>Ayimi</td>
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<td>El Balaury</td>
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<td>Aintab</td>
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<tr>
<td>Ashoori</td>
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<td>Traborella</td>
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<td>Bronte</td>
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<td>Sangurgha</td>
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<tr>
<td>Girasola</td>
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</tr>
<tr>
<td>Girasola Cappucia</td>
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<tr>
<td>Bianca Giardino</td>
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<td>Blanco Regina</td>
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<tr>
<td>Rappa di Sessa</td>
<td></td>
<td></td>
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<tr>
<td>Minnullina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gialla (three strains)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Aleppo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safax</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronte</td>
<td></td>
<td>California</td>
</tr>
<tr>
<td>Safax</td>
<td></td>
<td>Algeria</td>
</tr>
<tr>
<td>Lassen</td>
<td></td>
<td>Tunisia</td>
</tr>
</tbody>
</table>
1.3. With the commercial exploitation of Pistachio in the United States and Australia the loss of crop due to phytopathogens has become of immediate importance. In the Old World growing area a considerable number of diseases have been reported as pathogens of Pistachio (Table 3).

In Turkey (Bremer 1954) and Greece (Chitzandis 1956) the main disease leading to serious crop loss is a leaf spot caused by *Septoria* Sacc. spp, a pycnidial fungus. The disease *Septoria* leaf-spot or 'Septoriosis of Pistachio' (Rieuf 1964) has also been described from Morocco (Rieuf 1964), Tunisia (Chabrolin 1935), Greece (Sarejanni 1935), Sicily (Carracciolo 1934), Italy (Pupillo & Di Caro 1952) and Syria (Hallage 1927). In Iran the leaf spot is present but does not cause serious crop loss (Ershad pers. comm); however recently severe outbreaks have been reported in 1972 and 1977 in Turkey (Dinc et al 1979) and also as a new record in the United States from Brownwood, Texas where severe leaf spotting and defoliation occurred (Maas et al 1971).

The United States material of Pistachio was derived from various cultivations from Iran and it is probable that the disease was introduced along with the host plant. Fortunately there have been no recorded outbreaks in Australia.
Table 3  Major fungal pathogens of P. vera

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease Effect</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>Leaf spot</td>
<td>Egypt</td>
<td>Wasfy et al (1974)</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Nut spoilage</td>
<td>Iran; USA</td>
<td>Thomson &amp; Mehdy (1978)</td>
</tr>
<tr>
<td>Camarosporium pistaciae</td>
<td>Vascular necrosis</td>
<td>Greece</td>
<td>Zachos et al (1974)</td>
</tr>
<tr>
<td>Eremothecium ashbyi)</td>
<td>Stigmamycosis</td>
<td>Iran</td>
<td>Ershad &amp; Barkhordary (1974)</td>
</tr>
<tr>
<td>E. cymbalariae</td>
<td></td>
<td></td>
<td>Gerlach &amp; Ershad (1970)</td>
</tr>
<tr>
<td>Fusarium equiseti</td>
<td>Leaf spot</td>
<td>Iran</td>
<td>Ershad &amp; Barkhordary (1974)</td>
</tr>
<tr>
<td>Nematospora corgli</td>
<td>Stigmamycosis</td>
<td>Iran</td>
<td>Ershad (1977)</td>
</tr>
<tr>
<td>Phyllactinia imperialis</td>
<td>Powdery mildew</td>
<td>Iran</td>
<td>Ershad (1977)</td>
</tr>
<tr>
<td>Phytophthora spp.</td>
<td>Foot rot</td>
<td>Iran; Greece</td>
<td>Ershad (1977) Kouyas (1952)</td>
</tr>
<tr>
<td>Pileolaria terebinthi</td>
<td>Rust</td>
<td>Egypt</td>
<td>Assawah (1969)</td>
</tr>
<tr>
<td>Septoria pistacularum</td>
<td>Leaf spot</td>
<td>Greece; USA</td>
<td>Chitzandis (1956) Maas et al (1971)</td>
</tr>
<tr>
<td>S. pistacina</td>
<td>Leaf spot</td>
<td>Greece</td>
<td>Chitzandis (1956)</td>
</tr>
<tr>
<td>Verticillium albo-atrum</td>
<td>Wilt</td>
<td>California USA</td>
<td>Ashworth &amp; Zimmerman (1976)</td>
</tr>
</tbody>
</table>
1.4. The Genus Septoria Sacc.

The fungal genus Septoria is a form genus within the Deuteromycotina or Fungi Imperfecti. The Deuteromycotina can be divided into three large classes: 1) those members with sterile mycelia, the Agonomycetes; 2) those with spores borne on free conidiophores or mycelium, the Hyphomycetes and 3) those with spores produced within pycnidia or acervuli, the Coelomycetes.

Deuteromycotina (Deuteromycetes) = Imperfecti Fungi

Agonomycetes (Mycelia sterilia) Coelomycetes Hyphomycetes (Anamorph)

Ascomycetes Basidiomycetes (Teleomorph)

(Ainsworth 1977)

The conidia of Septoria are produced within pycnidia thus Septoria is placed in the Coelomycetes (Sutton 1980).

Some species of Septoria represent the conidial phase of species of fungi within the Class Loculoascomycetes. The Loculoascomycetes form a large natural class within the Euascomycetes (Table 4)
Table 4  Classification of the Teleomorphs associated with Septoria based on Kendrick (1979) and Barr (1983).

 LOCULO-ASCOMYCETES
  (Loculoparenchymatomy cetidae)*
    Pleosporales
      IA
        Pleosporaceae (Phaeosphaeriaceae(Barr 79)
          IIA1
            Phaeosphaeria(1)
            Leptosphaeria(4)
            Ophiobolus(1)
            CesendNot.

    Anamorph
      Septoria
      Septoria - Anamorph

  (Loculoedaphomyetidae)*
    Dothideales
      IB
        Mycosphaerellaceae
          IIB1
            Mycosphaerella(25)
            Sphaerulina Sacc. (2)

  (Barr 1983)

The figure in parenthesis gives the number of Septoria anamorphs associated with each genus.
The terms teleomorph - anamorph and holomorph were introduced by Hennebert & Weresub (1977).

Holomorph is a term which covers the whole organism. The characteristic form that is involved in producing meiotic diasporas may be termed the teleomorph or perfect state. Thus in Ascomycetes the teleomorph is the ascocarp or its equivalent at maturity producing asci and ascospores.

The form characteristic of the asexual state i.e. production of the mitotic diasporic expression of the fungus may be termed the anamorph or imperfect state.

The conventional system of nomenclature (Donk 1960a) or the anatomical system (Hennebert 1971) allows the naming of an imperfect state using an unbotanical scheme of nomenclature which enables form genera to be delimited.

Thus in fungi with known teleomorphic and anamorphic forms, the holomorphic name refers to the teleomorphic stage with inclusion of the appropriate anamorphic name indicating the genomic relationship (Carmichael 1979).

Many fungi classified in the Deuteromycotina are known only as imperfect asexually reproducing forms and are accordingly identified and named using the anamorphic states, and in these cases the anamorph represents the holomorph, though the Code of nomenclature does not recognise the names of anamorphs as holomorphic names (Hennebert 1979).
In the Kananaskis II conference (Kendrick 1979), several authors (e.g. Nag Raj, Luttrell, de Hoog) discussed the problems of genus limitation in terms of teleomorph/anamorph relationships. Many ascomycete teleomorphic genera were associated with several different anamorphs and inversely many anamorphic form genera were associated with several different teleomorphs.

Thus the present methods for the recognition of teleomorphic generic limits based on teleomorph characters alone may be too restrictive and anamorphic states as a partial statement of genomic information should form part of teleomorph descriptions. The non-botanical system of form-genus delimitation to separate discrete groups within a form-genus is likewise imprecise, but in forms without a teleomorphic connection the method reveals a failure to fully describe the potential of the genome.

This may be exemplified by the anamorphic genus Septoria and its associated teleomorphs (Tables 5, 6). The genus Septoria is regarded as a heterogeneous form-genus of fungi with over 400 species (Raj N, 1979). However Sutton (1980) refers to 2,000 and various ascomycete genera have been described as the teleomorphs. Luttrell (1979) regards the Septoria species with a Mycosphaerella teleomorph as a natural grouping. However various species of Mycosphaerella have been described with anamorphic states not apparently related to Septoria (Table 7).
Table 5  Teleomorphs associated with Septoria from Kendrick 1979

<table>
<thead>
<tr>
<th>Anamorph</th>
<th>Teleomorph</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycospherella (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.aceris (Lib) R&amp;Br.</td>
<td>M. latebrosa (CKe.) Schrot</td>
<td>Stone(1916)</td>
</tr>
<tr>
<td>S. aurea Ell. &amp; Ev.</td>
<td>M. aurea Stone</td>
<td></td>
</tr>
<tr>
<td>S. ceptidis Berk &amp; Curt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. coptis Berk &amp; Curt</td>
<td>M. coptis (Schw.) House</td>
<td></td>
</tr>
<tr>
<td>S. hederae Desm.</td>
<td>M. hedericola (Desm.) Lindau</td>
<td></td>
</tr>
<tr>
<td>S. hippocastani B&amp;Br</td>
<td>M. maculiformis Schrot. Var.</td>
<td></td>
</tr>
<tr>
<td>S. ligustri Kickx</td>
<td>M. ligustri Fckl.</td>
<td></td>
</tr>
<tr>
<td>S. linicola (Spg.) Gar.</td>
<td>M. linorum (Wr.) Gracia-Rad</td>
<td></td>
</tr>
<tr>
<td>S. musiva PK.</td>
<td>M. populorum Thom.</td>
<td></td>
</tr>
<tr>
<td>S. piricola Desm.</td>
<td>M. pirri (Auersw.) Bower.</td>
<td></td>
</tr>
<tr>
<td>S. populicola Desm.</td>
<td>M. sentina (Fr.) Schrot.</td>
<td></td>
</tr>
<tr>
<td>S. populicola PK.</td>
<td>M. populii Schrot.</td>
<td></td>
</tr>
<tr>
<td>S. quercina Desm.</td>
<td>M. populicola Thom.</td>
<td></td>
</tr>
<tr>
<td>S. querceti Thum.</td>
<td>M. psilospora Cilm. &amp; kadl.</td>
<td></td>
</tr>
<tr>
<td>S. ribis Desm.</td>
<td>M. grossulariae (Auersw.) Lindau</td>
<td></td>
</tr>
<tr>
<td>S. ribis Desm.</td>
<td>M. ribis (Fckl.) Feltg.</td>
<td></td>
</tr>
<tr>
<td>S. rubi (Durby) West.</td>
<td>M. joerstadii Arx</td>
<td>Von Arx(1957)</td>
</tr>
<tr>
<td>S. rubi West</td>
<td>M. ligea Sacc.</td>
<td></td>
</tr>
<tr>
<td>S. rubi West</td>
<td>M. rubi Roark</td>
<td></td>
</tr>
<tr>
<td>S. stellariae Rob &amp; Desm.</td>
<td>M. isariophora Johans</td>
<td></td>
</tr>
<tr>
<td>S. sp.</td>
<td>M. impatientis (PK. clinton) House</td>
<td></td>
</tr>
<tr>
<td>S. triticici Rob. opud Desm.</td>
<td>M. graminicola (Fucker) Sanderson</td>
<td></td>
</tr>
<tr>
<td>S. sp.</td>
<td>M. ascocephyllii cotton</td>
<td></td>
</tr>
</tbody>
</table>

Reference:
- Stone (1916)
- Grove (1935)
- Barr (1972)
- Hoore (1959)
- Shaw (1973)
- Thompson (1941)
- Grove (1935)
- Thompson (1941)
- Grove (1935)
- Grove (1935)
- Thompson (1941)
- Grove (1935)
- Grove (1935)
- Thompson (1941)
- Roof (1921)
- Von Arx (1957)
- Grove (1935)
- Roark (1921)
- Sanderson (1976)
- Webber (1967)
<table>
<thead>
<tr>
<th>Anamorph</th>
<th>Teleomorph</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phaeosphaeria</strong> (1)</td>
<td><strong>Phaeosphaeria nigrans</strong> (Rob.ex Desm.)Holm</td>
<td>Hughes (1949)</td>
</tr>
<tr>
<td>S. alopecuri Syd.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leptosphaeria Ces</strong> &amp; deNot. (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sorbi Lasch</td>
<td>L.Sorbi Jacz</td>
<td>Grove (1935)</td>
</tr>
<tr>
<td>S. zeae Stout</td>
<td>L.maydis Stout</td>
<td>Stout (1930)</td>
</tr>
<tr>
<td><strong>Ophiobolus</strong> (1)</td>
<td>O.herpotrichus(Fr.)Sacc.</td>
<td></td>
</tr>
<tr>
<td>S. sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sphaerulina Sacc.</strong> (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(phloeospora)</td>
<td></td>
<td>Barr (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sp.</td>
<td>S.rubi Desm.&amp;Wilcox.</td>
<td>Barr (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shaw (1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 7

Anamorphic genera associated with *Mycosphaerella Johanson*.

(Figures in parenthesis indicate number of species involved).

<table>
<thead>
<tr>
<th>Mycosphaerella Johanson</th>
<th>Aschochyta Lib (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asteroma DC. ex. Fr., (1)</td>
</tr>
<tr>
<td></td>
<td>Asteromella Pass et Thum. (4)</td>
</tr>
<tr>
<td></td>
<td>Cercoseptoria Petr. (1)</td>
</tr>
<tr>
<td></td>
<td>Cercospora Fres. (16)</td>
</tr>
<tr>
<td></td>
<td>Cercosporella Sacc. (4)</td>
</tr>
<tr>
<td></td>
<td>Cercosporidium Earle (1)</td>
</tr>
<tr>
<td></td>
<td>Cladosporium Link ex Fr. (4)</td>
</tr>
<tr>
<td></td>
<td>Cylindrosporella Höhnel (1)</td>
</tr>
<tr>
<td></td>
<td>Fusicladiella Höhnel (1)</td>
</tr>
<tr>
<td></td>
<td>Heberosporium Cooke (1)</td>
</tr>
<tr>
<td></td>
<td>Lecanosticta Syd. (1)</td>
</tr>
<tr>
<td></td>
<td>Ovularia Sacc. (1)</td>
</tr>
<tr>
<td></td>
<td>Passalora Fr et Mort (1)</td>
</tr>
<tr>
<td></td>
<td>Phaeoisariopsis Ferraris (1)</td>
</tr>
<tr>
<td></td>
<td>Phloeospora Schultz (2)</td>
</tr>
<tr>
<td></td>
<td>Phoma Sacc. (6)</td>
</tr>
<tr>
<td></td>
<td>Phyllosticta Pers ex Desm (13)</td>
</tr>
<tr>
<td></td>
<td>Placosphaeria Sacc. (1)</td>
</tr>
<tr>
<td></td>
<td>Poly thrincium Kunze &amp; Schm ex Fr. (1)</td>
</tr>
<tr>
<td></td>
<td>Ramularia Unger (10)</td>
</tr>
<tr>
<td></td>
<td>Septocylindrium Bon. et Sacc. (2)</td>
</tr>
<tr>
<td></td>
<td>Septogloeum Sacc. (1)</td>
</tr>
<tr>
<td></td>
<td>Stenella Syd. (1)</td>
</tr>
<tr>
<td></td>
<td>Sterigna Sacc. (1)</td>
</tr>
<tr>
<td></td>
<td>Toxosporium Vuill (2)</td>
</tr>
</tbody>
</table>

Kendrick (1979)
Sutton (1980) attempted to devise a new artificial arrangement of the Deuteromycotina on the basis of conidiogenesis and the type of conidium. He stressed that the system was experimental with many anomalies but that it had proved workable.

*Septoria* as presently defined would therefore be in the major sub-division Blastodeuteromycetes. At the next division however, the genus would have to be separated into the two groups Holoblastomycetidae and the Enteroblastomycetidae. The final location for members of the genus would be either in the Blastopycnidiineae or the Phialopycnidiineae (Table 8).

However reexamination of "conidiogenesis as a total process involving conidial ontogemy, conidial delimitation, conidial secession, proliferation and regeneration suggests that the scheme may have to be discarded since the terms phialidic, blastic and thallic are at present ill defined and cannot be applied to fundamental division of conidial fungi and that if a new system of classification is to be used for these fungi it should not rest on such terms" (Minter et al 1982,83).

This view will require such an extensive reworking of conidiogenetic patterns and consequent revision of generic and higher relationships that at this time the position of *Septoria* as a form genus within the Class Coelomycetes remains uncertain. The main characteristics by which the genus *Septoria* is recognised is a combination of pycnidial morphology coupled with the long slender curved or straight septate conidia.
The definitions of conidiogenesis set out at Kananaskis II (Kendrick 1979) allows some division of the genus *Septoria* into three groups (Sutton 1980). Conidial formation in most fungi can be described by one of three terms, holoblastic, enteroblastic or thallic (Madelin 1979). Conidiogenesis in *Septoria* is regarded as blastic (Sutton 1980). Blastic development can either be holoblastic or enteroblastic with a special sub-division of the latter being described as phialidic.

Holoblastic was defined by Cole & Samson (1979) Cook and Kendrick (1979) as 'the mode of blastic conidium ontogeny in which all wall layers of the conidiogenous cell are involved in the formation of the conidial wall'. The apical growth of the acroauxic conidiophore gives way to conidiogenesis with so little pause that irreversible changes in wall structure do not occur. In enteroblastic development there is a pause sufficiently long to allow the wall at the conidiogenous locus to lose direct involvement in further growth and differentiation and new wall material has to be laid down (Fig 1).

![Diagram of Blastic Spore Development](redrawn from Madelin 1979)
Using this simple system the following three groups have been devised, but the sample of the genus is small and many more species would have to be studied before such groups could be regarded as finalised:

**Group I** Phialidic development.
- *S. apiicola* Speg
- *S. tritici* Rob
- *S. avenae f.sp. avenae* Frank
- *S. nodorum* Berk

**Group II** Holoblastic development.

IIa Holoblastic - simple
- *S. adanenis* Petrak
- *S. leucanthemi* Sace et Speg.
- *S. socia* Pass
- *S. lactucae* Pass
- *S. glycines* Henrici
- *S. lycopersici* Speg.

IIb Holoblastic - sympodial.
- *S. cytisi* Desm. Type Spec (Ann. Sci. Nat ser 3.8:24 (1847)).
- *S. chrysanthemella* Sace
- *S. obesa* Syd
- *S. passerinii* Sace
- *S. helianthi* Ell et Kell.
1.5. Septoria as a Pathogen

The genus *Septoria* Sacc is composed entirely of pathogens of flowering plants. They cause necrotic spots and blotches on stems, leaves and fruits and in severe infections defoliation occurs. Most of the major agricultural and horticultural crop plants have one or more *Septoria* spp which cause significant crop losses. Amongst the world's important food crops, wheat, barley, oat, cucumbers, pistachio, soybean and sunflowers and amongst flower crops the genus *Chrysanthemum* are severely affected.

A list (Tables 9, 10) of important *Septoria* spp for which recent descriptions are available which includes cultural characteristics, symptoms, geographical distribution and control measures has been prepared from publications from the Commonwealth Mycological Institute, Kew, and the National Mycological Herbarium, Canada.
## Table 9 Important *Septoria* Diseases

<table>
<thead>
<tr>
<th>CMI Number</th>
<th>Pathogen</th>
<th>Host</th>
<th>Disease</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td><em>S. adanensis</em> Petrak</td>
<td>Chrysanthemum spp</td>
<td>Leaf spot of Chrysanthemum</td>
<td>Turkey (1953)</td>
</tr>
<tr>
<td>88</td>
<td><em>S. apiicola</em> (Speg)</td>
<td>Celery and celeriac (cultivated or wild) (Apium sp)</td>
<td>Leaf spot (or late blight)</td>
<td>Italy (1890)</td>
</tr>
<tr>
<td>312</td>
<td><em>S.avenae f.sp. avenae</em> Frank</td>
<td>Oat (Avena sp)</td>
<td>Speckle blotch of oat</td>
<td>First reported by Weber 1922.</td>
</tr>
<tr>
<td>668</td>
<td><em>S. cannabis</em> (Lasch) Sacc.</td>
<td>Cannabis sativa (hemp)</td>
<td>White leaf spot or leaf blight of hemp</td>
<td>Italy (75, 97, 39, 471).</td>
</tr>
<tr>
<td>669</td>
<td><em>S. carthami</em> (Murashkinskij)</td>
<td>Carthamus tinctorius and <em>C. marencticus</em></td>
<td>White leaf spot of safflower</td>
<td>West Siberia</td>
</tr>
<tr>
<td>137</td>
<td><em>S. chrysanthemella</em> Sacc.</td>
<td>Chrysanthemum spp</td>
<td>Leaf spot</td>
<td>Italy 1878</td>
</tr>
<tr>
<td>740</td>
<td><em>S. cucurbitacearum</em> Sacc.</td>
<td>Cantaloupe, cucumber, melon, pumpkin, squash and vegetable marrow (Cucurbita spp)</td>
<td>Leaf spot</td>
<td>Denmark (25 m364) U.S.A. (17, 364).</td>
</tr>
<tr>
<td>339</td>
<td><em>S. Glycines</em> Hemmi</td>
<td>Soybean (Glycinemax)</td>
<td>Brown spot of soybean</td>
<td>Canada.</td>
</tr>
<tr>
<td>709</td>
<td><em>S. liniocola</em> (Speg.)</td>
<td>Flax (Linum usitatissimum) and Linum spp.</td>
<td>Pasmo disease of flax-spots on leaves, stems, penduncles</td>
<td></td>
</tr>
<tr>
<td>CMI Number</td>
<td>Pathogen</td>
<td>Host</td>
<td>Disease</td>
<td>Note</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------</td>
<td>-------------------------------------</td>
<td>--------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>335</td>
<td><em>S. Lactucae</em> pass.</td>
<td>Lettuce (<em>Lactuca satira</em>)</td>
<td>A minor leaf spot of lettuce</td>
<td>Australia (1961)</td>
</tr>
<tr>
<td>138</td>
<td><em>S. Leucantheim</em> Sacc. speg.</td>
<td>Ox-eye daisy (Chrysanthemum spp)</td>
<td>Leaf spot of ox-eye daisy</td>
<td>Italy (1878)</td>
</tr>
<tr>
<td>89</td>
<td><em>S. Lycopersia</em> speg.</td>
<td>Tomato (<em>Lycopersicum esculentum</em>)</td>
<td>Leaf spot of tomato</td>
<td>Argentina (1882)</td>
</tr>
<tr>
<td>86</td>
<td><em>S. nodorum</em> Berk.</td>
<td>Wheat (<em>T. aestivum</em>)</td>
<td>Glume blotch of wheat</td>
<td>England (1845)</td>
</tr>
<tr>
<td>139</td>
<td><em>S. obesa</em> Syd.</td>
<td>Chrysanthemum spp.</td>
<td>Brown spot of cultivated chrysanthemum</td>
<td>Japan (1914)</td>
</tr>
<tr>
<td>670</td>
<td><em>S. passiflorae</em> Syd.</td>
<td>Passiflora spp.</td>
<td>Leaf, blossom, fruit and stem spot</td>
<td>South Africa 1938-1939</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Spring)</td>
</tr>
<tr>
<td></td>
<td><em>S. passerini</em> Sacc.</td>
<td>Barley (<em>Hordeum spp</em>)</td>
<td>Leaf blotch</td>
<td>Fungi Canadiensis 1983 No.243 (Binett et al 1983)</td>
</tr>
<tr>
<td>140</td>
<td><em>S. socia</em> pass.</td>
<td>Ox-eye daisy (Chrysanthemum spp)</td>
<td>Leaf spot of ox-eye daisy</td>
<td>Italy</td>
</tr>
<tr>
<td>91</td>
<td><em>S. triciti</em> (Rob + Desm)</td>
<td>Wheat (<em>Triticum aestivum</em>)</td>
<td>Leaf spot (or speckled leaf blotch)</td>
<td>France (1842)</td>
</tr>
<tr>
<td></td>
<td><em>S. pistaciae</em> Caraco</td>
<td>Pistacia vera</td>
<td>Leaf spot</td>
<td>Chitzandis (1957)</td>
</tr>
<tr>
<td></td>
<td><em>S. pistaclina</em> (Allesh)</td>
<td>Pistacia vera</td>
<td>Leaf spot</td>
<td></td>
</tr>
</tbody>
</table>

CMI = CMI descriptions of pathogenic fungi and bacteria. Number = Commonwealth Mycological Institute, Kew, U.K.
1.6. **Septoria leaf spots of Pistachio**.

The first description of a *Septoria* sp. causing a leaf spot of *P. vera* was based on material from the north of France, and given the specific name *S. pistaciae* Demaziere (1842). Leveille (1842) described a leaf spot of *Pistacia* sp. caused by a perithecial fungus *Dothidea pistaciae*. This was reworked by Cooke (1883, 1885) and transferred to *Septoria pistaciae* (Lev) Cooke. Allescher (1901) recognised the occurrence of *S. pistaciae* Desm in Germany and also proposed the new binomial *S. pistacina* to replace *S. pistaciae* (Lev) Cooke.

Caracciolo (1934) described *S. pistaciarum* as a new leaf spot of Pistachio from Sicily.

The symptoms as described by Chitzandis (1956) and Maas et al (1971) show that the two species *S. pistaciarum* and *S. pistacina* cause leaf spots which can be distinguished on rate of growth and form.

**Symptoms:**

* S. *pistacina*

The leaf spots were first visible in June but were not markedly necrotic, the pycnidia which were produced on both upper and lower surfaces of the leaf were the first symptoms to appear as dark dots. These were confined to small angular zones confined between two secondary veins. Later growth of the lesion led to an increase in size of
up to 2.5 cm and individual lesions coalesced into larger units. Finally chlorosis and neurosis occurred later in the season.

**S. pistaciarum**

Symptoms began to appear in May and consisted of small, 1 mm diam, round brown necrotic spots. Rapid enlargement of the lesion occurred up to 2.5 mm and pycnidia are produced on both leaf surfaces. The spots are necrotic and coalesced into large necrotic angular spots.

The positive connections of the anamorphic Septoria spp to new teleomorphic Mycosphaerella spp were reported by Chitzandis (1956) using material from Greece. She examined *S. pistacina* Allescher and *S. pistaciarum* Caracciola using fresh, cultive and herbarium material. The descriptions of both species are recorded in Table II.

The present taxonomic relationships of Septoria spp on *Pistacia* can be summarised as:

<table>
<thead>
<tr>
<th>Teleomorph</th>
<th>Anamorph</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycosphaerella pistaciarum Chitzandis</td>
<td>Septoria pistaciarum Caracciola</td>
<td>none</td>
</tr>
<tr>
<td><em>M. pistacina</em> Chitzandis</td>
<td><em>S. pistacina</em> Allescher</td>
<td>Dothidea pistaciae Lev. <em>S. pistaciae</em> (Lev) Cooke</td>
</tr>
<tr>
<td>Not known</td>
<td><em>S. pistaciae</em> Desmazieres</td>
<td>none</td>
</tr>
</tbody>
</table>

The last species is distinguished from the others on the basis of conidia size (i.e. 20-30 um x 1.6 um).
Table 11 COMPARISON OF S. PISTACINA ALLESCHER AND S. PISTACIARUM CARACCIOLI

<table>
<thead>
<tr>
<th></th>
<th>S. pistacina</th>
<th>S. pistaciurum</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYMPTOMS:</td>
<td>Dark dots (pycnidia) appearing scattered over a rather large (1-2 cm) area of the leaves not forming definite spots from the beginning.</td>
<td>Numerous, small (2.5 mm), angular, brown, necrotic spots on the leaves.</td>
</tr>
<tr>
<td>Pycnidia :</td>
<td>Globose-depressed to irregularly globose with irregular, large, up to 70 µm, ostiole</td>
<td>Irregularly globose with typical ostiole up to 40 µm</td>
</tr>
<tr>
<td>Size:</td>
<td>96-160 x 160-240 µm</td>
<td>138-218 x 128-192 µm</td>
</tr>
<tr>
<td>Pycnidiospores:</td>
<td>Shape and septation; curved, almost falcate with 1 septum always.</td>
<td>Irregularly curved with 2 to 5 septa.</td>
</tr>
<tr>
<td>Size:</td>
<td>31.8-47.7 x 3.6-4.8 µm</td>
<td>47.7-84.3 (97) x 2.2-4.3 µm</td>
</tr>
<tr>
<td>Spermogonia:</td>
<td>Size: 80-118 x 77-106 µm</td>
<td>96-150 x 90-122 µm</td>
</tr>
<tr>
<td>SpERMATIA:</td>
<td>Size: 3.9-5.6 x 1.0 x 1.4 µm</td>
<td>2.8-4.9 x 1.0-1.4 µm</td>
</tr>
<tr>
<td>PerITHECIUM:</td>
<td>Size: 90-112 x 80-106 µm</td>
<td>96-128 x 86-118 µm</td>
</tr>
<tr>
<td>ASCI:</td>
<td>Number per perithecum: About 20.</td>
<td>50-60.</td>
</tr>
<tr>
<td>Size:</td>
<td>44.8-54.4 x 12.8-14.4</td>
<td>47.7-60.4 x 8.0-11.9 µm</td>
</tr>
<tr>
<td>ASCOSPORES:</td>
<td>Size: 26.2-40 x 3.2-4.8 µm</td>
<td>18.3-30.2 x 3.3-4.8 µm</td>
</tr>
<tr>
<td>CULTURAL CHARACTERS:</td>
<td>Colony: black, stromatic, forming numerous spermogonia but no pycnidia.</td>
<td>Colony reddish, not stromatic, forming numerous pycnidia but no spermogonia.</td>
</tr>
<tr>
<td>INCUBATION PERIOD:</td>
<td>30 days</td>
<td>10 days</td>
</tr>
</tbody>
</table>

ex: Chitzandis (1956)
Infection is presumably due to germination of the conidia and entry either directly through the host cuticle or by stomatal penetration.

The formation of a germ-tube from a spore normally involves three basic phases: (i) the activation of the spore from a resting level of metabolism to a high enough level to enable the synthesis of cellular components to begin (Van Etten et al 1983). This process for most conidial fungi requires the presence of free available water (Van Etten et al 1983). Associated with the water requirement may be a requirement for an exogenous supply of nutrients though many fungal pathogens do not show such an exogenous dormancy. (ii) The initiation of a germ-tube involves the formation of a hyphal-building apex with the local accumulation of vesicles (Grove 1978). The insertion of such an apex into a spore usually follows an initial phase of volume increase by swelling and growth and the subsequent insertion at an appropriate locus. This locus should be predictable and a pattern of development, following a predetermined sequence, should occur. The pattern will be genotypically governed and will be species specific, though modifications of such patterns may occur due to nutrient shifts or temperature differences. The formation of the hyphal building apex must involve the establishment of polarity (Trinci & Banbury 1967). This must develop de novo within the swollen spore or be related to an already pre-existing polarity. Such a polarity is potentially inherent in a spore which has a base and an apex, though this type of polarity may be lost during the process of swelling (Elwy 1980).
(iii) The growth of the first germ-tube and the insertion of subsequent germ-tubes should also follow a predictable pattern (Trinci 1979). During the increase in size of a fungal spore by swelling and subsequent growth the number of nuclei in the swollen spore and germ-tubes is related to a volume of cytoplasm i.e. a nucleocytoplasmic ratio is established. The relationship is constant throughout the growth or duplication cycle and again is species specific. Division of the nuclei is often synchronous and septum formation partitions the cytoplasm into equal portions (Trinci 1979). The pattern of germ-tubes and hyphal branches has been described in terms of a hyphal growth unit (HGU). This is the relationship between the number of branches or hyphal tips and the total length of the mycelium. During early growth of the germ-tube the rate of increase is exponential but later becomes arithmetic. Subsequent hyphal branching however maintains the exponential growth relationship when expressed as a function of total mycelial length (Trinci 1974, Prosser 1983).

Penetration of the host by a germ-tube may be via an intact surface or through a natural opening e.g. stomatal aperture. Entry either through the cuticle or stomatal opening may involve the formation of an appressorium with the subsequent development of an infection peg (Aist 1981). Direct penetration of stomatal aperture may also occur without the development of an appressorium. In some pathogens e.g. Cercospora stomatal penetration is by germ-tube entry during periods when the stomata are
open and by appressorium formation when the stomata are closed (Rathaiah 1976). Similar responses may be influenced by chemicals leached from the plant or by other microorganisms on the plant surface (Preece & Dickinson 1971).

Necrotrophic pathogens are often facultative parasites (Lewis 1973) and can be grown on standard laboratory media. The investigation of germination and pattern production can therefore be examined in vivo and in vitro.

The invasion of host tissues by necrotrophic fungal pathogens will lead to either inter and/or intra cellular hyphal growth. Intercellular hyphal growth is characteristic of biotrophic pathogens (Lewis 1973) e.g. rusts and downy mildew fungi and intracellular haustoria are typically produced by these organisms. Some necrotrophic pathogens grow intercellularly e.g. Cladosporium fulvum, Tomato leaf blight (Lazarovits & Higgins 1976) though several others have been shown to ramify extensively through host cell walls. Such ramification is associated with the production of large amounts of polysaccharase enzymes which degrade the pectin, cellulose and hemicellulose components of the cell wall (Aist 1981).

The initial host tissue response to invasion by fungal pathogens is governed by whether the pathogen is biotrophic or necrotrophic. In the former photosynthesis is slowly diminished but CO₂ fixation continues at nearly the same rate. In both biotrophic and necrotrophic diseases an increase in host metabolic rate is characteristic.
This rise is associated in necrotrophic disease with the onset of necrosis but in biotrophic disease may provide a more favourable environment for growth (Daly 1976).

The universal final response to infection is the lysis and breakdown of host cellular organelles, cells, or tissues (Aist 1981). The breakdown may be uniform within a lesion or may be localised even to the extent of adjacent host cells showing one with lysis and organelle disruption and the other with an apparently undisturbed cellular organisation (Lazarovits & Higgins 1976).

Apart from the gross symptomology, distribution and effect on yield the Septoria leaf spots of Pistachio have not been investigated. There are no published accounts of the processes of spore germination, host penetration and host response to infection.

The aim of this work is to compare the two leaf-spots of Pistachio caused by S. pistacearum and S. pistacia of the behaviour of the spore during germination and the host-pathogen interaction.
MATERIAL AND METHODS

2.1. Host Plant, Pistacia Vera

Fruits of Pistachio, variety Badami, were kindly supplied by Mr. A. Shahrokhi from Iran (Rafsanjan Province). They were stored at 10°C, in a coldroom in the botany department of Royal Holloway College, until required.

The fruit has a fleshy outer layer (husk) and an extremely hard bipartite endocarp (shell) enclosing the seed.

To effect germination the fruit has to be soaked in water for up to 48 hours. This soaking leads to softening of the layer cementing the two halves of the shell, permitting their separation, which in turn allows the embryo to grow and develop. (Plate 1,a).

In practice the husks were removed from the fruits before soaking them in water containing benlate (0.6g/litre), the fungicide was present to prevent fungal infection. After 48 hrs soaking, the fruits were removed from the benlate solution and kept moist in muslin sacks to allow them to germinate. The temperature was between 20 - 22°C throughout the germination period. The seedlings were inspected daily and any diseased seeds or seedlings were removed. When the radicle reached approximately 2 cm, each seedling was planted singly, either in pots containing Levington's seedling compost or in conical flasks containing vermiculite. The planted seedlings were now kept at a
plate 1

_Pistacia vera_

a) germinating seeds (x1)

b) young seedling (x1/3)
temperature of 18 - 21°C. When the 2 to 4 leaf stage was reached, usually in 2-3 weeks after planting, the plants were transferred to a greenhouse, where the temperature was 27 ± 2°C. To have a regular supply of young plants 10 seedlings were planted every month. (Plate 1,b).

2.2. Pathogens

(i) Stock Material.

(a) Septoria pistaciarum

A freeze dried culture was obtained from the American type culture collection, No. ATCC 22201, October 1970.

To maintain a supply of spores, the organism was subcultured on oat agar every 2 weeks and kept at 25°C. Reserve cultures were kept on silica gel (Perkins 1962).

(b) Septoria pistacina

Leaves of P. vera, infected with P. pistacina were kindly supplied by Prof. Dr. Kosker (University of Ankara, Turkey). The leaves were kept clean and dry in the dark at room temperature.

(ii) Preparation of Spore Suspensions.

(a) Septoria pistaciarum

5 - 7 day old cultures were flooded with sterile distilled water (containing 0.01% tween 80, to aid dispersal of the spores). The spore suspension obtained was transferred to a sterile vial.
(b) *Septoria pistacina*

Infected leaves, containing pycnidia, were washed with sterile distilled water and placed in a sterile petri dish containing moist tissue and a small amount of surplus water. After 12 hours spores exuded as spore tendrils from the pycnidia. They were collected by means of a wire loop and transferred to sterile distilled water in a sterile vial.

With both fungi, the concentration of spores in a suspension was counted using a haemocytometer. When washed spores were required the suspensions were centrifuged, resuspended in sterile distilled water and recentrifuged. The number of resuspensions in sterile distilled water varied depending on the amount of washing required.

2.3. **Media.**

All sterile media were autoclaved for 15 minutes at 121°C and 15 lbs/sq.inch; the pH was adjusted to 5.7 - 6.0, with NaOH or HCl, before autoclaving. Agar media were solidified by adding 15-20g agar/litre.

(i) **Natural Media.**

(a) **Oatmeal agar (OA)**

40g oatmeal/litre.

(b) **Potato dextrose agar (PDA)**

potato 200g

<table>
<thead>
<tr>
<th>dextrose</th>
<th>20g</th>
<th>per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{KH}_2\text{PO}_4 )</td>
<td>1g</td>
<td></td>
</tr>
</tbody>
</table>
(c) **Carrot agar** *(CTA)*

- Carrot 200g/litre

(d) **Oatmeal extract** *(OE)*

- Oatmeal 40g/litre

(ii) Synthetic Media.

(a) **Basic Standard Synthetic Medium** *(SS)*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>0.2g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.0g</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>0.5g</td>
</tr>
<tr>
<td>Asparagine</td>
<td>1.0g/litre</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0g</td>
</tr>
<tr>
<td>Trace element solution</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

**Trace Elements Solution**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_3$BO$_3$</td>
<td>2.86g</td>
</tr>
<tr>
<td>ZNSO$_4$ 7H$_2$O</td>
<td>0.22g</td>
</tr>
<tr>
<td>CuSO$_4$</td>
<td>0.08g</td>
</tr>
<tr>
<td>MnSO$_4$ 4H$_2$O</td>
<td>1.81g</td>
</tr>
</tbody>
</table>

The SS medium was used both as a liquid and an agar medium.

The following modifications were made to SS medium in experiments investigating spore germination:

(b) **SS + Vitamin**

- Yeast extract 0.5g/litre added.

(c) **SS + Casein**

- Casein 0.5g/litre added.
- Asparagine reduced to 0.5g/litre.
(d) **SS + Yeast + Casein**  
Casein 0.5g/litre added.  
Yeast 0.5g/litre added.  
Asparagine reduced to 0.5g/litre.

(e) **SS + A** (Where A = a specific amino acid eg. proline, leucine, and so on).  
Specific amino acid 0.5g/litre added.  
Asparagine reduced to 0.5g/litre.

(f) **SS - P**  
$\text{NaH}_2\text{PO}_4$ replaced by $\text{NaCl}$ 0.2g/litre.

(g) **SS - N**  
No asparagine.

(h) **SS - C**  
No glucose.  
No asparagine.  
$\text{KNO}_3$ 1g/litre added.

2.4. **Microscopy**

(A) **Light Microscopy**

Epidermal strips, sections and cleared whole specimens of healthy and diseased leaves of the host plant were used either fresh or fixed and stained.  
Ungerminated and germinated spores were mounted in water or in various stains.

(i) **Fixative and Clearing Agents.**

(a) Chlortal hydrate.  
Saturated solution, 5g in 2 ml water (McBryde 1936).
(b) F.A.A.

Formalin 13 ml
Acetic acid 5 ml
(glacial)
Alcohol (ethanol) 200 ml
(50%)

(c) Ethanol 70%.

(i) Stains

(a) Cotton Blue.

0.05 - 1% in Lactophenol.

This was the most frequently used stain for both fungal spores and fungi in infected host tissue. Material to be stained was usually fresh and was immersed directly in the stain.

(b) Acid Fuchsin.

4% in 70% ethanol.

(c) Toluidine Blue.

0.05% in 70% ethanol.

Stains (b) and (c) were used to stain fungi in host tissue.

(d) Safranin - aniline blue.

i) Safranin 1% in water

ii) aniline blue 1% in ethanol.

(e) Malachite green - acid fuchsin - martius yellow.

i) Pianez's

(malachite green 1% in water 50 ml
acid fuchsin 1% in water 10 ml
martius yellow 1% in water 1 ml
(distilled water 89 ml)
(95% ethanol 50 ml)

ii) Acetic acid (glacial) 1 ml
Alcohol 1% in water 99 ml.

(f) Safranin - light green in clove oil.
   i) 1% light green in clove oil.
   ii) 1% Safranin in water.

Stains (d) to (f) were used to stain section of wax embedded material, the staining procedures used were taken from Johansen (1940).

(g) Photine HV. (Hickson & Welch Ltd).

This stain was used to stain fungal spores. The spores were mounted directly in the stain and examined with fluorescent microscopy.

(h) Giemsa.

5% in phosphate buffer pH = 7. This stain was used to stain fungal nuclei.

(iii) Techniques

Epidermal strips were prepared as described by Sharp & Emge (1958). The strips were stained with either cotton blue or toluidine blue (Ghemawat 1977).

Whole leaves were cleared in chloral hydrate and stained with acid fuchsin (McBryde 1936).

Sections of leaf material were cut, either by hand with a razor or by microtome. Microtomes used were:
(a) freezing microtome - for fresh material sections were cut 10μm thick.

(b) rotary microtome - for wax embedded material sections were cut 5μm thick.

(c) electronmicroscope microtome for material prepared as for transmission electron microscopy but cut much thicker, 1-2 μm thick.

Sections were usually stained before examination.

Fungal nuclei were stained with Giemsa as follows:
Spores, either in suspension or on celophane, were washed in water and transferred to 5 ml of a 3:1 ethanol: acetic acid mixture and left for 30 mins when the fixative was removed. Normal HCl at 60°C was then added and the temperature kept at 60°C in a water bath. After 12 minutes ice was added, to cool the acid quickly, the acid was removed and the spores were washed twice in distilled water, followed by M/200 phosphate buffer pH 6.8-7.0. Giemsa was then added to the spores and left for 1½ hrs. The spores were then ready for examination to see their nuclei. They were mounted in the stain.

(B) Scanning electron microscopy (SEM)

(i) Preparation of material.

Host leaves were cut into pieces (5-10mm²) and washed in distilled water.

Spores were washed with 0.05% tween 80.
(ii) Technique for fixing and coating.

Note: - the buffer used was 0.1M sodium cacodylate pH 7.2-7.4. The temperature was room temperature (18°C - 20°C) unless otherwise specified.

The specimens, host tissue and fungal spores, were treated as follows:

1. Fixed in (a) or (b) for the appropriate time.
   a. Osm  (1% in buffer) for 2 hrs.
   b. Glutaraldehyde (4% in buffer) for 2-3 hrs at 4°C.

2. Washed in buffer for 15 mins.

3. Dehydrated in an acetone series, 30%; 50%; 70%; 80%; 90%; 15 mins in each, 100% two changes 30 mins each.

4. Transferred to Polaron E 3000 CPD and critically point dried at 36°C, at 1200 psi (84 Kg/sq cm).

5. Mounted, appropriately orientated, with a thin coat of adhesive, on aluminium rivets. (Spores were mounted on a No.2 coverslip before mounting on the aluminium rivets).

6. Coated with a standard (48mm) coating of gold paladium, by transferring them to a Polaron E 5100 series II 'Cool' sputter coater for 2 mins at an accelerating voltage of 2.5 KV and a current of 20m A. The specimens were now ready for examination.

(iii) Technique for freeze fracturing.

Goldsworthy (1979) technique was used. Dehydrated specimens from step (3) above were immersed in liquid
nitrogen and then rapidly fractured with a sharp razor blade, which had been pre-cooled in liquid nitrogen, before being transferred to a Polaron E for critical point drying as in Step (4). The procedure continued from Step (4) as previously described.

(C) Transmission electron microscopy (TEM)

(i) Preparation of materials.

Leaves were cut into pieces (1-2.5 mm²) and washed several times in distilled water.

Spores were washed with 0.05% tween 80 for 1 hr and then concentrated by filtration using Hamilton-type syringe. At some stage in their preparation for TEM spores have to be embedded in agar blocks to facilitate their manipulation in the resin in which they are sectioned. This step, based on the method of Kellenburgh et al. (1958) was usually carried out prior to fixation, but occasionally spores were fixed first (for fixation see later). The spores were embedded in agar as follows:

Washed spores were transferred to molten 2% water agar. The agar was then poured as a thin layer on a slide and allowed to set; 1 mm³ blocks were then cut out.

(ii) Technique for fixing, embedding in resin and sectioning.

Note: the buffer used was 0.1M sodium cacodylate (pH 7.2-7.4). The temperature was room temperature (18°C - 22°C) unless otherwise specified.
The specimens, leaf pieces, or agar embedded spores (or stores) were treated as follows:

1. Fixed in (a), (b) or (c) for appropriate time(s).
   - (a) Glutaraldehyde (4% in buffer) for 12-18 hrs.
   - (b) Glutaraldehyde (3-6% in buffer) under vacuum of 15 lbs/sq.inch, for 1-2 hrs, then washed in buffer and transferred to osmium (1-2% in buffer) for 1-2 hrs.
   - (c) Potassium permanganate (2-3% in buffer) for 2 hrs.

2. Washed in buffer 3 times. 10 mins each.

3. Dehydrated in an ethanol series 30%; 50%; 70%; 90%, 15 mins in each, 100% 2 changes, 30 mins in each.

4. Transferred to a 50:50 mixture of propylene oxide and ethanol for 30 mins, then to 100% propylene oxide for 30 mins.

5. Transferred to a 50:50 mixture of propylene oxide and Spurr's resin and left overnight, the first 2 hrs under vacuum of 15 lbs/sq.inch. The container was left unstoppered to allow the solvent to evaporate.

6. Transferred to 3 changes of resin over 48 hrs.

7. Transferred to a small plate containing resin which had been left in the oven for the resin to harden, fresh resin was added to cover the specimens and the plate transferred to a pre-heated oven and left for 6 hrs at 45°C followed by 36 hrs at 60°C. The embedded specimens were now hard enough to be sectioned.
(8) The blocks of resin were trimmed, transferred to an LKB ultratome III microtome and sectioned 50 nm thick or 500 Å.

(9) Sections transferred to small grids and are now ready for staining and examination.

(iii) Stain and Staining Procedure.

(1) Uranyl acetate 0.05% in water.

This stain was used after Step 2 in the fixing, embedding procedure. The specimens were left in stain for 2 hours before dehydration.

(2) Ruthenium Red 0.01% in water.

This stain was used for spores, the spores were stained for 15 min before they were embedded in the agar block. The stain performed two purposes, it stained the spores and also enabled them to be easily located in the agar block.

(3) Double Stain 10% uranyl acetate in 50% methanol followed by Reynold's lead citrate.

Double staining is a standard staining for sections on grids.

2.5 Spore Germination.

(A) The course of germination in liquid media was studied in two ways:

(i) A spore suspension was washed and 10^6 spores added to 250 ml conical flasks each containing 50 ml of the appropriate medium. The flasks were incubated
at 25°C on an orbital shaker at 180 rpm. Samples of spores were removed at intervals with a wire loop, and examined under the light microscope. The state of the spores, and their percent germination was recorded. If details of cell walls and nuclei were required the spores would be mounted in an appropriate stain.

(ii) Coulter Counter.

A series of flasks were prepared and incubated as in (i). At intervals single flasks were removed and their contents diluted with 0.5% NaCl solution to give a spore concentration of $4.6 \times 10^4$ ml. Before dilution the % germination was determined as in (i). 300 ml of the diluted samples were assessed using a coulter counter type ZB (Kubitschek 1969).

(B) The course of germination on solid media was also investigated in two ways:

(i) Cellophane discs (2 cm diameter) were sterilised by autoclaving and placed on the surface of the appropriate agar media in petri dishes. The cellophane was then inoculated with a drop of washed spore suspension. At intervals pieces of the inoculated cellophane were removed and examined. The state of the spores and their nuclei and the percent germination were recorded as already described.
(ii) Micro manipulation technique.

To follow the germination of individual spores, single spores were isolated using micromanipulation. The method used was adapted from that of Dickinson (1929) which has been described as "Isolation by micromanipulation below the air gel interface" and Johnstone (1969). The moist chamber of Dixon (1958) was used.

The chamber was prepared as follows: two thin strips of glass were cut from a No.2 coverslip, sterilised by flaming in alcohol, and placed on the surface of an entire sterile No.2 coverslip. A second sterile No.2 coverslip was then placed on top of the strips. The edges of the 2 coverslips on either side of the glass slips were precisely lined up. The other 2 edges were overlapped, so that the pair of coverslips could be lifted with forceps by holding on to the bottom one only. The coverslips were transferred to a warm aluminium plate, kept warm by a very low bunsen flame. A few drops of appropriate molten agar were then run in between the coverslips. The coverslips were carefully removed from the warm plate, the agar allowed to set. When the agar had set, the two coverslips were gently separated so that the agar remained attached to one of them. The coverslip containing the agar was placed on a sterilised U shaped plastic chamber (previously cleaned and sterilised by wiping over with acetone). A second coverslip was placed next to the first, to close the top of the chamber. The
Fig 2  **Surface view of apparatus for single spore isolates**

View in section

1. **Inoculation of host.**

To study host-pathogen interaction leaves were inoculated with spore suspensions of the pathogen. The age of leaves was found to be very important in determining...
base of the chamber was formed by a glass slide which could be drawn out to open the chamber from below. The top of the coverslip carrying the agar was marked with rows of small dots using Indian ink.

Spores were introduced into the chamber by drawing back the bottom slide, sufficiently far to expose the agar surface. A small drop of spores was placed at the edge of the agar surface and then using the micromanipulator equipped with a glass needle and x 400 magnification, individual spores were transferred from the edge to the positions on the agar marked by the Indian ink spots on the coverslip. When the required number of spores had been isolated the chamber was closed by pushing back the basal slide. The whole apparatus was placed in a sterile Petri dish and incubated at 25°C. At intervals the spores were examined under the microscope and their behaviour during the course of germination was recorded and some of the stages were photographed.

When studying spore germination in this way it is very important to ensure that the agar layer is firm, has a smooth surface and is clear.

2.6. Host-Pathogen interaction

1. Inoculation of host.

To study host pathogen interaction leaves were inoculated with spore suspensions of the pathogen. The age of leaves was found to be very important in determining
whether the pathogen became established. It was therefore necessary to study the growth and development of host leaves.

i) Development of Leaves
   a) Plastochron Index.

   10 potted seedlings were grown in a Weyco climatic condition cabinet (25°C arid).

   As the leaves expanded they were measured at 5 days intervals (using a mm ruler). Leaf number and measurement of the first 16 leaves of all 10 plants were recorded. The experiment was carried out from November to January.

   b) Scanning electron microscopy.

   Young plants with 12 leaves were taken. The leaves were labelled 1-12, with 1 being the oldest leaf. The length of each leaf was measured and recorded. One leaf from each pair was subsequently prepared for scanning with E.M. The development of the plant up to the 12 leaf stage had been previously recorded therefore the age of each leaf was known.

ii) Inoculation

   Two methods were used for inoculating the host plant:

   a) Attached leaves. Plants, either in pots or in flasks, were washed thoroughly with tap water and inoculated with a spore suspension. The suspension
was usually in water, but occasionally in 0.05% gelatin. The suspension was applied either as a drop, which was spread over the surface by hand, or as a spray using a spray gun. The inoculated plant was either completely covered by a polythene bag, which was removed after 48 hours, or the inoculated leaves were each surrounded by a plastic Petri dish containing a small amount of water. The Petri dish was left in position until infection was apparent. After 48 hours the plants were transferred to a bench glass cabinet with a temperature of 25 ± 2°C and a R.H. of 80-100. (Plate 2 a,b).

b) Detached leaves. The leaves were removed from the plant and washed thoroughly with tap water and placed in petridishes containing moist tissue. They were then inoculated as above with a spore suspension. The petridishes were kept at 25 ± 2°C.
Plate 2

Pistacia vera

a) inoculation chamber for inoculation of selected leaves of seedlings grown in pots (x 1/3).

b) inoculation of whole plant in vermiculite culture (x 1/2).
2.7. Measurements of Spore Germination and Hyphal Growth Kinetics

Spores were isolated from 5 day old culture onto SSM using the micromanipulation chamber. The chamber was used as a growth chamber and incubated at 25°C. The isolated spores and subsequent germlings were either, drawn with a camera lucida, or photographed using a Leitz photomicroscope and prints made at a suitable enlargement. Eleven sequential observations were made at intervals over a period of 31 hours incubation.

The total mycelial length was measured from either the drawings or photographic print and converted to μm. The numbers of hyphal tips were counted and recorded as tips/mycelium.

The H.G.U. was estimated from these two values:

\[
\text{H.G.U.} = \frac{\text{length of hyphae (μm)}}{\text{no. of hyphal tips}}
\]

2. Statistical methods (Zar, 1974)

1. mean $\bar{x} = \frac{\Sigma x}{n}$ or $\frac{\Sigma fx}{\Sigma f}$

2. Sum of squares $SS = \frac{E x^2 - (\Sigma x)^2}{n}$ or $\frac{\Sigma f x^2 - (\Sigma f x)^2}{n}$

3. Variance $S_x^2 = \frac{SS}{n-1}$

4. Standard Deviation $S_x = \sqrt{S_x^2}$

5. Standard Error $S_{\bar{x}} = \frac{S_x}{\sqrt{n}}$

6. 95% Confidence Limits $= \bar{x} + t \cdot S_{\bar{x}}$ $\Rightarrow 2\alpha = 0.05$, $n-1$

7. Comparison of means: $H_0 : \mu_1 = \mu_2$

$$t_{2\alpha, n_1 + n_2 - 2} = \frac{|\bar{x}_1 - \bar{x}_2|}{S_d}$$

$$S_d = \sqrt{S_1^2 + S_2^2 \over n_1 + n_2}$$

8. Product-moment correlation coefficient $r$

$$r = \frac{SP_{xy}}{\sqrt{SS_x \cdot SS_y}}$$

$$SP_{xy} = \frac{\Sigma xy - (\Sigma x)(\Sigma y)}{n}$$
9. Significance of $r$

**ANOVA**

<table>
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<th>Source</th>
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<th>SS</th>
<th>MS</th>
<th>$\alpha F$, n-2</th>
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</thead>
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<td>$r^{2SSy}$</td>
<td>CMS/RMS</td>
</tr>
<tr>
<td>Residual (R)</td>
<td>n-2</td>
<td>$(1-r^{2})SSy$</td>
<td>RSS/n-2</td>
<td></td>
</tr>
</tbody>
</table>

This result is in contrast to the use of the method for scoring herbaceous plants, e.g., tomato where the plastochron intervals and the plastochron index (PI) can be used as a substitute for a time reference base, at least for the first few leaves. A plot of the plastochron index in tomato is linear up to leaf 9 but becomes non-linear as soon as flowering is initiated (Coleman & Crayson 1976).
3.1. **Plastochron interval** (Erickson & Michelini 1957)

In an attempt to establish reference points related to plant development rather than to actual age in days, the plastochron intervals between successive leaves were estimated for a set of seedlings. There were practical constraints on measuring young leaves. The leaves were tightly rolled and did not open until >1.2 cms long. All measurements were made on unrolled leaves. The data represent the mean values for ten plants and are shown in Fig 3. The plot of log leaf length versus time showed that all leaves grew exponentially for the first 10-15 days after unrolling, but the growth rate slowly declined until maximum size was reached. The graph reveals that the first formed leaves, 1-8, form a cluster with no convenient measure to separate them. Later leaves, >8, show uneven plastochron intervals and no convenient reference length could be determined from which to estimate the plastochron intervals.

This result is in contrast to the use of the method for seedling herbaceous plants, e.g. tomato where the plastochron intervals and the plastochron index (Pl) can be used as a substitute for a time reference base, at least for the first 8-9 leaves. A plot of the plastochron index in tomato is linear up to leaf 9 but becomes non-linear as soon as flowering is initiated (Coleman & Greyson 1976).
The plastochron method failed to provide a convenient or reliable system for relating Pistachio leaf development to a PI value. The technique was discontinued and developmental periods of both host and pathogen are given in days with either a planting date or an infection date as the origin. Reference to leaf position in subsequent experiments is always related to the youngest available leaf which is identified as Leaf 1 and all older leaves are numbered in sequence downwards from the apex.
Fig 3 The plot of log leaf length versus time.

Time


mean log leaf length (mm)

Leaf no.

1, 2

3

4

5, 6

7, 8, 9

10

11

12

13

14

(in order to avoid confusion some symbols are open and the lines pecked).
3.2. **Stomatal maturation**

The maturation of the leaf of *P. vera* was only investigated in terms of stomatal maturation. Three leaves, leaf 1, leaf 4 and leaf 8 in sequence below the apex of a seedling grown in full daylight were cut off and immediately prepared for S.E.M. The youngest leaf (1) had no open stomata, leaves 4 and 8 had fully open stomata. Leaf 1 was 1.9 cm long and 4 days had elapsed with reference to the time of leaf unfolding. The stomata on the abaxial surface were fully differentiated from the epidermal cells and had raised prominent guard cells. The aperture was closed and appeared to be occluded. The cuticular rim was not fully differentiated (Plate 3). In leaves 4 and 8 the stomata were fully mature with prominent cuticular rims surrounding the aperture (Plates 4, 5).
Plate 3

SEM

Stomatal maturation of uninfected leaves of *Pistacia vera*. distribution and aperture structure of Leaf 1 (4 days old) of a seedling with 11 leaves.

a) distribution of stomata (x 490).
b) enlarged view of a (x 990).
c) aperture structure of stomata (x 1400).
d) aperture structure of stomata (x 3400).
Plate 4

SEM

stomatal maturation of uninfected leaves of *Pistacia vera.*

distribution and aperture structure of Leaf 4 (24 days old) of a seedling with 11 leaves.

a) distribution of stomata (x 520).
b) enlarged view of a (x 1151).
c) aperture structure of stomata (x 2735).
d) aperture structure of stomata (x 5470).
Plate 5

SEM

stomatal maturation of uninfected leaves of *Pistacia vera*.

distribution and aperture structure of Leaf 8 (45 days old) of a seedling with 11 leaves.

a) distribution of stomata (x 500).
b) enlarged view of a (x 1192).
c) aperture structure of stomata (x 4167).
d) aperture structure of stomata (x 3607).
4. **Result**

4.1 **Growth in Culture**

*S. Pistacia*rum developed on all four media, OA, PDA, CTA, SS, but the most growth occurred on oat agar. After 5-7 days on oat agar pycnidia were formed and conidial masses were extruded shown in plate 6.

A mycelium was formed on all media but pycnidial production was not observed on SS agar, pigment production on oat was visible after 2 days, the colour was initially pink but turned to dark brown after 3-4 days. In PD agar the pigment was dark brown to black in colour.

Comparison of the growth on four different media is shown in Table 12.

The optimum temperature 22°-26°c, and pH between 5.7-6.8 were checked as being appropriate for growth of the fungus in these studies.

4.2 **Morphology of Conidia Produced in Culture.**

The conidia of *S. Pistacia*rum grown on OA were multicelled, hyaline, curved and septate. The number of cells were estimated (Table 13(C) Fig 4) and ranged from 3-11 with a mode of 6 cells (5 septa).

The overall spore lengths are shown in Table 13(b) Fig 5 with a mode of 60-70μm. The lengths of the individual cells was measured using a sample of 6 celled conidia i.e. the modal cell number.
The proximal cell with the flat basal attachment scar was identified as cell no.1 and the distal terminal cell was number 6. Each cell was uninucleate. The proximal cell was longer than the other five cells (mean = 15.13 μm) which were approximately the same size with mean lengths within the range of 10 - 12.15μm (Table 13(a) Fig 6). The taper of the spore was confined to the proximal and distal cells. The main axis being cylindrical with a diameter of mean = 3.62 μm.
Plate 6

*S. pistaciarum*

5 days old culture on oat at 25°c.

aerial mycelium (Am), Pycnidia with slimy spore masses (sm) (x 40).
Table 12  Growth on four different media.

<table>
<thead>
<tr>
<th>medium</th>
<th>mycelial density</th>
<th>sporulation</th>
<th>observation of culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat Agar</td>
<td>++</td>
<td>+++</td>
<td>pink turned into black</td>
</tr>
<tr>
<td>PD Agar</td>
<td>+++</td>
<td>++</td>
<td>dark brown turned into black</td>
</tr>
<tr>
<td>Carrot Agar</td>
<td>+</td>
<td>+</td>
<td>red to dark brown</td>
</tr>
<tr>
<td>SS Agar</td>
<td>++</td>
<td>-</td>
<td>white turned into black</td>
</tr>
</tbody>
</table>

+++ excellent
++ very good
+ good
- none
Table 13  Spore characteristics.

a) mean length + standard error of individual cells of six celled spores (n=115)

<table>
<thead>
<tr>
<th>Position of cell</th>
<th>mean ± standard error (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (base)</td>
<td>15.13 ± 0.17</td>
</tr>
<tr>
<td>2</td>
<td>12.15 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>10.64 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>10.54 ± 0.19</td>
</tr>
<tr>
<td>5</td>
<td>10.86 ± 0.1</td>
</tr>
<tr>
<td>6 (apex)</td>
<td>12.06 ± 0.17</td>
</tr>
</tbody>
</table>

b) mean overall length + standard error of six celled spore

<table>
<thead>
<tr>
<th>number of spore = n</th>
<th>mean ± standard error (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>75.98 ± 0.75</td>
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</table>

c) mean ± standard error of cell number in spore

<table>
<thead>
<tr>
<th>number of spore=n</th>
<th>mean ± standard error of cell number per spore</th>
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</thead>
<tbody>
<tr>
<td>334</td>
<td>6.03 ± 0.08</td>
</tr>
</tbody>
</table>
Fig 4  Cell number per spore
Frequency (%) histogram.
Fig 5  Spore length
Frequency (\%) histogram.
Fig 6  Individual cell length of 6 celled-spore
Frequency (%) histogram.
4.3. Relationship of spore length and the number of cells.

A graphical analysis of spore length and cell number shows that there is a fixed relationship between the two variables. The data from 100 spores from an oat agar culture (Table 14) shows that for each cell number/spore a small approximately normal distribution of cell length is found (Table 14) i.e. the data shows a bivariate normal distribution. The overall data can be fitted by the regression line

\[ y = 0.81 + 0.074 \times \]

where \( y = \) cell number and \( x = \) spore length.

A sample of 100 spores for a single pycnidium produced on SS agar but adjacent to the original inoculum which came from an oat agar culture (Table 15) showed the same \( v \) value (\( v = 0.7 \)) and a plot of the mean cell number values against cell length (Fig 7) shows the slope of the two lines are approximately the same.

Since the modal number of cells lies between 6 and 7 cell/spore an extended analysis of 6-celled spore was made.
Fig 7  Relationship between spore cell and number of cells for cultures grown on oat and SS agar.

\[
\begin{align*}
\text{oat agar} & : \quad y = 0.81 + 0.074x \\
n & = 100 \\
r & = 0.73^{**} \\
R^2 & = 54\% \\
\text{SS agar} & : \quad y = 0.14 + 0.065x \\
n & = 97 \\
r & = 0.70^{***} \\
R^2 & = 49\%
\end{align*}
\]
Table 14  Spore length and cell number of conidia from oat agar

<table>
<thead>
<tr>
<th>length (um) (x)</th>
<th>cells/spore (y)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>44</td>
<td>2</td>
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<tr>
<td>49.5</td>
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</tr>
<tr>
<td>55</td>
<td>1</td>
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<td>60.5</td>
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<td>66.0</td>
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<td>71.5</td>
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<td>82.5</td>
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</tr>
<tr>
<td>88</td>
<td>0</td>
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<tr>
<td>93.5</td>
<td>2</td>
</tr>
<tr>
<td>99.0</td>
<td>2</td>
</tr>
<tr>
<td>104.5</td>
<td>2</td>
</tr>
<tr>
<td>111.0</td>
<td>1</td>
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</tbody>
</table>

mean spore length = 76.7 ± 2.2 um
mean cell number = 6.12 ± 0.2
\[ y = 0.7335 + 0.074x \]

ANOVA Ho: p=0

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<td>Total</td>
<td>99</td>
<td></td>
<td></td>
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</table>
Table 15  Spore length and cell number of conidia from SS agar

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<th>8</th>
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<td>95.3</td>
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<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>7.8</td>
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</tbody>
</table>

mean spore length = 78.68 ± 1.02 um
mean cell number = 6.59 ± 0.18

r = 0.70
R² = 49%
y = 0.81 + 0.065x.

ANOVA Ho: p=0

<table>
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<tr>
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<th>MS</th>
<th>F</th>
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<td>Total</td>
<td>96</td>
<td></td>
<td></td>
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</table>
4.4. Spore germination on natural and synthetic media.

Spore germination was measured on various semi-solid media and the same media without agar addition. The work of Chitzandis (1957) had shown that oat meal agar was suitable for growth and maturation and this medium was compared with potato dextrose, carrot juice and a synthetic medium (SS) in which the carbon was supplied as glucose and the nitrogen as asparagine.

Spore germination was estimated as the production of a germ-tube; the fate of the germ-tube i.e. whether it became a hyphal initial or a secondary conidium, was not determined. Results are the mean of two determinations of 2-300 spores/sample.

On solid media all the germination curves showed the general ogival form with short lag times of 3-4 hours. The G50 times for the natural media oat, PD and carrot ranged between 7-8 hours (Fig 8).

In liquid media the ogival curve was translated by +14 hours on natural and synthetic medium. In oat and carrot media the G50 was 20-24 hours respectively. The SS curve though not showing the same maximum rate as the natural media supported effective germination with a G50 of approx 29-30 hours. The addition of yeast extract or casein hydrolysate or both to SS showed a fast rate of germination (Fig 9) comparable to the natural media.

The removal of phosphate from the SS medium showed an
increased lag phase on both solid and liquid SS medium. The G50 on semi solid was 21 hrs, in liquid the G50 was reached after an estimated 46 hours (Fig 10). The growth of the spore axis on SS-P was extensive and the long lag phase represented this period of axis growth before lateral germ-tubes were produced.
Fig 8 (8) germination of S. pustulatum in solid media at 25°C.
Fig 9  (%%) germination of *S. pistaciarum* In liquid media at 25°C.
Fig 10 (% ) germination of S. pistachiarum in liquid media at 25°C.

(○) SS + yeast  (□) SS + yeast + casein
(●) SS + casein  (■) SS - p
4.5. Spore germination in synthetic medium with various nitrogen sources.

The successful germination of conidia in SS asparagine and SS + casein hydrolysate was further investigated using SS asparagine as a control and 12 amino-acids as single nitrogen sources. In the first experiment a 5 day old culture was used as a source of inoculum, in the second an old culture (120 days) was used. Values are expressed as the mean of two determinations. Germination was shown in all media (Table 16, Fig 11). The response to the various amino-acids measured against SS asparagine showed that 3 or 4 groups could be recognised. Group A proline and isoleucine matched the enhanced germination in SS + casein. Group B, citrulline, phenylalanine and methionine were equivalent to SS asparagine. Group C histidine lysine threonine glycine tryptophane and hydroxyproline were less efficient than SS asparagine and Group D arginine which showed a long lag phase. The two experiments differed in the rate of germination the older spores showing a uniform increase for all nitrogen sources tested.

The growth pattern shown by histidine was altered with respect to Group A and Group B nitrogen sources and further experiments were set up to explore the differences shown.
Table 16  % germination of conidia of *S. pistaciarum* in synthetic medium with various nitrogen sources.

a

<table>
<thead>
<tr>
<th>medium</th>
<th>Germination time (hrs)</th>
<th>Group</th>
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<td>18</td>
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<tr>
<td>casein</td>
<td>25</td>
<td>35.5</td>
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<tr>
<td>citrulline</td>
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<td>22</td>
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<td>hydroxy proline</td>
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b

<table>
<thead>
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<th>medium</th>
<th>hrs</th>
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<th>21</th>
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<td>3</td>
<td>14</td>
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<td>1</td>
<td>3</td>
<td>14</td>
<td>D</td>
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</tr>
</tbody>
</table>
Fig. 11 S. pistaciarum % germination of conidia in synthetic liquid medium with various nitrogen sources at 25°C.
4.6. Spore Germination on Different Media

The germination of spores was followed on four different media SSM, SS-P, Histidine, Oat using the micro-manipulation chamber (Method 2.5 & ii). A sample of 10 spores from a 6 day old culture was isolated onto each of the four different media and incubated at 25°C, drawn with a camera lucida and photographed using a Leitz photomicroscope. The number of cells and the number of germ tubes were counted (at 0, 22-24, 36-38 hours).

On SSM the main spore axis increased in length, this increase was due to an increase in cell number (5.6-19.06-23.9). The number of lateral germ tubes increased over the last two intervals from (0-9.8-14.9).

On SS-P the main spore axis also increased in length and cell numbers (5.6-16.10-29.90); lateral germ tube production was low and only occurred after 36 hours (0-0.1-2.10).

On Histidine the main axis increased in length, and cell number (5.3-9.10-14.4).

On Oat the main axis increased in length and cell number (6-15.5-10), germ tube production was low (0-1.8-6.3). A comparison of germ tube production showed that in SS-P, Histidine, Oat media germ tube production was lower than in SS (Table 17a,b) Fig 12.

On all media there was an increase in spore axis length (Table 17). That was due to the division of the axis cells. In SSM the number of cells increased from (5.7-23.9) with 36 hours period of incubation.
In contrast the number of cells along the main axis was lower than SS, on Histidine and Oat Agar. The maximum reduction occurring on histidine; the number of cells were reduced by 40% on SS-P, the number of cells were increased by 25% with respect to SS. The number of germ tubes was reduced in comparison to SS. The lowest reduction by 86% was shown by SS-P. Histidine was reduced by 46% and Oat by 58%.

The relationship between the number of cells in the axis and the number of germ tubes produced showed:

\[
\text{% success} = \frac{\text{mean number of germ tubes}}{\text{number of cells per spore}} \times 100
\]

Percentage of success in Histidine was the highest with 90.22% with respect to SS and SS-P was the lowest with 11.29% after 36-38 hours incubation (Table 18c). Fig 12.

A selection of illustrations of the stages of growth, of 3 sets of four spores grown on oat, SS, SS (Histidine) are shown in Plates 7, 8, 9. The mode of axis growth, axis curvature and insertion of germ tubes conform to the values set out in Tables 17, 18.
Table 17: Spore germination data in different media at 25°C

a) Mean ± standard error for values of cells' number (n=10)

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>SS</th>
<th>SS-p</th>
<th>Histidine</th>
<th>Oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.60±0.16</td>
<td>5.6±0.37</td>
<td>5.3±0.3</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>22-24</td>
<td>19.06±0.6</td>
<td>16.1±0.43</td>
<td>9.1±0.64</td>
<td>14.4±0.88</td>
</tr>
<tr>
<td>36-38</td>
<td>23.9±0.53</td>
<td>29.9±1.04</td>
<td>14.4±0.88</td>
<td>19.0±1.02</td>
</tr>
</tbody>
</table>

b) Mean ± standard error for values of germ tubes (n=10)

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>SS</th>
<th>SS-p</th>
<th>Histidine</th>
<th>Oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22-24</td>
<td>9.8±0.96</td>
<td>0.1±0.12</td>
<td>4.6±0.37</td>
<td>1.8±0.47</td>
</tr>
<tr>
<td>36-38</td>
<td>14.9±0.77</td>
<td>2.1±0.25</td>
<td>8.10±0.43</td>
<td>6.3±0.62</td>
</tr>
</tbody>
</table>

c) % of success with the % value of SS as control

<table>
<thead>
<tr>
<th>Time</th>
<th>SS</th>
<th>SS-p</th>
<th>Histidine</th>
<th>Oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-24</td>
<td>100</td>
<td>1.19</td>
<td>98.05</td>
<td>22.50</td>
</tr>
<tr>
<td>36-38</td>
<td>100</td>
<td>11.29</td>
<td>90.22</td>
<td>53.18</td>
</tr>
</tbody>
</table>
(Contd.) Table 18 spore germination data in different media at 25°C

a) % germ tube (% values of SS as the control)

<table>
<thead>
<tr>
<th>Time</th>
<th>SS</th>
<th>SS-p</th>
<th>Histidine</th>
<th>Oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-24</td>
<td>100</td>
<td>1</td>
<td>46.93</td>
<td>18.36</td>
</tr>
<tr>
<td>36-38</td>
<td>100</td>
<td>14.89</td>
<td>54.36</td>
<td>42.28</td>
</tr>
</tbody>
</table>

b) % cell number (% values of SS as the control)

<table>
<thead>
<tr>
<th>Time</th>
<th>SS</th>
<th>SS-p</th>
<th>Histidine</th>
<th>Oat</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-24</td>
<td>100</td>
<td>84</td>
<td>47.74</td>
<td>81.58</td>
</tr>
<tr>
<td>36-38</td>
<td>100</td>
<td>125</td>
<td>60.25</td>
<td>79.49</td>
</tr>
</tbody>
</table>
Fig 12

spore germination on different media at 25°C (S. pistaciarum).

a) number of cells/spore
b) number of germ tubes/spores
c) ratio of percentage success (SSm as control).

- SSm
- SS-p
- Oat
- SS (Histidine)
Plate 7

a-d mode of germination of 4 spores of S. pistaciarum on Oat at 0 hours, 24 hours and 38 hours. (x 550).

Twin germ tubes (TG)

½ Twin germ tubes (G)

Septate hyphae (H. j)
Plate 8

a-d mode of germination of 4 spores of *S. pistaciarum* on SS at 0 hours, 24 hours and 38 hours. (x 430)

Twin germ tubes TG.

½ Twin germ tubes G.

Septate hyphae H.
Plate 9

a-d mode of germination of 4 spores of *S. pistaciaraum* on SS (Histidine) at 0 hours, 24 hours, 38 hours. (x 650) a-0, b-0, c-0, d-0 (x300).

Twin germ tubes TG.

½ Twin germ tubes G.

Septate hyphae H.
4.7. Volume Increase during Germination.

The size and volume increase during germination was determined by using the coulter counter "Industrial Model ZB with coincidence adaptor with the 100 m aperture tube".

Fresh spores were measured as the control and germination was followed in three different media (SSM, SS-P, SS (Histidine). The suspension was made as in method 2, the cultures were grown at 25°C in the orbital shaker. The manometer was filled with a sample which was stirred continuously to prevent the spores settling out and 0.5 ml of the sample was counted immediately to prevent the possibility of shrinkage occurring in the 0.5% NaCl. Two replicates for each treatment were set up. The spore number for each size category (size window) was estimated using a range of volumes (0.05 x 10^{-3} \mu m^3 to 60.00 x 10^{-3} \mu m^3). The number of spores in each volume window were estimated and recorded as a percentage of the total spore number (Table 19). The data were plotted as a log percentage against volume category and samples were taken at four time intervals. The percentage germination was also measured (Table 20). The stages of spore development examined in this experiment were fresh spores (dormant), swollen spores and germinated spores.

SSM: at 0 time sample was taken as the control Fig 13, 14. The volume lay between 0.1-0.8 \mu m^3 x 10^{-3} and the peak volume lay between 0.2-0.4 \mu m^3 x 10^{-3}. After 18 hours the spores started to swell and the volume increased, the peak shifted upward to 0.8-1.6 \mu m^3 x 10^{-3} (swollen spores). After 24 hours
the volume increased and volume range was between 0.4-1.6 μm$^3$ x 10$^{-3}$ and the volume peak has increased to 1.6-3 μm$^3$ x 10$^{-3}$. At this time the 20% has occurred (germinated spores). After 30 hours the percentage germination was greater than 70% and secondary spores were produced; at 42 hours (92% germination) a bimodal distribution of volume size was observed. Volume range was between 0.1-0.8 μm$^3$ x 10$^{-3}$ and a peak at 0.2-0.4 μm$^3$ x 10$^{-3}$ representing secondary spores and a peak at 1.6-3 μm$^3$ x 10$^{-3}$ which represented the swollen and germinated spores. The replicate in this experiment shows approximately the same result. (Fig 13, 14).

SS-P: after 18 hours spore volume increased and the volume ranged between 0.05-3 m$^3$ with a peak volume at 0.8-1.6 μm$^3$ x 10$^{-3}$. After 24 hours volume ranged between 0.4-3 μm$^3$ x 10$^{-3}$ and peak volume was the same as at 18 hours, at this time only 4% germination occurred. After 38 hours the volume range increased 0.4-15 μm$^3$ x 10$^{-3}$ and the volume peak shifted upward to 1.6-3 μm$^3$ x 10$^{-3}$. No indication of secondary spores were found. The percentage germination at this time was 15%. After 42 hours the volume increased and the volume peak shifted upward to 0.8-1.6 μm$^3$ x 10$^{-3}$. (Fig 15, 16).

SS (Histidine): after 18 hours the volume increased and the volume peak shifted upward to 0.8-1.6 μm$^3$ x 10$^{-3}$. After 24 hours (18% germination occurred) the volume increased and ranged between 0.8-5 μm$^3$ x 10$^{-3}$ but the volume peak lay between 0.8-1.6 μm$^3$ x 10$^{-3}$. After 38 hours the peak volume increased to 3-5 μm$^3$ x 10$^{-3}$. At this time percentage germination was greater than 73%. After 42 hours 89% of the spore had germinated.
There was no indication of a bimodal distribution as in SSM after 42 hours, i.e. no secondary spores were being produced. The volume range was between $0.1-10 \, \mu m^3 \times 10^{-3}$ with a peak volume between $3-5 \, \mu m^3 \times 10^{-3}$. The peak volume had increased fractionally over the 38 hour value. (Fig 17, 18).
Table 19 and 20 coulter counter data

Volume distribution of Fresh Spores

<table>
<thead>
<tr>
<th>n</th>
<th>volume $m^3 \times 10^{-3}$</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>19</td>
<td>0.2</td>
</tr>
<tr>
<td>27</td>
<td>1.6</td>
<td>8</td>
<td>0.1</td>
</tr>
<tr>
<td>174</td>
<td>0.8</td>
<td>147</td>
<td>1.8</td>
</tr>
<tr>
<td>3204</td>
<td>0.4</td>
<td>3030</td>
<td>38.48=38</td>
</tr>
<tr>
<td>7130</td>
<td>0.2</td>
<td>3926</td>
<td>49.86=50</td>
</tr>
<tr>
<td>7832</td>
<td>0.1</td>
<td>702</td>
<td>8.9</td>
</tr>
<tr>
<td>7873</td>
<td>0.05</td>
<td>41</td>
<td>0.5</td>
</tr>
</tbody>
</table>

(%) germination of spores in different media at 25°C

<table>
<thead>
<tr>
<th>Time</th>
<th>SS Set 1</th>
<th>SS Set 2</th>
<th>SS-p Set 1</th>
<th>SS-p Set 2</th>
<th>Histidine Set 1</th>
<th>Histidine Set 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>22</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>38</td>
<td>79</td>
<td>80</td>
<td>15</td>
<td>14</td>
<td>69</td>
<td>75</td>
</tr>
<tr>
<td>42</td>
<td>82</td>
<td>89</td>
<td>28</td>
<td>25</td>
<td>80</td>
<td>84</td>
</tr>
</tbody>
</table>
Figs 13, 14, 15, 16, 17, 18 show spore germination and changes in spore volume at time intervals in different media.

Fig 13, 14 SS
Fig 15, 16 SS - p
Fig 17, 18 SS (Histidine).
Fig 13 (SS) Set 1

0 hours

18 hours

24 hours

38 hours

Number of spores (%)

spore volume ($m^3 \times 10^{-3}$)

0.05 0.1 0.2 0.4 0.8 1.6 3 5 7.5
Fig 15 (SS-p)  Set 1

0 hours

18 hours

24 hours

42 hours

Number of spores (%)

spore volume (m$^3 \times 10^{-3}$)
Fig 17 SS (Histidine) 98

0 hours

18 hours

24 hours

38 hours

Volume (μm$^3 \times 10^{-3}$)

number of spores (%)
Fig 18 SS (Histidine)\textsuperscript{99} Set 2

0 hours

18 hours

24 hours

38 hours

Number of spores (x)

spore volume (\textmu{}m$^3$ x 10$^{-3}$)

0.05 0.1 0.2 0.4 0.8 1.6 3 5 7.5 10

In order to present the overall size the spore volume\textsuperscript{1} was calculated and the shown in Fig 20.

The mean spore length at time 0 was 79.2-84.3\textmu{}m and the length increased to 172.9\textmu{}m after 28 hours.

The number of spores showed a step-wise progression. The protective steps increased 28 hours after 28 hours and the number of tips increased in the same step.

We thank the University of Texas for the use of their facilities.

1 The ratio of the total number of spores at each 0.05 indicates the number of spores at each time point.
4.8. Kinetics of Spore Germination

Individual measures of hyphal length, number of tips, HGU and initial time of germ tube formation showed variation in all measures between each spore, depending on the time of germination. In a sample of four individual spores, spore length varied between 76-80 μm. The initial times of germ tube production were between 12-17 hours. After 31 hours hyphal length ranged from 1367-2400 μm. The total number of tips was between 29-46.

A plot of the total hyphal length for the four individual spores shows that the slopes of the lines are approximately parallel and indicates the same specific rate, the position of the line depending on the time of germination. (Fig 19).

In order to present the overall data the mean value for all ten colonies were calculated and are shown in fig 20. and table 21. The H.G.U. varied 30-60 μm, the overall growth of the mycelium became exponential after 22 hours. The mean spore length at time 0 was 79.2-84 μm and increased to 205.40 μm when all ten spores had germinated. The mean total hyphal length increased to 1729.1 μm after 31 hours. The number of tips showed a step-wise progression. Tip production was slow and step wise up to 22 hours and became continuous & exponential after 22 hours. The total hyphal length and the number of tips increased exponentially but the slopes of the two measurements were not parallel.
The initial time of germ tube initiation showed that one spore started to germinate after 10 hours, after 14½ hours 7 of the 10 spores had germinated. All spores had germinated after 18 hours with a G50 of approximately 13 hours.

In order to relate the exact behaviour of each of the 10 hyphal systems to each other, the data for each mycelium was plotted as an individual graph. A standard hyphal length of 200 μm was selected and the graphs were then superimposed by lateral translation to this standard point, all the values were then plotted for hyphal length and number of tips as a master graph (Fig 21). The data showed that hyphal length became exponential after 22 hours and the number of tips also became exponential after 22 hours, also the slopes of the two measurements were not identical.

A selection of illustrations of mycelial growth are shown in Fig 22.
Table 21 Increase of mycelial length and number of hyphal tips and number of germinated spore/10 spores on SS at 25°C

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mycelial length in μm</th>
<th>No of Tips</th>
<th>H G U</th>
<th>No of germinated spore/10 spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>79.20± 2.32</td>
<td>2.00±0.0</td>
<td>39.60±1.16</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>92.60± 2.18</td>
<td>2.00±0.0</td>
<td>46.20±1.13</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>113.40± 3.55</td>
<td>2.00±0.0</td>
<td>54.53±1.97</td>
<td>1</td>
</tr>
<tr>
<td>12.20</td>
<td>129.00± 3.50</td>
<td>2.10±0.10</td>
<td>59.66±2.43</td>
<td>2</td>
</tr>
<tr>
<td>14.35</td>
<td>143.65± 3.94</td>
<td>2.20±1.3</td>
<td>49.16±4.72</td>
<td>7</td>
</tr>
<tr>
<td>17.45</td>
<td>205.40± 15.78</td>
<td>3.20±0.33</td>
<td>42.55±5.22</td>
<td>10</td>
</tr>
<tr>
<td>20.45</td>
<td>339.40± 47.16</td>
<td>5.80±1.04</td>
<td>34.50±2.07</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>479.29± 66.45</td>
<td>10.20±1.41</td>
<td>34.88±2.71</td>
<td>10</td>
</tr>
<tr>
<td>26.30</td>
<td>890.22±124.92</td>
<td>13.90±1.61</td>
<td>40.77±3.96</td>
<td>10</td>
</tr>
<tr>
<td>28.45</td>
<td>1191.80±160.19</td>
<td>21.40±1.93</td>
<td>44.20±4.21</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>1729.10±182.27</td>
<td>34.70±1.96</td>
<td>49.30±3.18</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig 19

growth of total mycelial length of four spores on SS agar medium at 25°C.

Total mycelial length (μm log scale)

Time (hours)
Figure 2: Mean growth of 10 spores on SS agar medium at 25°C.

- (●) mycelial length
- (□) number of hyphal tips
- (○) hyphal growth units
Fig 21

Growth of 10 spores on SS agar medium at 25°C

(○) mycelial length

(●) number of hyphal tips

- A standard point for hyphal length of 200 μm
Fig 22 The mycelial growth of *S. pistaciarum* on SS (20 hours after incubation).
4.9. Pattern of Growth Germination

12 single spores of various sizes i.e. 4-8 cells/spore were isolated onto SS using the micromanipulation chamber. These were incubated at 25°C and the growth followed photographically from 0-32 hours. Six of the spores are shown in Fig 23 a-f. Spores (a), (e) show the growth of eight-celled spores. Nuclear division in the distal and proximal cells did not occur coincidentally with division in the intermediate cells. Increase in spore length occurred by growth in length of all cells. Nuclear division occurred approximately synchronously in the intermediate cells whilst the proximal and distal cells showed no signs of division. Cell septation was asynchronous. Little or no increase in cell length occurred during the next round of asynchronous division leading to a spore axis with seventeen cells, at which time a germ tube was produced. After 28 hours spore (a) and (e) had 17 cells and two germ tubes produced from intermediate cells, the proximal and distal cells appeared to have produced germ tubes. The number of cells after 32 hours was 20 in spore (a) and 17 in spore (e).

Spore (b), a seven-celled spore, shows the nucleus division in proximal and distal cells occurred coincidentally with the intermediate cells and after 28 hours had increased to 14 cells without any germ tubes in either the intermediate cells or proximal and distal cells. After 32 hours the number of cells was 17 and two germ tubes in intermediate cells and two germ tubes in distal and proximal cells.
Spore (c) shows the growth of a 6-celled spore; nuclear division followed the same pattern as the 8th and 7th celled spore but in (c) almost all cells divided at the same time and the number of cells doubled before a germ tube emerged (11 cells). After 32 hours the number of cells was 21 with one intermediate germ tube and a distal germ tube.

Spore (d) shows a 5-celled spore, the nuclear division was not clear because of two missing stages but after 28 hours the number of cells had increased to 12 with one intermediate germ tube. The distal cell was in nuclear division state. After 32 hours the number of cells increased to 20 and three germ tubes from the intermediate cells had been produced.

Spore (f) a 4-celled spore, shows each cell had the nuclear division and after 18 hours the number of cells were 7 and after 28 hours the germ tubes produced from intermediate and from the distal and proximal cells but no nuclear division was observed between 16 and 18 hours. After 24 hours the number of cells increased to 13 and the number of intermediate germ tubes was three, plus two distal, proximal germ tubes.
Germination of 6 conidia of *S. pistaciarum* on ss agar at 25°C over 0-32 hours Incubation.
4.10. Polarity of Spore Germination

A sample of 42 germinated spores was taken from a population grown at 25°C in liquid SSM for 32 hours.

The number of cells/spore and the number of germ tubes/spore were counted and recorded. Each spore was divided into two parts with respect to the point of attachment. A proximal half extending from the flattened basal end and to the midpoint and a distal half extending from mid-point to the apex. The number of cells and the number of germ tubes were counted in each part separately.

Each half spore was divided into two resultant quarters numbered from 1-4 starting at the basal attachment of the spore (Fig 24).

![Diagram of spore with basal and apex](image)

Fig 24  Spore with basal and apex.

The means of all data were calculated and recorded - table22.

The number of cells per spore ranged between 16-28 with the mean of 21.59 and the number of germ tubes between 9-19 with the mean of 13.35. The number of germ tubes in first and fourth quarter part of spore were added together to make the total number of germ tubes in 1+4 and the same in the middle part to make the total of 2+3.
The mean number of germ tubes for 1+4 was 4.95 and for 2+3 was 8.44. The number of cells in both parts was the same with the mean of 5.36.

Means of part 1 and part 4, 1+4 and 2+3 were compared using a t-test. There was no significant difference between the number of germ tubes produced by part 1 and part 4.

A comparison of part 1+4 vs part 2+3 showed a significant difference, a larger number of germ tubes being produced by the central two quarters of the spore (table 22).
4.10a. Spore axis growth on leaves of P. vera.

The observation that axis growth occurred during growth in liquid cultures suggested that if pistachio leaves were excessively wet during the infection period the spores might behave as in liquid culture, i.e. extensive axis growth would occur. To test this plants were inoculated in the usual manner and the disease allowed to progress for thirteen days. The leaves were then washed and the residual spores, which had not effected entry, were examined for cell number/spore. A frequency histogram of the range of cell number is shown in (Fig 26). The cell number/spore in the inoculum ranged from 4-10 with a mode of 6 and a mean of $6.03 \pm 0.08$. The spores washed from the leaves showed a range of 5-17 with a mode of 13.
Table 22 mean ± standard error of cell number and germ-tubes per 4<sup>th</sup> spore and comparison of means by t test.

<table>
<thead>
<tr>
<th>/spore</th>
<th>1+2+3+4 (Total)</th>
<th>1+2, 3+4</th>
<th>1</th>
<th>2+3</th>
<th>4</th>
<th>1+4</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of cell</td>
<td>21.59±0.47</td>
<td>10.72±0.24</td>
<td>5.36±0.12</td>
<td>10.72±0.24</td>
<td>5.36±0.12</td>
<td>10.72±0.24</td>
</tr>
<tr>
<td>no. of germ-tube</td>
<td>13.35±0.42</td>
<td>6.75±0.23</td>
<td>2.73±0.12</td>
<td>8.44±0.29</td>
<td>2.24±0.17</td>
<td>4.95±0.21</td>
</tr>
</tbody>
</table>

1+4 vs 2+3  \( t = 10.05 \)  \( p = 0.001 \)

\( t_{1+4} \)  \( t = 2.3 \)  \( p = 0.05-0.02 \)
Fig 26 Cell number per spore
Frequency (%) histogram.

a) Fresh spores from culture
b) Unerminated spores from leaf surface
(13 days after inoculation).
4.11. Fluorescence studies of growing spores

The brighteners, Photine and Tinopal, gave essentially the same levels and distribution of the fluorescence. Plates 11, 12, 13 a-i show Photine treated sporelings after 18-30 hours in SS shaken culture.

The brightener was taken up by the spore wall and internal cross-walls. The uptake was uneven within and between spores. The brightness increased with time. Sporelings photographed immediately after staining showed that the cross-walls (chitin) fluoresced more strongly than the lateral walls (K) and that a discoidal bright zone appeared where a lateral was being produced (a,b).

As staining time increased cells of the spore axis increased in brightness (a,b,c,d,e,h) and individual cells from which laterals were being produced became uniformly fluorescent. At this stage the apices of the spore axis and the apices of laterals did not fluoresce brightly (a,c,e). In spore c, two cells show brighter lateral tips. Laterals produced from uniformly bright cells also showed uniform brightness. One spore (i) produced a terminal germ-tube and this fluoresced brightly. The pattern of fluorescence shown by the illustrated spore showed single active cells (a,b,c) pairs (a,b,d,e,f,h,j) and continuous regions (d,f,g,j,l).
S. pistaciarum. Spore germination of 12 spores after 18 - 30 hours in SS at 21°c.

(i) Epifluorescence using brightener. Regions of high B. glucon synthesis show high fluorescence.

(ii) Bright-field microsopy. (x 909).
4.12. **Nuclear behaviour during spore germination**
(Plate 14a-h and Plate 15a-i).

The conidium is multiseptate with a single nucleus in each cell (14a). Nuclear division occurs in the central region of a cell of the spore axis and the division figure is longitudinal (14 d,e; 15 c,d,e). No clear details of mitosis were shown by the Giemsa techniques used. The daughter nuclei were partitioned into two daughter cells by a cross-wall. The nucleus was located in the central region of the cell during active axis growth; but was located adjacent to the oldest cross-wall during lateral production (14 g,h; 15 a,b,c,d,e,h). The nucleus divided into the lateral (16 d,e,h). A lateral which formed a germ-tube (14 h) gave rise to a uninucleate septate hypha (14 g,h,i). Hyphal germ-tubes were produced from central axis cells or from the terminal cells (14 h). The first branch of a germ-tube was formed when 4-5 cells had been produced (14 h).
Plate 14  S. pistaciarum

Giemsa staining of nuclear distribution in ungerminated and germinating spores.

a) ungerminated 6-celled spore with a single nucleus in each cell. Basal end in the right hand side. (x 5000).

b)–f) axis growth and nuclear division with uninucleate axis cells. (x 2600).

i)–h) uninucleate mycelium arising from germ-tubes. (x 2800).

H = hypha
N = nucleus
Nd = nuclear division
Sa = spore axis.
4.13. Nuclear Behaviour During Secondary Spore Production. (Fig 25)

During the formation of secondary spores, the division of the nucleus in the conidiogenous cell was not initiated until the secondary conidial initial was well established (a-h). There was no apparent relationship between the length of the secondary conidium and the onset of nuclear division.

<table>
<thead>
<tr>
<th>Fig25</th>
<th>length of secondary conidium (µm)</th>
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<tbody>
<tr>
<td>a-h</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>4.3</td>
</tr>
<tr>
<td>b</td>
<td>9.1</td>
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<tr>
<td>c</td>
<td>10.8</td>
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<tr>
<td>d</td>
<td>13.9</td>
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<tr>
<td>e₁</td>
<td>6.0</td>
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<tr>
<td>e₂</td>
<td>10.4</td>
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<tr>
<td>f</td>
<td>12.2</td>
</tr>
<tr>
<td>g</td>
<td>19.1</td>
</tr>
<tr>
<td>h</td>
<td>19.5</td>
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</table>

Table 23 length (µm) of secondary conidia (data ex Fig 25).

a-h shows secondary conidia of increasing length with conidiogenous nuclei at approximately the same stage of division; e₁ shows a very short secondary conidium 6 µm in length with the daughter conidial nucleus already established in the conidium.

Nuclear division occurred through the isthmus joining the conidiogenous cell and the secondary conidium (f). No examples were seen of division of the nucleus within the conidiogenous cell i.e. no conidiogenous cells were formed with two nuclei prior to the immigration of one of them into
the secondary conidium shows conidia with a single nucleus and shows the process of nuclear division and septation into two cells prior to release from the conidigenous cell. The migration of the daughter nucleus into the conidium never exceeded mid point (Fig hj). In most conidia the subsequent nuclear division occurred at the mid point Fig (1). though in some the nucleus divided whilst in the basal half of the secondary conidium Fig (k). The septation of the secondary conidium typically resulted in a conidium with two equal sized cells (m) but in some the basal cell was the smaller (n).

The nuclear in each cell of the secondary conidium migrated into a central position, Fig (m & n).

Fig 25a-h represents a summary drawing made from the series of photographs in Plate 7, in which the various stages are shown using Giemsa stain.
Fig 25
Cytology (Giemsa) of secondary conidium production.
Plate 15  S. pistaciarum

Giemsa staining of nuclear distribution during secondary spore formation. (x 1600).

a) b) e) production of single and twin-pairs of spores.

c) single spores and initial stage of twin-spore production.
left hand single spore with daughter nucleus migrated into central position
right hand spore without nucleus; the nucleus divided in the conidiogenous cell.

d) nuclear division in two adjacent axis cells.

f) uninucleate secondary spore prior to cross-wall formation.

g) three secondary spores just prior to secession.

h) i) nuclear division and migration into secondary spore.

\[ H = \text{hypha} \]
\[ N = \text{nucleus} \]
\[ Nd = \text{nuclear division} \]
\[ Sa = \text{spore axis} \]
\[ Sc = \text{secondary spore} \]

Plate 16 shows the stages of formation of secondary spores by germlings of *S. pistaciarum* in SS at 25°C in shaken culture. Secondary spores were produced either as twin-pairs (a + b) or as single spores (a + c) from a zone adjacent to the cross walls. Spore secession left a prominent abstriction scar at the locus of spore formation. The production of more than one conidium from a conidiogenous site was not observed but abstriction scars (d) showed that a conidiogenous zone was formed adjacent to a cross wall. The abstriction scar was 3-20μm in diameter and formed a raised collar. The collar was formed from the outer layer of the conidiogenous cell wall. The SEM's show that the cross wall formed in the central region of a cell which was producing conidia at each end was not so rigid as the primary cross wall and the method of preparation produced a series of small longitudinal undulations at the site of the cross wall (b + d).
Plate 16

SEM: secondary spore formation during germination.

a) twin pair (ss) and half-twin modes of spore formation (x 1107).

b) twin pair with cross-wall (cw) (x 5000).

c) spore with half-twin (s) spore formation (x 1106).

d) spore with half-twin secondary spore initiation (SI), half twin spore attachment scars (S) (x 3218).
4.15 **Resting Spore** (Plate 17)

Fixation of spore in this stage was poor either with KMnO₄ or with Glutaraldehyde +

The spore was multicellular, the hyphal wall was uniform in thickness (150 nm) and consisted of three layers: an outer layer (o) which consists of a thin 19 nm electron dense layer and two inner layers I₁ and I₂ with a combined thickness of 100 nm.

In most electron micrographs the resolution of the inner layer into its two compartment layers was not revealed, the interpretation is based on evidence of inner wall layering at the position of septum insertion (PL 20b).

The primary septum was simple with a central perforation. Associated with the septum were electron dense Woronin bodies on either side of the septum.

The fixation of organelles and membranes in the resting spore was also very poor but in some preparations organelle distribution was determined.

Vacuoles were present and they were large with electron dense material within, lipid bodies were present in large numbers, only one nucleus was present in each cell. Numerous ribosomes were observed. ER (Endoplasmic reticulum) was not observed.

**Spore Structure during Germination** (Plates 18-23)

Fixation was improved with the onset of germination, the spore wall was 255 nm in thickness and consisted of two distinct layers 0.1.
An outer layer (0) which was electron dense and was clearly trilaminar; a less dense electron layer demarcated internally and externally by an electron dense margin. Outer layer 0 in some preparations showed apparent discontinuation in electron density but this was not a consistent observation. Plate 20c shows a spore in which the right hand side shows apparent discontinuities while the left hand side shows continuous electron density.

The inner layer (I) was composed of layers $I_1$ and $I_2$. There was a small difference in electron density between the two layers in some preparations with $I_1$ (the outer of the two layers) showing slightly increased electron density. The layering into two distinct zones was revealed clearly at the junction of the primary septum (Plate 20d) but was also detectable along the hypha away from the primary septum (Plate 20d). $I_1$ was 52 nm thick $I_2$ was 89 nm.

In longitudinal section the triangular space formed between the spore wall layer $I_1$ and the layer $I_2$ at a primary septum (Plate 20e) was filled with electron dense material. The electron lucent layer within the primary septum was continuous with the triangular space.

**Septum**

The transverse primary septum was perforated with simple pore. In structure septum was composed of a duplex layer separated by an intermediate electron lucent layer. The intermediate layer was delineated from the layers of the duplex by a thin electron dense line which marked the outer margin of the
wall layer $l_2$. Simple pore plugged with Woronin bodies each side of the pore (Pl 18d). The margin of the pore typically showed a sharp taper (a.c.d.) but in some preparations the pore was not tapered but had a squared off margin (b).

The development of new (secondary) septa showed that the crosswall material was derived from wall layer $l_2$. (PL 20) Development was centripetal and the wall was characteristically thin and presented an undulate outline in some sections (c+d). The septum was initially 179 nm thick but the electron lucent layer appeared to be present and the layer was therefore duplex. A Woronin body was associated with the septal pore (c+d).

The addition of a pre-fixation soak in ruthenium red demonstrated a feature not shown in material which had only $\text{KMnO}_4$ or glutaraldehyde + $\text{OsO}_4$ fixation. This took the form of patches of electron dense material external to the plasmalemma but incorporated into wall layer $l_2$ which was well...
differentiated from \( I_1 \) (Plates 21 and 22) and suggested that they were discoidal with a diameter of 4.75 nm. No evidence was found of a continuous red positive layer. The cross walls, both primary and secondary showed similar patches.

A germination zone was either initiated by germ tube inception at a point of maximum swelling adjacent to a cross-wall (Plate 23b) or was initiated at the tip of the spore C.

Germination zones (gz) showed that the term tube wall was formed from wall layer \( I_2 \), wall layer \( I_1 \) and \( O \) were disrupted and remained as a neck around the germination point (a c d e f). Plate 23a shows a single germ tube produced adjacent to a primary septum at the junction of two cells p.f. shows twin germ tubes.

Germ tubes produced by the terminal cell of the spore showed the same structure. Well established germ tubes did not show the sharp demarcation at the spore/germ tube junction between wall layers \( O + I_1 \) and \( I_2 \). The junction between the new wall layer of the germ tube and the spore wall appeared continuous.
Plate 17

*S. pistaciarum*

Spore a-e showing organelles of spores.

a) (x 19800)
b) (x 2200)
c) (x 14800)
d) (x 13200)
e) (x 24100)

$I_1$ = first inner layer
$I_2$ = second inner layer
$L$ = lipid
$N$ = nucleus
$O$ = outer spore layer
$SP$ = septum
$TS$ = triangular space
$V$ = vacuole
$W$ = woronin body
Plate 18

*S. pistaciarum* TEM

swollen spore a-d is showing perforate primary septa of swollen spores.

a) - (x 15166).
b) - (x 21200).
c) - (x 14500).
d) - (x 22000).

0 - outer spore layer
I₁ - first inner layer
I₂ - second inner layer
ML - middle layer
W - woronin body
CP - central perforation
Plate 19

*S. pistaciarum* TEM

Swollen spore in SS after 12 hours at 25°C. Organelles present in swollen spore.

a) - Ls (x 14000).

b) - Ls (x 12895).

c) - Ts (x 35000).

d) - Ts of primary septum (x 28000).

e) - Ts (x 34086).

f) - Ts (x 33000).

L - lipid

M - mitochondrion

N - nucleus

O - outer spore layer

V - vacuole

W - woronin body

a, d, e, f, potassium permanganate fixation

b, c, glutaraldehyde/ osmium fixation.
Plate 20

S. pistaciarum TEM

swollen spore in SS after 12 hours at 25°C.

a) 3 celled spore with dividing central cell (x 4463) PS₁, PS₂, primary septum DS developing septum DS.

b) enlarged view of primary septum PS₁ (x 23698)

c) spore cell with young transverse septum (x 21254)

d) developing transverse septum in dividing cell shown in c) (x 56806)

e) primary septum PS₂ (x 55676)

CP - central perforation
L - lipid body
V - vacuole
W - woronin body
SS - secondary septum
I₁, I₂ - inner layer
O - outer layer
HW - host wall
ML - middle layer
Plate 21

*S. pistaciarum* TEM Ruthenium red treatment.

Swollen spore in SS after 15 hours at 25°C pretreated with Ruthenium red (RR) prior to fixation.

a) T.S. spore with marginal deposits of RR + islets (arrow) associated with plasmalemma (PM) and cell wall. (x420).

b) L.S spore with marginal RR + islets. (x472).

M = mitochondria
L = lipid bodies
Plate 22

*S. pistaciaeuropum* TEM Ruthenium red treatment (cont).

swollen and germinating spore show RR + islets.

a) T.S. spore axis (x15100)

b) T.S. spore axis (x16200)

c) germinating spore with deposits of RR + material in spore axis and neck of germ-
tube (x1564)

d) L.S. spore axis with transverse young undulate septum. RR + material along wall but not on septum. (x14200)

e) T.S. spore axis with transverse primary septum. RR + material associated with longitudinal wall and septum. (x15500).

f) oblique section of apex of spore axis showing shrunken protoplast with RR + material associated with the plasma-
lemma (x27200)
Plate 23
S. pistaciaeum spores after 24 hours

Development of germination points.

a) L.S. half-pair germination site with centrally perforate septum leading to adjoining cell of main spore axis. (x13714)

b) L.S. presumptive site of twin-pair germination (x10000)

c) L.S. through terminal cell of swollen spore with terminal germination zone. (x3612)

d) same cell as c). (x6200)

e) L.S. through young secondary spore attachment to mother spore. (x16363)

f) T.S. of a twinned pair of germ-tubes. (x9100)

g) T.S. through germination zone of either one of a twin pair or half-twin germ tube. (x12800)

cp - central perforation

gz - germination zone

I - inner spore layer (I₁+I₂)

ms - mother cell

o - outer spore layer

sy - young secondary spore
5. Results

5.1 Symptoms of leaf spots on *P. vera* by *S. pistaciarum* and *S. pistacina*.

*S. pistaciarum*

The symptoms developed during a 55 day period of infection agreed with symptoms described by Chitzandis (1956) and Maas et al (1971). Necrotic lesions were first observed as distinct small necrotic flecks 20-22 days after inoculation. The spots were 1-2 mm in diameter after 30 days. The lesions were not confined by the major veins of the leaf and necrosis of the tissue over the vein was more intensely pigmented. A narrow zone of chlorosis was observed at the margin of the lesion. After 50-55 days the lesions ranged in size from 2-3-4 mm and adjacent lesions had coalesced. The zone of chlorosis around the necrotic zone had extended to 1-1.5 mm around the spot. 1-3-6 black pycnidia were produced in each lesion and from the ostioles of the pycnidia spore cirrhi were extruded (Plate 24, Plate 25B).

*S. pistacina*

The symptoms agreed with those described by Chitzandis (1956). The necrotic lesions did not form as darkly pigmented necrotic spots but as a wide scatter of pycnidia on a pale green/yellow/brown necrotic region. The lesions were large 1-2-2.5 cm and were of irregular outline without a distinct margin. Numerous pycnidia were present in the lesion. After 50-60 days the surface was covered with extruded spore cirrhi (Plate 25A).
Plate 24

P. vera infected with S. pistaciarum. (30 days infection)

a. plant with uninfected healthy apical region and infected basal zone showing leaf spots (x 1/3).

b. enlarged view of leaf spot symptoms. (x 1).
Plate 25

(A and B) symptoms of *S. pistaciarum* and *S. pistacina* on infected leaves of *Pistacia vera*, after 30 days infection.

A) *S. pistacina*, (x1)
B) *S. pistaciarum*, (x 05)
5.2. Progress of Infection on Leaf Surface

Plates 25-35 show the germination, penetration and production of spores on the surface of the leaf. Infected leaves (plant) were maintained at a temperature of 24 ± 2°C and at a light intensity of about 300 lumens for 8 hours per day. Infected leaves were collected and fixed at intervals of 5, 7, 12, 21, 29, 35, 45 - 55 days.

Plate 26 shows the germination of *S. pistaciarum* and *S. pistacina* on the abaxial surface of the leaf. The leaf surface was undamaged by the inoculation procedure. The three celled spore of *S. pistacina* (Plate 26 b) (5 days) started germination by producing two germ tubes from the terminal cells but not from the intermediate cells (Plate 26 a) (7 days). The diameter of ungerminated spore was 2.8 μm and germinated was 3.3 μm.

Plate 26 d shows a multicellular spore of *S. pistaciarum* after 5 days, inoculation has produced two twin pairs of germ tubes from the intermediate but no germ tubes from the terminal cells, then produced more germ tubes and a surface mycelium (Plate 27 a,b) (5 days) which consisted of fine filamentous (0.9 - 1.6 μm) branched hyphae. Plate 27c,d, Plate 28 a,b shows the distribution of the fine surface mycelium, Plate 28d shows the beginning of penetration of surface mycelium through the stomata with the diameter of 1.3-1.4 μm. Plate 26c shows filamentous material which was occasionally found as surface debris.
Plate 29 a-c shows the penetration of surface hyphae through a stomatal aperture and epidermal penetration was not observed. Later stages of the infection progress during which extensive surface hyphae and pycnidial formation occur are shown in Plate 34, 35 adaxial surface of the infected leaf was examined.

After 35 days of inoculation the occlusion of stomata was observed (Plate 30a,b,c) by the emergence of the internal hyphae (Plate 30d) from the stomatal aperture and producing the branches on the adaxial surface. Diameter of branches was 7 \(\mu m\) which divides into two branches of 2.2 \(\mu m\) diameter and then spreads as a finer hyphae (1.6-0.9 \(\mu m\)). Plate 31,32,33.
Plate 26

SEM

germination of spores on abaxial leaf surface.

a) spores of *S. pistacia* (7 days) (x 1205).

b) spores of *S. pistacia* (5 days) (x 1600).

c) debris on leaf surface (x 2156).

d) germinated spores of *S. pistaciarium* (5 days) (x 1205).
Plate 27

*Pistacia* sp. SEM

Spores on abaxial leaf surface after 5-7 days.

a) - germinating spore (x 485).
b) - germinating spore (x 660).
c) - surface hypha (x 2671).
d) - surface hypha (x 3600).

a) + b) - fresh material
c) + d) - fixed material for SEM.
Plate 28

*S. pistaciarum* SEM

spores on abaxial leaf surface after 7 days.

a) - surface hyphae (x 346).
b) - stomata with surface hyphae (x 1100).
c) - stoma with two different hyphae (x 2900).
d) - stomatal penetration by surface hyphae (x 3450).

a) - Fresh material
b) + c) + d) - Fixed material for SEM.
Plate 29

S. pistaciarum SEM

spores on abaxial leaf surface after 12 days.

a) stomatal penetration by surface hyphae (x 5454).

b) stomatal penetration by surface hyphae (x 5555).

c) stomatal penetration by surface hyphae (x 3550).

d) surface hyphae (x 802).
Plate 30

SEM of adaxial surface view of stomata and epidermal cells of leaves inoculated on the abaxial surface 35-45 days previously showing development of surface hyphae associated with the formation of pycnidia.

a) b) c) occlusion of stomatal aperture.
   a) (x 4649) b) (x 2400) c) (x 3000).

d) emergence of hypha from stomatal aperture (x 3428).

sa - stomatal aperture
eh - emerging hypha
SEM of adaxial surface view of stomata and epidermal cells of leaves inoculated on the abaxial surface 35-45 days previously showing development of surface hyphae associated with the formation of pycnidia.

a)-d) variation in amount branching of emerged hyphae.

a) (x 1704 ).
b) (x 3428 ).
c) (x 2173 ).
d) (x 3382 ).
Plate 32

*S. pistaciarum* SEM of adaxial surface view of stomata epidermal cells of leaves inoculated on the abaxial surface 45 days previously, showing development of surface hyphae associated with the formation of pycnidia.

a) occlusion of stomatal aperture (x 2400).
b) occlusion of stomatal aperture (x 2327).
c) occlusion of stomatal aperture (x 7391).
d) emergence of hypha from stomatal aperture (x 3100).

sa - stomata aperture
ev - emerging hypha
Plate 33

SEM of adaxial surface view of stomata and epidermal cells of leaves inoculated on the abaxial surface 50 days previously showing development of surface hyphae associated with the formation of pycindia.

a) occlusion of stomatal aperture by internal hyphae, formation of surface mycelium (x 4775)

b) surface mycelium produced from two different stomata (x 1582).
Plate 34

*S. pistaciarum* SEM

Pycnidium maturation in leaves infected for 55 days.

a) emergence of first formed conidia through the pycnidial ostiole from the adaxial epidermal surface (x 2250).

b) ostiole of a pycnidium beneath the adaxial epidermal surface (x 802).

c) collapsed adaxial epidermal surface associated with pycnidial development (x 2201).
Plate 35  S. pistaciarum SEM

Pycnidium maturation in leaves infected for 55 days.
Emergence of conidial mass through ostiole, abaxial epidermis.

a) surface hyphae and emerged conidia (x 523).
b) same view (x 805).
c) conidial spore mass (x 2050).
d) conidial spore mass (x 1688).

⇒ base of spore
⇒ apex of spore
5.3. Progress of Infection within the Leaf

*S. pistaciarum* and *S. pistacina* were used in this part of the experiment. Healthy leaf sections were used as a control leaf section.

Healthy Leaf.

Mesophyll cells of *Pistacia vera* had the usual complement of organelles, cell organelles were found in a narrow band of peripheral cytoplasm. They had a large central vacuole which occupies almost all the cell space, the nucleus was large with a double membrane and contained a nucleolus and condensed chromatin (Plate 37 a, b and Plate 36 a, b, e). Chloroplasts were arranged in the narrow band of peripheral cytoplasm containing a number of starch grains (Plate 36 d, f, g). The plasmalemma was smooth and showed a continuous apposition to the cell wall (Plate 36 c, d, e and Plate 37 c, d).

Mitochondria were apparent as circular or ellipsoidal sections. Endoplasmic reticulum was also present though sparsely distributed (Plate 37 b, c).

The intercellular spaces were clearly defined but electron material was found to have accumulated at some junctions. Plate 36 f, d shows a large triangular space with one tapering junction with accumulated material. Plate 36 e shows a small triangular intercell space filled with electron dense material. The plasmalemma shows the usual feature of a narrow channel linked by plasmalemma (Plate 37 d).
Plate 36

Sections through uninfected leaf of *P. vera*

TEM cell ultrastructure (1)

a) mesophyll cells (x 2590).

b) mesophyll cells (x 2652).

c) mitochondrion, cell wall, plasmalemma, tonoplast membranes and a part of chloroplast (x 24240).

d) chloroplast with starch grain, nucleus, cell wall and mitochondrion (x 38250).

e) nucleus and nucleolus with two membranes (x 10161).

f) chloroplast with large starch grains (x 10200).

g) cytoplasm, chloroplast and tonoplast membranes (x 10111).

C - chloroplast
CW - cell wall
Is - intercellular space
M - mitochondrion
N - nucleus
PM - plasmalemma
S - starch
Section through uninfected leaf of _P. vera_

**IEM cell ultrastructure (II)**

(a) arrangement of chloroplast, nucleus, membrane in mesophyll cells. (x 3417).

(b) nucleus (with double membrane), nucleus, mitochondrion and endoplasmic reticulum (x 14000).

(c) cell wall, mitochondrion, endoplasmic reticulum, plasmalemma and tonoplast membranes (x 3341).

(d) two adjacent cells showing plasmodesmata, plasmalemma, tonoplast membranes and mitochondria. Cell debris is present in left hand cell (x 9800).

- **Db** - cell debris
- **ER** - endoplasmic reticulum
- **IS** - intercellular space
- **M** - mitochondrion
- **N** - nucleus
- **Pd** - plasmodesmata
5.4. Membrane Complex

A complicated membrane system of unknown nature was formed in various shapes and places in the infected leaf section.

Plate 38 a-f shows the varied shapes of membrane complex material in various positions in the cell. A, f shows the complex membrane associated with chloroplast; b,c with the cytoplasm. C was the section of the Pathogen S. pistaciarum with the membrane complex associated with nucleus.

Plasmodesmata

Plate 39 shows the section of the interface between two cells of infected leaf. (a) shows a heavy deposit of electron-dense material in contact with the cell plasmalemma and the vesiculation of the host plasmalemma in the adjacent cell. (b) shows the healthy part of the section of two adjacent cells with plasmodesmata.

A shows two adjacent cells with different degrees of infection response. The lower cell shows massive tonoplast thickening and folding with collapse of the cytoplasm and the contiguous association with the plasmalemma. The upper cell shows a quantitatively reduced response; the cytoplasm is showing some vesiculation. The plasmodesmata show the presence of several strands of undisrupted membrane material traversing the plasmodesmatal canal. (c) shows a later stage of infection with both cells with disrupted tonoplast membranes and accumulation of electron-dense material within the vacuole. The
The plasmodesmata show disrupted membrane within the canal. (d) shows two adjacent cells, a membrane complex was present in both cells and tonoplast and plasmalemma in this stage of infection were present and undamaged. The membrane complexes were either associated with nucleus or vacuole, vesiculation of the host vacuole were present in the upper cell associated with a membrane complex.
Plate 38

Section through leaf of *P. vera* infected with *S. pistaciarum* TEM

a) host cell with membrane complex associated with chloroplast (x 11200).
b) host cell with membrane complex associated with chloroplast (x 11200).
c) hyphal cell with membrane complex associated with nucleus (x 30240).
d) host cell with membrane complex in disintegrated cytoplasm (x 10889).
e) host cell with membrane complex associated with chloroplast (x 10889).
f) host cell with membrane complex associated with chloroplast (x 10889).

C - chloroplast
HCW - host cell wall
m - complex membrane
M - mitochondrion
n - nucleus
Plate 39

Section through uninfected leaf of P. vera and leaf of P. vera infected with S. pistaciarum TEM plasmodesmata ultrastructure.

a) 20 days after infection. Cell wall between two adjacent cells, showing two plasmodesmata. The upper cell shows vesiculation of the cytoplasm and lower cell shows thickening and folding of the tonoplast membranes (x38500).

b) uninfected cells (control) with two plasmodesmata, intercellular space, plasmalemma and tonoplast membranes all present. Cell debris shown in lower cell (x8000).

c) 25 days after infection. Cell wall between two adjacent cells showing 3 plasmodesmata, the lower cell shows collapsed in which plasmalemma has fragmented and disintegrated, chloroplast bounding membrane has disrupted and electron dense material are present (x7985).

d) 20 days after infection (1 mm away from centre of lesion). Showing the swollen chloroplast with enlarged starch grains, cell membrane complex and vesicole are present in upper cell. In lower cell also membrane complexes associated with nucleus are present. (x 9800).

Db - cell debris
HP - host plasmalemma/tonoplast
is - intercellular space
M - mitochondrion
Pd - plasmodesmata
vs - vesicle
Wt - cell wall thickening
5.5. Progress of infection within the leaf \((S.\text{pisiyarum})\)

Distribution of Hyphae.

The early stage of infection (20 days) shown in SEM freeze fracture preparations showed intercellular hyphae ramifying through the region adjacent to the inoculated epidermis (Plate 41b). The palisade tissue had not been invaded, no hyphae were observed in the upper palisade region and the section appeared (Plate 41c) as the control (Plate 41a).

In maturing lesions the hyphae were sparsely distributed in the intercellular spaces. No penetration of the host cells was observed. The hyphae were either immediately adjacent (Plate 50a,b,c) to a host cell wall or passed through the intercellular space without any apparent host cell contact (Plate 46c,d). The number of hyphae in any particular space appeared to be low. The orientation of the hyphae was random and sections of hyphae cut in all planes could be found. Plate 47e shows hyphae in T.S. and L.S. Granular intercellular material was also found in some intercellular spaces (Plate 48a,e). External hyphae were present in favourable sections particularly in material which had been infected for 30+ days \( (n) \). (48b).

These hyphae were not attached to the epidermal surface and in Plate 48b were distributed immediately above a stomatal aperture.
5.6. Hyphal/Cell Wall Attachment (S. pistarum)

The hyphal which were associated with the cell wall of the host mesophyll were always surrounded by a layer or extracellular sheath of electron-dense material (Plate 50 a-d) which appeared to attach the hyphae to the cell wall. In early stages of the infections as indicated by host-cell responses (host response section) the amount of material was small. Plate 47d shows an L.S. with a single point of contact with a small pad of material. Plate 47a shows cells in contact without any extracellular sheath material developed, (b) shows a hypha within host intercellular space region without sheathing material. Hyphae in contact with each other (e-f) show no indication of material deposited between the hyphae.

In older infection the development of sheathing material was enhanced and some hyphae showed a complete sheath with large deposits of material accumulated between the host cell and the intercellular hyphae Plate 51a, b,d, Plate 50 a,b,c,d. Where more than one hypha was passing through a small intercellular space the sheath extended between the two hyphae (Plate 50 a). When a hypha was in a narrow intercellular space the extracellular sheathing material was present as a dense layer over the hyphal surface (Plate 50 c). The material in glutaraldehyde/osmium double fixation showed as very small granules.

Hyphae without contacts with host cells, at least in the plane of section, showed uneven deposits of extracellular
sheathing material (b) leading to an uneven profile of the hyphal section.

In SEM freeze fracture observation the material which acted as an adhesive showed that the material was only revealed at points of contacts between host cell and hypha. The uniformity of the material seen in TEM was not shown in the SEM the material appeared to be fibrillar or stranded in nature (Plate 42 a,b).
5.7. Host Response

The host-pathogen interface was varied within any one section but as the infection process continued and the lesion matured a generalised disorganisation of the host-cell cytoplasm occurred.

Early stages of infection (11 days after inoculation) showed a vesiculated appearance of the cytoplasm adjacent to the nucleus (Plate 45). The marginal cytoplasm showed normal electron density. The chloroplast showed no apparent changes in the organisation of the photosynthetic lamellae, but small electron-dense bodies, the plastoglobuli appeared to be more numerous than in uninfected material, the plastoglobuli (g) varied in diameter and in number in any one section. Plate 45 a shows a chloroplast with 12 pg/section; b) with 27 pg.

The plasmalemma did not show a uniform response from cell to cell (Plate 45). The response varied from a series of small undulations to major folding. The tonoplast response was quantitatively greater than the plasmalemma and folding, leading to major discontinuities or fragmentation of the tonoplast was shown by many sections. Plate 45 b-e shows the variation in tonoplast response with the major folding shown in (d) and the discontinuities in e.

After 25 days, in the centre of the lesion host cells showed variable responses. Guard cell contents and
epidermal cells showed complete disorganisation, with fungal hyphae attached to the guard cells (Plate 46 a+b). In mesophyll cells the chloroplasts were swollen with larger inter-lamellar spaces and enlarged plastoglobuli. The plasmalemma was separated from the host-cell wall but was intact.

After 35-55 days many of the host cells showed complete disorganisation (Plate 49 a,b,c) with electron-dense material being distributed throughout the disorganised vacuole. Though the lesion was fully developed some host cells had not fully disorganised (Plate 48 a-e, Plate 47 a-f). Some host cells showed a small lobe-like folding of the cell wall (Plate 47 a,b). Intercellular material, consisting of small electron-dense granules enclosed in an electron-dense boundary layer, was distributed patchily in some sections (Plate 48 a,e; Plate 49 a,b,c).
5.8. **Hyphal Structure**

In early stages of infection there was no evidence of short-order branching hyphae or very dense hyphal population in any one intercellular space, Plate 48 a-e.

The hypha was smooth and continuous. Simple centrally perforate septae are formed (c,e) with Woronin bodies associated with the pore. The hyphal wall in contact with the host cell showed a small amount of electron-dense sheath material at the point of contact (d), this material was not observed to cover the hyphae. The vacuoles were large, mitochondria were present and appeared as oval-round section and the nuclei had a double-membrane. Lipid bodies were not observed.

Plate 50 a-d shows very fine structure of hyphae in later stages. (a) shows the structure of two fungus hyphae in contact with host cells by sheathing material nucleus and membrane complex associated with the nucleus were present; one lipid body was found in each of the hyphae. (b) shows the section of two single separated hyphae, the one adjacent to the host cell had two large vacuole, mitochondria were present. Plasmalemma membrane was well defined. The hypha was surrounded by an amorphous electron-dense sheathing material. (c) was shown in Plate 51 a-d. Plate 51 a-d shows the section of intercellular hyphae and typical component of the hyphal cell with incomplete septum like infolding of the hyphal wall. The number of mitochondria was increased in the tips of the hyphae, they were circular or ellipsodal in section; the vacuoles were smaller but more numerous. Lipid bodies were present in large numbers. Ribosome were clearly defined in Plate 51 c.
SEM of freeze-fractured infected leaves inoculated 20 days previously with *S. pistaciarum* showing ramification of the intercellular hyphae.

a) uninfected leaf, palisade mesophyll. (x3846).

b) infected leaf after 15 days with hyphae ramifying through abaxial intercellular spaces. (x 2692).

c) infected leaf after 15 days showing no hyphae present in the palisade mesophyll. (x 3460).
Plate 42

*S. pistaciarum* SEM

Freeze fracture of infected leaf after 20 days.

a) adhesion of hypha to the wall of palisade cell (x 10952).

b) same as a) with lower magnification (x 5524).

h - hypha
pc - palisade cell
Plate 43

SEM of freeze-fractured infected leaves inoculated 20 days previously with S. pistaciarum showing ramification of the intercellular hyphae.

a) branched hyphae in intercellular spaces (x2692)
b) branched hyphae in intercellular spaces (x3300)
c) aspect of Plate a). (x3076).
SEM of freeze-fractured infected leaves inoculated 20 days previously with \textit{S. pistaciaeum} showing ramification of the intercellular hyphae.

a) clusters of hyphae in sub-stomatal space. (x1852)

b) hyphae lining sub-stomatal cavity. (x729).

c) initial stages of penetration showing two hyphae ramifying over the inner surface of the abaxial epidermis. (x1401).
Plate 45

Section through leaf of *P. vera* infected with *S. pistaciarum* TEM after 11 days.

Tonoplast changes in host cells.

a) vesiculation adjacent to tonoplast (x ).

b) folding of tonoplast (x 10733).

c) increased electron density of tonoplast (x 10585).

d) increased folding of tonoplast (x ).

e) disruption or fragmentation of tonoplast shown by two arrows (x 10705).

Tonoplast folding shown by single arrow.

C - chloroplast
HCW - host cell wall
M - mitochondrion
V - vesicule
N - nucleus
Plate 46

Section through leaf of *P. vera* infected with *S. pistaciarum* after 25 days TEM

a) §s of stomatal apparatus showing fungal hyphae present in sub stomatal space. (x 2800).

b) §s through guard cell showing undisrupted cuticle, fungal hypha in contact with host guard cell wall (x 10182).

c) sections through spongy mesophyll cell showing the plasmalemma pulled away from cell wall (arrows), chloroplasts disrupted and swollen. Fungus hyphae in intercellular spaces c (x 10000), d (x 9722).

Cu - cuticle
EP - epidermal cell
F - fungus
GC - guard cell
HCW - host cell wall
HP - host plasmalemma
iS - intercellular space
Plate 47

Section through leaf of *P. vera* infected with *S. pistaciarum* TEM after 35 days.

Distribution of intercellular hyphae of *S. pistaciarum*.

a) Hypha in contact with host cell but without extracellular sheathing material (x 12216).

b) Intercellular hypha without sheath (x 12000).

c) Septate hypha in contact with host cell but without sheathing material (x 12000).

d) Hypha showing contact with host cell with extracellular material at point of contact (x 2450).

e) Two hyphae in contact without sheath material (x 12450).

f) Two hyphae in contact without sheath material (x 12450).

CP - central perforation
F - fungus cell wall
F - fungus
hc - host chloroplast
HC - host cell
HV - host vacuole
IM - intercellular material
IS - intercellular space
L - cell wall loop
N - nucleus
NC - necrotic cell
sp - septum
T - tonoplast
W - woronin body
Section through leaf of *P. vera* infected with *S. pistaciarum* TEM after 45 days.

Distribution of hyphae and intercellular material in intercellular spaces.

a) sub-epidermal and intercellular hyphae (x 2924 ).

b) sub-stomatal and external hyphae (x 3850 ).

c) sub-epidermal and intercellular hyphae (x 3550 ).

d) spongy mesophyll and intercellular hyphae (x 3495 ).

e) spongy mesophyll and intercellular hyphae, intercellular granular material is present (x 3130 ).

**EP** - epidermal cell  
**F** - fungus  
**Gc** - Guard cell  
**iM** - Intercellular material  
**iS** - Intercellular space  
**N** - nucleus.
Sections of leaf of *P. vera* infected with *S. pistaciarum* after 45 days. TEM.

**Host cell reaction**

a) Differential host response adjacent to intercellular hyphae. Upper left host cell with enlarged chloroplast, upper central cell and lower two cells electron dense material accumulating in vacuole ($D_m$) intercellular granular material is present. (x 3261)

b) Hypha adjacent to intercellular granular material. (x 13851).

c) Hypha adjacent to intercellular granular material. (x 13750).

CW - cell wall
HCW - host cell wall
HP - host plasmalemma
im - intercellular granular material
is - intercellular space
L - lipid
sp - septum
V - vacuole
F - fungus
FCW - fungus cell wall
Plate 50

Section through leaf of P. vera infected with S. pistaciarum TEM after 55 days.
Adhesion of intercellular hyphae to host cell wall.

a) two hyphae with extracellular sheath. Upper host cell totally disorganised, lower host cell showing no response. Intercellular granular material arrowed. (x 16500).

b) two hyphae with extracellular sheath material (x 12833).

c) single hypha with partial septum and extracellular sheath. Host cell showing folded tonoplast (x 11400).

d) detail of b). Extracellular material forming adhesion pad to host cell wall. (x 39200).

FCW - fungal cell wall
FP - fungal plasmalemma
HC - host cell
HCW - host cell wall
HP - host plasmalemma
HT - host tonoplast
iS - intercellular space
L - lipid
M - mitochondrion
V - vacuole
WT - cell wall thickening
X - extracellular material
Plate 51

Section through leaf of P. vera infected with *S. pistaciarum* TEM after 55 days.

Ultrastructure of intercellular hyphae.

a) b) d) section through a hyphal lobe showing a septal-like structure separating the two compartments. The cell wall shows the two layers of the cell wall. Hyphae surrounded by extracellular sheathing material. (*x1455*) (*x37750*) (*x35200*)

c) LS through hypha with extracellular sheath. Host cell showing plasmalemma/tonoplast folding. (*x26800*)

- **I** - I₁, I₂
  - I₁ - inner layer one
  - I₂ - inner layer two
- **L** - lipid body
- **M** - mitochondrion
- **O** - outer layer
- **R** - ribosome
- **X** - extracellular material

**in** = infolding
5. Distribution of Hyphae. (S. pistacina)

Plate 55 shows the different stages of the infection, early stages (a,b) and later stages (c,d).

(a) shows the distribution of hyphae in the intercellular spaces either adjacent to the host cells or to each other. No penetration was observed into the host cell wall. The contact of the pathogen and host cell was by hyphal wall to host cell wall contact. A layer of electron-dense material formed a sheath over the hyphae. The number of hyphae in any particular space appeared to be high and in the later stages of infection, hyphae occupied almost 50-75% of the intercellular space.

Branch frequency was high. The sections through the hyphae (c) shows lobed branches. (d) shows the growth of hyphae in a small intercellular space in contact with host cells.
5.10. Hyphal/Cell Wall Attachment. *(S.pistacina)*

The hyphae which were associated with the cell wall of the host mesophyll had very little electron-dense material as a sheath or layer (Plate 53 a,c,d and Plate 54 a,b,c,d). The contact between the hypha/host cell wall was essentially a wide plane contact, sheathing material formed a small fillet which filled the triangular space between the curved region of the hyphal wall and the region of plane contact.

In early stages of infection (Plate 53c) the amount of electron-dense sheath material was very small and even in some there was no evidence of the sheath material between the host cell and hyphae or between two hyphae. Plate 54 a,b,c,d showed the attachment of hyphae to the host cell wall without any sheath material, only in (a) a small amount of sheath material was observed in the attachment of hyphae in the triangular space and a pad of electron-dense material was found between the two cells in the upper part of the picture.

Plate 54b shows no sign of the electron-dense material either between one hyphae with another or between the host cells and the intercellular hyphae. (c) shows the intercellular hyphae in contact with each other with a small amount of sheathing material and with host cells without any sheathing material. (d) shows the same feature as (c).

Plate 53 a shows the intercellular hyphae in intercellular space, a small amount of sheathing material was observed in
the point of two hyphae contact but it was not continuous and did not make a complete sheath of material around the hyphae.

(b) shows the triangular space filled with electron-dense sheathing material. (c) shows the heavy deposits of electron-dense material associated with the tonoplast and plasmalemma membrane as droplets. Intercellular hyphae were present, very little sheathing material was observed around the hyphae not clearly in contact with a host cell wall. A small amount of electron-dense sheathing material was found in the triangular space formed by three adjacent host cells.
5.11. **Host Response. (S. pistacina)**

Early stages of infection (11 days) showed the accumulation of electron-dense material. This material was associated with the tonoplast of the host cell as a discontinuous layer of discrete flattened dome-shaped dark bodies (Plate 52 a, b, c, d, e, f, g) and in some parts of the cell it was more continuous (Plate 52 d, e, f). This electron-dense material was sparsely distributed in the vacuole of the epidermal cells; but in mesophyll cells it was only associated with the tonoplast. The plasmalemma was identifiable in some preparations and was intact (Plate 52 f).

Some small plastoglobuli (pg) were found in some chloroplasts but the starch grains appeared to have increased in size and ranged between 0.1–0.5 μm in diameter, but the other host cell components appeared to be normal.

In the later stages of infection (Plate 55) with many hyphae within the intercellular space, the host cells in contact with the pathogen showed various degrees of collapse and disintegration. The amount of fungal hyphae was large; 50–75% of the intercellular spaces.

Plate 53 a (29 days after inoculation) shows the intercellular space with three fungal hyphae. The upper host cell shows no sign of disintegration but the plasmalemma appeared to be undulate in the host adjacent to the hyphal contact and the host wall also showed thickening in that area. The chloroplast
was swollen and the starch grains were large, the nucleus and mitochondria had a normal boundary membrane.

The lower cell shows massing of electron-dense material which had combined together from the disrupted tonoplast, the remainder of the tonoplast was shown as small discontinuous fragments of electron-dense material. The plasmalemma was not defined except in a small region which was separated from the chloroplast. The host cell wall immediately underlying the attached hypha showed a distinct though small increase in thickness. This is also shown in Plate 54 a,d and Plate 53c,d.

Plate 53 b shows the section of host cell 1 mm away from the centre of the lesion. The electron-dense material was observed in the large vacuole and the tonoplast had disintegrated. The triangular intercellular space was filled with electron-dense material, (c) shows the same features as (a) and (b). The mitochondria membrane was intact. (d) shows two hyphae in small intercellular space, the lower hypha filled a triangular intercellular space and electron-dense sheath-material was present around the hyphae. Plasmalemma membrane was present in all three host cells but it was rough in some places. A thickened wall was observed in host cell wall.

Plate 54 a,b 29 days old infected leaf shows the host cells in contact with the fungal hyphae but were slightly affected by the pathogen; some of the cells had the feature of the healthy cell in which all organelles were in a narrow band of peripheral cytoplasm which surrounded a large central vacuole.
In some of the host cells massive amounts of the electron-dense material were observed. Plate 55 (c,d) a 55-day old infected leaf shows the centre of lesion, a large number of hyphae were found in the intercellular space.

The host cells were completely collapsed and had disorganized cytoplasm with no defined structure of the cell components. The cell wall was entire and thickened in some cells. In all preparations no cell wall penetration by fungal hyphae was observed and also no intercellular granular material was found.
5.12. Hyphal Structure. (*S. pistacina*)

The early stages of infection showed no evidence of short-order branching or very dense hyphal populations in intercellular spaces. (Plates 53, 54).

The hyphae had little or no electron-dense sheath material, fixation was poor therefore the structure of the hyphae was not clearly defined but as shown in Plate 54 a-d the inner layer was thick and at the point of attachment to the host cell the inner layer was thickened (a), also shown in Plate 53 a and d. Vacuoles were large in some preparations covered all cytoplasm, Mitochondria were present and a small number of lipid bodies. A primary septum is shown in Plate 54 c, Hyphal growth was not confined to the larger intercellular spaces and hyphae were found in small intercellular spaces (Plate 53 a, d).

In later stages of infection fixation improved and more components of the hyphae were clearly defined. Plate 55 a-e shows the structure of intercellular hyphae. In this stage hyphae were branched and simple centrally perforate septae are formed, a,b,c,d with Woronin bodies associated with the pore (b,d). The hyphal wall showed a small amount of external electron-dense material. Large numbers of vacuole were present and few lipid bodies were observed. In some preparations a discontinuous layer of electron-dense material was present associated with the plasmalemma (b). Ribosomes were scattered in the cytoplasm associated with ER. Only one nucleus was observed in each hypha with a typical double membrane (e).
Plate 52

Leaf of *P. vera* infected with *S. pistacina* TEM

Sections of mesophyll tissue 11 days after infection.

a) abaxial epidermis and mesophyll cells with electron-dense material as droplets in vacuole and associated with the tonoplast. Note not all cells show the same length of response. Mesophyll cell in l.v. corner with zero response. Epidermal cells with maximal response. (x2500)

b) as a). Note absence of intercellular deposits (x(x2400))

c) d) mesophyll cells with continuous zone of electron-dense droplets associated with the tonoplast membrane. Chloroplasts with large starch grains (x8800)

e) junction of two mesophyll cells with deposits on the tonoplast membrane. Note extracellular electron-dense material at the junction of the two cells (x 8100)

f) junction of three cells: two cells with large deposits on the tonoplast, one cell without dense deposits on the tonoplast membrane (x 8000)

g) section through two adjacent cells with large and small deposits of dense material associated with the tonoplast (x 9200)

cu = cuticle
PM = plasma membrane
DM = dense material
S = starch grain
N = nucleus
M = mitochondrion
Section through leaf of *P. vera* infected with *S. pistacina* TEM after 29 days.

a) section through showing intercellular hyphae all in contact with host cells, also showing vesiculation of host cell and cytoplasm adjacent to plasmalemma (x 9800).

b) mesophyll cell from the same infected leaf as a) but section taken from 1 mm distance from a) associated with tonoplast, showing dense material, deposition of electron-dense material in the cytoplasm and intercellular material are present in intercellular space between three cells (x 9852).

c) showing a section of host cell with electron dense material close to the infected area of the leaf. Fungus hyphae are present. (x 9732)

d) hyphae in close contact with host cells in restricted intercellular space, the lower hypha occupying the triangular prism between three host cells. (x 9979).

E - extracellular deposit
F - fungus (I)
DM - dense material
H - host
im - intercellular material
IS - intercellular space
PV - plasmalemma vesicule
V - vacuole
Plate 54

Section through leaf of *P. vera* infected with *S. pistacia* : TEM after 29 days.

a) section through lesion showing intercellular hypha in contact with host cell (x 9722).

b) showing two host cells in contact with one hypha (x 9864).

c) d) showing attachment of host cell to hypha x 8892) (x 9625).

A - attachment

H - host cell

F - Fungus

N - nucleus
Plate 55

Section through leaf of P. vera infected with S. pistacia TEM after 35-55 days.

Distribution of hyphae in intercellular space.

a) fungus hyphae in intercellular space and also in contact with host cells (x 3500).

b) host cell intact (x 3617).

c) mass of hyphae, host cell plasmalemma has fragmented and disintegrated (x 2450).

d) section of host cells in contact with fungus hyphae, showing disintegration of host cells plasmalemma. Host cell wall is still present but the host cell itself has collapsed. (x ≤200).
Plate 56

Section through leaf of *P. vera* infected with *S. pistacina* TEM after 45 days.

a) b) Transverse section of the intercellular hyphae showing septum, thick wall, mitochondrion, lipid bodies and vacuoles. (x 40121) (x 32580).

c) Transverse section through intercellular hyphae, mass of fungus hyphae in contact with host cells (x 9947).

d) Transverse section through an intercellular hypha with central perforation (cp) (x 4593).

e) Transverse section through an intercellular hyphae showing hyphal contact (x 9582).

*cp* - central perforation

*FCW* - fungus cell wall

*FP* - fungus plasmalemma

*HC* - host cell

*M* - mitochondrion

*N* - nucleus

*SP* - septum

*V* - vacuole

*W* - woronin body
The conidia of *S. pistaciarum* produced in culture were elongate curved phragmospores, with a modal cell number of six cells and a modal spore length of 60-70 μm. The straight line regression of cell number on cell length showed that though there was considerable variation the culture nevertheless showed this basic morphometric causal relationship (Fig 27). Within the genus *Septoria* no other data of this type is available, however an analysis of measurements made from a series of illustrations on spore shape and size in *Septoria* spp from *Chrysanthemum* cvs (Punithalingam & Wheeler 1965) show that this relationship is demonstrable even though only a few spores for each isolate have been illustrated. The type of illustration and resultant graph is shown in Fig 27 for one isolate of *S. chrysanthemella*, and Fig 28 shows 45 spores derived from several isolates combined. Fig 29 shows the general relationship for 4 species. This fixed relationship between spore size and septation has been demonstrated for the analogous macroconidia of *Fusarium culmorum* (Marchant 1965). This was constant even under different conditions of nutrition. It was suggested that the relationship could be used as an additional taxonomic feature and could also be employed for studies within the genus *Septoria*.

The modal number of six cells/spore in *S. pistaciarum* hides a considerable variation which is characteristic of
Fig 27 plot of spore length against number of cell/spore for 11 spores of *Septoria chrysanthemella* (illustration ex Punithalingam 1966)
Fig 28 Plot of spore length against number of cell/spore for 45 spores of *Septoria chrysanthemella*

\[ o = Septoria chrysanthemella. \]
Fig 29  plot of spore length against number of cell/spore for Septoria SPP. on chrysanthemella

○ Septoria chrysanthemella

• S. obesa

□ S. leucanthehi

▼ S. adarensis

spore length (μm)

number of cell/spore
the genus. This is shown for the species on *Chrysanthemum* (Fig 29) and variation in other species is similar e.g. *S. alni* with septal numbers (1-) 3-4 (-7-9) (Constantinescu 1984). In some species septal number is low 1-2-3 though the spore relatively long e.g. *S. betulae-odorata* (32-) 40-55 (-78) um.

The variation in spore size, measured as length and width, and the associated number of cells would possibly be most usefully integrated as a correlation between cell number and volume. If a nucleo-cytoplasmic ratio is a constant feature for closely related species a study of a large example of the 2000 species of *Septoria* might reveal the existence of natural groups of species.

6.2 The process of spore germination in *S. pistaciarum* showed that on semi-solid media germination was a slow process: initially, the spore axis increased in volume by swelling leading to an increase in diameter and an increase in length due to longitudinal extension growth of each individual cell. This was followed by the division of the individual cells of the spore axis one or more times and germ tubes were eventually produced after this period of spore growth. The growth in liquid media showed a similar stage of spore axis extension growth and the production of either germ-tubes or secondary conidia spores.

The increase in spore volume is a characteristic feature of spore germination in many fungi in which an external source of nutrient is required for germination.
(Bartnicki-Garcia 1981). The use of the coulter counter has enabled volume measurements to be made with accuracy (Barnes & Parker 1966) though the major investigations in which volume changes have been monitored have used microscopical measurements. This latter technique is entirely satisfactory for spores with simple geometric shapes for which reasonable volume approximations can be made. For simple spores e.g. *Aspergillus nidulans* where the conidium is approximately spherical, swelling leads to spherical enlargement prior to the insertion of the germ tube. There is a constant volume increase within a species but between species and genera the rate and amount of swelling is very different (Table 24). In species with more complicated spore shapes the estimation of percentage volume changes has been neglected though for many phragmospores similar in shape to *Septoria* swelling is recorded as being a normal morphogenetic stage prior to germ-tube isolation. It was not possible during the volume increase in *S. pistaciaeum* to clearly define a passive swelling phase as a separate entity from an active growth phase. In a population of freshly produced conidia all the individual cells of the spores were viable and capable of growth. The increase in cell size, accompanied by nuclear division and septation lead to an increase in volume and length of the spore. In either liquid or on semi-solid media spore-axis growth always occurred.

In other fungi with phragmospores the normal mode of germination is the insertion of germ-tubes into the swollen
Table 24 Changes in spore diameter and increase in volume during germination of single celled spores.

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<th>Diameter (um)</th>
<th>Volume increase (times)</th>
<th>References</th>
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<tr>
<td>Hypocrea pulvinata</td>
<td>4.5x2.9-12.3</td>
<td>30</td>
<td>Dixon &amp; Speller (1983)</td>
</tr>
</tbody>
</table>
cells of the spore axis, without any predivision of the cells of the spore axis. As examples this type of germination is shown by the macroconidia of Fusarium culmorum (Marchant & White 1966) Ascochyta soudalis, Diplodinia teretiuscula (Cunnell 1956), Dithiostroma pini (Ivory 1967) and Cercospora spp (Oso 1972). In a series of Stagonospora spp (Fig 30) between which phragmospores varied in size germination was predominately by the central cells of the axis (Cunnell 1957). (Fig 31).

Increase in cell number along the spore axis has been recorded for S. api-graveolens (MacMillan 1962), S. nodorum (Dickinson & Skidmore 1976) and S. tritici (Jones & Lee 1974). The study by MacMillan (1942) was the most detailed study of germination and showed that the proximal and distal terminal cells increased in length and divided. The central cells enlarged to give the typical half dumbell shaped profiles and germ-tubes were produced, singly, from one or more of the central cells. The germ-tubes were inserted in the median position and not at either end of a cell i.e. adjacent to a cross-wall (Fig 32). The illustrations of S. tritici show germinating spores with ten axis cells, an increase from 4-5 cells, and several cells with germ-tubes and occasional twin-pairs. Thus axis growth during germination is not widely distributed within fungi with phragmoconidia but is probably the normal mode in the genus Septoria.

A central problem of interpretation of the process of germination in Septoria is whether axis growth by cell division is regarded as 'spore germination' or whether
Fig 30 Germination of conidia of phragmospores

Stagonospora SPP.

S. vitensis

S. caricina

S. carius

S. cylindrica

S. anglica

S. elegans

(redrawn from Gunnell G.J. 1956-1957)
Fig 31  Germination of conidia of various phragmospores

Diplodina teretiuscula

Ascochyta sodalis  
(redrawn from Gunnell 1956)

Fusarium culmorum  
(redrawn from Marchant & White 1966)

Dothistroma pini  
From New Zealand

Dothistroma pini  
From Kenya

Dothistroma pini  
From Canada  
(redrawn from Ivory 1967)
Fig 32
Germination of conidia of *S. apii-graveolentis*
(redrawn from MacMillan 1942)
germination is confined to the production of a germ-tube. Manners (1966) considered a spore to have germinated if the germ-tube was as long as it was broad. This definition is acceptable for a spore in which no cell division occurred prior to germ-tube insertion. A convenient manner in which a descriptive process of spore germination in Septoria can be recorded is to define germination as a two stage process: Germination I (GI) in which axis growth and cell division occurs.

Germination II (GII) in which germ-tubes are produced. The fate of the germ-tubes produced in GII depends upon the cultural conditions i.e. whether a hyphal tip is produced or a secondary conidium.

During extension growth of the main axis in S. pistaciarum all the spore-axis cells were capable of growth and division and the production of laterals. The use of the term lateral is convenient in that it encompasses the production of germ-tubes and secondary conidia. The number of cells produced along the axis depends upon the nutrient status of the medium.

6.3. In a spore with a file of cells, each cell has a basic location relative to the base (proximal) and the apex (distal) of the spore. The onset of cell division can therefore be located with respect to the poles and any polarity can be determined and be regarded as a response to a polar control. Growth in apparently random positions can be used as an indication of the absence of polar controls.
In *S. pistaciarum* the axis showed no polarity and division occurred evenly along the length of the spore. Each cell increased in length and width. The growth of the spore wall was however uneven with an enhanced rate of growth on the convex side of the spore curve. This leads to an increasingly pronounced curvature of the spore usually in one plane, with germlings showing U-shaped or circular forms. This is also shown in the illustrations of *S. tritici* (Jones & Lee 1974) though the authors do not comment on this.

Associated with the enhanced one-sided growth was increased wall growth at the septal poles of the cell so that in profile the cells took on the appearance of a half-dumbell (Fig 33).

![Fig 33 Polarised Growth of Septal Zone](image)

The new cross-wall dividing the cell was located at the central point. Longitudinal growth of the daughter cells continued followed by further divisions. The apical cells increased in length with the maintenance of a tapering
profile. The septa produced could be identified as members of a cohort (Fig 34).

An idealised growth pattern of a five-celled spore showing progressive reduction of cell size and the polar distribution of nuclei is shown in Fig 35.

The insertion of laterals was associated with septa i.e. polarised insertion. Laterals are often produced as twin-pairs, one from each cell, adjacent to a common septum. The septum involved in any one cell was always the oldest (Fig 36).

A model of the main events predicted to occur during twin-germ-tube production including nuclear division and migration, septation and local concentrations of vesicles is set out in Fig. 37.
Fig 35  Idealised growth pattern of a five-celled spore
spore cell

swollen spore
nuclear division

nuclear migration

septation into
two daughter cells

daughter cell growth
nuclear division

nuclear migration
septation

twin germ-tube

○ nucleus
● woronin body
▼ vesicle concentrations

Fig 37 Model of polar responses during spore germination
Twin pairs are also shown by *S. tritici* (Jones & Lee 1974).

The production of laterals was not evenly distributed but tended to begin in the central region of the spore axis but all cells could eventually produce laterals or in the case of the terminal cells a terminal germ-tube. The amount of division was dependent on the nutrient status but cell size gradually reduced since cell extension growth rate was not maintained though cell division was continued.

The production of hyphal germ tubes from a U or circular shaped axis would automatically orientate the laterals at divergent angles to each other. The characteristic feature of young mycelia is divergent growth (Gregory 1984). This is also shown by germ-tubes produced from a cluster of spores (Gooday 1983). In *Geotrichum candidum* tandem-pairs of arthrospores have been shown to germinate at the opposite poles; chains of arthrospores still held in their original formation show that germ-tubes are never produced in twin-pairs and that production of a germ-tube on one side of a septum appears to inhibit the production of a germ-tube on the other side (Robinson 1973, 1980). These chains could be regarded as the equivalent of a phragmospore, this shows that, in Septoria, such inhibition i.e. early negative autotropism does not occur. The divergent growth in *Septoria* therefore shows two conflicting processes: angular separation of germ-tubes by curvature of the axis but associated with twin-pairs of germ-tubes which then have to adopt a divergent growth pattern (Fig 38).
The negative autotropism shown by *G. candidum* has been interpreted as being a response to an oxygen concentration gradient which is maximal at the poles of a tandem pair but low in centre. The possible transmission of signals between two adjacent cells through a common septum would be enhanced if the septum was perforate. In *G. candidum* the septum during development is multiperforate but becomes occluded during maturation, though the disarticulation of the arthroconidia is retarded by the presence of thin strands of material (Cole & Samson 1979); which may represent connective strands which originally passed through the septum. These observations suggest that using
a chain of arthrospores as a phragmospore analog is superficially attractive but the open perforate septa in *Septoria* would allow some communication between adjacent cells.

6.4. The patterned growth process leading to axis extension and the insertion of laterals can be described by means of simple model. There are few references to pattern formation in discrete files of short-order cells. A regular spacing of morphological features has been described in *Anabaena* spp, *Polysphondylium* spp and *Dipodascopsis* sp.

In *Anabaena*, a filamentous aquatic cyanobacterium under conditions of an adequate combined nitrogen source produces only vegetative cells. On removal of nitrogen source there is an intercalary insertion of thick-walled cells, the heterocysts, which fix atmospheric nitrogen and vegetative growth can continue. The insertion pattern is initially at random but each heterocyst induces a zone of inhibition 5 cells in extent on either side, in which no other heterocyst can form (Mitchison et al 1976). The zone of exclusion develops during the early developmental stage i.e. proheterocyst, prior to nitrogen fixation (Bradley & Carr 1971) but it is postulated that control is due to the production of an inhibitor by the pro-heterocyst which diffuse along the filament until a minimal concentration is reached which allows new heterocyst formation to be initiated (Wolk 1982). The morphogen was originally considered to be an ammonium compound but recent work suggests that glutamine may be the morphogen (Wolk 1982).
In *Polysphondylium pallidum*, a cellular slime mould (Spiegel & Cox 1980) the vertical stalked sorocarp consists of an erect stalk bearing whorls of branches spaced out at regular intervals along the stalk. The stalk is composed of a file of equal-sized cells, thus there is an equal number of cells between the branches. However in diploid strains, though the stalk cells were of increased size the branches were the same distance apart. The spacing of the whorls is therefore distance determined. The authors suggest that this is a timing phenomenon though an alternative explanation could involve the existence of a morphogen diffusing either basipetally or acropetally which allows whorl initiation at a critical concentration. The presence of a whorl then acts as a new base (Elwy 1980; Elwy & Dixon 1985).

In the hemiascomycete, *Dipodascopsis uninucleata*, asci are initiated in a file of cells at each alternate septum. The ascus is formed from the fusion of two isogametangia produced, from adjacent cells, on each side the common septum. These can be regarded as twin-pairs of specialised laterals which arise in a similar to the twin-pairs in *Septoria*. The insertion point is always 4-5 cells from the tip of the file. Three control models were discussed (Elwy 1980; Elwy & Dixon 1985) but a model involving an increasing metabolic gradient established behind the tip was suggested as the mode of control of first ascus insertion; the ascus eroded the concentration and this was only re-established by growth of the tip.
In **Septoria** an analogous simple model can be erected. The production of twin germ-tubes by adjacent cells from the zone close to the common cross-wall is the equivalent of twin isogametangia. The initiation of germ-tubes in the central cells of the spore-axis (Fig 39 C,D) which would be several cells proximal to the distal tip is equivalent to the proximal insertion of an ascus. In a spore all the cells are the same age. Each cell undergoes one or more divisions and thus a file of equal-aged cells is produced. If each cell has the same capacity to produce a germ-tube then insertion should be random. However, germ-tube production is concentrated in the central zone which implies that the central zone is different from the poles of the spore axis. To explain such differences it is postulated that a gradient of either a morphogen or a metabolite is set up between the poles (Fig 39 A & B) and that a maximum is reached in the centre after sufficient growth has occurred. Once a critical threshold level has been reached lateral production ensues (Fig 39 B,C). The production of twin germ-tubes adjacent to the oldest septum implies that sub-cellular organisation is polarised but is not influenced by the activities on the other side of the septum i.e. there is no transfer of a negative-antotropic-factor through the septum.
Fig 39  A-D  Single gradient model of twin germ-tube production in *S. pistacia rum*.

A & B = GI
C & D = GII

\[ \text{postulated gradient} \]

\[ T = \text{threshold value} \]
The simple model does not adequately explain the insertion of twin germ-tubes. This defect is also shown by the model for *D. uninucleata* in which twin gametangia are produced.

It could be postulated that a cell, in isolation, has two poles at the cross-walls; and a potential symmetrical gradient representing a polar component can be inserted into the cell (Fig 40).

\[ \text{high} \quad \text{----- gradient} \quad \text{low} \]

*Fig 40* Intra-cellular gradient

A cell, in a file of cells with a clearly defined tip has, with reference to the tip, a proximal and a distal cross-wall and to represent this an asymmetric gradient can be inserted (Fig 41).

\[ \text{high} \quad \text{----- gradient} \quad \text{low} \]

*Fig 41* Asymmetric intracellular gradients

Each cross-wall will have two identities: a distal identity and a proximal identity and a gradient can be inserted into each cell (Fig 42).
There is a high value at each cross-wall and a progressive reduction as the tip is approached. This reduction can be summarised as an interactive model incorporating two gradients (Fig 43 a-h): an intra-hyphal gradient and an intra-cellular gradient with high concentration at the poles. This can be represented as a sine curve (a). The same wave can be overlaid into a single cell (b). During division the gradient is stretched (c) and re-established when the cross-wall is completed (d). The intra-hyphal gradient can be represented as a gradient of increasing concentration with the lowest value at the tip (e). The individual cell sine wave can be linked into a continuous sine wave and these two gradients can be combined by addition to give a resultant curve with peaks at the cross-walls. The peaks diminish in height as the tip is approached (g). A threshold value can be drawn (T) and a morphological response can be expected when the threshold level is reached (h). The twin germ-tubes are formed as a result of the approximately symmetrical concentration on each side of the cross-wall. As the tip advances the model is drawn forward and cells respond in order.
Fig 43: An interaction model of polar cell growth and proximal insertion of laterals in a germinating spore of *S. pistaciarum*.

**a-d, cell division**

a: sine wave representing a concentration gradient.

b: sine wave overlaid into a cell with growth concentrated at the poles.

c: cell division with a stretched gradient.

d: re-established gradient in the two daughter cells.

**e-h, germ tube insertion**

e: intra-hyphal concentration gradient established behind the tip.

f: sinusoidal intra-cellular concentration gradient.

g: resultant combined concentration gradient. Threshold value (T).

h: morphological response to the threshold value i.e. proximal insertion of germ-tubes in positions adjacent to the cross-walls.
This model can also be deployed to explain the analogous formation of twin gametangia in D. uninucleata. The twin gametangia fuse immediately to produce the ascus initial, whereas in S. pistaciarum the germ-tubes show negative autotropism and grow away from each other. This indicates a fundamental difference between specialised laterals as gametangia and lateral germ-tubes. In the former there must be an anastomosis factor or positive autotropism factor which overrides the basic negative autotropism shown by most fungal hyphae, except in the older parts of a colony where anastomosis is a common phenomenon (Gregory 1984).

The threshold value postulated in the interactive model is the basic control of the germination response. A germination sequence in which the threshold is never reached confines germination to GI; alternatively a sequence in which the threshold is reached rapidly i.e. a short GI phase will lead to the rapid induction of GII events. The experiments in which germination was followed in media of various components showed considerable variation in GI and GII responses.

6.5. The fundamental pattern of germination in S. pistaciarum always involved GI axis growth. No swelling or growth occurred in water culture. This conflicts with the evidence of Chitzandis (1956) who reports the germination of both conidia and ascospores in water. S. nodorum also showed germination of washed spores after storage for 7, 24 hours
when placed on cellophane on wet filter paper (Griffiths & Perrett 1980). A problem with pycniospores is the carry-over of extracellular exogenous mucilaginous matrix during the process of spore release in the cirrhus. Cirrhus material of *S. nodorum* has been shown to contain a germination inhibitor i.e. to prevent spore germination in the cirrhus prior to disposal (Griffiths & Perrett 1980) and matrix material has been shown to improve long-term viability of a spore washed free of matrix material in *Sphaerellopsis filum* (Louis & Cooke 1983).

Spore germination in many fungi requires the presence of exogenous nutrients (Van Etten et al 1983) and the basic requirements are a readily available carbon source e.g. glucose (C) a nitrogen source (N) and a source of phosphate (P) (Table 25). The original work of Ekundayo & Carlisle (1964) on *Rhizopus arrhizus* showed the need for C, N and P for complete germination though swelling occurred with glucose alone.

The larger macroconidia of *Fusarium culmorum* required C and N (Marchant 1966), the internal reserves of P apparently being sufficient. The very small ascospores of *Hypocrea pulvinata* required C, N and P for germination which involved a 30x increase in spore volume and three rounds of nuclear division before a germ-tube was inserted (Dixon & Speller 1983). In *Syncephalastrum racemosum* only glucose is required. Some spores contain sufficient endogenous reserve nutrients to enable them to germinate in distilled water e.g. aedospores of rusts (*Puccina*
Table 25 Nutrients required for germination by some fungi.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Nutrients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrytis cinerea</td>
<td>Simple sugars, amino acids</td>
<td>Blakeman 1975</td>
</tr>
<tr>
<td>Ceratocystis adiposa</td>
<td>glucose</td>
<td>Skone 1982</td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>carbon, nitrogen</td>
<td>Marchant &amp; White 1966</td>
</tr>
<tr>
<td>Glomerella cingulata</td>
<td>glucose, MgSO₄, KNO₃, KH₂PO₄</td>
<td>Lin 1945</td>
</tr>
<tr>
<td>Phylllosticta lycopersici</td>
<td>glucose, nitrogen, phosphate MgSO₄</td>
<td>Onesivosan 1976</td>
</tr>
<tr>
<td>Penicillium atrovenetum</td>
<td>glucose, nitrate phosphate</td>
<td>Gottlieb &amp; Tripathi 1968</td>
</tr>
<tr>
<td>Penicillium griseofulvum</td>
<td>glucose, nitrate phosphate</td>
<td>Fletcher &amp; Morton 1970</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>carbon, nitrogen</td>
<td>Martin &amp; Nicolas 1970</td>
</tr>
<tr>
<td>Rhizopus arhizus</td>
<td>glucose, nitrogen phosphate</td>
<td>Ekundayo 1966</td>
</tr>
<tr>
<td>Syncephalastrum racemosum</td>
<td>glucose</td>
<td>Hobot &amp; Gull 1977</td>
</tr>
<tr>
<td>Trichoderma lignorum</td>
<td>carbon, nitrogen</td>
<td>Martin &amp; Nicolas 1970</td>
</tr>
<tr>
<td>Hypocrea pulvinata</td>
<td>carbon, phosphorus, nitrogen</td>
<td>Speller 1969</td>
</tr>
</tbody>
</table>
graminis (Allen 1955 and 1957) large ascospores e.g. Neurospora tetraspoma (Lowry & Sussman 1968).

The failure of the spores of S. pistaciarum to germinate in water indicated a need for exogenous nutrients. A problem inherent in all spore germination studies is accounting for a small percentage of spores which are capable of germination in media apparently deficient for the compound(s) required by the majority of spores. A partial explanation may lie in the adequate provision of internal reserve in a few spores and/or in the provision of micro-amounts of essential compounds being released from other spores due to the death of a fraction of the spore population. In the studies of S. pistaciarum a small fraction of spores were capable of germination in media lacking glucose or nitrogen. The basal medium (SS) selected for analysing the basic pattern contained glucose, nitrogen as asparagine, sodium and potassium phosphates, magnesium and sulphur and trace elements. Effective germination i.e. GI and GII required the C, N and P components of the medium.

In media lacking phosphate the spore was capable of mobilising internal \( \text{PO}_4 \) reserves to produce extensive spore axis growth (20 cells) but the GII response was markedly reduced. On transferring long, \( \text{PO}_4 \), GI type spores to complete medium, almost synchronous production of GII events was induced within 5-6 hours. In terms of the interactive model failure to produce GII events suggest that the threshold values were not reached due to phosphate
Starvation. An examination of phosphate reserves in spores of *Septoria* in comparison to other spore types would be appropriate as well as an examination of the main carbohydrate reserves; which is assumed to be trehalose as in many other fungi (Van Etten et al 1983).

The failure to germinate in the absence of N suggested that an examination of various amino-acids as a substitute for asparagine would be appropriate, particularly as the surface of plant leaves normally has an accumulation of essential mineral, organic compounds including sugars and sugar-alcohols, amino-acids, pectic substances, vitamins and phenolics (Godfrey 1976). The material arrives by diffusion from the epidermal cells through the micro-pores in the cuticle (Macauley & Waid 1981).

Germination in media containing single amino-acids showed support of germination, though the absolute rate of response varied considerably. Proline was more effective than asparagine, an observation also shown by a strain of *Rhizopus arrhizus* used by Weber & Ogawa (1965). In this work individual amino-acids, sugars, organic acids, vitamins and inorganic nitrogen sources were tested. An interesting feature was that proline was effective in the absence of phosphate. In the presence of P ornithène and arginine as well as proline were stimulatory. An examination of a possible phosphate/proline interaction in *S. pistaci arum* would be of interest.
A major difference in pattern response was observed with histidine when used as a source of nitrogen. The GI response was reduced and the spore axis growth was overtaken by the onset of GII. In terms of the model the internal threshold was reached rapidly i.e. the axis was shorter.

A multiseptate spore is regarded as germinated if one or more germ-tubes are produced; but each cell has the potential to produce a germ-tube 'success' i.e. the realisation of the potential can be measured as the ratio or percentage of the number of germ-tubes to the number of cells in the axis. Thus a simple spore with one germ-tube is 100% successful.

The capacity of spores of S. pistaciarum to respond to different media by altered GI and GII responses showed that not all cells produced germ-tubes during the course of the experiment. A maximum success rate of 60% was found with SS and substitution of the aspartagine by histidine did not reduce this significantly. If an equal number of spores is considered the reduction of GI response under histidine to 60% of SS coupled with the same GII response i.e. 60% the overall germ-tube production would be reduced to 60% by the value achieved by SS.

In a plant pathogen the production of 'many germ-tubes' would represent a greater opportunity for successful infection i.e. the inoculum potential of a single spore would be increased. The balance between rapid germ-tube insertion i.e. short GI rapid GII and a slower but greater
potential response i.e. longer GI period, may be critical in the survival of a propagule during the infection period; though a capacity to vary the length of the GI/GII stages may be important if conditions vary during the period in which spores are disseminated and infection proceeds. The latter property may also be important if the GII response is of two possible outcomes, either a germ-tube or a secondary conidium.

A major difference in the GII response of *S. pistacia-larum* is the production of germ-tubes on semi-solid media and the production of either germ-tubes or secondary conidia in shaken liquid culture.

6.6. The production of secondary conidia by germinating spores has been recorded in several plant pathogens. Jones & Lee (1974) illustrate twin conidial-pairs produced from strongly curved axis in *S. tritici*, and this also occurs in *S. linicola* (Sackston 1970). *Ramulispora* spp, common leaf spot pathogens of Gramineae, also produce secondary conidia in short lateral outgrowths inserted in the cells of the axis of the tapering curved phragmospore. The spore axis may bear two or three tapering branches but secondary spores are not produced on these. In the phragmospores genus *Cercospora* secondary conidia are produced on lateral conidiophores in *Carachidicola*. The conidiophores are produced singly from cells of the spore axis. The outgrowths have a greater diameter than the hyphal germ-tubes. Spore axis cells can produce both germ-tubes and secondary conidia (Oso 1976).
Similar secondary conidiation has also been shown in C. bourgainvilleae, though in this species the conidia are sessile and the cells of the spore axis act as the conidiogenous cell (Sobers & Martinez 1966). In contrast C. personata and C. nicotianae do not form secondary conidia (Oso 1966). The conidia were produced from spores dried onto a glass surface and then allowed to germinate in humidity chambers.

In S. pistaciarum no secondary conidia were observed other than in liquid culture. The secondary conidia were produced from the same region of the cells as lateral hyphae and often as twin-pairs. Thus the fate of a lateral depended upon the cultural conditions i.e. the type of lateral eventually produced did not influence any change in the basic responses as set out by the model. This implies that control of the fate of the lateral is under a different set of controls, than insertion. No attempt has been made in this work to explore the differential controls that may be involved. The production of a secondary conidium leaves a scar, which is easily seen in SEM as a raised ring and which can also be seen as a bright fluorescent spot using the brightener Tinopal. The kinetics of secondary spore production could easily be followed by fluorescence analysis of spore scars coupled with spore counts, using both direct visual counts, and as has been done, using the coulter counter. The peaks in volume plots of germinating spores showed the production of large numbers of secondary conidia after 38 hours in SS media.
The production of secondary conidia is similar to microcycle conidiation. This phenomenon has been observed in several fungi (Table 26) but always in response to alterations in the growth conditions leading to growth limitation. A feature of these organisms in which microcycle conidiation has been induced is the suppression of germ-tube→hyphal growth and the substitution of a germ-tube→conidiophore growth sequence. Temperature control is shown by A. niger in which growth at 44°C leads to the conidium swelling into a giant multinucleate cell, from which arises a conidiophore on being transferred to the normal growth temperature of 30°C (Anderson & Smith 1971, 1972). In N. crassa 46°C (Cortat & Turian 1974) and in P. urticae 37°C are the critical temperatures. Nutritional controls have been shown for N. crassa, growth on acetate; a mixture of amino-acids in Penicillium digitatum and a shift-up of nutrition from a low to a high sugar/urate medium in Trichoderma harzianum (Zuber & Turian 1981).

An interesting feature of secondary conidium production in S. pistaciarum is the behaviour of the nucleus. No standard relationship between conidial size and the immigration of a daughter nucleus from the conidiogenous cell was observed. Similar observations during primary conidiogenesis within the pycnidium can be made using the illustrations of conidiogenesis in S. leucantheum (Punithalingam 1974). This suggests that the establishment of a fixed nucleo-cytoplasmic relationship is only
### Table 26: Fungi with Microcycle Conidiation

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>Anderson &amp; Smith (1971)</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td>Rossier et al (1975)</td>
</tr>
<tr>
<td></td>
<td>Cortat &amp; Turian (1980)</td>
</tr>
<tr>
<td>Penicillium urticae</td>
<td>Sekiguchi et al (1975)</td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>Anderson et al (1978)</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>Park &amp; Robinson (1969)</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>Zuber &amp; Turian (1981)</td>
</tr>
<tr>
<td>Acremonium diospyri</td>
<td>Saviour (1981)</td>
</tr>
</tbody>
</table>
controlled loosely within rather wide control limits.

The alteration in morphogenetic responses with altered nutrition does not invalidate the interactive model.

Relevant to this are the observations of Jaffe (1981) on electrical inward current flow of positive charges through the growing tips of pollen tubes and fucoid embryos i.e. a polar inward flow of protons exists. A similar flow has been demonstrated in fungal hyphae and this has been correlated with pH differences in the medium immediately surrounding a hypha. In Achlya (Gow et al 1984) it is shown that in the apical 180 ± 130 μm region of the tip an inward flow of protons can be demonstrated leading to a lowering of the external concentration of H⁺ and subsequent rise in pH. Post-apically the medium is acid due to proton release. The differences are small 0.01-0.06 pH units. Associated with this is an electrical current flow. A site scheduled for branch initiation shows the same phenomena. Post-apical release of H⁺ into the medium is associated with the plasma membrane proton-pumping ATPase system. The protons flow into the hyphal tip by means of amino-acid symport. Current flow was prevented by withdrawing amino acids from the medium or raising the pH from pH6.5-8.5; however recovery of current flow occurred after one hour. In phosphate deficient media branching was prevented.
These observations suggest that the differential responses in _S. pistaciarum_ to histidine was possible due to the establishment of proton flow more rapidly than in asparagine media. The responses with other amino-acids indicating differential responses. Gow et al. (1984) indicates that methionine as a single amino-acid inhibits current flow in Achlya. The low level of lateral formation in phosphate deficient media may be related to the failure to meet the intra-hyphal demand for phosphate i.e. ATP requirements involved in proton flow are not satisfied, resulting in failure to initiate a lateral growth point. Since the insertion of germ-tubes in _S. pistaciarum_ can be predicted and the individual cells also exhibit polarity it could be predicted (i) that the convex side of a germling would show a higher pH than the concave side and (ii) that the poles of the cells would show a higher pH than the central region and that this would be enhanced when the laterals are inserted.

The growth of a hyphal apex is controlled by the co-ordinated flow of vesicles to the tip i.e. against the proton flow; and the longitudinal orientation of microtubules (Gooday 1983). In a cell showing localised growth inward proton flow should be associated with the growth pole; a cell showing diffuse growth should show no net flow.

6.7. Investigation of the basic kinetics of spore germination were complicated by the heterogenous nature of the spore suspension. The spore suspensions used were obtained
from the spore output of many pycnidia and no evidence was obtained to explore inter-pycnidial variation. It could be assumed that local variations in nutrient levels would lead to variations in mean spore size between pycnidia. Variation in spore size of *S. tritici* has been shown to vary with the season when grown on the host, showing a reduction in length of 10-12 um between the Spring and Autumn collections (Shearer & Wilcoxson). This probably reflects differences in the nutritional status of the host. This reflects the general observations that spore size is influenced by the nutritional status of the medium (Williams 1959). The cells varied in length, but cell size, though variable, showed a small deviation and thus cell number varied directly with spore length. All measurements whether of cell length or spore volume are population averages. The data obtained from the coulter counter is from a minimum sample of $5 \times 10^3$ spores and shows the considerable variation within spore suspensions.

In many conidial fungi with simple small spores e.g. *A. nidulans* (3.5 um diam) the variation in spore size is small and spore germination tends to be synchronous in behaviour (Bainbridge 1971) at least for first two or three nuclear divisions. Suspensions of the arthrospores of *G. candidum* show reasonable synchronicity (Park & Robinson 1970) even though the population has spores with 1-2-3 nuclei though the majority have only one nucleus. In fungi with a long swelling phase before germ-tube insertion, e.g. in the ascospores of *H. pulvinata* (Dixon & Speller 1983)
synchrony is lost during the three rounds of nuclear division prior to germ-tube production. A loss of synchrony can also be shown by slow growing organisms. In *D. uninucleata* the small uninucleate ascospore secretes a capsule during the first stage of spore swelling and the spore population shows poor synchrony of germ-tube insertion and growth of the germling (Dixon & Elwy 1985).

The growth of mycelia and the formation of colonies involves the replication of a basic growth unit (Trinci 1974). The co-ordinated process of hyphal tip growth, insertion of lateral branches, nuclear division and septation form a cycle of events, the duplication cycle (Trinci 1979). Individual hyphae extend linearly but growth of the whole mycelium is exponential (Trinci 1969). The relationship between the length of mycelium and the number of hyphal tips is the hyphal growth unit (HGU) (Caldwell & Trinci 1973). This represents the mean length of hypha required to support hyphal tip growth. The HGU increases exponentially immediately following germination, oscillates as branches are produced and stabilises when tip production is continuous. The total mycelial length and number of tips show the same specific rate and this rate is found to be equal to the specific growth rate during exponential growth in liquid media.

In *D. uninucleata* the establishment of the basic kinetics involved the measurements of mycelial length and hyphal tips as a sample of young germlings growing on semi-solid media. The asynchrony was overcome by overlaying
the plotted data of one mycelium on to another et seq. using one constant length as the critical overlay point (Dixon & Elwy 1985).

In S. pistaciarum individual spore germlings show identical slopes if total mycelial length is plotted against time but the absolute values due to asynchrony are different. When the data are overlaid the values still show a considerable variation about a trend line. The use of mean values however produces the same trend lines with the added convenience that the slope is easily determined.

The early stage of germination in A. nidulans and G. candidum show that growth of the first germ-tube is exponential and slowly becomes arithmetic (Trinci 1974). In Candida albicans during the dimorphic shift from γ-μ the mother cell produces a germ-tube instead of a bud. This germ-tube shows arithmetic linear growth (Gow & Gooday 1982) and shows that a yeast cell cannot be equated with a resting fungal spore.

The previous work has been confined to those fungi with simple spores. In phragmospores there is the complication of several cross-walls and the propagule will produce several germ-tubes together. It is a reasonable assumption that the overall kinetics of the germinating phragmospore would be similar to simple spores though there is no quantitative evidence available.
The data for *S. pistaciaram* shows an overall resemblance to the simple spore examples. The early stages of germination, up to 15 hours, shows no parallel with the behaviour of simple spores; since axis elongation and cell division is taking place once germ-tube production has been initiated, though the specific rates of hyphal length and tip production are not equal. The rate of tip production is lower than the rate for hyphal length and the slope of the two lines diverge.

The divergence may be due to the response of the germling to growth through an arena which has already produced nutrient for spore axis growth i.e. GII events follow considerable GI exploitation of the medium. The data does not allow a firm conclusion to be drawn with regard to matching the kinetic behaviour of simple spores with multiseptate spores. A comparative examination of the kinetic behaviour of phragmospores in which no axis division takes place e.g. *Fusarium* spp and *Cercospora* spp would be revealing.

The HGU showed a value between 30-60 µm and this compares with values for other septate ascomycete/hyphomycete fungi e.g. *A. nidulans* 160 µm, *G. candidum* 102-112 µm, *P. chrysogenum* 25-100 µm, *N. crassa* 130 µm (Trinci 1974).
6.8. The cells of the conidia of Septoria spp. are typically uninucleate (MacNeill 1950, Shaw 1953, Punithalingam 1967) as are the cells of the spore of S. pistaciarum and GI events always involve nuclear division and the maintenance of a uninucleate condition. The formation of a lateral either as a germ-tube or a secondary spore leads to the formation of, in the former a uninucleate mycelium or in the latter a multicellular secondary spore with uninucleate cells. In the spore subsequent nuclear division and septation maintain the uninucleate status of each cell. In other fungi the most frequently observed mode of nuclear behaviour during spore germination is the coincidental formation of the germ-tube and the division of the nucleus/nuclei, and immigration of some of the daughter nuclei into the germ-tube. Uninucleate spores in which this takes place are Aspergillus fumigatus (Baker 1945), Verticillium spp (McGavvceIsaac 1968), Penicillium griseofulvum (Fletcher 1969) and multinucleate spores e.g. Trichoderma viride (Rosen et al 1974), Neurospora crassa (Loo 1976).

In the phragmospore of Fusarium oxysporum each cell is uninucleate and nuclear division and germ-tube formation from individual cells of the spore shows the same phenomenon (Kumari et al 1975). There are few observations of fungi in which more than one division of the nucleus/
nuclei takes place before germ-tube insertion. In the uninucleate species *Aspergillus clavatus* 2-6 nuclei are produced before germ-tube initiation (Baker 1945). In *Fusarium culmorum* Marchant (1966a) showed that up to four nuclei are produced before germ-tube insertion. In the *sporangiospores* found in the *Mucorales* e.g. *Mucor hiemalis*, *Phycomyces blackesleeanus* several nuclear divisions occur before germ-tube insertion irrespective of whether the spores are uninucleate e.g. *M. hiemalis* or multinucleate e.g. *P. blackesleeanus* (Robinow 1957). In multinucleate *simple spored hyphomycete fungi* division occurs prior to germ-tube insertion in *Aspergillus echinulata*, 4--8, (Baker 1945); in the *Acremonium* type of conidia of *Hypocrea pulvinata* 2-3 8-12, (Dixon & Speller 1983). In *Monilia fructicola* germ-tube production occurs before nuclear division (Hall 1963) and only involves the migration of 2-3 nuclei from the multi-nucleate spore. However this is followed by synchronous division of the nuclei and further migration as the germ-tube elongates.

The GI events in *S. pistaciarum* can be equated with the divisions in uninucleate spores, in which several rounds of division occur and the observation of nuclear division and nuclear migration show that germ-tube and nuclear division are associated but there is a wide range of germ-tube size achieved before the nuclei divided. This contrasts with the observation of spore germination in *A. nidulans* (Clutterbuck 1969, Fiddy &
Trinci 1976), Phytophthora drechsleri (Hooly et al 1982), Hypocrea pulvinata (Dixon & Speller 1983) in which a constant relationship was observed between the cytoplasmic volume and nuclear division.

The uninucleate mycelium is similar to species of Verticillium (McGarvie & Isaac 1966) (Puhalla & Mayfield 1974), Fusarium oxysporum (Buxton 1954), F. Culmorum (Punithalingam 1972).

In the mycelium of Verticillium, F. oxysporum, F. culmorum some multinucleate cells have been observed but the spacing and frequency needs to be re-examined particularly in Fusarium spp. It is assumed from the observations on S. pistaciarum that the mycelium of Septoria spp is uninucleate as also shown for S. triciti (Punithalingam 1974).
6.9. The light microscopic studies were supplemented by a study of the ultrastructure of the spore in an attempt to locate features which might reflect the different polarities which were predicted by the interactive model; also to relate the fine structure to other simple and septate spore types.

The fixation of the resting spore was poor. This is a common feature found by other workers and must be related to the slow penetration of the fixative through the spore wall. Shaking with glass beads to crack the spore prior to fixation (Greenhalgh & Evans 1971) did not effect any improvement and observations of internal structures were perforce limited.

The conidial wall resolved into two clearly defined zones, an outer layer 0, an inner layer I. The outer layer was more electron-dense than the inner. In some sections it was possible to identify an electron-dense fine demarcation zone which divided the inner layer, I, into two $I_1$ and $I_2$. This layered appearance of spore walls into 2-3-4 layers is the common observation and is related to the mode of conidiogenesis i.e. to the number of layers in the wall of the conidiogenous cell and whether the spore is holoblastic or enteroblastic in origin, and whether additional layers are laid down during spore maturation.
The hyphal wall of many hyphomycete fungi, with ascomycete connections, resolves into two layers with the inner layer being composed of two. These would correspond to the three layers demonstrated in *N. crassa* as glucan/glucan protein/chitin (Hunsley & Burnett 1970). Holo-blastic conidia are derived from all wall layers and enteroblastic conidia from the inner layer (Kendrick 1971, Cole & Sampson 1979, Sutton, 1980, Minter et al 1982). The wall of the simple conidium of *Botrytis cinerea* has two clearly defined zones D1 and D2 (Gull & Trinci 1971), the arthroconidia of *Geotrichum candidum* has three layers, two derived from the hyphal wall and one inner conidial wall. In *Fusarium culmorum* the wall has three zones, when fixed with permanganate, a central electron-dense zone with a less dense layer on each side (Marchant 1966) and in *F. sulphureum* the wall shows only two layers with glutaraldehyde/osmium fixation, but if the conidium is pretreated with KOH the wall shows three layers (Schneider & Wardrop 1979). The interpretation of the wall layers is clearly seen in the regions adjacent to a cross-wall. In the recent work on conidial and hyphal wall structure in *S. nodorum* (Karjalainen & Lounatmaa 1984) the conidial wall was composed of two layers, an electron-dense outer layer and an inner transparent layer. The author's illustrations show an outer coating which, in TEM sections showed an amorphous layer. In freeze-etched surface preparations this was interpreted as a fibrillar layer
which was perhaps associated with attachment in the host. The hyphal wall was also described as having two layers, but can be interpreted in two ways. Fig 44 A & B1 & B2, B1 shows an outer fibrous layer with three wall layers, with electron-dense material demarcating the second and third layer. In B2 the electron-dense material is regarded as a distinct layer and the wall has four layers. The authors interpret B1 layers 1 and 2 as a single layer, giving a two layered wall. The authors attempt to clarify the interpretation by freeze fracture did not clearly define more than two layers. An alternative approach would be to use sequential enzyme stripping (Hunsley & Burnett 1970) or alkali softening/stripping which might clarify the nature of the electron-dense demarcation zone.

In order to avoid the problem of layer interpretation it has been suggested (Griffiths 1973a & b) that unless fibrillar organisation is known it is more convenient to use the term 'zone' and reject osmophilic demarcation regions as distinct layers.

The main problem in wall zone or layer interpretation is caused by the preparation of the material. Howard & Aist (1979) showed that in the hyphae of Fusarium acuminatum the wall resolved as two layers with conventional fixation, but resolved as four layers with freeze-substitution.
Fig 44 Interpretation of the layers in TEM median sections of the wall of A, the pycnidiospore and B1 & B2 hypha of Septoria nodorum (redrawn from Karjalainen & Lounatmaa 1984).

f - fibrous layer.
1-4 - wall layers.
p - plasmalemma.
The poor fixation of the conidium of *S. pistaciarum* allowed only a limited interpretation of the number and state of the organelles. The individual cells of the spore, each contained a single nucleus; the most abundant feature was the large number of lipid bodies. These were distributed throughout the cytoplasm, in contrast to *F. culmorum* where the lipid bodies were peripheral (Marchant 1966) but similar to *S. nodorum* (Karjalainen & Lounatmaa 1984). The distribution of lipid was the same in all cells, a feature shown by *F. culmorum* (Marchant 1966), *F. sulphureum* (Schneider & Wardrop 1979) and *Diplodia maydis* (Murphy et al 1976). Vacuoles were few in number and irregular in shape and size, again similar to *F. culmorum* and *S. nodorum*.

The septum separating each cell was perforate with associated plugging Woronin bodies. This condition is a characteristic feature of septation in multisepulate euseptate conidia (Cole & Samson 1979) where the perforations in the septa remain open until spore maturation.

With the onset of swelling the most obvious ultrastructure wall changes occurred adjacent to the primary cross-walls. As the spore swelled the apparent euseptation appeared to change to distoseptation (Luttrell 1963) with a clear separation of the outer and inner wall layers and the development of a region of amorphous material, which, in median section appeared triangular. This may represent an annular zone of lysis which would reduce the
rigidity of the spore and allow bending to occur at the primary septum. (Fig 45).

![Diagram of spore germination](image)

**Fig 45** Development of a lytic zone LZ at the primary septum during germination.

A ungerminated spore  
B swollen spore with lytic zone LZ  
C swollen spore with axis curvature.  

(arrow represents the direction of spore axis).

The pronounced bending at each primary septum is always associated with enhanced growth of the cell wall on the convex side of the basic curvature of the spore. A similar response at the septa in other *Septoria* spp would be expected.

The insertion of secondary septa in the germinating (GI) spore showed that the septum was derived from the innermost wall layer, grew centripetally and presented in the young phase a typical undulate profile. This was consistent with the development of septa in hyphae of many
other fungi. If the innermost wall was chitin then the septum will essentially resemble the septum of *N. crassa* (Hunsley & Gooday 1974, Cole & Samson 1979).

In the early stage Woronin bodies are associated with the central perforation as in *F. oxysporum* (Wergin 1973).

A feature of the outer layer of the wall of the swollen spore is the uneven electron-density shown in some micrographs. This may represent new material being inserted into the extending cell wall or change in electron-density as the wall was stretched.

To locate the centres of axis growth fluorescent brighteners were used, which complex with wall polysaccharides and give enhanced fluorescence in regions of active synthesis (Preece 1971).

Photine and Tinopal were used in the present study and both stains showed that though the whole spore wall gave a generalised fluorescence it was possible to detect slightly enhanced brightness at the poles of the cell; all the cross-walls fluoresced brightly, a characteristic feature of fungal hyphae both young and mature (Dixon pers comm). Cells which were producing laterals (GII) fluoresced brightly either the whole cell, or a disc-shaped zone adjacent to the septum.
To further differentiate possible areas of enhanced polysaccharide synthesis, ruthenium red (RR) was used as an indicator of acidic polysaccharides (Luft 1971). RR has been used successfully to locate the secretion and nature of extracellular capsular layers in various bacteria (Cagle et al 1972) and the capsule of _D. uninnucleata_ (Elwy 1980). When RR was used as a prefixation treatment in _S. pistaciarum_ RR + ve material was identified as discrete islets of material dispensed around the periphery of the cell at the cell wall/plasmalemma interface. It appeared that the material was first secreted within the cytoplasm and then exported into _I_2 layer of the wall (Fig 46 A). The fate of the material was not determined but could represent local accumulations which eventually disperse at the surface of the O layer to give external RR + ve fibrils. When RR was incorporated as a post permanganate fixation treatment the outer layer of the wall stained densely but in irregular small patches, but no clear islets of RR + ve material was observed (Fig 46 B).

Regions of the wall in which the initiation of a cross-wall could be predicted showed a diffuse zone of RR + ve material. In the permanganate fixed material the membrane bound organelles and endoplasmic reticulum were well defined but discrete vesicles could not be identified.
Fig 46 Distribution of Ruthenium red (RR) +ve material in the wall of S. pistaciarm.

A RR → Glut/OsO₄ treatment
B KMNO₄ → RR treatment
O = outer layer
I = inner layer
PM = plasmalemma
DI = discrete islets

The preservation of internal organelles was improved as germination began. With permanganate fixation the membrane bound organelles showed the expected structure, the mitochondria showed clear cristae, the endoplasmic reticulum increased in amount, individual vacuoles increased in number and showed a more regular profile. In glutaraldehyde/osmium fixation preservation of organelles allowed their identification and location but the TEM images were of low contrast. The deficiencies of both techniques did not allow clear identification of local vesicle concentrations in areas of the cell where these were predicted to be by the interactive model. The growth of
hyphal and spore walls by the accretion of vesicle-bound material being transported to regions of growth (Bartniki-Garcia 1973) was assumed to be valid for the model and it is unequivocal that in hyphae the apical cluster of vesicles is involved in apical growth (Gooday 1983). This cluster is present in growing hyphae and absent in non-growing hyphae. The definitive work of Howard and Aist (1980) on the hyphal tip of *Fusarium acuminatum* shows an apical cluster of vesicles 70-90 mm in diameter enclosing a cluster of microvesicles, 30 mm diameter and microtubules and subtending a dense cluster of ribosomes. This apical aggregation is the "spitzenkorper" of Girbardt (1969) and the sequence of event leading to hyphal growth suggests that vesicles are derived from port-apical dictyosomes, migrate to the apex and fuse with the plasma membrane. The contents of the vesicles contain matrix material, enzymes for maintaining wall elasticity and polymerases (Bartniki-Garcia). The only vesicle-like structures were found in the tips of lateral (GII) secondary conidia. Microtubules were not visualised.

The work of Howard & Aist (1980) shows that freeze substitution would be a preferred technique to reveal vesicle and microtubule distributions. An additional technique to detect regions of enhanced wall synthesis would be the use of a pulse of labelled wall precursors added to germinating spores, followed by autoradiography. The materials selected could be $^{3}\text{H}$ glucose to show apical
or polarised incorporation or N-(\(^3\)H)-acetyglucosamine to show septal incorporation (Gooday 1971, Hunsley & Gooday 1974). Counts of silver grains and estimates of the distribution and numbers of vesicles would enable some parameters to be added to the model. Such estimates have been made for hyphal growth in *N. crassa* and *Penicillium chrysogenum* in the former 38,000 vesicles/min fused with the plasma membrane, in the latter only 480 (Collinge & Trinci 1974), Collinge et al 1978).
6.10. The formation of the secondary conidium showed clearly that the conidial wall was derived from the inner layer of the wall of the conidiogenous cell, i.e. entero-blastic (sensu Kendrick 1971, Cole & Samson 1979, Sutton 1980, Minter et al. 1982). The abstriction scar was formed of a raised collar composed of the ruptured outer layer which surrounded the remaining single layer of the initially duplex spetum (Fig 47 A-D). The spore secession was two-celled.

Fig 47  Diagrammatic interpretation of secondary spore formation in S. pistaciarum.


O = outer layer. I = inner layer. W = Woronin body.
If the conidiogenous cell continued to produce secondary conidia from the same locus then the conidiogenous cell could be regarded as a phialide. The concept of the phialide has in recent years been redefined (Kananaskis I 1971). Minter et al (1983) has surveyed the range of conidial production from 'phialide-like' cells and decided that the term 'phialide' was ambiguous and could only be used as a general descriptive term. Conidiogenesis, sensu Minter et al (1983) is regarded as an entire process, i.e. conidium ontogeny, delimitation, secession, proliferation and regeneration; rather than placing the whole emphasis on conidium ontogeny (Minter et al 1982). In the present work only ontogeny, delimitation and secession has been studied for the first formed conidium at any one locus. The subsequent events at the locus have not been identified and whether more than one secondary conidium is produced at the same locus requires further investigation. The secondary conidia were only observed up to the two celled secession stage, whether further septation occurs during subsequent culturing was obscured by the onset of GI germination event, i.e. axis growth. Secondary conidial production at a similar locus adjacent to the transverse septa has been shown in S. passerini (Green & Dickson 1957).
6.11. The relationship in plant disease between the host and pathogen based on the host-pathogen interface was discussed by Bracker & Littlefield (1973) and they considered that at any one time or place the interface may consist of any or all of the following:

(i) Normal host cell components (i.e. those in a form and functional state characteristic of a healthy or non-invaded cell).

(ii) Normal components of the pathogen (symbiont) cell.

(iii) Structures of host or pathogen that are modified as a result of the interaction.

(iv) Intimate combinations or mixtures of material or structures derived from both host and pathogen.

(v) Newly formed structures or materials that are not normally present in either host or pathogen when either organism is grown separately.


The basic interface was regarded as a post entry property of the disease syndrome. In a discussion of the development of parasitic conidial fungi in plants, Aist (1981) considered the whole process i.e. ingress, ramification and egress leading to propagule production. A pathogen which gains entry by active penetration of a plant surface could be considered to have an interactive phase which will begin at spore germination.

Germ tubes produced by germinating conidia of different fungi show various modes of entry. In many pathogens
direct penetration by a germ-tube of the cuticle and outer wall of the epidermal cell occurs. McKeon (1974) in an elegant study of the penetration of epidermal cell walls of *Vicia faba* by *Botrytis cinerea* showed that a conidial germ-tube was attached to the host surface by a mucilage pad. A pore was formed at the tip of the germ-tube and allowed the plasmalemma to be appressed against the cuticle. The cuticle appeared to be enzymically degraded and the germ-tube advanced as a narrow infection peg. This expanded into the lumen of the epidermal cell as an infection hypha from which intracellular hyphae were produced. The classic work of Brown & Harvey (1927) suggested that penetration was mechanical and it is assumed that a combination of enzymic softening and mechanical force are involved in breaching the cuticle and epidermal cell walls.

Many plant pathogenic fungi gain entry by cuticular/epidermal cell penetration following the development of an appressorium at the tip of a germ-tube. An appressorium is either a simple direct swelling of the germ-tube tip or a lobed branched structure; plane contact surfaces are formed between the cuticle and the appressorium. The two surfaces are firmly attached by a mucilagenous gum and a penetration peg is formed at the contact surface (Emmett & Parberry 1975). Subsequent events may depend on whether the fungus penetrates through the cuticle and then ramifies sub-cuticularly or penetrates further. The classic example of entry and sub-
cuticular ramification is shown by Venturia spp. V. inaequalis on apple (Nasbaum & Keitt 1936), V. rumicis on Rumex sp. (Kerr 1966). Penetration of epidermal cells from appressoria is a feature of the genus Colletotrichum. The appressoria are heavily melanised and experiments using inhibitors of melanin biosynthesis have shown that in C. lindemathianum if melanisation is inhibited penetration of a Formvar film is prevented (Wolkow et al 1983). A feature of the penetration of the epidermis of Lettuce (Lactuca sp.) by Bremia lactucae is the adhesion of an appressorium at the depression formed at the junction of two epidermal cells but the penetration peg does not force a way down between the anticlinal walls but drives through the adjacent outer periclinal wall. (Sargent et al 1973). Chou (1970) showed penetration of anticlinal walls by Peronospora parasitica.

Penetration of stomata either by direct germ-tube penetration or following appressorial development is a common mode of penetration by many phytopathogenic fungi. This type of penetration is a feature of the rusts. In Puccinia graminis on wheat (Allen 1923) the germ-tube grows over the surface with directional growth until a stoma is reached. An appressorium is produced and a penetration peg pushes down between the guard cells. A sub-stomatal vesicle is formed, from which arises the intercellular mycelium. Feeding haustoria are produced after local penetration of the mesophyll cell walls. Most powdery mildews do not form an intercellular mycelium
but remain as a surface mycelium with feeding haustoria in the epidermal cells as the only host penetration; however in *Leveillula taurica* on green pepper, which forms an intercellular mycelium germ-tubes form an adhesion body from which arises an infection hypha which then grows as a secondary germ-tube before direct penetration of a stoma. (Kunoh et al 1979). In the phragmosporous fungus *Cercospora beticola* two modes of stomatal penetration were observed (Rathaiah 1976). When the stomata were closed an appressorium was formed, when the stomata were open penetration was by direct germ-tube growth. Rathaiah (1977) shows a twin pair of germ-tubes produced from the central region of the spore penetrating a single stomatal aperture. The two modes were correlated with night or daytime infection. Directed growth of germ-tubes has been shown in *Scirrhia acicola* on pine (Patton & Spear 1978) in which direct stomatal entry is effected without an appressorium being formed and also for *Dithiostroma pini* (Peterson & James 1978).

The germination of the conidia of *S. pistaciarum* showed that germ-tube growth could lead to rapid location and direct penetration of a stoma, or to a rather diffuse branching superficial hyphal system with some hyphae growing over stomatal apertures, eventually some hyphal tips showed direct penetration. There was no evidence of appressorial development or direct entry by cuticular/epidermal penetration. In other species of *Septoria* stomatal penetration has been shown. In *S. passerini*, on
barley, Green & Dickson (1957) showed infection from primary and secondary two-celled conidia by germ-tubes produced from either the ends or central region of the spore; no appressoria were observed though a period of up to 72 hours was required for stomatal penetration. Covey (1962) made similar observations on S. linicola in which 36 hours was required for direct stomatal penetration. In contrast the penetration of the cereal host by S. nodorum is by epidermal penetration from appressoria. The infected cell shows lignification and cell collapse (Bird & Ride 1981). In the other important Septoria pathogen of cereals, S. tritici, conidia germinate most frequently near or on stomatal cells. Germ-tubes penetrated either directly through the stomata or rarely through the epidermal cells, without forming appressoria. Penetration took 24-36 hours (Peresypkin & Kovalenko 1981).

The direct penetration of stomata by S. pistaciarum was similar to S. linicola and S. passerini but different to S. nodorum and S. tritici. Though no long distance directed growth of germ-tubes was observed towards stomatal apertures but since growth into a stoma was the only method found it is assumed that short distance directed growth must occur. These results and the absence of appressoria suggests that there are several modes of entry within the genus Septoria and mode of entry may be another useful criterion for classification. The failure to effect infection of P. vera in young leaves must be
related to the failure of germ-tubes to penetrate stomatal apertures. This is probably related to the apparent immaturity of the stomatal apparatus in young leaves and a more detailed examination of stomatal maturation in *P. vera* and should be undertaken. It would be of interest to explore infection of young leaves by other *Septoria* spp with stomatal penetration in relationship to stomatal maturation.

A similar response was shown by *Pseudoperonospora humuli* on young hop leaves. In hop the young stomatal aperture is closed by a layer of cuticle which ruptures during maturation to expose the stomatal vestibule surrounded by a clearly defined rim. Penetration of stomata, with the cuticuled layer complete, did not occur (Royle & Thomas 1971).

The stomatal penetration by *Septoria* spp and *Cercospora* spp, with tropic responses towards stomatal apertures, imply that with excessive leaf moisture, when the whole leaf surface is wet, such tropisms will not be shown (Wynn 1981). Random growth of germ-tubes under such conditions is shown by *C. beticola* (Rath 1977) *C. musae* (Meredith 1970) and *C. zeae-maydis* (Beckman & Payne 1982). The conditions under which the infection of *P. vera* by *S. pistariarum* were established probably maintained an extended period of leaf wetness. In liquid culture the conidia show long spore axis growth and this response can be demonstrated in conidia washed off from the leaf after 13 hours. In *Septoria*
such long axis growth may replace random germ-tube growth and the concentration of local germ-tubes produced by the sporeling would be an efficient way to effect stomatal entry. If the local humidity decreases during the latter part of this process directed germ-tube tropic growth should lead to multiple penetration of a stoma. To explore this possibility an SEM study of germination of spores at various humidities should be undertaken both in vitro and in vivo.

6.12. Ramification of fungal phytopathogens represents the principal parasitic phase of the parasite (Aist 1981). Mycelia can be located either in an epicuticular, subcuticular or intra and/or inter cellular position.

The most important groups of pathogens producing an epicuticular mycelium are the powdery mildews. Penetration is by feeding haustoria in the epidermal cells (Bushnell & Gay 1978) and the surface mycelium ramifies as a discoidal colony around the point of infection. This group is clearly separate from those fungi in which a certain amount of mycelium is produced on the surface, often not as a preliminary phase of growth but a secondary phase from which will arise the reproductive structures. The most extensive of these surface developments is shown by the ectostroma formed as an investment of the ovary in ergot infection of grasses. The surface growth is initiated from an intercellular mycelium (Luttrell 1977).

Ramification in a sub-cuticular position is common but is often only a prelude to extensive intercellular ramification within the host tissues. In the conidial
fungus Gloeocerospora sorghi penetration is by cuticular penetration leading to sub-cuticular mycelium from which arises an intercellular mycelium. Stomatal penetration may also occur whether stomatal penetration leads to sub-cuticular growth as well as intercellular growth was not shown (Myers & Fry 1978). Other fungi in which limited sub-cuticular mycelium is formed are B. cinerea on V. faba (McKeen 1974) and Colletotrichum gloeosporioides on orange (Brown 1977).

The major pathogens in which an extensive sub-cuticular mycelium forms the vegetative phase are Venturia spp and Diplocarpon rosae. In Venturia sp the mycelium forms a large sub-cuticular stroma but without host cell penetration, whereas in D. rosae haustoria are inserted into the host epidermal cell (Aist 1981).

Intercellular ramification occurs in many phytopathogens either with the development of haustoria, as in the rusts and downy mildews or as hyphae which grow in the intercellular spaces with intermittent contact between hyphae and host cells. The physiological results of these two types of growth are in the former a biotrophic response in which the host cells do not die until late in the infection cycle; and in the latter a necrotrophic response in which after a short interval host cells collapse and die.

In the rusts and downy mildews, an intercellular mycelium with feeding haustoria is the normal mode of
growth. In the rusts colony size varies from minute, with only one suprastomatal sorus to mark the small infection e.g. Desmella aneimiae; through _Hemileia vastatrix_ with an expanding radial thallus of thin primary marginal hyphae and thicker 'feeder' hyphae in the older parts of the thallus, from which arise a series of interconnected suprastomatal sori; to the larger spreading thalli of _Puccinia graminis_ and _P. recondita_ in which a young circular thallus occupies the entire depth of the leaf and produces large sub-epidermal sori. As the colony enlarges concentric rings of sori are produced. The amount of tissue occupied is small, 1% of the leaf volume was occupied by hyphae and 60-70% of the mesophyll cells were occupied by haustoria in _H. vastatrix_ (McCain & Hennen 1984).

In necrotrophic lesions two types of invasion are possible: a) where intercellular hyphae produce branches which invade host cells intracellularly, but which are not specialised feeding haustoria and b) where all the hyphae are intercellular. An example of the former is the necrotic chocolate spot of _Vicia faba_ caused by _Botrytis fabae_. After infection and abaxial epidermal cell penetration an intercellular mycelium was developed within the mesophyll. The intra cellular invasion of the mesophyll was not common but penetration along the middle lamella of adjacent mesophyll cells was common. Only three days was required for occupation throughout
the thickness of the leaf and the adaxial epidermal cell walls were swollen and occupied by hyphae. A typical feature of this pathogen was the induction of swollen cell walls in advance of the mycelial front. The hyphae in the intercellular space were often surrounded by fibrillar material which adhered to the host cell walls (Mansfield & Richardson 1981). This type contrasts with the advance through tissues of vascular wilt pathogens. Bishop & Cooper (1983) describe the penetration of tomato roots by Fusarium oxysporum f.sp lycopersici (FOX) and Verticillium albo atraum (VA-a). In these there was local degradation of host cell walls where intercellular hyphae were attached to the host cell walls by means of a mucilaginous sheath. Other hyphae ramified down the middle lamella and short lateral branches penetrated adjacent host cells through a small pore. In these two fungi no degradation action in advance of the mycelial front was observed. A marked difference between the two fungi was found in the mode of intra-cellular occupation. FOX was frequently found in necrotic moribund host cells and VA-a was found in healthy host cells but were essentially extracellular since they were surrounded by host plasmalemma. In FOX, f.sp. radicis-lycopersici (FORL) Charest et al (1984) have shown that though the basic inter/intra cellular relationships were similar to FOX, in some cells where a host cell wall had been thickened by apposition or papilla formation these wall thickenings were breached by the enzymic activities of the pathogen.
A typical leaf-spot in which intracellular penetration is confined to moribund cells is shown by *Cercospora beticola* in leaves of *Beta vulgaris* (Steinkamp et al 1979). The infection was by stomatal penetration and after 5 days a sparse intercellular mycelium was produced. The hyphae traversed the intercellular spaces and were attached to mesophyll cells, in young lesions, by a very small deposit of granular matrix material which in older lesions increased in amount until a thick layer was formed.

In tomato leaf blight caused by *Cladosporium fulvum*, Lazarorits & Higgins (1976) showed that after stomatal penetration intercellular hyphae spread throughout the leaf tissue with early colonisation of the mesophyll and late colonisation of the palisade region. The hyphae were closely appressed to the host cell wall and a localised deposit of electron-dense material was formed at points of contact. This development of adhesion pads or complete hyphal investments is a feature of other intercellular pathogens. Net blotch of barley caused by *Pyremophora teres* (Kean & Hargreaves 1983) developed an intercellular mycelium after penetration by appressorial/penetration peg of the epidermis. An intracellular vesicle was formed in the cell and an infective hypha penetrated the lower wall of the epidermal cell; all subsequent mycelium was intercellular. The contacts between hyphae and host cell walls showed an electron-dense granular vein of material similar in appearance to the very substantial hyphae sheaths shown
by Wheeler & Ganz (1979) produced by Helminthorium spp. In the latter however a similar sheath was found on hyphae grown in distilled water and may not be part of the attachment system apparently developed de novo between host and pathogen observed in other combinations.

In Septoria infections there are limited accounts of mycelial distributions in infected tissues. In all the mycelium is distributed intercellularly once either epidermal or stomatal penetration has occurred, and there are no reports of intracellular occupation. In S. passerini on barley (Green & Dickson 1957) the mycelium is sparse and confined to the mesophyll. In S. nodorum on wheat, after epidermal penetration, the mycelium was sparsely distributed in the mesophyll (Bird & Ride 1981). This is in contrast to S. triciti in which lesion is slow but 12-13 days after infection all the intercellular spaces were filled with mycelium (Peresypkin & Kovalenko 1981).

It has been suggested that the sparse mycelium of S. nodorum is attached to the host mesophyll cells by the fibrillar material which is apparent as an external layer on the intercellular hyphae, but the published illustrations (Kajalainen & Loutma 1984) do not show a significant pad of material at the point of contact.

The two Septoria spp on P. vera show some similarities to other Septoria spp. The mycelium in S. pistaciarum is comparatively sparse in its distribution whereas S. pistacina is densely distributed. The hyphae
cross intercellular spaces in both species though the branches in *S. pistacina* are short-order branches in contrast to the long-order branching in *S. pistacierum*. Both species show deposits of matrix material at points of contact. In *S. pistacierum* the amount is large, often forming a discrete pad and in some cases formed a complete sheath. In *S. pistacina* the amount was less and never formed a complete sheath. The nature of the material is not known but may be part of a host/pathogen interaction product. In other fungi this material has been variously identified as being either polysaccharide particularly in the case of pathogens producing degradative polysaccharidases, or proteinaceous or glycoproteins.

The variation shown by a series of leaf spots indicate that there are no generalisations that can conveniently be made to relate the amounts of mycelium which lead to the development of the disease syndrome. The use of the term 'sparse' is unfortunately not precise but must be regarded as a function of how much intercellular space is occupied by hyphae. Large intercellular spaces with few hyphae would be regarded as 'sparse'. Small spaces with the same amount of hyphae would be estimated as dense. In comparing the two *Septoria* spp in *P. vera* the comparison is valid though only on a two point scale. An additional method of comparison would be to estimate fungal biomass/unit tissue volume using a chitin assay. Fungal cell walls
after hydrolysis release N-acetyl glucosamine, the monomer of chitin, which can be assayed. Though fungal hyphae show variation of chitin levels with age, the method would enable a more precise estimate to be made (Sharma et al 1977).

6.13. The result of fungal invasion by a necrotic pathogen is the eventual death of the host cells leading to the external symptoms of necrosis. The rapidity of lesion formation depends on the death rate of individual cells and eventual tissue disorganisation. It would be expected that pathogens which invade by cell separation and intracellular invasion would be more effective than those in which tissue coherence is maintained. However if host cell death is due to pathogen produced toxins then that death could be a very rapid phenomenon.

Typical of necrotic disease in which pectolytic activity is involved in necrosis are the brown rots of apple and pear caused by Monilia spp (Byrde & Willets 1977). In these the mycelium is intercellular including middle lamella tunnels, and leads to massive middle lamella breakdown and accumulation of the products of breakdown as an electron-dense deposit in the intercellular spaces. The wall adjacent to hyphae is deformed and stained unevenly, indicating some enzymic breakdown. The principal response of the host cells is shown by protoplast collapse, though the protoplast membrane remains intact. The membrane however is non-
functional since electrolytes leak from the cells. Intracellular hyphae eventually penetrate into the space left by the shrunken protoplast. The browning reaction is caused by the oxidation of host phenols by host polyphenol oxidases due to the 'decompartmentalisation' of the protoplast.

Membrane responses and disruption are the first signs of physiological disorder in necrotic lesions. The endomembrane concept (Morre & Mollenhauer 1974) describes a functional and developmental continuum of internal cytoplasmic membranes in which the nuclear envelope, endoplasmic reticulum, dictyosomes, secretory vesicles, vacuole membranes, plasma membrane and outer membranes of mitochondria and chloroplasts are all associated (Bracker & Littlefield 1973) (Arias et al 1983).

The barley mesophyll reaction to P. teres was shown two days after inoculation. Host mesophyll cells showed various degrees of disruption. The initial events in the death of the cells appeared to be vesiculation associated with the tonoplast followed by disruption of this membrane. Following the loss of tonoplasts integrity the chloroplasts swelled and the grana showed vesiculation. With the disintegration of the chloroplast membrane the contents were released into the cell lumen. Serial sections showed that hyphae bridged intercellular spaces and that host cells which were undergoing disorganisation were in close contact.
with fungal hyphae. This implies that cells which were not in close contact were not responding in the same way i.e. the cells in the lesions were not behaving in a synchronous manner (Keon & Hargreaves 1983).

A similar lack of synchrony was shown by tomato leaf tissue infected with C. fulvum. Variation in rate of the response was also shown by various cultivars which had various degrees of resistance (Lazarovits & Higgins 1976b). A common feature in all combinations was the increase in crystal containing microbodies and lipid bodies. Susceptible host/pathogen combinations showed a minor response until pathogen sporulation was well established i.e. a tolerant host reaction. An interesting feature of host cell membrane response was the association of a strand of host ER parallel to the host plasmalemma in the region of fungal contact. The major ultrastructural changes only occurred in the mature lesion and here the tonoplast and plasmalemma showed many breaks, the chloroplast envelope became disrupted and the grana were dilated. Starch levels increased and occupied a large portion of even the damaged chloroplasts. Extra-cellular material was accumulated as a granular matrix with an electron-dense outer layer. The resistant combinations showed a rapid disorganisation of host cells around the site of infection with same final ultrastructural changes as in the susceptible combinations. The epidermal cells and stomatal guard cells showed necrosis and collapse in the centre of a resistant lesion.
The lack of synchrony of host response has also been observed by Steinkamp et al. (1979) in beet leaves infected with *Cercospora beticola*. In young lesions the first effects were shown by an increase in small vesicles and swelling of the ER, the mitochondria developed holes and thickened outer membranes. As the infection progressed the plasmalemma and tonoplast became disrupted and in the chloroplasts an increase in the number and size of the plastoglobuli was found. Other chloroplasts showed breakdown of the grana membranes. Adjacent to hyphal/cell contacts wall appositional material was laid down between the cytoplasmic remains and the inner surface of the host cell walls. The appositions were composed of electron-dense droplets, vesicles and membrane fragments in an electron-lucent ground material. An unusual feature of the final stage of necrosis was complete host cell collapse with loops of folded wall though breaks were rarely seen. Large amounts of extracellular material was accumulated in and occluded the intercellular spaces. The nature of the material was not identifiable but it did not react with callose reagents and was interpreted as probably of pectin origin.

In *Septoria* leaf spots there has been little ultra-structural investigation of host cell response. Bird & Ride (1981) have shown that the intercellular mycelium of *S. nodorum* in wheat induces collapse of the mesophyll cells with degeneration of the cytoplasm. Associated with the collapse was extensive lignification.
of the degenerating cytoplasm and accumulation of extracellular deposits and in the cell walls. The same lignin response could however be induced by wounding and suggests that this response is comparable to the wound response found in many host/pathogen combinations as a result of microbial penetration and resultant mechanical damage (Vance et al. 1980). Karjalainen & Loutmaa (1984) have initiated a further study of S. nodorum on wheat and the work published so far, has only examined the structure of spores, hypha, and the host/hyphal contact surface. The one illustration to show a host cell showed that the host plasma membrane appeared to form a layer of irregular thickness still in contact with the host cell wall.

The two pistachio leaf spots both show some of the necrotrophic features shown by other combinations. Both are characterised by lack of host synchrony and adjacent cells show massive disorganisation in one and apparently normal cytoplasm in the neighbouring cell. In both the plasmalemma and the tonoplast showed signs of vesiculation and infolding with the tonoplast showing more response. The major difference between the two spots was the massive accumulation of electron-dense deposits on the vacuolar face of the tonoplast in S. pistacina infections.

This is similar to the droplets developed in infections of Vicia faba by Pseudomonas phaseolicola.
Inoculation of monofoliolate leaves by the bacterium led to the formation of lesions in the trifoliolate leaves. The first effect of infection was an increase in starch and the chloroplasts became very swollen. There was an increase in ER and layers were formed in parallel to the host cell wall. Three major vacuolar responses were found: amorphous matrix material accumulated in the vacuole, densely fibrous matrix material lined the tonoplast membrane, and dense globular coalescent droplets formed on the inner face of the tonoplast. The identity of these deposits was considered to be phenolic in origin though the material did not react with phenolic reagents (Lallyett 1977). *P. phaseolicola* produces a phytotoxin, phaseotoxin, and the early responses in the trifoliolate was considered to be evidence of toxin translocation from the site of inoculation and primary lesion formation.

The chloroplast changes in both pistachio leaf spots were of the same type and order as observed in other host/pathogen combinations. The increase in starch content was difficult to quantify since the amounts appeared to be within the normal variation of control tissue. The same problem was found in *C. beticola* on Beet (Steinkamp et al 1981a). The increase in plastoglobuli was clearly seen as was the increase in chloroplast size. The increase appeared to be due to separation of grana membranes with a concomitant increase in the amount of matrix plus the starch increase.
No wall appositions were found but in some sections the wall adjacent to a point of hyphal contact could be interpreted as having an irregular profile, particularly on the inner face, which might represent a small deposit of material. The intercellular material in the form of small granules within a limiting layer is similar to that found in other combinations. It is unlikely to be pectic or wall material since no middle lamella or wall damage was observed and is probably phenolic in origin. The blackening of the lesion suggests that polyphenol oxidases are active in the lesion and some of the material might accumulate in the intercellular spaces. It could be assumed that the capacity of the two pathogens to degrade pectin in vitro would be limited if not absent. In necrotic pathogens in which middle lamella breakdown is found, pectolytic activity is usually high (Wood 1967). A comparative study of polyphenol oxidase activity in vivo and in vitro of the two Septoria spp might reveal differences in phenolic processing which could account for differences in tonoplast droplet accumulation and intercellular deposits.
6.14. The ultrastructural changes in necrotic diseases in which pectin and cell degrading polysaccharidases are involved have a different aspect to those in which no apparent host cell wall responses are found. The necrotic lesion due to cell death and possible collapse must be related to some other feature of pathogen metabolism and in many instances a phytotoxin is involved. The molecules of phytotoxin can either be required for pathogenicity i.e. an ability to cause disease (qualitative), or for virulence i.e. the amount of disease (quantitative) (Yoder 1980).

Toxins can be grouped into three classes: (i) host specific toxins (HST); (ii) selective toxins (ST); (iii) non-specific toxins (NST). HST have high biological activity to the specific host whereas NST have an effect on plants in general.

Examples of HST are found in the Alternaria alternata complex of six pathotypes (Nishimara & Kohmoto 1983). Each pathotype produces a unique phytotoxin which is active only against the precise host. AM toxin produced in blotch of apple will not affect tomato. AL toxin produced in tomato stem canker will not affect apple. The HST are produced in cultures and can be isolated as pure substances. AM is a cyclic depsipeptide with symptom induction in concentrations as low as $10^{-8} \text{M}$. AL is a $C_{17}$ long chain molecule with 2 ester side chains on $C_{13}$ and $C_{14}$ and activity is shown at 25 ng/ml.
As well as HST production several NST are produced: alternariol, a polyketide pyrone; tentoxin, a cyclopeptide. The former cause necrosis and chlorosis, the latter, chlorosis on several plant species. Tentoxin can easily be produced in high yields (50 mg/l) in fermenter culture (Lieberman & Oertel 1983). An NST tenuazonic acid produced by A. alternata is also produced by other phytopathogens, Phoma sorghi and Pyricularia oryzae (Iwasaki et al 1972). The biological activity of Ca++ tenuazonates are responsible for acute haemotologic disorders in people eating sorghum infected with P. sorghi (Steyn & Rabie 1976). The brown rot pathogens also produce NST e.g. monilidiol, an octaketide produced by M fruticola (Sugiyama et al 1983).

Several necrotic diseases of graminaceous hosts have been shown to produce HST. Species of Helminthosporium involved are H. victoriae on oats, H. carbonum on maize, H. maydis on maize, H. sacchari on sugar cane and Periconia circinata on sorghum (Yoder 1980). Synthesis of the H. maydis toxin has shown that C41 analogs are as active as the natural product, shorter chains are less effective (Suzuki et al, 1983).

It has been demonstrated that the toxins, both HST and NST, induce electrolyte leakage and disrupt cyto-membranes i.e. consequences of altered membrane permeability and also alter membrane bioelectric properties.

Park et al (1977) attempted to correlate changes in
permeability of apple tissue infected with *A. alternata* (apple pathotype) with changes in ultrastructure. The primary effect of the toxin was to cause invagination of plasma membranes and extension of the desmotubules from plasmodesmata; subsequent vesiculation and fragmentation of membranes occurred. Chloroplasts were also involved in the reaction and appeared to be a positive correlate with activity. Apple petals showed no necrosis but apple leaves became necrotic on treatment with the toxin, and chlorophyll levels were reduced in susceptible but not in resistant leaves. In addition, cell wall degradation was observed in both apple with AW toxin and pear with AK toxin. The changes in chloroplast structure were further examined (Park et al 1981) and after 1 hour of treatment disrupted membrane material derived from the grana were found within the swollem chloroplasts. The changes increased up to 12 hours of treatment.

The rapid onset of symptoms similar to the necrosis observed over several days in fungal infected tissue is a typical result of substituting toxin in place of the host/pathogen interaction i.e. a shortening of the response time from days to hours. The disease syndrome however may be the total interactive effect of all the HST and NST produced in vivo as well as host response to the other metabolic activities and demands of the pathogen. The advantage of using toxins in precise quantities either singly or in mixtures to describe the necrotic process might help model the host/pathogen interaction and establish some quantitative measures of the amounts of toxin.
secreted in vivo.

The production of toxin by *H. saccharum* leads to a runner or streak of necrotic tissue being formed away from the site of mycelial infection. This streak is also typical of *H. maydis* blight of maize. The addition of the toxin, helminthosporocide, a B galactofuranocide of a sesqui-terpenoid, to sugar cane leaves mimicked the disease syndrome. The first signs of chloroplast membrane response was observed after 1 hour after addition of the toxin. The granal membranes disassociated and lost identity, their position being filled with an electron-dense deposit of disorganised membrane and the whole chloroplast swelled. Both the bundle sheath and mesophyll chloroplasts showed lamellar disorganisation. After twelve hours the plasmalemma was separated from the cell wall. These symptoms were the same as those induced after three weeks by the pathogen (Strobel et al 1972). The symptoms induced in barley by toxins from *Helminthosporium teres* showed similar effects but the plastoglobuli increased in number and size and starch increased (Barrault et al 1982).

The most detailed study of an HST necrosis was of the effects of PC toxin from *P. circinata* on root tips of sorghum seedlings (Arias et al 1983a). An analysis was made of membrane changes by a morphometric technique using a set of serial sections through 3-4 root cap cells and measuring the amount of membrane and number of organelles in 100 μm³. The technique allowed a quantitative
estimate to be accurately made and allowed estimates of changes induced by brief (0.25 hr) toxin treatments (Arias et al 1983b). The feature of host cell response was a rapid decline of secretory activity and accumulation of material between the plasmalemma and the cell wall. Longer treatment (2 hrs) led to increased vacuolation, loss of starch, increased number of lipid bodies and stacked endoplasmic reticulum. The mitochondrial membranes remained normal but the tonoplast developed lesions before autolysis. The tonoplast showed tears with KMnO₄ fixation but not with glutaraldehyde/KMnO₄ fixation. Though this could be regarded as a fixation artefact it also reveals an essential tonoplast weakness and fixation only with glutaraldehyde/KMnO₄ would not reveal such a weakness.

The use of the morphometric technique would allow accurate comparisons to be made, whereas comparisons made on non-serial sections would allow accurate comparison only for features showing presence or absence.

The NST, cercosporin (Baylis & Payne 1971) a perylene quinone, produced by C. beticola, reproduced most of the necrotic symptoms caused by the fungus except that there were no electron-dense bodies in the vacuoles, the necrotic remnants lacked a well-defined boundary zone and the plastoglobuli did not increase in size or number (Steinkamp et al 1981b).

In the genus Septoria one NST mellein (ochrasin)
has been identified as di hydro-3, 4 hydroxy 8-methyl-3-isocoumarin and has been isolated from cultures of S. nodorum (Kent & Strobel 1976). Activity has been shown against mitotic index and 'S' phase labelling in root tip cells of wheat (Essad & Bousquet 1981) to reduce net assimilation of CO₂ and increase stomatal resistance (Bethenod et al 1982) and also to be positively correlated with aggressiveness in various isolates of S. nodorum (Griffiths & Ao 1980).

No attempt has been made in this study to explore the possible involvement of either an HST or NST in Septoria leaf spots of pistachio, but the histopathology suggests that these diseases conform to the general pattern of toxin induced host responses and that two different toxins might be involved. An investigation to explore whether phytotoxic substances are produced by the pistachio Septoria spp, and other Septorias would allow comparison with the production of mellein by S. nodorum to be made. In Cercospora, a large genus of phytopathogens comparable to Septoria, several species of the 1200 described species have been examined for cercosporin production. Some species do not produce cercosporin under the conditions of culture used and in others different strains show large, small or zero amounts (SteinKamp et al unpublished). If a similar situation exists in Septoria the use of toxin production as a possible taxonomic tool may be of some use, but if strain variation is great it's utility may be reduced.
Variation in the genus *Septoria* has been shown only in *S. nodorum*, in which each compartment of the conidium is uninucleate and is derived from a uninucleate conidiogenous cell (Shaw 1953). Variation in pathogenicity and cultural characteristics has been shown between single spore isolates from the same pycnidium and between a set of serial single spore isolates derived from a culture initiated by a single spore (Scharen & Krupinsky 1970). Griffiths & Ao (1980) showed that serial single spore isolates gained stability in cultural characters by the fourth serial transfer but pathogenicity showed considerable variation up to six transfers. The mechanism, other than mutation of either genetic or cytoplasm loci, whereby culture derived from a single haploid nucleus show such major variation has not been elucidated.

6.15. The formation of the pycnidia within the lesions caused by *S. pistaciaeum* appears to be initiated by sub-stomatal byphae. Many pathogens initiate spore production in such a position. In the rusts the uredinial stroma forms in a sub-stomatal position; whether the resultant output is confined to a few suprastomatal hyphae as in *H. vastrix* to the large sub-epidermal sori of *P. graminis* (McCain and Hennen 1984). A similar sub-stomatal stroma develops in *Cercospora zeae-maydis* in maize leaves and in other *Cercospora* spp. (Beckman & Payne 1982). In *Septoria* sub-stomatal hyphae are produced which penetrate
the stomatal aperture and form a tuft of external hyphae which presumably marks the site of pycnidal initiation. Many Sphaeropsidalean fungi form pycnidal initials in the sub-stomata spaces (Zeyen & Shearer 1974) and Punithalingam (1966) demonstrated this mode of formation in four species S. chrysanthemella, S. obesa, S. leucaanthemi and S. socia. All species formed pycnidia by meristogenously, i.e. repeated division and growth of adjacent cells of a single hypha. The small knot of hyphae so formed enlarge into multicellular globose solid structure. The outer layers become thickened and melanised. A pycnidal cavity, ostiole, and pycnidiospore are then formed. The pycnidia of S. avenae and S. nodorum (Zeyen & Steyn 1974) were prepared by vacuum infiltration with water and Tween 80 to remove the mucilage in which the spore mass is embedded within the pycnidium. TEM sections of a mature pycnidium showed that approximately 40-50% of the cavity is filled with mucilage in which the spores were embedded. Resin embedded material was sectioned and prepared for SEM observation and the method revealed clear details of the form of the pycnidal cavity. The spore cirrhus was removed from the leaf surface by this treatment. The critical point drying techniques for SEM preparation used in the present study would undoubtedly lead to the production of artefacts. In an extensive investigation of preparative techniques for the examination of the external morphology of fungal material with the SEM Read & Beckett (1983) suggest that the examination should be conducted using several techniques.
with samples in the frozen-hydrated (FH) state being used as a base line. Particular problems exist however where mucilage is involved in the structure.

6.16. Two major lines of investigation emerge: (i) the behaviour of the spore axis during germination in other Septoria spp GI events; coupled with a study of the factors which govern lateral production; GII events. Preliminary experiments on cell cycle events during the first round of axis division have involved the use of hydroxy urea (HU) to block DNA synthesis (Timson 1975) and benomyl to block tubulin formation during mitosis and prevent nuclear division after the S phase (Hassall 1982). The results suggest that cell growth is coupled to DNA levels to maintain a constant nucleo/cytoplasmic ratio and cell growth is inhibited by HU, with benomyl, at concentrations which prevent colony formation, cell enlargement occurs, but cell septation is restricted to a few cells in which an asymmetrical septum is inserted. Whether the small compartment is nucleated has not yet been determined.

(ii)a) the examination of S. pistaciarum and S. pistacina to determine whether phytotoxins can be isolated from the necrotic lesions and whether they can be produced in vitro. It is postulated that there are different toxins present in each species, based on differences in ultrastructure response shown by the host.

b) coupled with (ii)a an investigation of variation within the two species in terms of pathogenicity to various cultivars of P. vera should be undertaken.
Selection of *P. vera* cultivars has, so far, been confined to variation in nut size and quality. It is unlikely that susceptibility to the pathogens is correlated with nut type and variation of the host within a cultivar is possible. Reduced susceptibility is shown by wild species (Maas et al 1971). Pathotypes of *Septoria* on pistachio should be sought from the major growing areas in both the Old and New Worlds.
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