STUDIES ON

ALTERNARIA PORRI AND *STEMPHYLIUM VESICARIUM*

ON *ALLIUM* SPP.

by

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DECLARATION

I hereby certify that this research, unless specifically indicated to the contrary in the text, is the result of my own investigation.

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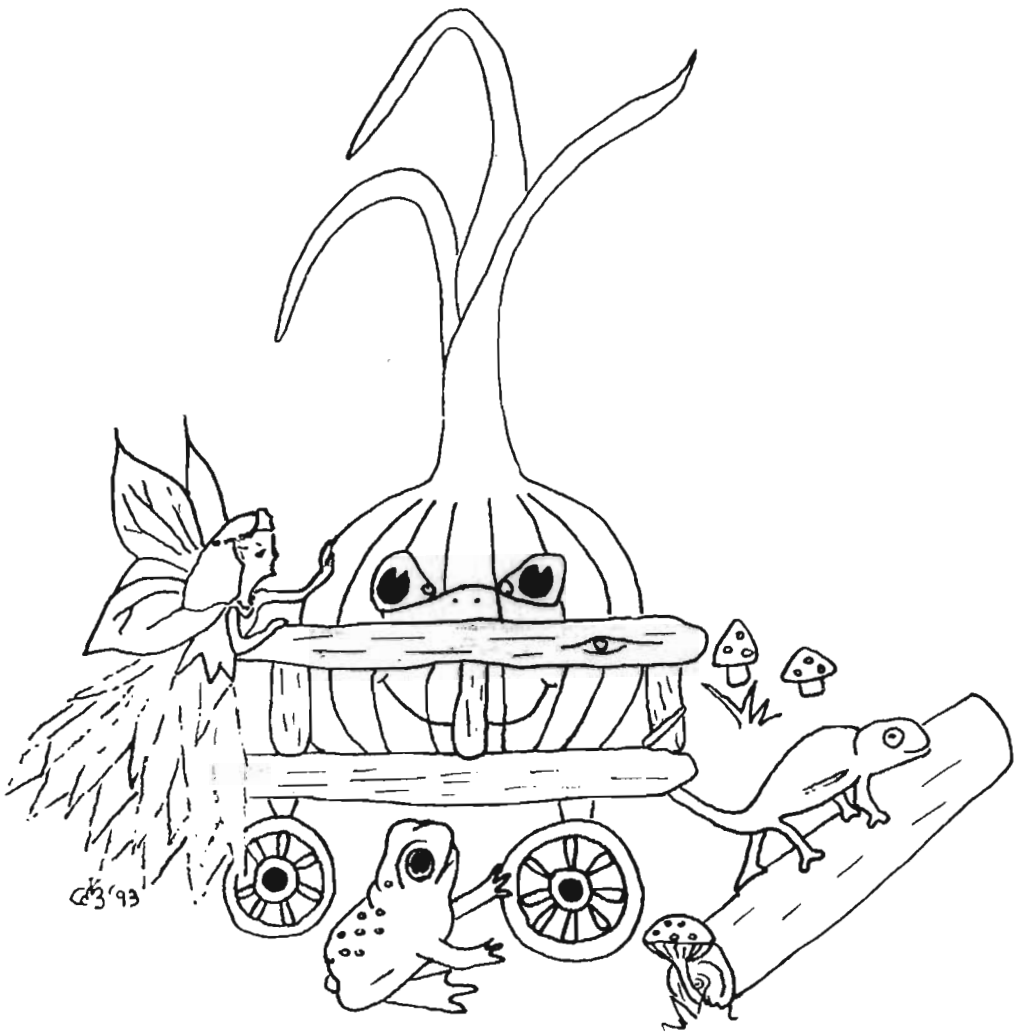

DEDICATED TO MY FRIENDS

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ABSTRACT

During surveys in South Africa, *Alternaria porri* (Ellis) Cif. and *Stemphylium vesicarium* (Wallr.) E. Simmons were found to be destructive seed-borne pathogens of onion (*Allium cepa* L.). These two pathogens are also reported on garlic (*Allium sativum* L.) in South Africa for the first time.

The development and morphology of conidiophores and conidia of the two pathogens on the onion leaf surface were examined using scanning electron microscopy. In both pathogens, solitary or fasciculate conidiophores emerged through the epidermis. Bud-like conidial initials were produced singly at the apex of conidiophores. As conidia of *S. vesicarium* matured, they became oblong to ovoid and densely verrucose. Those of *A. porri* showed slight growth in width but pronounced elongation.

Conidial germination, formation of pre-penetration structures, penetration of the onion leaf surface by *A. porri* and *S. vesicarium*, and the subsequent infection process by *A. porri*, were studied using light, scanning electron and transmission electron microscopy. Conidia of both pathogens usually germinated within 24 h of inoculation, forming several germ-tubes which often terminated in bulbous appressoria produced directly on the epidermal cells or on stomata. Following direct penetration of the outer epidermal cell wall or the stoma, bulbous primary hyphae developed below the appressoria. Secondary hyphae of *A. porri* developed from primary hyphae and grew within the intercellular spaces, penetrating mesophyll cells. The changes in ultrastructure of infected cells, and of

cells in close proximity to secondary hyphae, are described.

Six fungicides, anilazine, benomyl, carbendazim/flusilazol mixture, procymidone, tebuconazole and thiram, as well as a hot-water soak (50 C for 20 min) and sodium hypochlorite treatment, were evaluated for their efficacy in reducing both pathogens on seed and in culture. The effect of the various treatments on seed germination, and seedling emergence and growth, was determined. None of the treatments eradicated *A. porri* and *S. vesicarium* from onion seeds. The hot-water soak proved to be the best treatment for reducing these pathogens, although percentage germination and emergence of onion seeds were reduced when compared to the control.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

THE HOST: THE ONION PLANT

The information presented under this heading is not intended to be an extensive literature review of information pertaining to onions, but a brief summary of the host and its cultivation under South African conditions. This information is essential for the construction of ethographs.

Onion, the second most important vegetable crop in South Africa, is a R80 million per annum crop and is the most important bulb vegetable that is exported, with the European market being the prime destination. Onions, *Allium cepa* L., are believed to have evolved in the arid regions of Western Asia (Jones and Mann, 1963).

Morphology and Development

The onion seed, fairly smooth and plump while maturing, loses water and become wrinkled and irregular in shape once harvested (Jones and Mann, 1963) (Fig. 1). The curved embryo is almost completely surrounded by an endosperm. About one-tenth of the length of the embryo is hypocotyl; the rest is cotyledon (Hoffman, 1933). On germination, elongation of the base of the

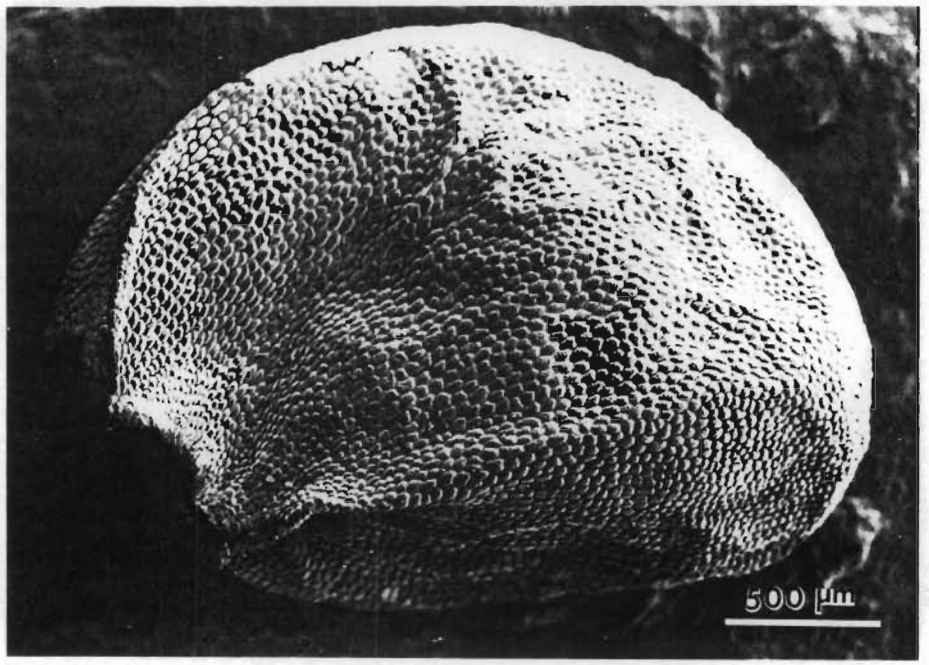


Fig. 1. Scanning electron micrograph of an onion seed

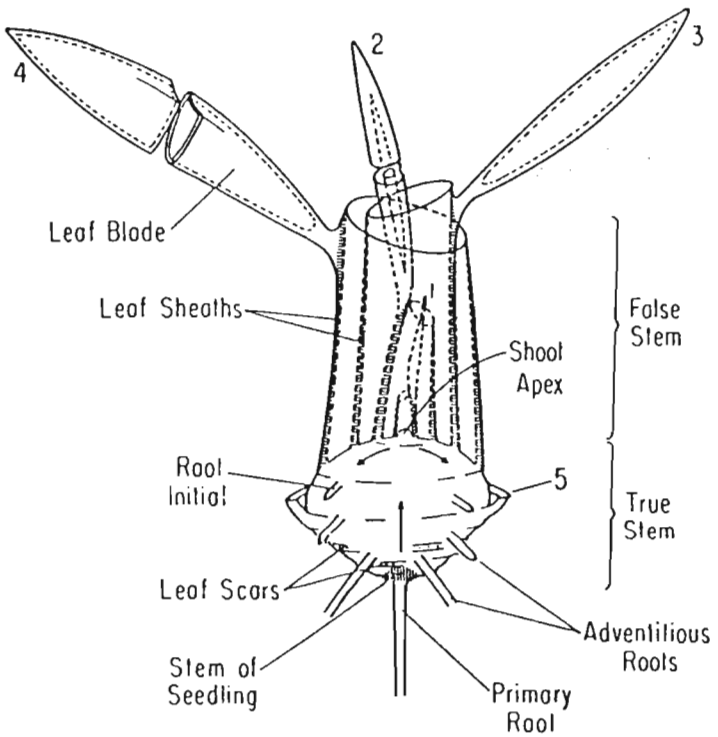


Fig. 2—A diagrammatic picture of the development of the stem, leaves, and roots, in a young plant of the common onion. Each new leaf arises at the stem or shoot apex, leaf 1 being the youngest and leaf 4 the oldest of the four leaves shown. Each leaf arises as a ring of tissue which grows upwards as a tubular sheath. The leaf-blade elongates from one side of the sheath's top. As the stem grows upwards, it also broadens, as is shown by the divergent arrows in the diagram. New roots continually arise in the younger (upper) part of the stem. In this figure, the space between adjacent leaf-sheaths is much exaggerated

(Jones and Mann, 1963)

cotyledon forces the root and the hypocotyl of the embryo out of the seed-coat, the cotyledonary tip remaining in the seed, where it absorbs nutrients from the endosperm (Hoffman, 1933; Jones and Mann, 1963). As the primary root begins to grow, the cotyledon continues to elongate forming a sharp bend, the knee, which pushes through the soil surface and eventually pulls the cotyledon free from the soil (Jones and Mann, 1963). The seedling is considered abnormal if it does not have a definite knee (International Seed Testing Association, 1985). Figure 2 illustrates the arrangement of the stem, roots and leaves of a young onion plant. At the center of the broad stem is the shoot apex from which the leaves arise. Each tubular leaf surrounds the successively younger leaves which follow it. The stem expands outwards and upwards providing room for the new leaves. The leaves alternate in position along the stem. The leaf blades arise from the leaf sheaths which are hollow tubes open at the top and project above the ground. The young leaf blade is solid but as it enlarges the central tissues fail to grow as rapidly as the surface tissues and, along with some cellular destruction, results in the formation of a large cavity within (Jones and Mann, 1963). When conditions of daylength and temperature favour bulb formation, the leaf bases, in which reserve nutrients are stored, enlarge a short distance above the stem and new leaves are produced in the bulb centre (Jones and Mann, 1963).

The flower stalk is an apical extention of the stem and like the leaf blade is at first a solid structure but, through

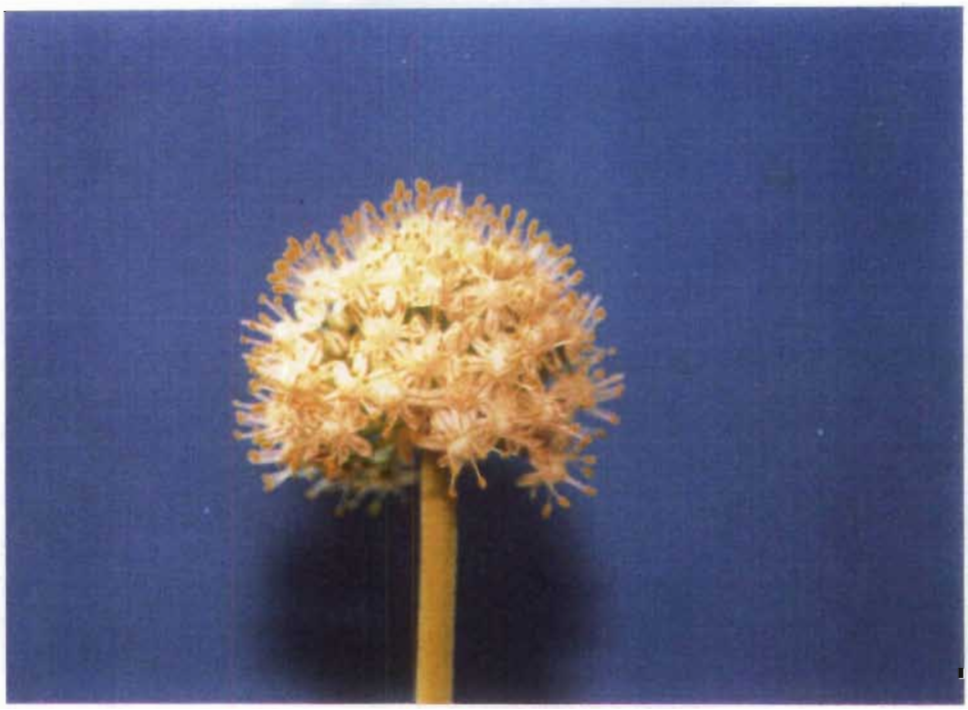


Fig. 3. The onion umbel inflorescence

Fig. 4. An individual onion flower

differential growth, becomes thin-walled and hollow as it increases in size (Jones and Mann, 1963). The flowers are borne in simple umbels on the top of a stem which normally grows to a height of one to two metres (Joubert, 1978) (Fig. 3). There are usually a few hundred individual flowers per umbel and the flowers on the same plant do not open simultaneously but over a period of about 30 d (Joubert, 1978). The flowers are made up of five alternating whorls of three organs each, which starting from the center, are; carpels (united into a single pistil), inner stamens, outer stamens, inner perianth segments and outer perianth segments (Fig. 4) (Jones and Mann, 1963).

Cultivars

The onion plant is very sensitive to temperature and daylength or photoperiod. The optimum temperature for onions is between 18 and 22°C (Joubert, 1986). During bulbing, however, higher temperatures (25-27°C) accelerate the bulbing process. Low temperatures (8-13°C) near bulbing time not only retard growth, but can trigger bolting (going to seed). According to their daylength requirements, onion cultivars are divided into three groups: early, medium and late. In the Transvaal, onions are grown as a winter crop between latitudes 22-28° south of the equator (Joubert, 1986). Cultivars must therefore be grown which are able to form bulbs when the daylength is less than 12 hr. Good examples in this connection are the Australian Brown and

Caledon Globe cultivars which only produce bulbs successfully at latitudes above 29° i.e., anywhere south of Bloemfontein. In the Western Cape the drier areas of Ceres and Caledon are ideal for producing export onions and Caledon Globe is the most important South African export onion cultivar (Joubert, 1986). A summary of the cultivars and their adaption to different regions in South Africa is given in Table 1.

Table 1. Summary of cultivars adapted to different regions in South Africa

CULTIVAR	EARLINESS	REGIONS WHERE ADAPTED*
Pyramid	very early	Tvl, Natal, OFS & Cape
Bon Accord	medium early	Tvl, Natal, OFS & Cape
Hojem	medium early	Tvl, Natal, OFS & Cape
Texas Grano	medium early	Tvl, Natal, OFS & Cape
Caledon Globe	late	Cape & all areas south of Kroonstad
Australian Brown	late	Cape & all areas south of Bloemfontein

* Tvl = Transvaal, OFS = Orange Free State, Cape = Cape Province. Table adapted from Joubert (1986).

Onion Production

The information presented under this heading was obtained from Comrie (1990). There are currently three main methods of producing onions in South Africa, namely: 1) Producing seedlings in seedbeds for later transplanting; 2) sowing seed

direct in the field and; 3) planting small bulbs or "sets".

1) Producing seedlings - Although this method requires considerable labour for transplanting, it is still the most commonly used system in South Africa, particularly in the Cape Province. The date on which the seed is sown is one of the most important factors determining yield and quality in onions. The purpose of a specific sowing date is to produce a seedling of optimum size on the envisaged transplant date.

2) Direct seeding - Sowing seed direct in the field has two main advantages over transplanting seedlings. Firstly, direct-seeded onions mature earlier than transplanted seedlings and bulbs can be marketed up to 6 wk earlier. Secondly, the labour-intensive transplanting is eliminated. However, one disadvantage is that the entire field is used as a seedbed instead of only one-tenth of the area to be planted and the soil requires more attention than before transplanting. As high temperatures play an important role in promoting bulbing of early onions this system is more suited to warmer regions with mild winters such as Natal and the Transvaal.

3) Onion sets - The planting of onion sets is another method used mainly in the Transvaal for the production of onions for the market. The seed is sown out of season when days are already long enough to cause the seedlings in the beds to immediately form bulbs. Since their energy is concentrated in the small bulbs, the plants do not grow strong leaves resulting in the bulbs remaining small. Disadvantages of planting sets are the

cost of storing the sets before planting and the high production cost because of the long growing season of 10 mo, compared with the normal season of 7 mo for transplanted onions.

Harvesting and Storage

The onion is mature when the leaves fall over. Growers usually start harvesting when 30-50% of the foliage, which is still green, has fallen over (Joubert, 1977). If premature death of the leaves is brought about by severe thrips attack, downy mildew, stemphylium blight or purple blotch, the plant continues to produce new leaves and forms a large, thick neck which holds the top erect. These thick necks do not shrink on drying, and such bulbs do not keep well in storage (Jones and Mann, 1963). Diseases and pests can lower yields considerably and it is therefore impossible to forecast yields. A yield of from 2 000 to 3 000 pockets (i.e., 20-30 tons/ha) is considered satisfactory although yields of 45 and 50 tons per hectare are not unusually high (Joubert, 1977).

When the crop is marketed soon after lifting, the onions are not dried. Only dry onions are stored (Joubert, 1977). During harvesting and drying the onions are placed in long windrows or in small heaps in such a way that the leaves of one onion shade the bulb of the one next to it (Fig. 5). Sometimes the tops are cut off immediately after harvesting and the onions are placed in hessian bags which are left in the sun to dry (Joubert, 1977).

Fig. 5. Onions placed in small heaps during harvesting in such a way that the leaves of one onion shade the bulb of the one next to it

Fig. 6. The Oudtshoorn - Willowmore area where most of the onion seed in South Africa is produced

Onions are not usually stored in the Transvaal and Natal but are sold directly. In the Cape Province onions are usually stored with the stems attached which are then plaited together and the bunches hung under cover, however, often the dry foliage can be cut off on the lands and the bulbs packed in shallow crates in the store (Joubert, 1977). In the Caledon and Ceres area onions are often dried artificially and stored in a building equipped with controlled temperature and air circulation systems (A.G. Comrie, personal communication). During the entire storage period the onion looses moisture and therefore mass as a result of drying and respiration. The most serious loss, however, is in bulbs that rot or sprout (Joubert, 1977). As mentioned earlier, purple blotch and stemphylium blight damage during the growing season results in the neck not drying which allows entrance sites for the rotting pathogens such as *Aspergillus* and *Fusarium* (Boelema, 1982; Jones and Mann, 1963).

Seed Production

The following information was obtained from Joubert (1978) except where otherwise stated. The Southern Cape Province is the most important production area and most seed is produced in the Oudtshoorn - Willowmore area (Fig. 6). Seed is produced on a smaller scale in the Northern Cape near Venterstad and Douglas and in the Transvaal in the Brits, Rustenburg and other Western Transvaal areas. A major problem in the Transvaal area is the

danger of late frost.

The onion is a biennial crop, i.e. bulbs are produced during the first year and planted during the second year to produce seed. The bulbs are grown in the same way as a crop normally grown for marketing. The bulbs are planted from March to May in the Oudtshoorn area and a low flower-inducing temperature of between 7-12.5°C is necessary for the development of seedheads. Another method, planting seed and allowing the onion plant to grow until it forms a seed head and then collecting this seed (seed-to-seed method), which reduces the period of production by half, may also be used.

Seed is harvested from November to December in the Transvaal and from December to February in the Cape Province. The seed heads are cut off or picked and spread over a tarpaulin or concrete floor to dry. The seed is usually cleaned by machine-threshing and winnowing. A good average yield is approx. 600-1 000 kg seed per hectare.

THE PATHOGENS: *ALTERNARIA PORRI* AND *STEMPHYLIUM VESICARIUM*

***Alternaria porri* (Ellis) Ciferri**

History and Range

According to Angell (1929), the genus *Alternaria* was founded by Nees in 1817 on the single species *A. tenuis*. Fries did not recognise the genus in his Systema Mycologicum in 1832, but cited

Nees's species as a synonym of *Torula alternata* (Angell, 1929). In 1879, Cooke and Ellis first described *Alternaria porri* on leaves of leek collected in New Jersey as *Macrosporium porri* Ellis, and Thaxter (1890) gave a more authentic description of this fungus on onion leaves and scales collected in Connecticut and Maine. Elliot (1917), while working on the taxonomic characters of *Alternaria* and *Macrosporium*, concluded that all obclavate, ovate, or elongated-pointed conidia of the *Macrosporium-Alternaria* type should be placed under *Alternaria*. Using this criterion of classification, Nolla (1927) described a new species *Alternaria allii* Nolla. He reported a severe outbreak of the disease in 1924 in three different places in Puerto Rico. Nolla's transfer of *Macrosporium* into *Alternaria* was widely accepted, however, the erection of the new species was not recognized by Angell (1929) who retained the name *Macrosporium porri*. Wiltshire (1933) examined the available type specimens and descriptive literature which were fundamental to the then current concepts of *Alternaria* and *Macrosporium*. One of his major conclusions was that *Macrosporium* should be suppressed in favour of *Alternaria*, typified by *A. tenuis* Nees. According to Skiles (1953), Ciferri (1930) used the binomial *A. porri*, and hence the present name of the pathogen is *A. porri* (Ellis) Ciferri. Common names of the disease caused by this pathogen include purple blotch first used by Angell (1929) and which is used most frequently (Bock, 1964; Skiles, 1952, 1953), alternaria blight (Husain and Newhall, 1959), alternaria blotch

(Boelema and Ehlers, 1967) and purple leaf spot (Nolla, 1927).

Its presence has been reported in many parts of the world, for instance in Bombay, India, in 1922 (Ajrekar, 1922), in the West Indies, Japan, Denmark (Neergaard, 1945) and in Australia (Anonymous, 1944 according to Husain, 1960). Purple blotch of onions was first recorded in Tanganyika by Wallace and Wallace (1944) and in Kenya in 1949 (Nattrass, 1951 according to Bock, 1964). The fungus is distributed throughout the United States, Canada, the West Indies, India, Western Europe, South America and many parts of Africa (CMI Map 350, ed.3; Sherf and Macnab, 1986).

In 1967 Boelema and Ehlers diagnosed a disease in South Africa, on the leaves of onion, as caused by *A. porri*. Whereas in previous years the disease occurred on the stems of the seed crop only, in the autumn of 1967 it was found on the leaves of small seedlings and older plants where it had never been troublesome before.

Apart from onion, purple blotch occurs on Egyptian onion (Angell, 1929), Welsh onion (Angell, 1929), leek (Cook and Ellis, 1879; Gladders, 1981), shallot (Nolla, 1927), garlic (Black *et al.*, 1985) and possibly other members of the onion family (Ellis and Holliday, 1970; Sherf and Macnab, 1986). *A. porri* has not been found to cause diseases on plant species other than *Allium* spp. (Ellis and Holliday, 1970; Sherf and MacNab, 1986). *A. porri* was reported for the first time on garlic in South Africa by the present author (Chapter 6).

Morphology

Mycelium in the lesions is hyaline or brown and the diameter of the hyphae ranges from 2-10 μm, but sometimes attains a thickness of 18 μm in culture (Angell, 1929; Nolla, 1927). The mycelium is composed of smooth, septate, short, simple or branching, subfasciculate hyphae (Nolla, 1927). Conidiophores arise singly or in groups through the stomata or the epidermis as terminal branches of hyphae (Nolla, 1927). Conidiophores are straight or flexuous, septate, pale to dark brown, 5-10 μm in diameter and up to 120 μm long (Ellis and Holliday, 1970). Obclavate conidia are usually 100-300 μm in length, 15-20 μm in diameter and are borne singly at the apex of the conidiophore (Ellis and Holliday, 1970). Conidia apically attenuate to form a simple, tapering beak, 2-4 μm in diameter, which is commonly about the same length as the body, but may be shorter or longer (Ellis and Holliday, 1970). The beaks of conidia produced in artificial culture are occasionally forked (Angell, 1929). Conidia are at first hyaline becoming dark brown when mature and have 8-12 transverse and no to several longitudinal or oblique septa (Ellis and Holliday, 1970; Nolla, 1927).

Cultural characteristics

Angell (1929) and Nolla (1927) described the cultural characteristics of *A. porri* in detail. These authors found that although the fungus grew well on carbohydrate media, it grew poorly on sugar media that did not not contain starch or protein.

Nolla (1927) found that growth was best on oatmeal agar, cornmeal agar, Czapek's agar, Cook's II agar and potato dextrose agar. Angell (1929), Fahim (1966) and Raju and Mehta (1982) found that growth on onion agar and potato dextrose agar was good. Gupta *et al.* (1987a) found that Czapek's agar proved best for growth of five different isolates, but that none of the isolates sporulated on any of the media they tested. Nolla (1927) reported that the best growth in liquid media was observed in Czapek's solution and that maximum growth in agar media occurred at slightly acid concentrations, but that the fungus could tolerate higher concentrations of alkali than acid. Similarly, Angell (1929) and Raju and Mehta (1982) found that the pathogen could grow over a wide range of pH from pH 3.8-9 and 4.0-8.0, respectively, with an optimum of 6.0. Angell (1929) and Nolla (1927) reported equally good mycelial growth when cultures were exposed to continuous light or darkness. Raju and Mehta (1982), however, found that the pathogen favoured continuous darkness. The optimal temperature for mycelial growth was found to be between 22 and 30°C and growth rate dropped sharply between 30 and 34°C (Angell, 1929), and Raju and Mehta (1982) found that the thermal death point of mycelium ranged between 55 and 60°C.

It is well known that *A. porri* does not sporulate consistently well on artificial media and several studies have been conducted on this topic. Angell (1929) and Skiles (1953) reported that sporulation occurred on cornmeal agar after using a modification of Rands' (1917) method in which the mycelial mat was macerated

and exposed to direct sunlight for 4 d. Nolla (1927) failed to induce sporulation using the technique of Rands (1917). Neergaard (1945) found that maximum sporulation was achieved at 26°C and 100% relative humidity. Husain (1960) used a culture that had been in storage for one year to try several methods to induce sporulation but was unsuccessful. He suggested that the culture had possibly lost its ability to sporulate in culture. Fahim (1966) found that sporulation was abundant on potato agar and moderate on onion and onion leaf agar in cultures which had been exposed to direct sunlight for 2 hr. The optimum temperature and relative humidity for sporulation were 25°C and slightly less than 100%, respectively (Fahim, 1966). Gupta and Pathak (1988a) induced sporulation by inoculating pearl millet leaf pieces placed on calcium carbonate agar medium with mycelial discs and incubating the plates at 25°C. The present author found that the pathogen sporulated well on cornmeal agar when petri dishes were incubated under a 12 hr dark/12 hr near-ultraviolet light regime.

Stemphylium vesicarium (Wallroth) Simmons

History and range

The genus *Stemphylium* was founded by Wallroth in 1833 on the single species *S. botryosum* Wallr. collected on asparagus stems. Attention was first drawn to *Stemphylium vesicarium* on onions by von Thumen, who described it as a new species (*Macrosporium*

parasiticum) in his Mycotheca Universalis, No. 667, in 1877. The specimens had been collected in Bavaria, where the fungus was stated to be parasitic, for the most part, on onion *Peronospora destructor* (Berk.) Casp. However, Rabenhorst (1857), according to Wehmeyer (1961), had identified the perithecial stage of a fungus on onion as *Sphaeria allii* almost twenty years before von Thumen's description of the imperfect state. Cesati and de Notaris (1861), after studying another collection from this host, transferred it to the genus *Pleospora* as *Pleospora allii*. The connection between the imperfect and perfect states was not, however, made. In 1889 Miyabe proved the genetic connection between the conidial stage, named *Macrosporium parasiticum* by von Thumen, with *Pleospora herbarum*, by pure cultures. Thaxter (1890) reported a fungus on onions and sets grown in Connecticut and Maine and identified it as *Macrosporium sarcinula* Berk. In 1915 Hanzawa, according to Wiltshire (1938), suggested that the seven species of *Macrosporium* recorded by Saccardo on *Allium* (including *M. sarcinula*) were all perhaps identical with, or closely related to *M. parasiticum*. Teodoro (1922) referred throughout his paper to the onion pathogen in Wisconsin as *M. parasiticum*, which he confirmed as the conidial stage of *Pleospora herbarum*. However, in 1931 Verwoerd and Du Plessis stated that *Macrosporium sarcinula* was the conidial stage of *P. herbarum* on onion.

Wiltshire (1933, 1938) concluded that *Macrosporium* should be suppressed in favour of *Alternaria* and that the limits of

Stemphylium should be modified to include two sections, *Eustemphylium* and *Pseudostemphylium*. Wiltshire (1938) suggested that *Stemphylium botryosum*, the conidial stage of *Pleospora herbarum* Rabenh., should be the synonym of *Macrosporium parasiticum*. Groves and Skolko (1944) agreed with, and retained, Wiltshire's sectional treatment of *Stemphylium* in their study on seed-borne species. Simmons (1969) renamed Wiltshire's (1938) synonym of *Macrosporium parasiticum* (i.e., *Stemphylium botryosum*) calling it *S. vesicarium* (Wallroth) Simmons, and demonstrated the misleading relationship between *Stemphylium* and *Pleospora*. He maintained the classic *P. herbarum-S. botryosum* states as a unique and fundamental combination and found that differences in the patterns of ascospore development and septation were sufficient to distinguish *P. herbarum* from the perfect state of *S. vesicarium*. Furthermore, he suggested that the perfect state of *S. vesicarium* should be called *Pleospora allii* (Rabenh.) Ces. & de Not.

Common names suggested for this disease caused by *S. vesicarium* include brown blotch (Skiles, 1953) and stemphylium leaf blight (Rao and Pavgi, 1975), the latter being the more popular name.

S. vesicarium was reported for the first time in South Africa on onions by Verwoerd and Du Plessis (1931) (although it was referred to as *Pleospora herbarum*), and on garlic by the present author (Chapter 6). Skiles (1953) reported the pathogen on onions in the Arkansas Valley of Colorado. The pathogen was

first reported on onion and garlic in India (and given the common name of stemphylium blight) by Rao and Pavgi (1973, 1975), on onion in Texas by Miller *et al.* (1978) and in New York by Shishoff and Lorbeer (1987). To date, to the author's knowledge, the pathogen has not been reported on other *Allium* spp. although it is most likely that it will also infect these species as it has a wide host range. It has been reported on alfalfa and lucerne (Lamprecht *et al.*, 1984), and asparagus (Johnson and Lunden, 1986; Lacy, 1982).

Morphology

The pale brown, septate mycelium closely aggregates within the host to form cuboid, stromatic cells. The medium to dark brown stroma gives rise to the conidiophores, usually in groups of 8-10 (Rao and Pavgi, 1975). Conidiophores are straight to variously curved, simple, cylindrical but enlarging apically to the site of conidium production, dilute yellow-brown darkening at the swollen apex, smooth throughout except sparsely punctate roughened on the apical cell, 5-8 X 33-47 μm and 1-4 septate (Simmons, 1969). Conidiophores are characteristically proliferated 3-4 times at the distal region (Rao and Pavgi, 1975). Conidia are produced singly on the swollen apex of the conidiophore. Mature conidia are oval to ellipsoidal with transverse and longitudinal septa, light to dark brown, densely verrucose and measure 12-22 X 25-42 μm (Simmons, 1969). Asci develop in globose, somewhat flattened perithecia with pronounced

apical beaks (Rao and Pavgi, 1975). The asci are octosporus, cylindrical to clavate, measure 110-150 X 24-38 μm with characteristic bitunicate walls (Rao and Pavgi, 1975). Ascospores are ellipsoidal, light to olive brown in colour, measure 33-43.5 X 15-19.5 μm and have 3-7 and 6-14 transverse and longitudinal septa, respectively (Rao and Pavgi, 1975).

Cultural characteristics

S. vesicarium has been found to grow on a wide range of media: potato-dextrose agar (Falloon *et al.*, 1987; Johnson and Lunden, 1986; Rao and Pavgi, 1975), malt extract agar (Lamprecht and Knox-Davies, 1984; Lamprecht *et al.*, 1984), V8-juice agar (Falloon *et al.*, 1987; Heiny and Gilchrist, 1991), potato carrot agar (Lamprecht and Knox-Davies, 1984), and, onion leaf agar (Shishkoff and Lorbeer, 1989). There is no information available on the pH range for optimum growth. Cultures have been incubated at 18°C (Shishkoff and Lorbeer, 1989), 20°C (Falloon *et al.*, 1987; Lamprecht and Knox-Davies, 1984) and 20-23°C (Johnson and Lunden, 1986). Shi and Kuang (1991) studied the cultural characteristics of *Stemphylium botryosum* Wallr., which they called the onion leaf blight fungus, and found that the optimum temperature for growth was 25°C. The pH range was 3-11 and the best medium for sporulation was PDA supplemented with 0.5% $MgSO_4$ and 0.5% $CaCO_3$. Conidial formation was inhibited by light. Light conditions that have been used to induce sporulation in *S. vesicarium* include an 8 or 16 hr photoperiod under fluorescent

light (Falloon *et al.*, 1987), continuous fluorescent light (Johnson and Lunden, 1986) and a 12 hr photoperiod of near-ultraviolet radiation (Shishkoff and Lorbeer, 1989). The present author induced profuse sporulation by growing the pathogen on potato dextrose agar in a 12 hr dark/12 hr near-ultraviolet light or fluorescent light regime. Simmons (1969) reported that the perithecial stage of *S. vesicarium* developed in artificial culture when exposed to a few hours of light daily and matured within 3-6 mo at refrigeration temperatures. Rao and Pavgi (1975) found that perithecia formed abundantly within 3 mo on pieces of onion leaf and inflorescence stalks and that light did not appear to be a conditioning factor, but low temperatures were favourable for their maturation.

Conidiogenesis of *Alternaria* and *Stemphylium*

The ways in which conidiogenous cells and conidia develop are now accepted almost universally by mycologists as features of great significance in classifying Deuteromycetes (Minter *et al.*, 1982). Conidia of *Alternaria* and *Stemphylium* spp. have been called porospores (Hughes, 1953; Simmons, 1967) however, as will be seen below, there is some controversy surrounding this concept.

The term porospore was coined by Hughes in 1953 for a conidium which develops through a pore in the wall of the conidiophore. Among the example of genera in which porospores are formed he

gave *Helminthosporium*, *Torula* and *Alternaria*. In 1963, Luttrell applied the adjective porogenous to conidia originating as protrusions through pores in the conidiophore wall.

At the Kananaskis workshop on criteria and classification in the Fungi Imperfecti held in 1969, a critical evaluation of Hughes's system was attempted (Kendrick, 1971). Particular attention was given to the mode of spore initiation. There it was recognized that conidia previously classified as "porospores" might arise in either of two fashions: holoblastically, by a simple blowing out of all wall layers of the conidiophore; or tretically, by the extrusion of the inner wall of the conidiophore through a preformed channel in the thickened outer wall (Ellis, 1971; Kendrick, 1971).

Several authors have carried out fine-structural studies of "poroconidium" formation and have concluded that the process is indeed enteroblastic tretic (Campbell, 1969; Ellis and Griffiths, 1977; Honda *et al.*, 1987). However, holoblastic conidiation has been demonstrated unequivocally in *Stemphylium botryosum* Wallr. (Carroll and Carroll, 1971), *Ulocladium atrum* Preuss (Carroll and Carroll, 1974), and *Helminthosporium maydis* Nisik. & Miyak. (Brotzman *et al.*, 1975), all supposedly "poroconidium"-producing fungi. Further, Carroll and Carroll (1971, 1974) criticized the three previous reports of enteroblastic-tretic conidiation as being unconvincing and concluded that as this mode of conidiation had not been convincingly demonstrated for any Hyphomycete, the term should be dropped from use until experimental evidence in

support of the concept was forthcoming.

In Hughes's initial diagnosis (1953) and in general discussions which have followed (Luttrell, 1963; Simmons, 1967) authors have stressed that, although poroconidia might be formed by a budding process, a sharp discontinuity is maintained between the conidium and conidiophore walls. Campbell (1969) claimed that the secondary wall of the conidiogenous cell of *Alternaria brassicicola* (Schw.) Wiltshire was continuous with the primary wall of the conidium. However, Carroll and Carroll (1971) interpreted Cambell's "primary wall" to be a superficial zone of pigment deposition within his "secondary wall". These authors provided the evidence that, at least in *Stemphylium botryosum*, the primary walls of conidiophores and conidia were continuous until the time of conidium secession. However, Honda *et al.* (1987) found that at the pore of the conidiophore of *Alternaria solani* Sorauer the outer and inner layers of the conidiophore cell wall dissolved completely and the cytoplasm, covered only with plasma membrane, protruded through the pore to form the conidium initial. Later on a cell wall was formed around the conidium, but discontinuity between the walls of the conidiogenous cell and a conidium was maintained through all stages of conidium development in accordance with Hughes's initial diagnosis of the porospore.

Everts and Lacy (1990a) studied the effect of dew on the ontogeny of *Alternaria porri* using scanning electron microscopy. The present author also used scanning electron microscopy to

study condial ontogeny of *A. porri* and to supplement the work of Everts and Lacy (1990a)(Chapter 2). As scanning electron microscopy has not been used previously to study the conidial ontogeny of *S. vesicarium*, the present author used this technique to study conidial development, *in situ*, on onion leaves (Chapter 2).

INTERACTION BETWEEN HOST AND PATHOGEN

Interaction Between Onion and *Alternaria porri*

Symptomatology

Nolla (1927) gave an excellent description of the disease on both leaves and flower stalks of onion and shallot. The first symptoms are numerous tiny, white, circular or irregular spots, less than one millimeter in diameter. These gradually increase in size until in advanced stages the diseased areas cover several square centimeters of surface. As the spots increase in size, they become oval-shaped or irregular and the white colour eventually changes to violet. Later stages of development show the central portion of the spots changing to purple, immediately surrounded by a pale yellow orange to salmon band beyond which is a pale green zone. Dull violet-black zones within the lesions are also observed. The dark purple colour is the most distinctive symptom of the disease. A distinct yellowing usually extends from both ends of the spots, often reaching the tips and

bases of the leaves. Gladders (1981) described similar symptoms on the stems and spathes of leek. Bock (1964), Boelema and Ehlers (1967) and Naude (1988), in addition to the symptoms described by Nolla (1927), found that the leaves shrivel, usually from the tip, and that the plant draws on its bulb reserves to produce fresh foliage which is again attacked (Fig. 7). Bock (1964) also found that symptoms varied with different environmental conditions. Typically, the lesions are purple or brown. Associated with these, however, under all conditions, are few to many white lesions which remain small. The two types of lesions may be present in varying proportions, depending on the weather at the time of infection and the climatic environment afterwards. He found that extensive development of purple blotch lesions takes place only at comparatively high relative humidities and that the incidence of small white flecks is highest at the low relative humidities. Boelema and Ehlers (1967) and Gladders (1981) also reported similar white fleck symptoms on onions in Transvaal (Fig. 7) and on leeks in Britain, respectively. These white lesions, when numerous, coalesce into large white blotches and later turn light brown often with concentric dark and light brown rings (Boelema and Ehlers, 1967).

Lesions, similar to those on the leaves, are also produced on flower stalks of onion (Nolla, 1927). These lesions cause girdling, and as a rule the stalks are destroyed or break in two before the seeds mature (Angell, 1929; Nolla, 1927; Pandotra, 1964). These symptoms on seed stalks have also been observed in

Fig. 7. Symptoms of *Alternaria porri* on leaves of onion

Fig. 8. Symptoms of *Alternaria porri* on flower stalks of onion

South Africa (Fig. 8).

Angell (1929) and Walker (1921) described the infection of bulbs and sets. Infection usually occurs through the necks of topped bulbs or through wounds made during harvesting. The disease develops rather quickly producing a semi-watery decay. Secondary organisms readily invade the dead tissues. Once neck infection has taken place, shrinkage of the infected succulent scale leaves results in loosening the dry outer ones (Angell, 1929). The decay of bulbs is deep yellow at first, but gradually turns to a wine red. With age, the decay becomes dark brown to black (Angell, 1929; Husain, 1960; Sherf and Macnab, 1986; Walker, 1921). Naude (1988) reported that infection of bulbs in South Africa rarely occurs.

Gupta and Pathak (1988b) found that bulb and seed yields, as well as 1000-seed-mass, of onions are significantly reduced as a result of purple blotch.

Histopathology

Angell (1929) reported that germination of conidia of *A. porri* on onion leaves occurred within three hours, and Fahim and El-Shehedi (1966) found that the majority of conidia germinated within 24 hr. Everts and Lacy (1987b) found that after 3, 6 and 12 hr of dew, 73, 84 and 94% of conidia of *A. porri* on the onion leaf surface had germinated and 5, 34 and 44% had formed appressoria, respectively. Conidia germinated from more than one cell and germ-tubes grew in any direction across the leaf surface

usually terminating in appressoria over stomata and on epidermal cells (Everts and Lacy, 1987b; Fahim and El-Shehedi, 1966). Angell (1929), Bock (1964), Gupta *et al.* (1985) and Khare and Nema (1982) studied various factors that influenced conidial germination of *A. porri in vitro* and *in vivo*. Angell (1929), Gupta *et al.* (1985) and Khare and Nema (1982) found that maximum germination occurred at 100% relative humidity prevailing for 6 hr or longer at 25°C. Bock (1964) reported that optimum appressorium formation occurred at 20-25°C.

Van Dyke and Trigiano (1987) found that germ tubes of *A. cassiae* Jurair & Khan on cotyledons of *Cassia obtusifolia* L. usually terminated in appressoria although intercalary appressoria were also formed. Appressoria formed directly on epidermal cells or over stomata with about equal frequency. These authors and Allen *et al.* (1983) observed an extracellular matrix associated with germ-tubes and appressoria of *A. cassiae* and *A. helianthi* (Hansford) Tubaki & Nishihara, respectively.

Angell (1929) and Nolla (1927) reported that *A. porri* penetrated via stomata of onion leaves and that no direct penetration through the epidermal cells occurred. Walker (1952) reported penetration through stomata and through wounds in the epidermis. However, Everts and Lacy (1987b), Fahim and El-Shehedi (1966) and Sherf and Macnab (1986) found that penetration occurred through the stomata and directly through unwounded epidermal cells.

Changsri and Weber (1963) revealed that host penetration of

crucifers was stomatal by *A. brassicae* (Berk.) Sacc. and stomatal or direct by *A. brassicicola* and *A. raphani* Groves & Skolko. Saad and Hagedorn (1969), studying the host-pathogen interaction between *A. tenuis* and *Phaseolus vulgaris* L., found that, 24 hr after inoculation, the fungus made numerous penetrations through the stomata and developed primary hyphae in the substomatal cavities. Direct penetration by the fungus was less common and usually occurred after the 24 hr period. Allen *et al.* (1983), studying the host-pathogen interaction between *A. helianthi* and sunflower, found that the development of an appressorium and subsequent direct penetration through the cuticle and epidermis was the most common mode of penetration although penetration through stomata and wounds without the formation of an appressorium was also observed. Penetration appeared to be accompanied by a chemical degradation of the surrounding host tissue (Allen *et al.*, 1983). Germ-tubes of *A. longipes* (Ell. & Ev.) Mason on tobacco leaves penetrated directly through the epidermis with or without the formation of appressoria, and through stomata (Von Ramm, 1962). Everts and Lacy (1987b) found that after 12 hr dew, 13% of *A. porri* conidia had formed infection hyphae within the onion leaf and cell collapse was visible beneath 6% of conidia. After 24 hr dew, 23% of conidia had formed infection hyphae and 68% of those had caused visible lesions. The infection hyphae developed within the substomatal chambers, sometimes filling them, and penetrated the underlying tissues either inter- or intracellularly (Angell, 1929). Nolla

(1927) found that the invading hyphae spread through the intercellular spaces of the leaf. Angell (1929) also reported that other hyphae grew within the cell wall, parallel with the cuticle, at first separated from it by a thin layer of tissue which soon disappeared, leaving them in a subcuticular position. Several layers of mycelium often formed beneath the cuticle, the epidermal cell walls became laminated and the middle lamellae weakened. The hyphae then grew between the laminae, forcing them farther apart, then between and through the cells into the underlying tissues (Angell, 1929).

Saad and Hagedorn (1969), studying the host-pathogen interaction between *A. tenuis* and *Phaseolus vulgaris* L., found that an infection peg developed from the appressorium and penetrated the epidermal cell directly. Secondary hyphae grew from the bulbous primary hyphae in the substomatal cavity 48 hr after inoculation and progressed into the spongy parenchyma. Van Dyke and Trigiano (1987) reported that in cotyledon tissues of *Cassia obtusifolia*, collected 18 hr after inoculation with *A. cassiae*, guard cells and mesophyll cells were necrotic where appressoria had formed over stomata and on epidermal cells, respectively. A penetration peg occasionally formed under the appressorium but only rarely were hyphae found inside the spongy mesophyll and palisade cells. True leaves showed similar results. Von Ramm (1962) found that *A. longipes* formed pigmented thick-walled "microsclerotia" in substomatal vesicle of tobacco leaves. Hyphae developed from these and invaded the adjacent

intercellular space. The "microsclerotia" of von Ramm (1962) are probably enlarged primary hyphae which have been reported by other researchers (Fahim and El-Shehedi, 1966; Saad and Hagedorn, 1969).

Studies on a wide variety of pathogenic species of *Alternaria* have shown that toxins are produced by these fungi and are responsible for much of the disease syndrome on the host (Brian *et al.*, 1952; Cotty and Misaghi, 1984; Gilchrist and Grogan, 1976; Haggblom, 1987; Langsdorf *et al.*, 1990; Suemitsu *et al.*, 1990a). *A. porri* has been found to produce the metabolic pigments macrosporin, alterporriol A, B, C, D and E, altersolanol A and B, and the phytotoxins tentoxin, zinniol and porritoxin (Ohnishi *et al.*, 1991, 1992; Suemitsu *et al.*, 1988, 1989, 1990a, 1990b, 1991, 1992). The role of these toxins during the infection of onion leaves by *A. porri*, has not yet been determined. However, Suemitsu *et al.* (1992) found that porritoxin inhibited seedling growth in lettuce and stone-leek seedlings and Gupta *et al.* (1986b) reported that treatment of onion seeds with culture filtrates of *A. porri* caused reduction in seed germination and seedling vigour. Reddy *et al.* (1983), in an earlier study, found that *A. porri* induced metabolic changes in various parts of onion leaves. They found that dry matter, total chlorophyll, chlorophyll 'b', total carbohydrates, free amino acids and proteins declined whilst chlorophyll 'a' and total phenols increased.

To date, there have been no electron microscopy studies of the

infection of onion leaves by *A. porri*. The present author used light, scanning electron and transmission electron microscopy to study the penetration and subsequent infection of onion leaves by *A. porri* and the result of these studies is presented in Chapter 4.

Environmental factors

Nolla (1927) stated that the ideal field conditions for epidemic development of the disease appeared to be warm moist weather with occasional rains. Spore germination and invasion of the leaf occurred only during the night and early hours of the morning or during cloudy weather. Showers during the day followed by bright sunny weather were evidently not favourable to infection (Nolla, 1927). Bock (1964) found that lesions were produced over a wide temperature range with a broad optimum between 17 and 25°C. Khare and Nema (1984) reported that an average temperature of 25°C and a high relative humidity of 90% led to severe disease symptoms. Maximum disease development (75.1%) and shortest incubation period (4.93 d) were recorded by Gupta and Pathak (1986) in plants kept under high relative humidities (*c.* 100%) for 120 hr. Naude (1988) reported that free water on the plant surface, either as a result of dew or rain, was a pre-requisite for infection. Infection could occur between 6 and 34°C with an optimum of 25 to 27°C. Naude (1988) further suggested that rain at any time of the year would encourage the development of purple blotch provided the temperature was high.

Miller (1983) and Miller and Amador (1981) studied the relationships of onion leaf age, position of leaves and susceptibility to *A. porri*. They reported that levels of leaf damage were significantly lower on younger, apical leaves than on older leaves, except one week before bulb maturity. Individual onion leaves were more susceptible as they aged and emerging leaves became more susceptible as the bulbs approached maturity. Gupta and Pathak (1986) found that 60-d-old onion plants were most susceptible.

Everts and Lacy (1987a) reported that sporulation on onion leaves increased with increasing relative humidities. Leaf age had no effect on sporulation. On calm summer days, peak conidium concentration above an onion field was found to occur between 08h00 and 14h00, with few conidia being trapped between 20h00 and 06h00 (Meredith, 1966). On windy days, there were marked increases in concentration. Meredith (1966) postulated that, at night, high relative humidity and dew formation encouraged sporulation of *A. porri* and that few spores were released on account of relatively low wind velocity. The rapid decrease in vapour pressure between 07h00 and 10h00, and the disappearance of dew, induced hygroscopic movements which weakened the conidial attachments. As wind velocity increased during the morning and afternoon, increasing numbers of mature conidia were passively released until the source was exhausted or until wind velocity decreased (Meredith, 1966). Meredith (1966) further suggested that increased sporulation during rainy periods and irrigation

probably accounted for the increase in daily mean conidium concentration which occurred after such periods. Everts and Lacy (1990b) also studied the influence of environment on conidial concentration of *A. porri* in air above an onion field. Correlations between temperature and conidial concentration and between wind velocity and conidial concentration were not found to be significant. They did, however, find that the natural logarithm of numbers of airborne conidia sampled during the current day (D) was positively correlated with 1) the maximum hourly vapour pressure deficit (= saturation-ambient vapour pressure) on D, and 2) the logarithm of the conidial concentration sampled on D-1. Large concentrations of conidia did not always preceed increases in lesions (Everts and Lacy, 1990b).

The hours of leaf wetness were monitored by Amador and Miller (1985) and broadcasted twice daily for the benefit of onion growers in Texas. Purple blotch development increased following periods of 10-12 continuous hours of leaf wetness and thus the information helped producers to time fungicide applications.

Interaction Between Onion and *Stemphylium vesicarium*

Symptomatology

Rao and Pavgi (1975) gave a comprehensive description of symptoms of stemphylium blight of onion and garlic. According to these authors, the infection appears as small, yellow to pale

orange flecks or streaks in the middle of the leaf. These soon develop into elongated, spindle-shaped to ovate-elongate spots, often reaching the leaf tips and becoming surrounded by a characteristic pinkish margin (Fig. 9). They usually turn grey at the centre, later brown to dark olive-brown with the development of conidiophores and conidia of the pathogen. The spots frequently coalesce into extended patches, blighting the leaves and gradually the entire foliage (Rao and Pavgi, 1975). Miller *et al.* (1978) described the lesions as nondelineated, light yellow to brown, water-soaked and progressing from the tip to the base of leaves as symptoms develop. The lesions eventually turns light brown to tan as the lesions enlarge and become black when spores are produced (Miller *et al.*, 1978). Rao and Pavgi (1975) observed similar symptoms of infection on the central inflorescence stalks of onions (Fig. 10). *S. vesicarium* may often girdle seed stalks (Teodoro, 1922), as has been found in South Africa (Fig. 10), and leaves (Shishkoff and Lorbeer, 1987) of onion.

Rao and Pavgi (1973, 1975) described the symptoms of the perfect state, *Pleospora allii*, on onion. They found that the perithecia (pseudothecia) appear in the blighted areas of the leaves and the inflorescence stalks, being more pronounced on the latter. The perithecia often occur in broadly concentric, ovate rings covering the entire surface of the stalk (Rao and Pavgi, 1975).

Infection usually remains confined to the leaves and does not

Fig. 9. Symptoms of *Stemphylium vesicarium* on leaves of onion

Fig. 10. Symptoms of *Stemphylium vesicarium* on flower stalks of onion

extend down to the scales of the bulb (Rao and Pavgi, 1975). Bulbs of heavily infected plants remain underdeveloped and become subjected to decay by saprobes in the soil and/or rot in post-harvest storage (Rao and Pavgi, 1975).

Histopathology

Although several researchers have studied the host-pathogen interaction between asparagus and *Stemphylium* sp. (Falloon *et al.*, 1987; Sutherland *et al.*, 1990), alfalfa and *S. botryosum* (Borges *et al.*, 1976; Pierre and Millar, 1965), and red clover and *S. sarcinaeforme* (Cav.) Wiltsh. and *S. botryosum* (Higgins and Lazarovits, 1978), no infection studies have been conducted on the onion-*S. vesicarium* interaction. *S. vesicarium* has, however, been found to produce a toxin, stemtoxin, but its activity has not yet been determined (Heiny and Gilchrist, 1991). The present author used light and scanning electron microscopy to study the pre-penetration structures formed by, and the penetration of onion leaves by, *S. vesicarium* (Chapter 3).

Borges *et al.* (1976) and Falloon *et al.* (1987), studying the infection of alfalfa by *S. botryosum* and asparagus by *S. vesicarium*, respectively, found that germ-tubes of these pathogens penetrated exclusively through stomata without the formation of appressoria. These authors (Borges *et al.*, 1976; Falloon *et al.*, 1987) observed that direct penetration of the epidermal cells by germ-tubes, or by means of appressoria, did not occur. Higgins and Lazarovits (1978) found that *S. botryosum*

penetrated the leaves of red clover primarily via stomata whilst appressoria, formed on epidermal cells, rarely resulted in successful penetration. *S. sarcinaeforme*, however, was more versatile in its mode of penetration with some stomatal penetrations (25% of infection sites) and numerous direct penetrations between or through epidermal cells (75% of infection sites) (Higgins and Lazarovits, 1978). Pierre and Miller (1965) found that 85% of germ tubes of *S. botryosom* gained ingress of alfalfa leaves through stomata whilst direct penetration occurred primarily at junctures of epidermal cells after the formation of an appressorium-like structure. Germ tubes often passed over stomata without gaining ingress (Pierre and Miller, 1965; Shrestha and Bhatnagar, 1988).

Several authors have reported the formation of bulbous, septate primary hyphae by *Stemphylium* spp. within the different hosts (Heiny and Gilchrist, 1991; Higgins and Lazarovits, 1978; Pierre and Miller, 1965; Shrestha and Bhatnagar, 1988). These primary hyphae gave rise to narrower secondary hyphae which ramnified through the host cells.

Higgins and Lazarovits (1978) reported that the most obvious features of tissue of red clover infected by *S. botryosom* or *S. sarcinaeforme* were the death of guard cells at the point of ingress, the presence of conspicuous yellowish refractile bodies (possibly lipid bodies) in adjacent epidermal cells or mesophyll cells, and the severe plasmolysis and cytoplasmic disruption near the penetration site. Sutherland *et al.* (1990) described three

types of lesions on spears of asparagus infected with a *Stemphylium* sp.: limited, spreading, and fleck. In the limited lesion type there was a rapid degeneration of host cells, beginning close to the penetrating hyphae, but eventually also occurring at a distance from these. Electron-dense material accumulated in the intercellular spaces and enveloped the hyphae. Hyphae, the contents of which became disorganised, occupied only a small part of the central part of the lesion. Electron-dense material was absent in the intercellular spaces or enveloping hyphae in the spreading type of lesion and hyphae ramnified throughout the intercellular spaces of the lesion. The third type, a fleck lesion, developed in response to inoculation of *Stemphylium* isolates nonpathogenic to asparagus. Electron-dense material was produced in the substomatal cavity before stomatal penetration was completed and hyphae failed to penetrate beyond the substomatal cavity. Only a few host cells were degraded (Sutherland *et al.*, 1990).

Environmental factors

The effect of environmental factors on severity of stemphylium leaf blight on onion has largely been neglected. Shishkoff and Lorbeer (1989) studied the effect of leaf wetness of onion on disease severity caused by *S. vesicarium* and the effect of leaf age on susceptibility. They found that, in controlled inoculations, the fungus caused lesions on leaves of all ages of onion plants at seedling, vegetative and flowering growth stages,

especially on older leaves. Disease severity was greater when onion leaves were either rubbed with bleached, nonabsorbent cotton or exposed to longer periods of free moisture after inoculation. These results supported an earlier report by the same authors (Shishkoff and Lorbeer, 1987), and one by Miller *et al.* (1978), which stated that appreciable damage to onion leaves occurred only after at least 24 hr of exposure to free moisture. Studies on the effect of temperature, light intensity and photoperiod on severity of stemphylium leaf blight of onion, as have been done on stemphylium leaf spot of asparagus (Falloon *et al.*, 1987; Menzies *et al.*, 1991), have yet to be conducted.

Seed Pathology

Both *A. porri* and *S. vesicarium* are seed-borne in onion (Neergaard, 1945; Simmons, 1967). It has not yet been determined how the onion seed becomes infected with these pathogens or what part of the seed is infected or contaminated. Singh *et al.* (1977) found that, in a sunflower seed lot, profuse mycelium of *Alternaria tenuis* was found in all layers of the pericarp but once the pathogen broke through the thick cuticle of the endosperm, it quickly invaded the endosperm and embryo. Maude and Humpherson-Jones (1980) and Singh *et al.* (1977) reported similar observations after studying *Alternaria brassicicola* on brassica seed and *A. sesamicola* Kawamura on sesame seed, respectively. However, Halfon-Meiri *et al.* (1987) and Knox-

Davies (1979) found that an *Alternaria* sp. and *A. brassicicola* were restricted to the seedcoat and pericarp of *Ranunculus asiaticus* L. and *Brassica oleracea* L., respectively, and did not infect the endosperm or embryo. Neergaard (1977) reported that spores of a *Stemphylium* sp. were carried on the surface of the seed coat. Lamprecht and Knox-Davies (1984) isolated *S. vesicarium* from the embryos and testas of lucerne seed although infestation of the latter was more prevalent.

Currently, thiram is considered a standard treatment in South Africa for controlling seed-borne diseases of vegetables (Vermeulen *et al.*, 1990). Boelema and Ehlers (1967) and Naude (1988) recommended that onion seed should be treated with thiram prior to planting to control *A. porri*.

Maude (1966) found that thiram eliminated infection of carrot seeds by *Alternaria dauci* (Kuhn) Groves & Skolko and *Stemphylium radicinum* (Meier, Drechsl. & Eddy) Neerg. However, Maude *et al.* (1969) found that, although a thiram soak gave complete eradication of sixteen seed-borne fungal pathogens it did not completely eradicate *Alternaria brassicola* from brassica seeds. These authors also found that the thiram treatment adversely affected the germination of some vegetable seeds. Maude and Bambridge (1991) found that topical applications of iprodione (2.5-5 g a.i./kg) gave effective eradication of internally seed-borne *A. dauci* of carrot. However, Maude *et al.* (1992) found that this fungicide only reduced infection when added in polyethylene glycol for priming of carrot seeds. The present

author evaluated the effectiveness of several seed treatments, including thiram, for eradicating *A. porri* and *S. vesicarium* from naturally infected onion seeds (Chapter 5).

Control on Leaves

Control of *Alternaria porri*

Chemical control

Godfrey (1945) obtained good control with Bordeaux mixture. Pandotra (1965b) conducted field trials with eleven different fungicides to control *A. porri* and also found that Bordeaux mixture provided the best control. In spraying trials by Bock (1964) in Kenya, good results were obtained with weekly applications of mancozeb (Dithane M45) or dichloran (Allisan). Gupta *et al.* (1986a, 1987b) also found that mancozeb gave the best disease control. Husain (1960) reported that, of the fungicides he tested, anilazine (Dyrene) was most effective against *A. porri*. Mancozeb and daconil controlled purple blotch of garlic provided it was applied at the rate of 3 kg/ha (Black *et al.*, 1985). Zineb (Dithane Z78) and captafol (Difolatan) have also been found to effectively control the pathogen (Sharma, 1987). Ahmed and Goyal (1988) reported that a seedling dip followed by a foliar spray with 0.2% copper oxychloride gave the highest disease control and maximum bulb yield. It was followed by zineb, mancozeb and captafol.

Fig. 11. Chemical spraying of diseased onions in the Weenen area, Natal

In South Africa, two fungicides are registered for the control of purple blotch, namely a fentinacetate/maneb mixture (Brestan) and iprodione (Rovral) (Vermeulen *et al.*, 1990). According to Naude (1988), Brestan should be applied at 7-14 d intervals after the first signs of the disease have been observed (Fig. 11). Rovral, on the other hand, should be used as a preventative measure. Spraying should commence when plants are at the 3-5 leaf stage and should be repeated at 14-21 d intervals (Naude, 1988).

Removal of crop debris

Diseased debris left behind in the field after harvest appeared to be the most important source of infection for the succeeding crop (Husain, 1960; Nolla, 1927; Pandotra, 1965a). Gladders (1981) also suggested that crop debris was a possible source of infection of leek by *A. porri*. Pandotra (1965a) found that *A. porri* remained viable for 9 mo in dried debris left at laboratory temperatures and for 8 mo in debris left on the field surface. However, if the diseased debris was buried 5-15 cm deep in the soil, the pathogen lost its viability within 2 mo (Pandotra, 1965a). Large onion dumps have also been found to supply primary inoculum (Thomas, 1951). In this case, wind appeared to be the primary agent for dissemination (Thomas, 1951). Ajrekar (1922) indicated that in Bombay, India, thrips played an important part in dissemination of *A. porri* inoculum and Nolla (1927) suggested that spattering rain and possibly

thrips were responsible for primary inoculations from debris. Boelema and Ehlers (1967) and Naude (1988) recommended that dead foilage and rotten bulbs should be removed from the field and destroyed. Summer ploughing of onion fields reduced purple blotch severity and that field that were ploughed three times before planting had the lowest disease severity and highest bulb yield (Gupta and Pathak, 1987). Boelema and Ehlers (1967) suggested that crop rotation should be practised.

Resistant cultivars

Miller (1982) screened 117 onion breeding lines for resistance against *A. porri*. He found that progeny from crosses of Bet Alpha and New Mexico Yellow Grano resulted in one line with a high level of resistance and three lines with medium levels of resistance. Gupta and Pathak (1988c) screened 21 indigenous and exotic onion cultivars for resistance to purple blotch and found that all the exotic lines proved highly resistant whilst all the indigenous lines were susceptible. Among the latter-mentioned, 'Pusa Red' was the least susceptible. According to Jones and Mann (1963), onions such as 'Yellow Globe Danvers' and 'Red Creole', that have a good covering of wax on the foliage and seed stalks, are more resistant to *A. porri* than such cultivars as Yellow Bermuda, Early Grano and Sweet Spanish which have less wax.

Control of *Stemphylium vesicarium*

Chemical control

No research has been done on the control of *S. vesicarium* on onion and there are no fungicides registered in South Africa to control this pathogen on onion or garlic. However, it is possible that the fungicides used to control *A. porri* might also control *S. vesicarium* on onion and garlic.

Removal of crop debris

It is most likely that diseased debris left behind in the field after harvest may be the most important source of infection for the succeeding crop and that wind and, possibly, thrips, act as dissemination agents, as is the case with *A. porri*. Johnson (1990) found that conidia of *S. vesicarium*, formed on the previous year's asparagus fern debris, served as a source of primary inoculum. He also suggested that volunteer asparagus seedlings that became infected during the harvest season may act as a bridge to carry inoculum through to the next growing season.

Resistant cultivars

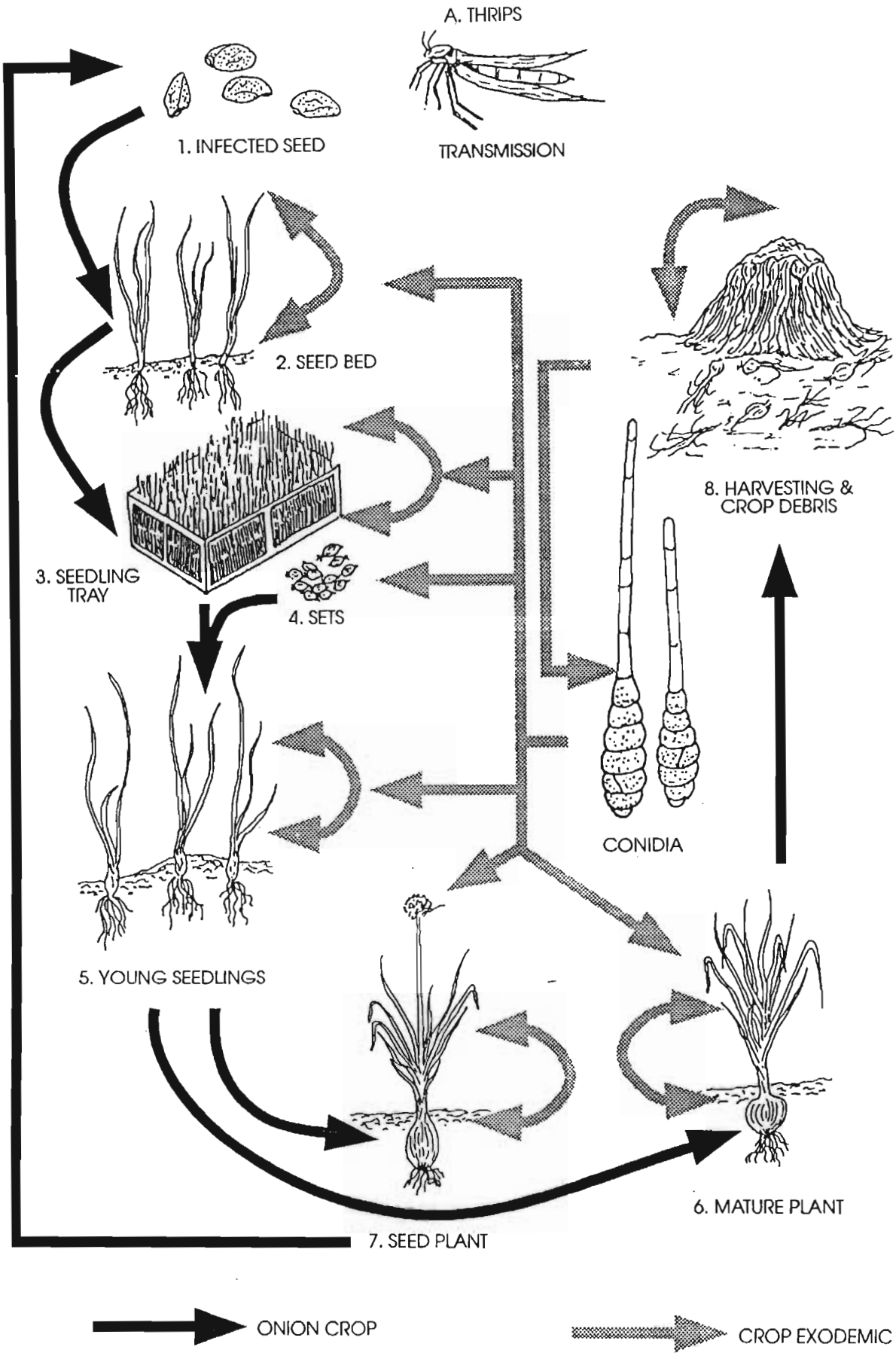
The National Bureau of Plant Genetic Resources in New Delhi, India, has 1200 accessions of onion which have been screened against stemphylium blight from 1986 to 1990 (Bisht *et al.*, 1990). These authors found that 40 lines, with disease intensities ranging from 1-5%, showed promising field resistance.

Some of these lines were also found to be disease resistant to *A. porri*.

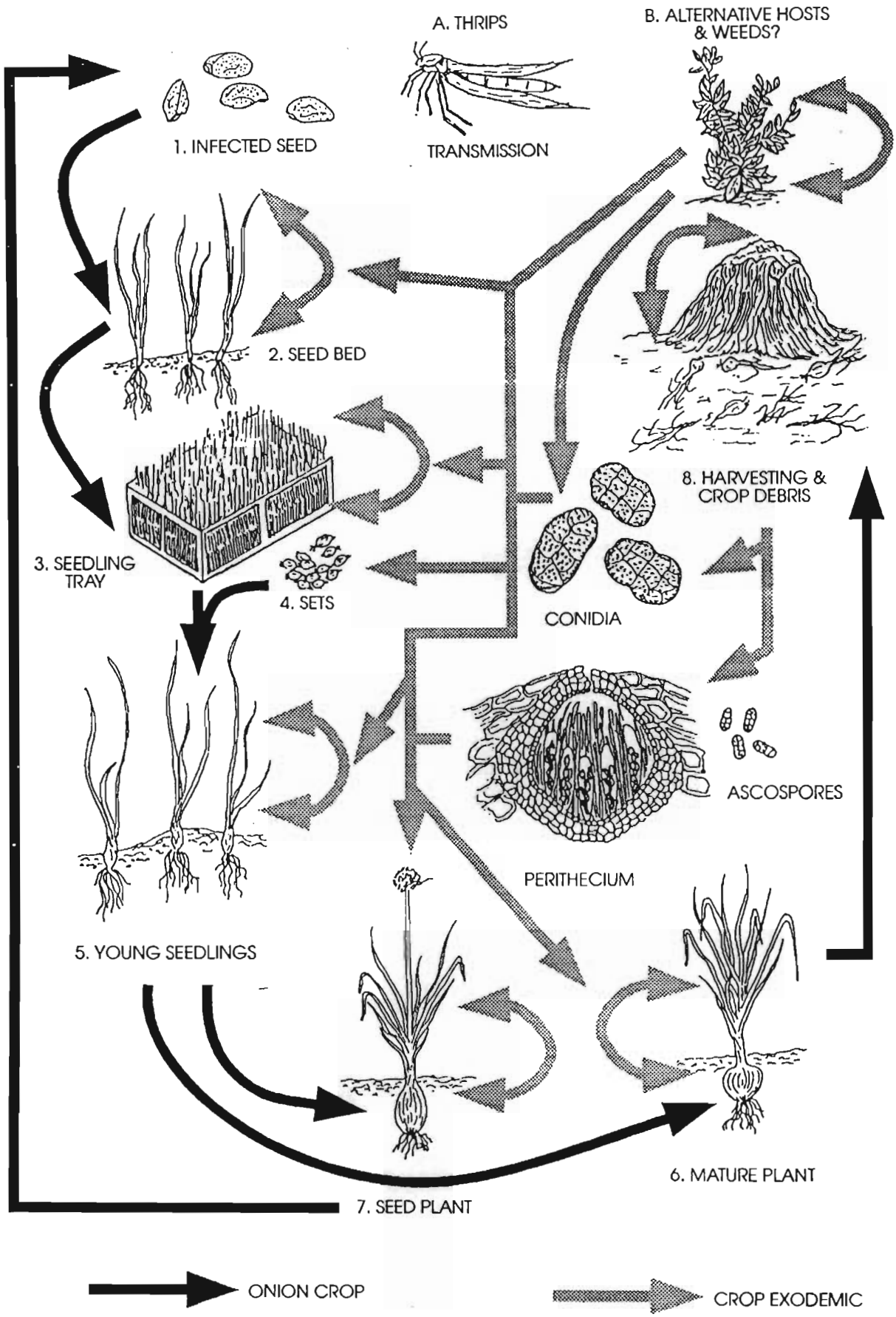
Ethographs

Using the information presented in this chapter, the author has constructed two ethographs; one for purple blotch of onion and the other for stemphylium leaf blight of onion.

Ethograph of purple blotch of onion



Ethograph of stemphylium leaf blight of onion



STRUCTURING OF THIS THESIS

This thesis consists of copies of manuscripts presented to scientific journals for publication. All the manuscripts have been accepted for publication and some have already appeared in press. In Chapter 2, the manuscript titled "Scanning electron microscopy of conidial formation of *Stemphylium vesicarium* on onion leaves" has been accepted for publication in the Journal of Phytopathology and the article titled "Conidial development of *Alternaria porri* on onion leaves" has been published in the Proceedings of the Electron Microscopy Society of Southern Africa. Chapter 3 has been published in Mycological Research and Chapter 4 has been accepted for publication in the Canadian Journal of Botany. In Chapter 5, the manuscript entitled "Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed" has been published in Plant Disease. In Chapter 6, the Disease Notes, "First report of *Stemphylium vesicarium* on garlic in South Africa" and "First report of *Alternaria porri* on garlic in South Africa", have been published in Plant Disease. The manuscript titled "Stemphylium leaf blight of garlic in South Africa" has been accepted for publication as a short report by the journal Phytophylactica.

The reader will notice some inconsistencies in author citation, referencing and manuscript compilation. Since manuscripts were submitted to different journals and had to comply with different requirements, such inconsistencies are

unavoidable. Author citation in Chapter 1 (Introduction and Literature Review) and Chapter 7 (General Discussion) and the citation of literature, pertaining to these chapters and presented at the end of the thesis, are according to the journal Phytopathology .

CHAPTER 2

CONIDIUM FORMATION BY *STEMPHYLIUM VESICARIUM* AND *ALTERNARIA PORRI* ON ONION LEAVES

Scanning Electron Microscopy of Conidium Formation of *Stemphylium vesicarium* on Onion Leaves

THERESA A. S. AVELING AND I. H. RONG

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With 8 figures

Abstract

Onion leaves were inoculated with conidia of *Stemphylium vesicarium* and the development and morphology of conidiophores and conidia on the leaf surface were examined using scanning electron microscopy. Occasionally solitary, but more usually fasciculate conidiophores emerged through the epidermis. Hyphae growing on or above the leaf surface also differentiated into conidiophores. Conidiophores were straight or flexuous, simple, smooth or verrucose and cylindrical but enlarged apically at the site of conidium production. Smooth, round, bud-like conidial initials were produced singly at the apex of the verrucose conidiophores. As conidia matured, they became oblong to ovoid and densely verrucose. Once the mature conidium seceded, a small pore was visible at the apex of the conidiogenous cell.

conidiophores proliferated percurrently at the distal region, forming secondary conidiophores and conidia.

Studies have shown that *Stemphylium vesicarium* (Wallr.) E. Simmons is a potentially serious pathogen of onion (*Allium cepa* L.) during wet periods (MILLER et al. 1978, RAO and PAVGI 1975, SHISHKOFF and LORBEER 1989). *S. vesicarium* was first reported to be a serious pathogen of garlic (*Allium sativum* L.) in South Africa by AVELING and NAUDE (1992). SIMMONS (1969) gave a detailed description of the morphological characteristics of conidiophores and conidia of *S. vesicarium* and RAO and PAVGI (1975) used light microscopy to study these characteristics in culture and on onion leaves. Furthermore, SIMMONS (1967) presented a general description of conidial formation of *Stemphylium* Wallr. and the closely related genera, *Alternaria* Nees : Fries, and *Ulocladium* Preuss, based on light microscopy observations of several collections and cultures, including the type specimens. CARROLL and CARROLL (1971) were the only authors to have used electron microscopy to study the ultrastructure of conidium formation by a *Stemphylium* sp. These authors used transmission electron microscopy to study the conidiogenesis of *S. botryosum* Wallr. grown in culture. The present study attempts to provide additional information on the development and morphology of conidia of *S. vesicarium in vivo* on the surface of diseased onion leaves using scanning electron microscopy (SEM).

Materials and Methods

Fungal cultures

S. vesicarium was isolated from diseased onion leaves. The leaves were surface-sterilised with 0.5 % (w/v) sodium hypochlorite for 10 min before lesions were excised and plated on potato dextrose agar (PDA) (Merck). An isolate of *S. vesicarium* was deposited with the National Collection of Fungi, Pretoria (PREM 50717). *S. vesicarium* was cultured on PDA and incubated at 24 °C. Sporulation was induced by exposing cultures to a 12 h dark/12 h cool white fluorescent light photoperiod for six days.

Plant material

Onion plants of the cultivar 'Texas Grano', were grown from bulbs and maintained in a greenhouse at 20 °C. Leaves grown from healthy, sprouted bulbs were used for inoculation.

Inoculation procedure

Inoculum was prepared aseptically by pouring sterile distilled water over the colonies, agitating with a glass rod and filtering the suspension through two layers of sterile muslin cloth. Onion leaves were spray-inoculated to run-off with conidia of *S. vesicarium* at a concentration of 2.3×10^4 conidia.ml^{-1}. Inoculated plants were placed in a dew chamber for

48 h and then in a greenhouse at 20 °C for a further 48 h. Plants with small white lesions were returned to the dew chamber for 24 h to induce sporulation.

Scanning electron microscopy

Leaf pieces (2 mm X 4 mm) excised 124 h after inoculation were vapour-fixed with 1 % (w/v) OsO_4 for 96 h in a closed container and air-dried for 48 h to prevent conidia from being dislodged. All specimens were mounted on SEM stubs, coated with gold in a Polaron Sputter Coater and examined with a Jeol JSM 840 scanning electron microscope operating at 8 kV.

Results

Ninety-six hours after inoculation, small pale yellow lesions were visible on the leaves. These lesions turned greyish-brown at 124 h after inoculation due to the development of conidiophores and conidia of the pathogen. After the plants had been placed in the dew chamber to induce sporulation, conidiophores were formed in two ways in and around the lesions. Firstly, ocassionally solitary but more usually fasciculate conidiophores emerged through stomata or directly through the epidermis (Fig. 1). Secondly, hyphae growing on or above the leaf surface differentiated into conidiophores (Fig. 2). The conidiophores were straight or flexuous, simple, smooth or verrucose (Figs 1, 2). Conidiophores were cylindrical but enlarged apically at the site of conidium production (Figs 3, 4,

5). Conidiogenous cells were terminal, verrucose and clavate to pyriform (Figs 3, 4, 6), often becoming cupulate with increasing age (Figs 5, 7).

A single conidium was produced at the apex of a conidiophore. The conidial initial was at first round, bud-like and smooth (Fig. 3). As the conidium matured, little growth in width but pronounced elongation occurred and the base of the conidium was broader than the apex (Fig. 4). The surface of the immature conidium was verrucose resembling the apex of the conidiophore (Fig. 4). Mature conidia were oblong to ovoid and densely verrucose (Fig. 5). Once the mature conidium seceded, a small pore (1-2 μm diam.), representing the conidiogenous locus, was visible at the apex of the conidiogenous cell (Fig. 6). The conidiophores proliferated percurrently at the distal region (Fig. 7), forming secondary conidiophores. Usually the mature conidium was dislodged before a secondary conidiophore and conidium were formed however, occasionally this occurred before the mature conidium was released (Fig. 8).

Discussion

The emergence of conidiophores of *S. vesicarium* through the onion leaf epidermis or the differentiation of hyphae, growing on or above the leaf surface, into conidiophores indicated the initiation of sporulation. The conidiophores were morphologically distinct from vegetative hyphal branches. The apex of the conidiophore where the single conidium was formed, was slightly to distinctly swollen, as reported by RAO and PAVGI

(1975) and SIMMONS (1967). CARROLL and CARROLL (1971) using TEM, found that the conidial initial of *S. botryosum* in culture was a simple, smooth, bud-like protrusion the wall of which was continuous with that of the conidiophore. The conidial initial of *S. vesicarium* was also smooth and bud-like. Furthermore, our observations correspond with those of CARROLL and CARROLL (1971), who noted that the surface of the conidia became verrucose as the spores matured.

RAO and PAVGI (1975) and SIMMONS (1967), using light microscopy, reported that in *S. vesicarium*, the apical pore of the conidiogenous cell was 4-7 and 5-6 µm in diameter, respectively. In this SEM study, the apical pore of *S. vesicarium* was only 1-2 µm in diameter, possibly due to shrinkage occurring during osmium tetroxide vapour-fixation for SEM.

Conidiophores of *S. vesicarium* became cupulate with increasing age as described in a light microscopy study by LAMPRECHT et al. (1984) of *S. vesicarium* on *Medicago* spp. Conidiophores of *S. vesicarium* were percurrently proliferated at the distal region as described by RAO and PAVGI (1975) and SIMMONS (1967). SIMMONS (1967, 1969) stated that the production of a single conidium at the apex of a conidiophore which had a percurrent method of proliferation, easily distinguishable among those of otherwise superficially similar genera, was the unique character which has been used in assigning isolates to *Stemphylium*. Occasionally a secondary conidiophore and conidium were formed by *S. vesicarium* before the mature conidium was dislodged, as described by SIMMONS (1969). SIMMONS (1969) reported that the mature conidium of *Stemphylium* spp. became lateral if its protoplasmic connective

withstood the thrust of the emerging secondary conidiophore, or it was dislodged if its connective broke.

In this study, SEM has proved to be a valuable technique for studying details of sporulation and of conidia of *S. vesicarium* formed on onion leaves and not artificially in culture.

The authors thank the Electron Microscope Unit, University of Pretoria for technical assistance and the Department of Agricultural Development for financial support. Part of a P.hD. dissertation submitted by the first author to the University of Natal, Pietermaritzburg.

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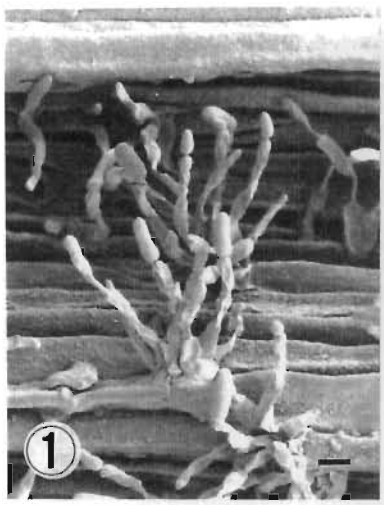
LEGENDS TO FIGURES

Figs 1-8. Scanning electron micrographs of the development and morphology of conidia of *S. vesicarium* on onion leaves. **Fig. 1.** Emergence of solitary and fasciculate conidiophores through the epidermis (bar = 10 μm). **Fig. 2.** A hypha (h), growing above the leaf surface, has differentiated into a conidiophore (Co) (bar = 10 μm). **Fig. 3.** Conidiophore (Co), distinctly verrucose at the apex, with a smooth-surfaced young developing conidium (C) (bar = 1 μm). **Fig. 4.** Maturing conidium (C) showing a slight growth in width but pronounced elongation. The surface of the immature conidium is verrucose resembling the apex of the conidiophore (Co) (bar = 1 μm). **Fig. 5.** Mature conidia (C) are oblong to ovoid and densely verrucose. Mature conidiophores (Co) have cupulate apices (bar = 10 μm). **Fig. 6.** A small pore is visible at the apex of the conidiophore (Co) once the mature conidium has been detached (bar = 1 μm). **Fig. 7.** Percurrently proliferating conidiophore (Co) with a secondary conidiophore (sCo)(bar = 1 μm). **Fig. 8.** Formation of a secondary conidiophore (sCo) and conidium (sC) before the mature conidium (C) has been dislodged from the primary conidiophore (Co). Arrow indicates where the secondary conidiophore emerges through the first (bar = 10 μm).



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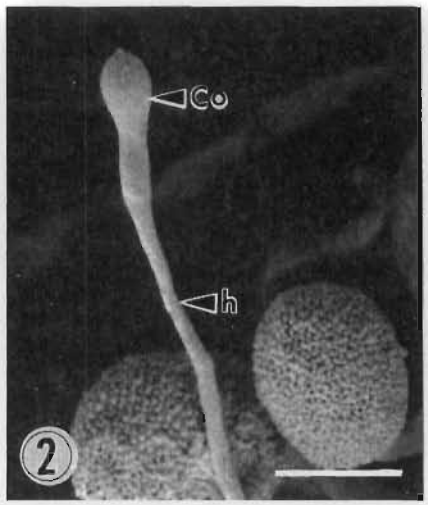

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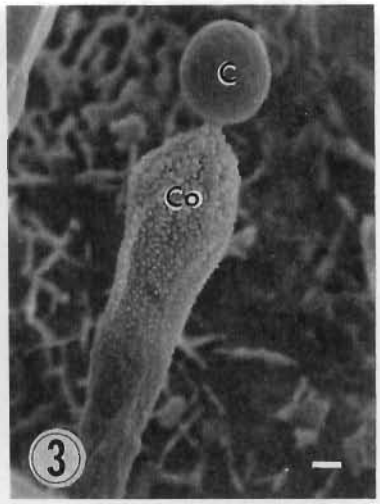

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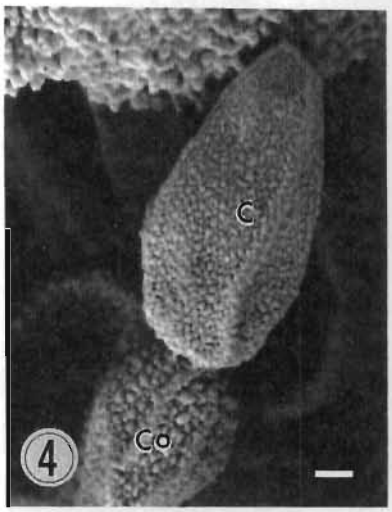

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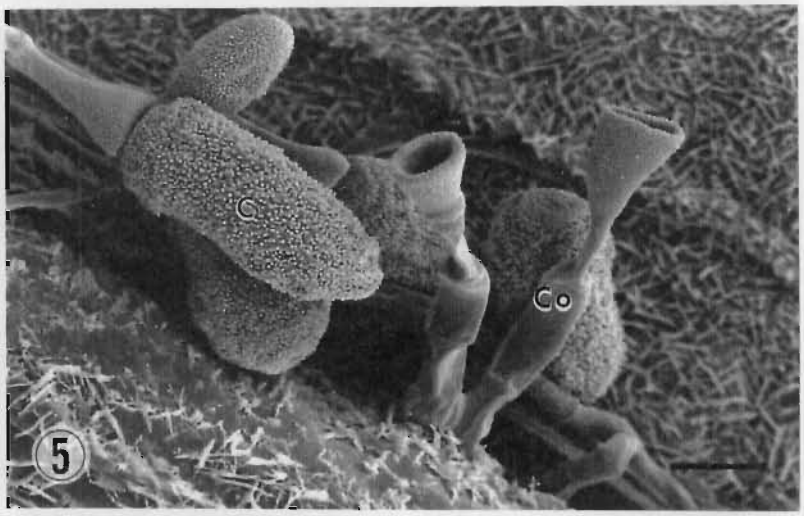

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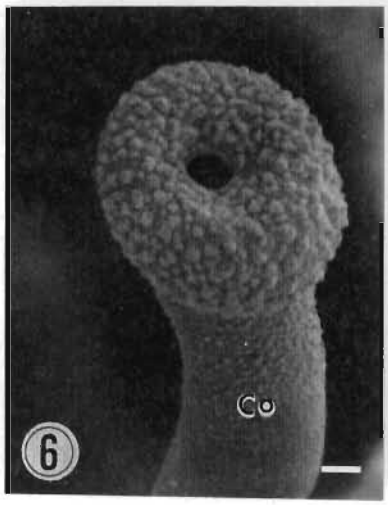

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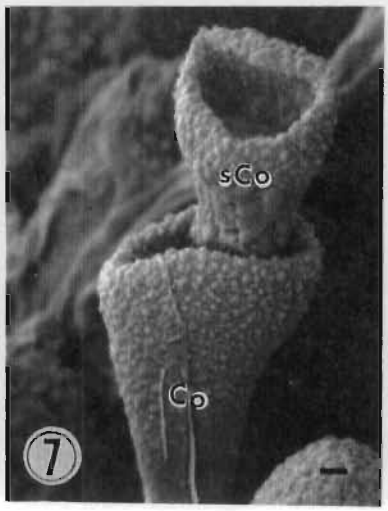

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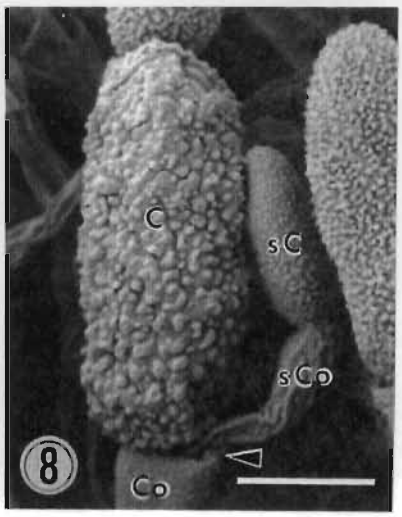

8

CONIDIAL DEVELOPMENT OF *Alternaria porri* ON ONION LEAVES

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Alternaria porri is the fungal agent of purple blotch of onion (*Allium cepa*) and other *Allium* spp. Purple blotch is a disease of worldwide occurrence and is responsible for extensive leaf damage. *Alternaria* spp. are placed in the Porosporae of the Hyphomycetes. Porospore is a term coined by Hughes[1] for a conidium which develops through a pore in the wall of a conidiophore. Campbell[2], conducting a TEM study of conidium structure and development in *A. brassicicola in vitro*, showed that the conidia are formed by budding through a fairly well-defined channel in the outer wall of the conidiogenous cell, apparently induced enzymatically. The aim of this research was to supplement earlier TEM and SEM work[2,3] by studying the structure and development of *A. porri* conidia, *in vivo* on onion leaves, using SEM.

Onion leaves were spray-inoculated to run-off with conidia at a concentration of 1×10^4 conidia ml^{-1}. Provided leaves were maintained at a high relative humidity, sporulation took place on leaf surfaces within 14 days. To prevent conidia from being dislodged, small tissue samples were cut, vapour-fixed with OsO_4 for 96 h in a closed container and air-dried for 48 h. The specimens were mounted on stubs, coated with gold and examined in a Jeol JSM 840 scanning electron microscope at 8 kV.

Solitary or fasciculate conidiophores emerged through the epidermis, the lengths usually varied from 12 µm to 46 µm. Conidia were borne singly at the apex of conidiophores. Figure 1 shows that the conidium was at first bud-like and appeared to be single-celled with the base of the conidium being broader than the tip. The next stage was marked by a slight growth in width and pronounced elongation (Fig. 2). This appeared to be followed by the formation of transverse septa (Fig. 3). The mature conidia were characteristic of *A. porri*, i.e., obclavate with a simple, tapering beak usually about the same length as the body but sometimes shorter or longer (Fig. 4). Transverse and often longitudinal or oblique septa were present. The overall length of the mature conidia varied from 100 µm to 270 µm. Once the mature conidium had been detached, a tiny pore, representing the conidiogenous locus was visible at the apex of the conidiophore (Fig. 5).

The obvious capabilities of SEM for the study of conidial development and structure have been presented in this paper.

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CHAPTER 3

INTERACTION BETWEEN THE ONION LEAF AND *STEMPHYLIUM VESICARIUM*

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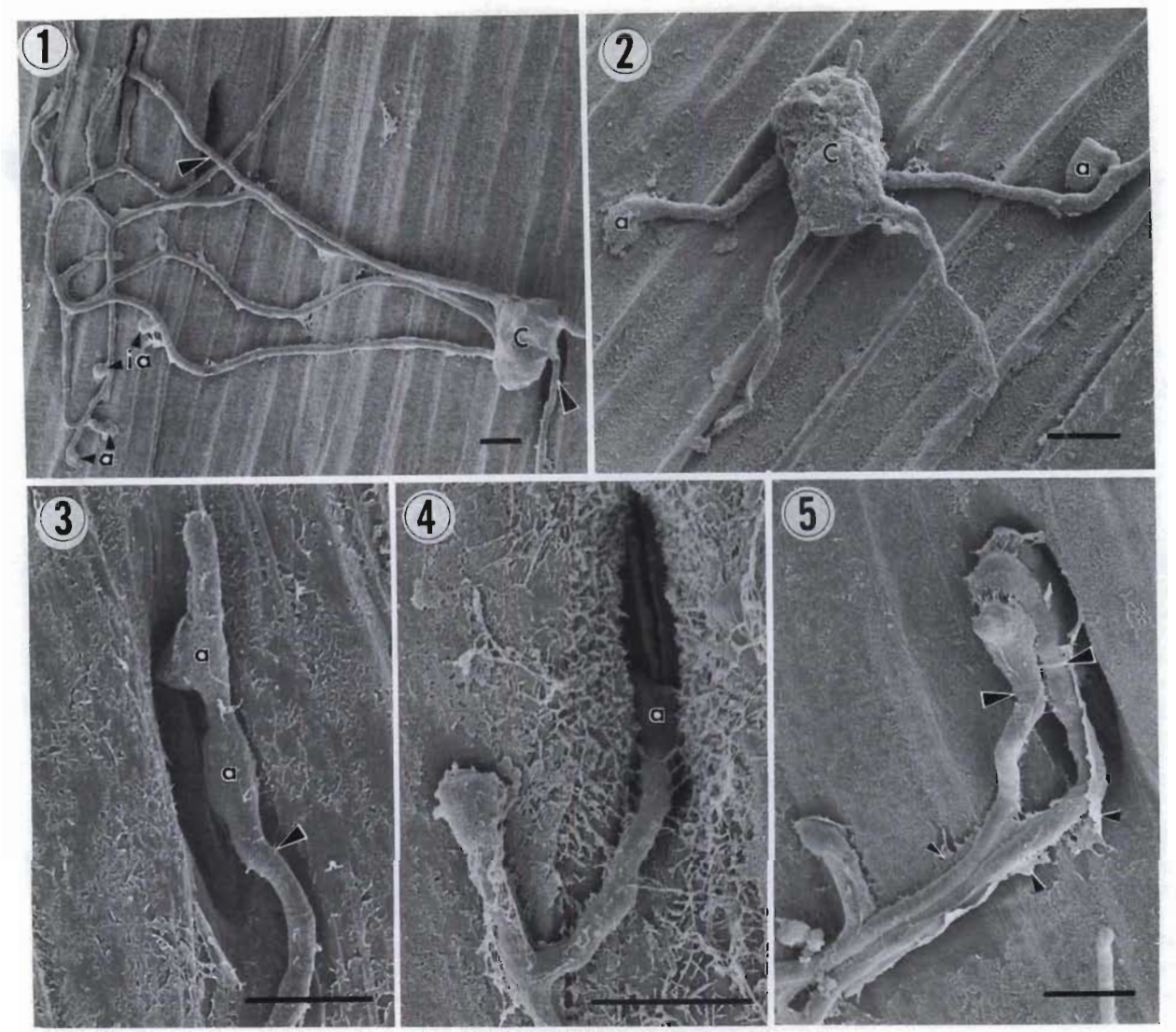

Figs 1–5. Scanning electron micrographs of the pre-penetration behaviour of conidia of *S. vesicarium* on onion leaves (bars, 10 µm). **Fig. 1.** Germinated conidium (C) showing extensive growth of germ-tubes. Terminal appressoria (a) and intercalary appressoria (ia) have formed on the leaf surface. Germ-tubes often pass over stomata without forming appressoria (large arrows). **Fig. 2.** Appressoria (a) formed by germ-tubes shortly after emergence from conidium (C). **Fig. 3.** Formation of two appressoria (a) over a stoma by a single germ-tube. A septum (arrow) appears to delimit the first appressorium. **Fig. 4.** The germ-tube does not grow directly into the open stoma but first forms an appressorium (a). **Fig. 5.** Germ-tubes forming terminal appressoria on top of one another over a stoma, producing a compound appressorium. Septa (large arrows) delimit the appressoria. Note the extracellular material associated with germ-tubes (small arrows).

coater and examined with a Jeol JSM 840 scanning electron microscope operating at 8 kV.

Light microscopy

To quantify the percentage of pre-penetration structures and penetration of the leaves, two pieces (2 × 5 mm) of leaf from two leaves of four plants were cut 36 h after inoculation. The leaf tissue was cleared using the technique of Herr (1971) and mounted on microscope slides. The different types of pre-penetration structures were expressed as a percentage of the total number of germinated conidia with pre-penetration structures on the leaf surface. Successful penetration by a pre-penetration structure was expressed as a percentage of the total number of that particular type of structure. Smaller leaf pieces (2 × 2 mm) were fixed in 2·5% (v/v) glutaraldehyde in 0·07 M phosphate buffer (pH 7·4) for 2 h and rinsed in the same buffer. Material was post-fixed in 0·25% (w/v) osmium tetroxide for 4 h, followed by three successive washing steps in distilled water. Material was then dehydrated in an ascending acetone series and embedded in Quetol 651 resin (Van der Merwe & Coetzee, 1992). Cross-sections of 2 µm thickness were made using a Reichert–Jung Ultracut E microtome and placed on drops of distilled water on microscope slides. After drying, the sections were stained with 0·5% (w/v) toluidine blue in 0·5% (w/v) borax and rinsed in water. Micrographs were made with a Nikon Optiphot photomicroscope.

RESULTS

Pre-penetration behaviour of conidia on the leaf surface

Germinated and ungerminated conidia adhered strongly to

Table 1. The percentage of various types of pre-penetration structures formed by *S. vesicarium* on the onion leaf surface and the percentage of successful penetrations by these structures

Pre-penetration structure	Occurrence (%)†	Successful penetration (%)‡
Appressoria on epidermal cells		
Terminal	29·7 a*	72·3 c
Intercalary	18·8 b	47·7 d
Appressoria above stomata		
Single terminal	35·9 a	96·5 a b
Double	12·5 b	89·4 b
Compound	3·1 c	100 a

† Expressed as a percentage of the total number of germinated conidia with pre-penetration structures formed on two leaf pieces from each of two leaves of four replicate plants.

‡ Expressed as a percentage of total number of that particular pre-penetration structure.

* Values within a column, not followed by the same letter, are significantly different ($P = 0{\cdot}05$).

various types of appressoria formed above stomata, terminal appressoria were formed most frequently (35·9%), followed by the formation of two appressoria on the same germ-tube (double appressorium) (12·5%) and lastly, the formation of compound appressoria (3·1%) (Table 1). Of these appressoria, 96·5% of terminal, 89·4% of double appressoria on the same germ-tube and 100% of compound appressoria successfully penetrated the leaf (Table 1). Figure 8 illustrates the penetration of a stoma by a primary hypha formed from a single, terminal appressorium above the stoma. Penetration of a stoma by two bulbous, septate primary hyphae each formed from an appressorium present on the same germ tube situated above the stoma is illustrated in Fig. 9.

DISCUSSION

Pre-penetration events, including adherence of conidia to the leaf surface, spore germination, germ-tube growth, adherence of germ-tubes to leaves, and the formation of appressoria during infection of onion leaves by *S. vesicarium* were effectively demonstrated using SEM. Successful penetration was determined using light microscopy.

Germ-tubes of *S. vesicarium* on the onion leaf surface usually grew for a short distance only before appressoria were formed. They were, however, capable of extensive growth prior to the formation of appressoria. Variation in germ-tube growth of *S. vesicarium* on asparagus has also been observed (Sutherland *et al.*, 1990; Falloon *et al.*, 1987). Emmett & Parbery (1975) discussed the possibility that extracellular material may play a role in adherence of appressoria to plant surfaces. The extracellular material associated with germ-tubes and appressoria of *S. vesicarium* possibly aided the adherence of these structures to the onion leaf surface.

Borges *et al.* (1976) and Falloon *et al.* (1987), studying the infection of alfalfa by *S. botryosum* and asparagus by *S. vesicarium*, respectively, found that germ-tubes of these pathogens penetrated exclusively through stomata without the formation of appressoria. Sutherland *et al.* (1990) studying the infection of asparagus by a *Stemphylium* sp. reported similar results. These authors (Borges *et al.*, 1975; Falloon *et al.*, 1987; Sutherland *et al.*, 1990) observed that direct penetration of the epidermal cells by germ-tubes, or by means of appressoria, did not occur. In some species, formation of appressoria might be obligatory for infection, while in others it might be optional or unnecessary (Emmett & Parbery, 1975). In *S. vesicarium*, penetration of the onion leaf only occurred after the formation of appressoria which may indicate that appressoria are obligatory for infection. Higgins & Lazarovits (1978) found that *S. botryosum* penetrated the leaves of red clover primarily via stomata whilst appressoria, formed above epidermal cells, rarely resulted in successful penetration. *S. sarcinaeforme*, however, was more versatile in its modes of penetration with some stomatal penetrations and numerous direct penetrations between or through epidermal cells (Higgins & Lazarovits, 1978). In this study, penetration of the onion leaf by *S. vesicarium* was primarily by means of appressoria formed above stomata, although penetrations by means of appressoria formed directly above epidermal cells also occurred. No evidence of long-distance attraction to stomata was detected and germ-tubes and hyphae often passed close to or over stomata showing no apparent tropic response.

The formation of bulbous, septate primary hyphae within the host, as found in this study, has also been reported by other researchers studying different host-*Stemphylium* sp. interactions (Pierre & Millar, 1965; Higgins & Lazarovits, 1978). The plasmolysis and cytoplasmic disruption of epidermal cells adjacent to, and mesophyll cells in the near vicinity of, penetrating hyphae may suggest a diffusion of toxic metabolites from the fungus. *S. vesicarium* has been found to produce a toxin, stemtoxin, but its enzymatic activity has not yet been determined (Heiny & Gilchrist, 1991).

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CHAPTER 4

INTERACTION BETWEEN THE ONION LEAF AND *ALTERNARIA PORRI*

Infection studies of Alternaria porri on onion leaves

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Conidial germination of Alternaria porri, formation of pre-penetration structures, penetration of the onion leaf surface and the subsequent infection process were studied using light, scanning electron and transmission electron microscopy. Most conidia germinated within 24 h of inoculation. Each conidium formed several germ-tubes which were initiated at random positions on the conidium and grew in any direction across the leaf surface. The germ-tubes usually terminated in bulbous appressoria formed directly on the epidermal cells or on stomata. Following direct penetration of the outer epidermal cell wall or the stoma, bulbous primary hyphae developed below the appressoria. Secondary hyphae developed from the primary hyphae and grew within the intercellular spaces penetrating mesophyll cells. The changes in ultrastructure of cells in close proximity to hyphae and of infected cells, are described.

Key words: Allium cepa, electron microscopy, infection process, purple blotch

Introduction

Alternaria porri (Ellis) Cif. is the causal agent of purple blotch of onion (Allium cepa L.) and is responsible for extensive leaf damage and yield losses (Bock 1964; Skiles 1953). The symptoms of infection of onion by this pathogen and the effect of various environmental factors on infection have been studied in some detail (Angell 1929; Bock 1964; Evert and Lacy 1990; Gupta et al. 1985; Khare and Nema 1982; Nolla 1927). However, little research has been conducted on the infection process, and the use of electron microscopy techniques to study this process has been largely neglected, despite its obvious advantages.

There have been some contradictive light microscopy studies on the mode of penetration of A. porri into onion leaves. Angell (1929) reported that A. porri penetrated via stomata of onion leaves and that no direct penetration through the epidermal cells occurred, whilst Nolla (1927) and Walker (1952) reported penetration through stomata and wounds in the epidermis.

Angell (1929) reported that infection hyphae developed within the substomatal chambers, sometimes filling them, and penetrated the underlying tissues either inter- or intracellularly. Nolla (1927) found that the invading hyphae spread through the intercellular spaces of the leaf.

The objectives of this study were to examine conidial germination of A. porri, formation of pre-penetration structures, penetration of the onion leaf surface and the subsequent infection process using light, scanning electron and transmission electron microscopy.

Materials and methods

Fungal cultures

A. porri was isolated from diseased onion leaves. The leaves were surface-sterilized with 0.5% (w/v) sodium hypochlorite for 10 min before lesions were excised and plated onto potato dextrose agar (PDA)(Merck). An isolate was deposited in the herbarium of the International Mycological Institute (IMI 339537) and with the National Collection of Fungi, Pretoria (PREM 50715). A. porri was maintained on PDA and cornmeal agar (CMA) (Difco) and incubated at 24°C. Sporulation was induced by exposing 4-day-old CMA cultures to a 12 h dark/12 h near-ultraviolet light regime for 4 days. These conidia were used for the inoculation procedure.

Plant material

Onion plants (cv. Texas Grano), were grown from bulbs surface-sterilized in 1% (w/v) sodium hypochlorite for 5 min. Plants were maintained in a greenhouse at ≈ 25/20°C day/night temperature. The leaves of healthy bulbs, sprouted for 6-8 weeks, were used for inoculation studies.

Inoculation procedure

Two procedures were used. In the one, inoculum was prepared by pouring sterile, distilled water over the colonies, agitating with a glass rod and filtering the suspension through two layers of sterile muslin cloth. Onion leaves were spray-inoculated to run-off with conidia of A. porri at a concentration of 5 X 10^4

conidia ml^{-1}. In the other, onion leaves were painted with conidia collected from sporulating colonies using a fine paintbrush. In each procedure, inoculated plants and uninoculated controls were placed in a dew chamber for 24 h and then in a greenhouse at 25/20°C day/night temperature for a further 24 h.

SEM

Leaf tissue was cut into 2 X 2 mm pieces 12 and 24 h after inoculation and was fixed in 2.5% (v/v) glutaraldehyde in 0.07 M phosphate buffer (pH 7.4) for 2 h. Material was rinsed in the same buffer and post-fixed in 0.25% (w/v) osmium tetroxide for 2 h, followed by three successive washing steps in distilled water. Material was dehydrated in an ascending acetone series, critical point dried in a Bio-rad critical point dryer and specimens were mounted on SEM stubs. The leaf epidermis of some of the specimens was removed using the stub method of Hughes and Rijkenberg (1985). The whole leaf and stripped epidermis specimens were coated with gold in a Polaron sputter coater and examined with a Jeol JSM-840 or Hitachi S-570 scanning electron microscope operating at 8 kV.

TEM

Leaf pieces (1 X 1 mm) were harvested 36 and 48 h after inoculation, fixed in 2.5% (v/v) glutaraldehyde in 0.07 M phosphate buffer (pH 7.4) for 2 h, and rinsed in the same buffer. Material was post-fixed in 0.25% (w/v) osmium tetroxide for 4 h, followed by three successive washing steps in distilled water.

Material was then dehydrated in an ascending acetone series and embedded in Quetol 651 resin (Van der Merwe and Coetzee 1992). Ultra-thin sections were cut using a Reichert-Jung Ultracut E microtome for transmission electron microscopy. Sections were stained with uranyl acetate and Reynold's lead citrate and examined with a Philips 301 transmission electron microscope operating at 60 kV.

Light microscopy

The semi-thin sections (2 μm thick) of resin-embedded material, prepared as for TEM, were placed on drops of distilled water on microscope slides and after drying, were stained with 0.5% (w/v) toluidine blue in 0.5% (w/v) borax and rinsed in water. Leaf pieces (2 X 5 mm) were removed 24 and 36 h after inoculation, for whole mount observations, mounted on microscope slides and stained as described above. Micrographs were made with a Nikon Optiphot photomicroscope.

Results

Most conidia of A. porri germinated within 24 h of inoculation. Each conidium formed several germ-tubes which were initiated at random positions on the conidium and grew in any direction across the leaf surface (Fig. 1). The germ-tubes usually terminated in bulbous appressoria but occasionally extensive growth formed a hyphal network. Appressoria were formed directly on the epidermal cells (Figs. 1, 5) or on stomata (Figs. 8, 9). There was no specific orientation by germ-tubes

towards stomata and they often passed near stomata without appressorium formation (Fig. 2). An extracellular material usually surrounded germ-tubes and appressoria (Fig. 1).

Penetration of the leaf surface occurred directly through the epidermis (Figs. 3, 5, 6) usually at the site of cell wall junctions (Figs. 3, 5) or through stomata (Figs. 4, 8, 9) but only after the formation of appressoria. Following direct penetration of the outer epidermal cell wall one, or occasionally more, bulbous primary hypha developed below the appressorium within the epidermal cell or between the cell walls of adjacent cells (Figs. 3, 5, 6). The epidermal cell wall became discoloured at the site of penetration (Fig. 6). Occasionally penetrations involved some subcuticular or lateral growth of the fungus within the host epidermal cell wall rather than immediate direct penetration (Fig. 7). This cell wall became swollen and discoloured in the vicinity of the invading hypha. When penetration occurred through a stoma, usually more than one bulbous primary hypha developed between the guard cells and in the substomatal cavity (Figs. 4, 8). The primary hyphae usually attained a larger diameter than the appressoria, from which they were usually separated by a septum (Fig. 9). The appressoria appeared to transfer most of their cytoplasm to the primary hyphae (Fig. 9). Figure 9 shows an electron-dense matrix in the cavity between the primary hypha and the guard cells. The cell walls of the guard cells became swollen and electron-lucent (Fig. 9) when compared to those of uninoculated control (Fig. 10). The cytoplasmic content of these cells and adjacent cells was disorganized and the cell membrane disrupted, often with only

remnants of membrane discernible (Fig. 9).

Secondary hyphae developed from the primary hyphae and grew within intercellular spaces (Fig. 12), penetrated mesophyll cells (Fig. 13), and often grew parallel to the cuticle within epidermal cells (Fig. 11). The transverse cell walls of such an epidermal cell were electron-lucent and the cell membrane was disrupted (Fig. 11). An extracellular matrix was often associated with hyphae in contact with mesophyll cells (Figs. 12, 13). Where the hyphae penetrated the mesophyll cell walls they were usually slightly constricted (Fig. 13). As the hyphae advanced into the adjacent cortical cells, infected cells, and cells in close proximity to or some distance from hyphae, showed changes in ultrastructure (Fig. 14). The plasmalemma retracted from the cell wall and often was also disrupted with only fragments of the membrane still present (Fig. 15). The cytoplasm was disorganized and granular when compared to that of uninoculated leaf tissue (Fig. 16). More severe disruption of host cells was characterized by chloroplast damage. Swelling of chloroplasts and vesiculation and disruption of membranes occurred (Figs. 13, 15). The membranes surrounding the chloroplasts in these cells usually disintegrated and the remains of the stroma and disrupted granum membranes were released into the cytoplasm of the cell (Figs. 13, 15).

Discussion

Our results confirmed those of Everts and Lacy (1987) and Fahim and El-Shedi (1966) who reported that conidia of Alternaria porri

germinated from more than one cell and that germ-tubes grew in any direction across the leaf surface. Fahim and El-Shedi (1966) found that germ tubes usually terminated in appressoria over stomata and on epidermal cells, but that these structures were not essential for successful penetration. We found that penetration of the onion leaf only occurred after the formation of appressoria, as described by Everts and Lacy (1987), which may indicate that appressoria are obligatory for infection. The extracellular matrix associated with germ tubes and appressoria of A. porri, as found in the present study, has also been reported for other Alternaria spp. (Allen et al. 1983; Van Dyke and Trigiano 1987), and may have an adhesive function.

We also found that penetration occurred through both stomata and unwounded epidermal cells as described by Everts and Lacy (1987) and Fahim and El-Shedi (1966). No evidence of long-distance attraction to stomata was detected and germ tubes often passed close to stomata showing no apparent trophic response. Saad and Hagedorn (1969) reported frequent stomatal penetration of bean leaves by Alternaria tenuis Nees during the first 24 h after germ-tube growth on the leaf surface, whereas direct penetration occurred more frequently after 24 h. In this study, however, penetration through stomata or the epidermis occurred with about equal frequency and was independent of time. Whether or not cuticular penetration was by mechanical force, or involved enzymic hydrolysis, was not determined in this study. However, the apparent electron-lucency of the cell wall at the infection site below an appressorium may indicate that enzymes play a role in direct penetration. The presence of hyphae growing parallel

to the cuticle within the outer epidermal cell wall, as described in this study, was also reported by Angell (1929).

The formation of bulbous, septate primary hyphae within the host, as found in this study, has also been reported by other researchers studying the same (Fahim and El-Shehedi 1966), and a different (Saad and Hagedorn 1969), host-Alternaria sp. interaction. Angell (1929) reported that infection hyphae developed within the substomatal chambers, sometimes filling them, and penetrated the underlying tissues either inter-or intracellularly

At most points of contact between the host cell and intercellular hyphae, an extracellular deposit between the hyphal cell wall and the host cell wall was clearly visible. This deposit may aid the binding of the hyphae to host cells. It was found in this study that the response, of mesophyll and epidermal cells in contact with an infection hypha, could occur before the cell wall was breached, which suggests that extracellular fungal metabolites affect the protoplast in advance of the hypha. Cytoplasmic changes in cells adjacent to the cell under attack may also implicate a metabolite of fungal origin as an incitant of these responses. Studies on pathogenic species of Alternaria have shown that toxins produced by these fungi and are frequently responsible for much of the disease syndrome on the host (Gilchrist and Grogan 1976; Langsdorf et al. 1990). A. porri has been found to produce the metabolic pigments macrosporin, alternsolanol, alterporriol A, B, C, D and E, and the phytotoxins tentoxin and porritoxin (Ohnishi et al. 1991; Suemitsu et al. 1990, 1991, 1992), but their role in disease development has not yet been determined.

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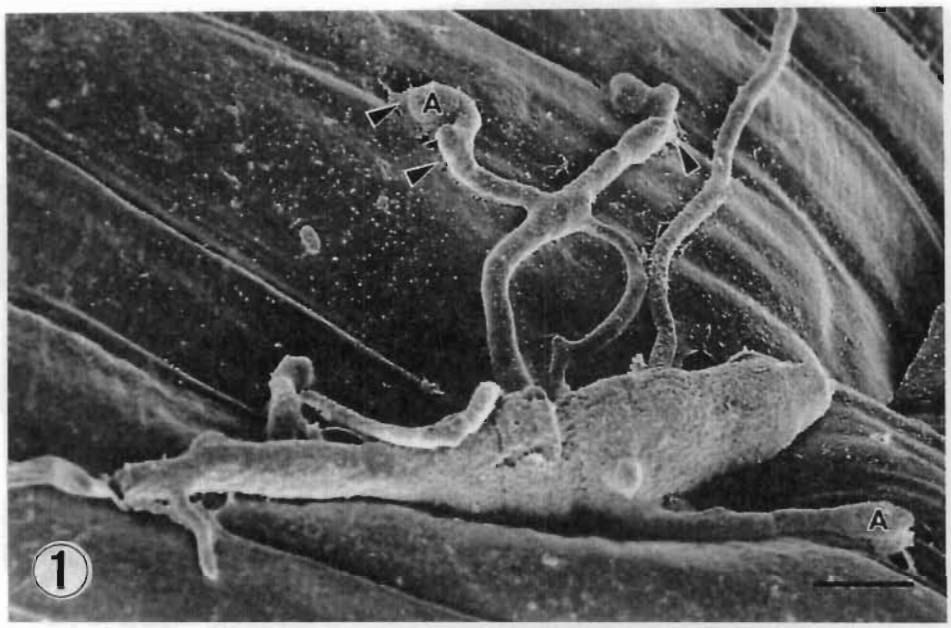
Figure captions

FIGS. 1 - 4. Scanning electron micrographs of the pre-penetration and penetration structures formed by A. porri on onion leaves. Scale bars = 10 μm. Fig. 1. Conidium forming several germ-tubes terminating in bulbous appressoria (A). An extracellular material surrounds germ-tubes and appressoria (arrows). Fig. 2. Germ-tubes showing no specific orientation towards stomata and often passing near stomata without forming appressoria. Fig. 3. The epidermis has been stripped off to show the formation of primary hyphae within the leaf after direct penetration of epidermis. Fig. 4. The epidermis has been stripped off to show the formation of primary hyphae within the leaf after penetration of a stoma.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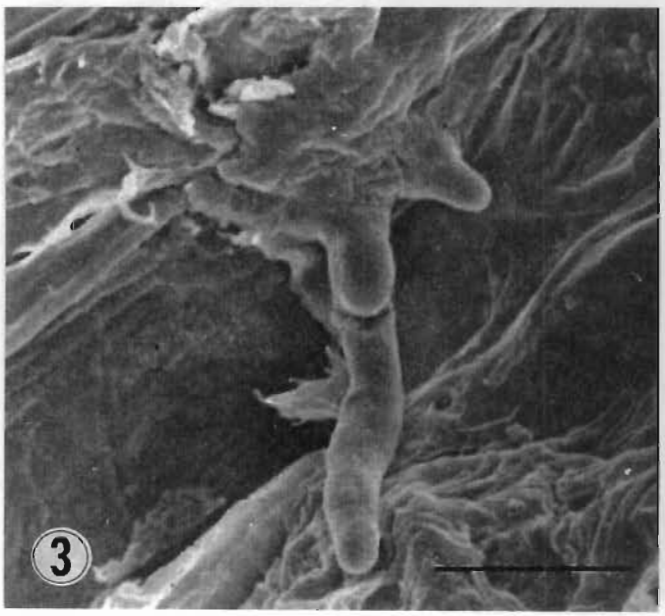
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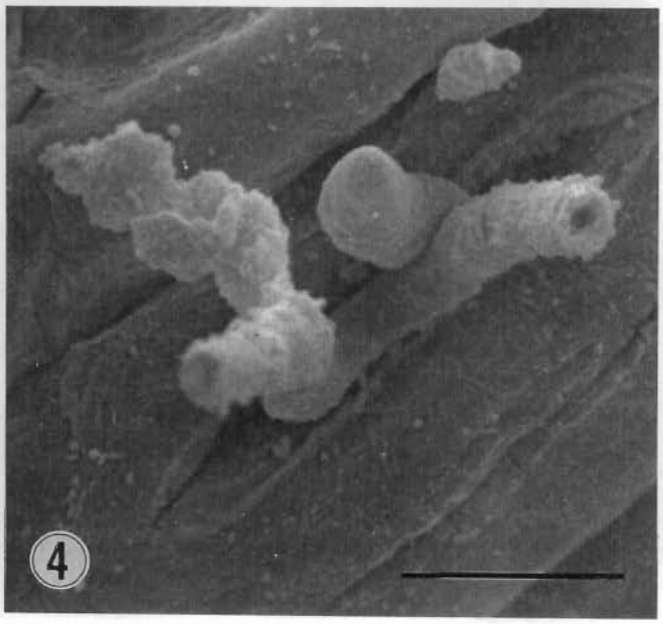
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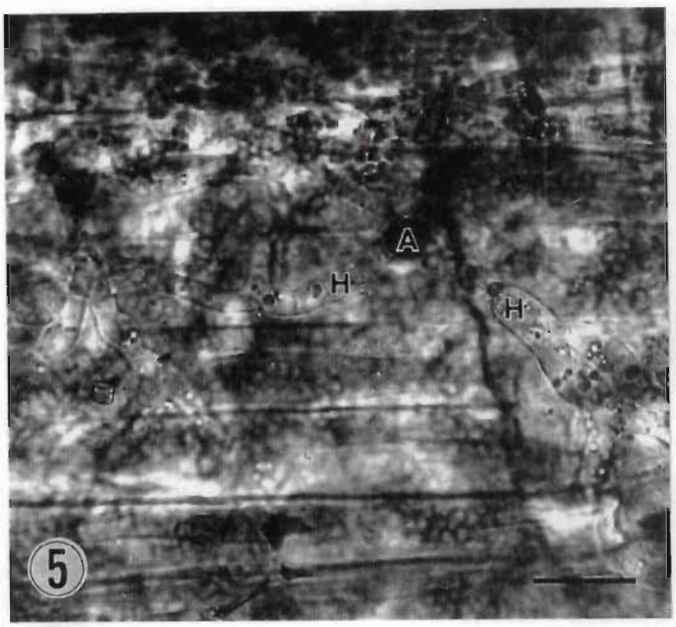
FIGS. 5 and 6. Light micrographs of direct penetration of the onion leaf by A. porri. A = appressorium, H = primary hypha. ig. 5. An appressorium above the junction of two epidermal cell walls has formed several bulbous primary hyphae within the leaf. Scale bar = 20 μm. Fig. 6. The formation of a primary hypha between the cell walls of adjacent epidermal cells. The epidermal cell wall is discoloured at the site of penetration (asterix). Scale Bar = 10 μm. Fig. 7. Transmission electron micrograph of the subcuticular and lateral growth of an infection hypha within the host epidermal cell wall which has become swollen and discoloured in the vicinity of the invading hypha (asterix). Scale bar = 1 μm. FIGS. 8 and 9. Light and transmission electron micrographs of penetration occurring through a stoma. A = appressorium, H = primary hypha, S = septum. Fig. 8. Several bulbous primary hyphae, developed from an appressorium situated on a stoma, are infecting the underlying tissue. Scale bar = 20 μm. Fig. 9. The primary hypha has attained a larger diameter than the appressorium, from which it is separated by a septum. An electron-dense matrix fills the cavity between the primary hypha and the guard cells (small asterix). The cell walls of the guard cells are swollen and electron-lucent (large asterix). Note that the cytoplasmic content of these cells and adjacent cells is disorganized and the cell membranes disrupted, often with only remnants of membrane discernible (arrows). Scale bar = 5 μm. Fig. 10. Transmission electron micrograph of a stoma of a healthy uninfected onion leaf. C = chloroplast, N = nucleus.



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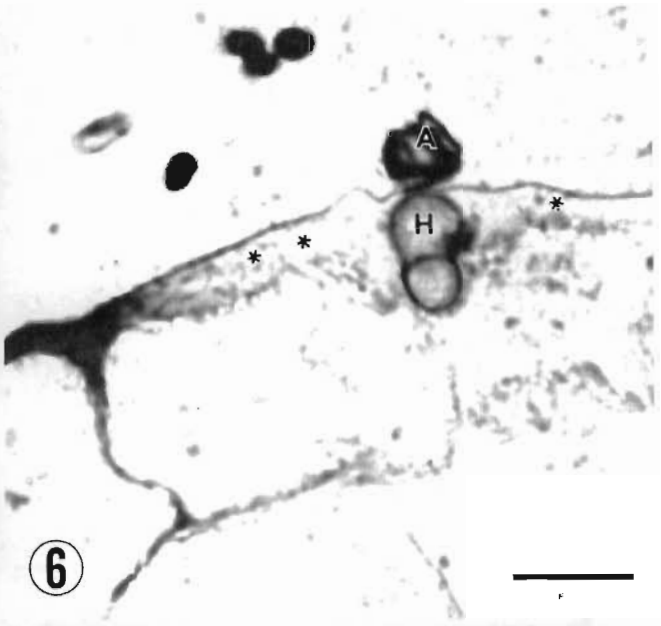

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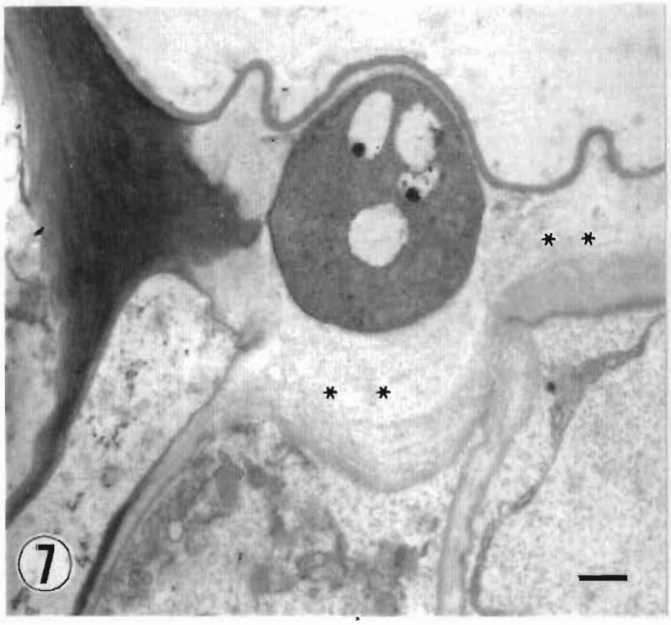

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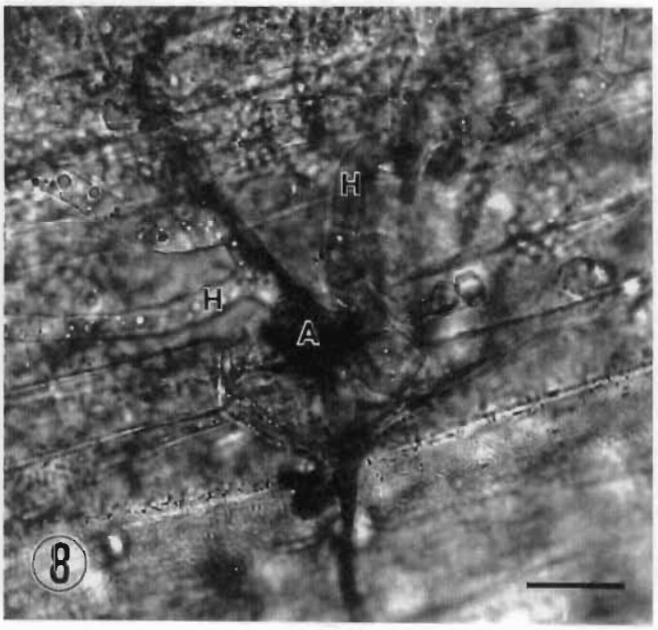

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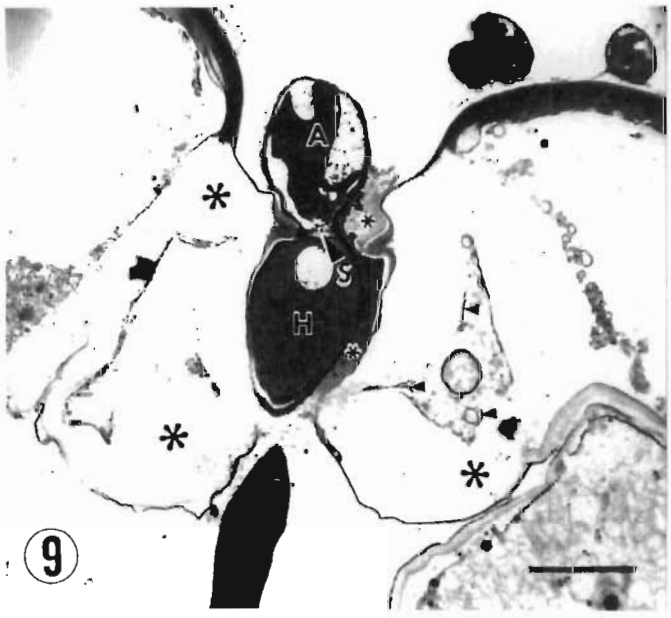

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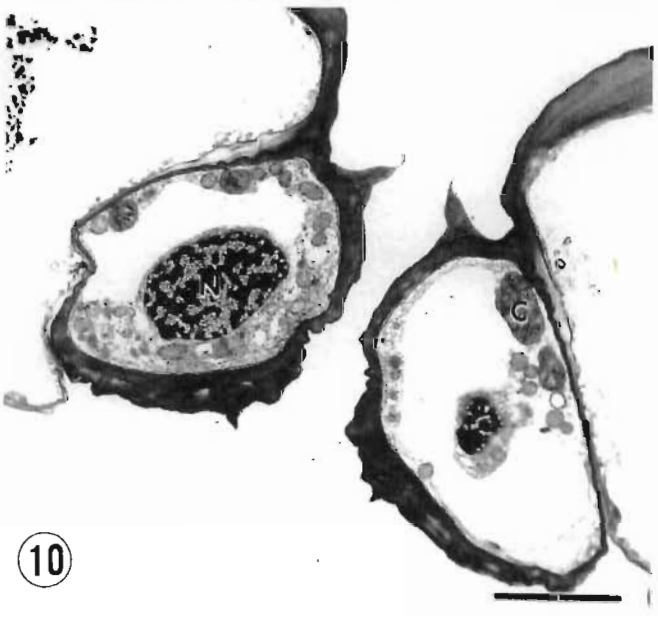

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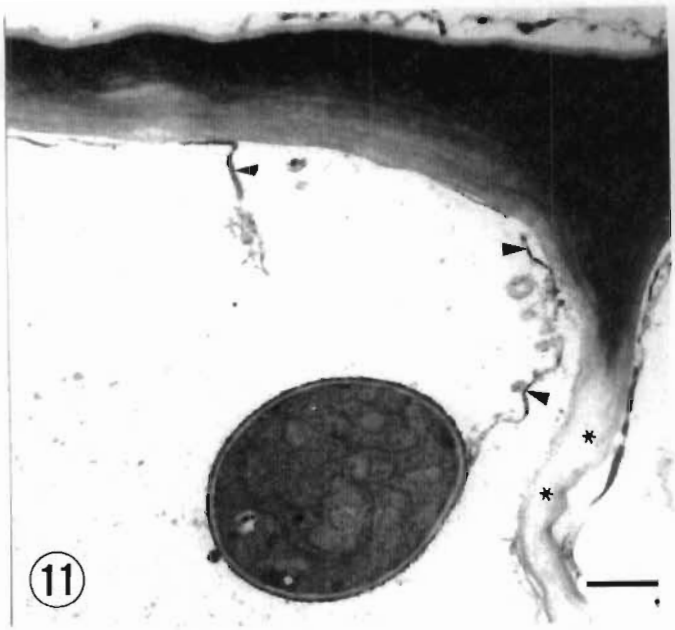
FIGS. 11 - 15. Light and transmission electron micrographs of the infection of the onion leaf tissue by A. porri. C = chloroplast, M = mitochondrion. Fig. 11. An infection hypha growing parallel to the cuticle within an epidermal cell. The transverse cell wall is electron-lucent (asterix) and the cell membrane is disrupted (arrows). Scale bar = 1 μm. Fig. 12. Intercellular growth of a secondary hypha. An extracellular matrix is associated with the hypha at the site of contact with surrounding mesophyll cells (asterix). Scale bar = 1 μm. Fig. 13. A secondary hypha is slightly constricted at the site of penetration of the cell wall of a mesophyll cell. An extracellular matrix is associated with the hypha at this site of penetration (asterix). Scale bar = 1 μm. Fig. 14. As the hyphae advance into the adjacent mesophyll cells, cells in close proximity to, and some distance from, hyphae and infected cells show changes in ultrastructure. These cells become plasmolysed and the swelling and disruption of chloroplasts are evident. Scale bar = 20 μm. Fig. 15. The cellular disruption of two mesophyll cells in close proximity to an secondary hypha. The plasmalemma of one of these cells has retracted from the cell wall (large arrows). In the adjacent cell, only fragments of the membrane still remain (small arrows) and the cytoplasm is disorganized and granular. The membranes surrounding the chloroplasts have disintegrated and the remains of the stroma and disrupted granum membranes have been released into the cytoplasm of the cell. Scale bar = 1 μm. Fig. 16. A transmission electron micrograph of a mesophyll cell of a healthy uninfected onion leaf. C = chloroplast, M = mitochondrion, N = nucleus. Scale bar = 2 μm.



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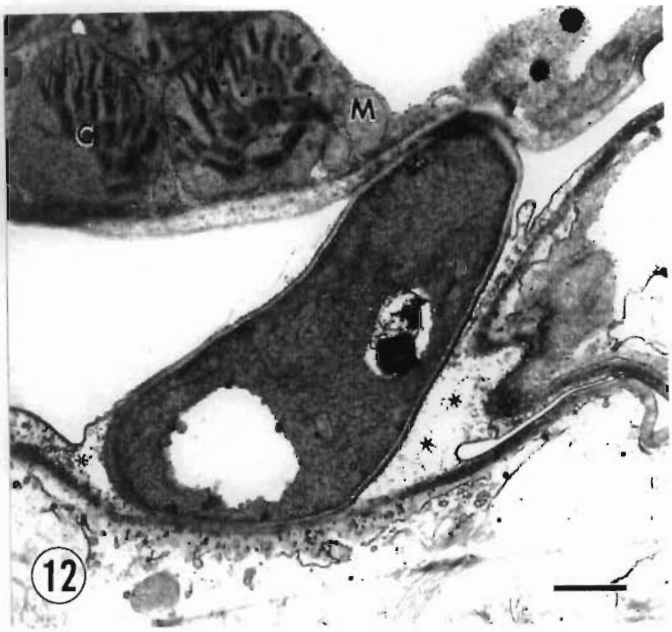

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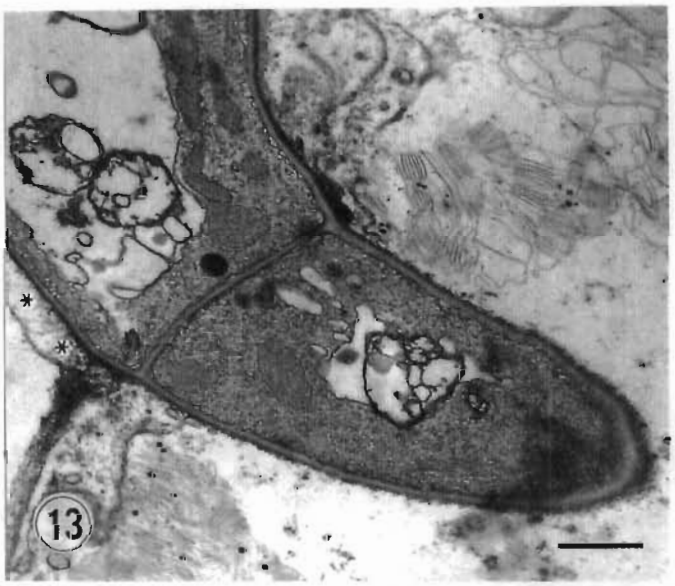

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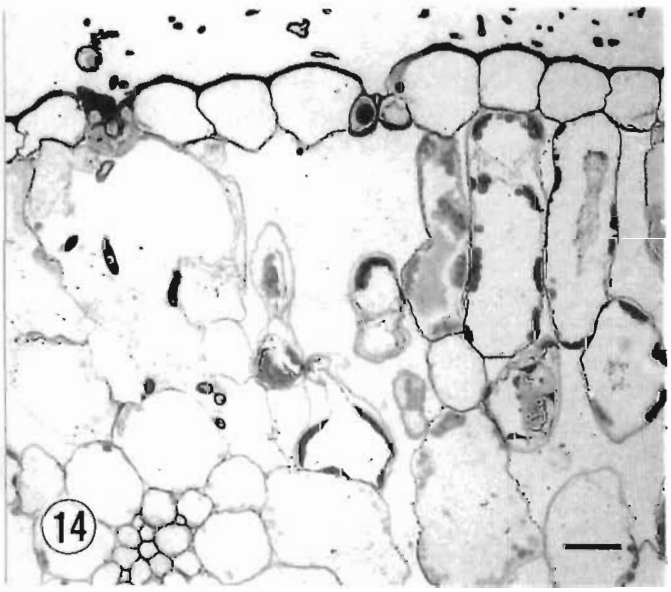

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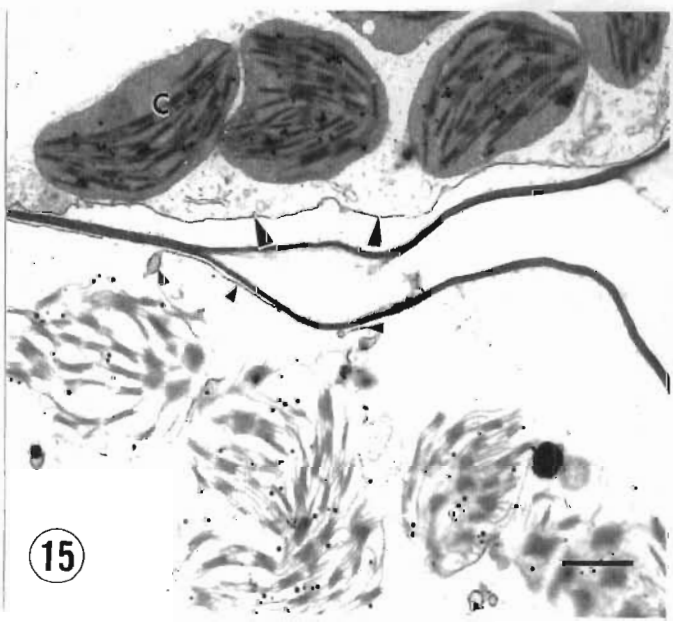

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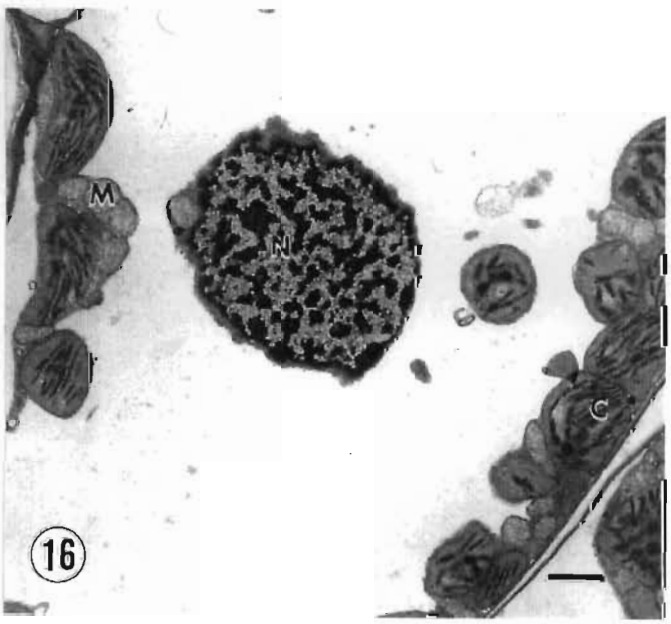

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CHAPTER 5

SEED PATHOLOGY OF *ALTERNARIA PORRI* AND *STEMPHYLIUM VESICARIUM* ON ONION SEED

Evaluation of Seed Treatments for Reducing *Alternaria porri* and *Stemphylium vesicarium* on Onion Seed

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ABSTRACT

Aveling, Theresa A.S., Snyman, H.G., and Naude, S.P. 1993. Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. Plant Dis. 77:1009-1011.

During surveys in the Cape Province of South Africa, *Alternaria porri* (Ellis) Cif. and *Stemphylium vesicarium* (Wallr.) E. Simmons were found to be destructive seed-borne pathogens of onion (*Allium cepa* L.). Six fungicides were evaluated for their efficacy in reducing both pathogens on seed and in culture. These included anilazine, benomyl, carbendazim/flusilazol mixture, procymidone, tebuconazole and thiram. An untreated control, hot-water soak (50 C for 20 min), and a sodium hypochlorite treatment were also included for comparison. Treated seeds were rated for germination using the blotter method and by emergence and seedling growth in seedling trays in the glasshouse. None of the treatments eradicated *A. porri* and *S. vesicarium* from onion seeds. The hot-water soak proved to be the best treatment for reducing these pathogens, although percentage germination and emergence of onion seeds were reduced when

compared to the control.

Purple or alternaria blotch caused by *Alternaria porri* (Ellis) Cif. is a common disease of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) occurring wherever these crops are grown in South Africa (1,6). First symptoms on onion leaves appear as numerous, white, irregular spots, which under favourable conditions gradually enlarge and become dark purple and brown (8). When seed stalks are girdled by the pathogen they may break and fall over before the seed matures (2). Recent surveys in the Cape Province of South Africa have shown that *Stemphylium vesicarium* (Wallr.) Simmons, in conjunction with *A. porri*, is also a destructive foliar and seed stalk pathogen of onion under warm, moist conditions (authors, unpublished). As both *A. porri* and *S. vesicarium* are seed-borne in onion (7), a seed treatment that could control or eradicate both pathogens simultaneously could be highly advantageous and would eliminate a potential source of inoculum in the field.

Currently, thiram is used as a standard treatment in South Africa for reducing fungal seed-borne pathogens of vegetables (9). Maude (4) found that thiram eliminated infection of carrot seeds by *Alternaria dauci* and *Stemphylium radicinum*. However, Maude, Vizor and Shuring (5) found that although a thiram soak completely eradicated 16 seed-borne fungal pathogens of vegetable, cereal and flower seeds, it did not completely eradicate *Alternaria brassicola* from brassica seeds. They also found that the thiram treatment adversely affected the

germination of some vegetable seeds (5).

The objectives of this study were to evaluate the effectiveness of several other fungicides, and hot water, for eradicating *A. porri* and *S. vesicarium* from naturally infected onion seeds and to determine the effects of these treatments on germination, emergence and seedling growth.

MATERIALS AND METHODS

Fungicide treatments. The following fungicides were used in *in vitro* and *in vivo* experiments (the active ingredient and recommended dosage rate per kilogram seed are given in parenthesis): Thiulin (thiram - 0.9 g a.i./kg); Benlate (benomyl - 1 g a.i./kg); Dyrene (anilazine - 1.6 g a.i./kg); Punch C (carbendazim/flusilazol mixture - 0.19/0.38 g a.i./kg); Raxil (tebuconazole - 0.05 g a.i./kg); and Sumisclex (procymidone - 0.5 g a.i./kg). Since no fungicides, other than thiram, are registered in South Africa for the treatment of onion seed, the authors relied on the expertise of the chemical companies, Agrihold, South Africa and Bayer South Africa (Pty) Ltd, to supply the fungicides and the respective recommended dosage rates. The above rates were also used as standard rates per liter medium in the *in vitro* experiment.

In vitro experiment. *A. porri* and *S. vesicarium* were isolated from leaves of diseased onion plants and a virulent isolate of each was maintained on potato dextrose agar (PDA) at 20 C in the dark. These isolates of *A. porri* (PREM 50715) and *S. vesicarium* (PREM 50717) were deposited with the National Collection of Fungi, South Africa.

Sodium hypochlorite (1%) and the test fungicides, at the standard rate, were each incorporated into PDA. Discs (3 mm in diameter) from actively growing cultures of *A. porri* and *S. vesicarium* were transferred aseptically to amended plates and to control plates. Six replicate plates of each treatment for each pathogen were incubated at 20 C in the dark. The diameter of the colony was measured after three, six and 10 days of incubation. Each assay was repeated once. From the results obtained from these experiments using the recommended dosage (1X), 0.5 or 1.5X the recommended dosage was also tested.

In vivo experiment. Four replicates of 100 onion seeds from two seed samples of the cultivar, Caledon Globe, were plated on PDA and incubated at 20 C in a 12 hr light/dark regime. Results from these experiments indicated that one seed sample was naturally infected with *A. porri* and *S. vesicarium* while the other was not infected with either pathogen. These two seed samples were used in *in vivo* experiments.

Based on the results obtained from the *in vitro* experiments, the treatments were applied to the two seed samples at 1, 0.5 and 1.5X the recommended dosage. Each of the chemicals was suspended in 1 ml sterile distilled water and applied as a slurry to a 20 g seed sample. The control was treated with 1 ml sterile distilled water. The treated seeds were allowed to air-dry overnight. Twenty grams of seed was also treated using a hot-water soak (50 C for 20 min).

Seed germination assays. Laboratory tests. The effect of the various treatments on percentage germination was determined according to the rules of the International Seed Testing

Association (ISTA) (3). Four replicates of 100 uninfected seeds from each treatment were plated on moist blotter paper in glass petri dishes and incubated at 20 C in a 12 hr light/dark regime. The experiment was repeated once. The efficacy of the various treatments in reducing seed infection by *A. porri* and *S. vesicarium* was also tested. Four replicates of 100 naturally infected seeds from each treatment were plated onto PDA. The untreated seeds yielded the level of infection of the seed. The petri dishes were incubated at 20 C in a 12 hr dark/12 hr near-ultraviolet light regime. Three and six days later, the number of *A. porri* and *S. vesicarium* colonies growing from the seeds was recorded. The experiment was repeated once.

Glasshouse tests. Four replicates of 50 uninfected seeds of each treatment (1 and 0.5X the recommended dosage) and four of 50 naturally infected seeds of each treatment (1 and/or 0.5 or 1.5X the recommended dosage) were planted in seedling trays containing a peat-based growth medium, and maintained in a glasshouse (25/20 C day/night temperature) and watered daily. Four weeks after planting, percentage emergence and shoot and root length of seedlings from uninfected seed were determined. The experiment was a randomised block design repeated once. Leaf samples from seedlings from treated, infected seed which showed symptoms of *A. porri* and/or *S. vesicarium* were surface sterilized with 1% NaOCl before plating on PDA or were placed directly onto moist blotter paper in glass petri dishes. The plates and petri dishes were incubated at 20 C in a 12 hr dark/12 hr near-ultraviolet light regime. Three and six days later, the tissues were examined for the presence of sporulation, and spores were

identified as either those of *A. porri* or *S. vesicarium*.

RESULTS

In vitro experiment. All of the fungicide and sodium hypochlorite treatments significantly inhibited mycelial growth of *A. porri* in culture when compared to the control, with the exception of thiram at the standard rate (Table 1).

Table 1. Diameter of colonies of *Alternaria porri* and *Stemphylium vesicarium* grown on potato dextrose agar amended with various fungicides and sodium hypochlorite

Treatment	Rate (g a.i./l medium)	Diameter of colonies (mm)[z]	
		A. porri	*S. vesicarium*
Control	-	21.62 a	37.52 a
Tebuconazole	0.05	6.22 f	2.53 i
	0.025	6.90 f	4.15 h
Carbendazim/flusilazol mixture	0.19/0.38	0.00 i	0.00 j
	0.095/0.19	0.00 i	0.00 j
Procymidone	0.5	1.30 h	0.00 j
	0.25	1.53 h	0.00 j
Thiram	0.9	21.13 a	23.75 b
	1.35	18.60 b	20.67 d
Anilazine	1.6	4.23 g	22.45 c
Benomyl	1.0	13.92 c	10.58 f
	1.5	12.30 d	8.90 g
NaOCl	1%	8.98 e	17.70 e
	1.5%	7.33 f	11.32 f

[z]Each value is a mean of six plates measured after six days of growth. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

Mycelial growth of *S. vesicarium* was significantly inhibited by all the fungicide and sodium hypochlorite treatments when compared to the control (Table 1). It is evident from the results presented in Table 1 that three fungicides, tebuconazole, the carbendazim/flusilazol mixture and procymidone, were most effective at inhibiting both pathogens in culture.

In vivo experiments. All treatments, with the exception of tebuconazole at half the recommended dosage, significantly reduced percentage germination of onion seed on blotter paper when compared to the control (Table 2). With the exception of tebuconazole and procymidone, at half the recommended dosage, all the treatments significantly reduced percentage emergence in glasshouse experiments when compared to the uninfected control (Table 2). Four treatments, tebuconazole, anilazine, the carbendazim/flusilazol mixture and procymidone, at the recommended dosage, also significantly reduced shoot growth (Table 2). Half the recommended dosage of tebuconazole, the carbendazim/flusilazol mixture and procymidone did not inhibit shoot growth. Five treatments stimulated root length significantly when compared to the control. These included thiram and anilazine at the recommended dosage and tebuconazole, the carbendazim/flusilazol mixture and procymidone at half the recommended dosage (Table 2). Only three treatments did not significantly reduce dry shoot and root mass when compared to the control, i.e., thiram and anilazine at the recommended dosage and tebuconazole at half the recommended dosage (Table 2).

Table 2. Percentage germination of treated onion seed in the laboratory and percentage emergence and seedling growth of onion in the glasshouse after various seed treatments

Treatment	Rate (g a.i. /kg)	Germination (%)[y]	Emergence (%)[z]	Shoot length (mm)[z]	Root length (mm)[z]
Control	-	75.5 a	75.0 b	186.6 bcd	47.79 a
Hot water	-	70.8 b	69.0 cd	181.5 bc	50.16 ab
NaOCl	1%	56.5 efg	65.0 def	176.9 abc	46.01 a
	1.5%	44.5 i	n/t	n/t	n/t
Tebucon-azole	0.05	58.5 de	65.0 def	164.9 a	48.08 a
	0.025	74.0 ab	80.0 a	197.3 d	56.56 c
Thiram	0.9	61.3 cd	66.0 def	181.0 bc	53.97 bc
	1.35	51.3 h	n/t	n/t	n/t
Anilazine	1.6	61.5 cd	67.0 de	166.6 a	53.51 bc
	2.4	57.3 ef	n/t	n/t	n/t
Benomyl	1.0	56.5 efg	68.0 cde	172.8 ab	46.45 a
	1.5	53.5 gh	n/t	n/t	n/t
Carbendazim/ flusilazol mixture	0.19/ 0.38	54.5 fgh	64.0 ef	163.2 a	49.88 ab
	0.095 /0.19	62.5 c	68.0 cde	188.6 cd	56.49 c
Procymidone	0.5	56.3 efg	62.0 f	163.2 a	49.29 ab
	0.25	63.3 c	72.0 bc	172.9 ab	56.64 c

[y]Each value is a mean of four replicates of 400 seeds.
[z]Each value is a mean of four replicates of 50 seedlings.
Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test, n/t = not tested as results from *in vivo* and germination tests indicated that these treatments were unsuitable for the treatment of onion seeds.

The percentage of onion seeds and seedlings infected with *A. porri* and *S. vesicarium* grown from treated and untreated naturally infected seed are presented in Table 3. Only three treatments significantly reduced the percentage infection of onion seed by *A. porri* when compared to the naturally infected

control. These were the hot-water treatment and the tebuconazole and the carbendazim/flusilazol mixture treatments at the recommended dosage (Table 3). These three treatments and thiram and anilazine (both at 1.5X the recommended dosage) also significantly reduced percentage infection by *S. vesicarium* (Table 3).

Table 3. Percentage infection by *Alternaria porri* and *Stemphylium vesicarium* of treated and untreated onion seed and of seedlings grown from this seed

Treatment	Rate (g a.i. /kg)	Infection of seed (%)[y]		Infection of seedlings (%)[z]	
		A. porri	S. vesicarium	A. porri	S. vesicarium
Control	-	6.5 cde	32.0 d	7.0 b	27.5 c
Tebuconazole	0.05	3.5 ab	23.0 bc	2.0 a	15.5 b
	0.025	4.0 abc	23.5 bcd	n/t	n/t
Carbendazim/ flusilazol mixture	0.19/0.38	3.5 ab	22.0 bc	1.5 a	14.5 b
	0.095/0.19	4.0 abc	25.5 bcd	n/t	n/t
Procymidone	0.5	4.0 abc	23.5 bcd	2.5 a	21.5 bc
	0.25	7.0 de	30.0 cd	n/t	n/t
Thiram	1.35	7.0 de	21.0 b	n/t	n/t
Anilazine	2.4	8.0 e	19.0 b	6.0 b	15.5 b
Benomyl	1.5	5.0 bcd	25.0 bcd	n/t	n/t
NaOCl	1.5%	5.0 bcd	21.0 b	n/t	n/t
Hot water	-	2.0 a	1.0 a	1.0 a	0.5 a

[y]Each value is a mean of four replicates of 100 seeds.
[z]Each value is a mean percentage of four replicates of 50 seeds. Values within a column not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test. n/t = not tested as results from *in vivo* and *in vitro* experiments indicated that these treatments were unsuitable for the treatment of onion seeds.

Of all the treatments tested in the glasshouse, only anilazine failed to control *A. porri* and procymidone failed to control *S.*

vesicarium. The hot-water soak was most effective in reducing both pathogens simultaneously in the laboratory and glasshouse (Table 3).

DISCUSSION

According to Neergaard (7), effective seed treatments must be able to eliminate pathogens and not be toxic to seeds. All the fungicides incorporated into PDA, except thiram at the standard rate, reduced growth of both pathogens, with the most effective being tebuconazole, the carbendazim/flusilazol mixture and procymidone. Only the former two fungicides, at the recommended dosage, also effectively reduced percentage infection of onion seed and seedlings by both pathogens in laboratory and glasshouse experiments, while only tebuconazole at half the recommended dosage did not significantly reduce germination in laboratory experiments. In glasshouse experiments, however, both tebuconazole and procymidone at half the recommended dosage did not significantly reduce percentage emergence although procymidone did have an adverse effect on seedling growth.

None of the seed treatments eradicated the two pathogens. The chemicals are either not fungitoxic to the pathogens or did not penetrate the seed tissues to kill internal mycelium. The hot-water soak was the most effective treatment for reducing both pathogens in laboratory and glasshouse tests. However, this treatment significantly reduced percentage germination and emergence when compared to the control. It is possible that by varying the temperature of the water and/or the submergence time, the percentage germination and emergence might be maintained

while still reducing the two pathogens.

The efficacy of the seed treatments was not tested under field conditions.

ACKNOWLEDGEMENTS

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CHAPTER 6

FIRST REPORTS OF *STEMPHYLIUM VESICARIUM* AND *ALTERNARIA PORRI* ON GARLIC IN SOUTH AFRICA

First Report of *Stemphylium vesicarium* on Garlic in South Africa.
T. A. S. Aveling, Margaretha Mes Institute for Seed Research, University of Pretoria, Pretoria 0002, and S. P. Naude, V.O.P.R.I., Private Bag X293, Pretoria 0001, South Africa. Plant Dis. 76:426, 1992. Accepted for publication 24 October 1991.

During surveys for diseases of garlic (*Allium sativum* L.), a leaf blight was repeatedly observed in the province of Transvaal. *Stemphylium vesicarium* (Wallr.) E. Simmons was consistently isolated from lesions varying from small and pale yellow to large, dark brown to black, and ovate-elongate, often with a pinkish margin. Immersed dark brown to black globose pseudothecia of the teleomorph, *Pleospora allii* (Rabenh.) Ces. & De Not. (1), formed on potato-dextrose agar in 3-mo-old cultures. Pathogenicity of an isolate from a garlic leaf (deposited with the National Collection of Fungi, South Africa, designated PREM 50634) was shown by inoculating leaves of 20 garlic plants (cv. Large Egyptian White) with conidia and maintaining plants in a mist chamber for 48 hr. After 8 days, leaves were severely diseased, with lesions similar to those seen in the field. Cultures of *S. vesicarium* reisolated from these plants were identical to the original. Our observations indicate that severe foliage damage of garlic caused by this pathogen, with subsequent yield loss, occurs only when leaf-wetness periods exceed 24 continuous hours and that warm, humid summers are conducive to development of severe epidemics.

Reference: (1) N. N. R. Rao and M. S. Pavgi. Mycopathologia 56:113, 1975.

First Report of *Alternaria porri* on Garlic in South Africa.
Theresa A. S. Aveling, Margaretha Mes Institute for Seed Research, University of Pretoria, Pretoria 0002, and S. P. Naude, V.O.P.R.I., Private Bag X293, Pretoria 0001, South Africa. Plant Dis. 76:643, 1992. Accepted for publication 30 December 1991.

A leaf disease of garlic (*Allium sativum* L.) causing severe foliage damage was recurrently observed in the Natal and Transvaal provinces of South Africa. Leaf symptoms varied from small, elliptic white lesions to large, sunken purple lesions with concentric dark and light zones where sporulation was heavy or sparse, respectively. *Alternaria porri* (Ellis) Cif., the causal organism of purple blotch of garlic and onion, was consistently isolated from both types of lesions and from diseased leaf tips that were dying back. Pathogenicity of an isolate of *A. porri* from a garlic leaf (deposited with the National Collection of Fungi, South Africa, designated PREM 50716) was shown by inoculating leaves of 25 garlic plants (cv. Large Egyptian White). Inoculated plants were placed in a mist chamber for 12 hr, then returned to the glasshouse. After 9 days, symptoms resembling those observed in the field were apparent. *A. porri* was reisolated from these plants and produced cultures identical to those of the original isolate. Our observations indicate that heavy dew or rain at any time of the year encourages the development of purple blotch of garlic.

STEMPHYLIUM LEAF BLIGHT OF GARLIC IN SOUTH AFRICA

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RESEARCH NOTE

ABSTRACT

Key words: *Allium sativum*, garlic, *Pleospora allii*, *Stemphylium vesicarium*

Stemphylium leaf blight disease of garlic (*Allium sativum*) in South Africa is discussed. *Stemphylium vesicarium* was consistently isolated from lesions and shown to be pathogenic to garlic. Severe foliage damage and subsequent yield loss occurred only when leaf-wetness periods exceeded 24 continuous hours. A new technique is also described to induce ascospore formation in the teleomorph, *Pleospora allii*.

UITTREKSEL

STEMPHYLIUM *BLAARSKROEI VAN KNOFFEL IN SUID-AFRIKA*

Stemphylium blaarskroei van knoffel (Allium sativum) *in Suid-Afrika, word bespreek.* Stemphylium vesicarium *is konsekwent vanuit letsels geïsoleer, en bevestig as patogenies op knoffel. Swaar blaarbeskadiging en gevolglike opbrengsverliese het slegs*

plaasgevind wanneer die blaarbenattingsperiode 24 u oorskry het. 'n Nuwe tegniek vir askospoor-induksie in Pleospora allii *word ook beskryf.*

Recently, a new leaf-blight disease of garlic (*Allium sativum* L.) was reported for the first time in South Africa by Aveling & Naude (1992). The causal organism was identified as *Stemphylium vesicarium* (Wallr.) Simmons. In a study of the perfect states of *Stemphylium* Wallroth spp., Simmons (1969) found the teleomorph of *S. vesicarium* to be represented by *Pleospora allii* (Rabenh.) Ces. & De Not. *S. vesicarium* has been reported on onion (*Allium cepa* L.) in Wisconsin (Teodoro, 1922), South Africa (Verwoerd & du Plessis, 1931), India (Rao & Pavgi, 1975), Texas (Miller *et al.*, 1978) and New York (Shishkoff & Lorbeer, 1989). Subsequent to the leaf-blight of onion, *S. vesicarium* has only recently been associated with symptoms on garlic in India (Rao & Pavgi, 1975). This paper reports on observations of *S. vesicarium* on garlic, the effect of various leaf-wetness periods on lesion development and the establishment of a new technique for ascospore induction in pseudothecia of *P. allii*.

Diseased garlic leaves were surface-sterilised with 0,5 % (w/v) sodium hypochlorite for 10 min and rinsed in sterile distilled water before lesions were excised. The excised leaf pieces were plated on potato-dextrose agar (PDA) and incubated at 20 °C in a 12 h dark/12 h light regime conducive to sporulation. Isolates were maintained on PDA and were identified at the Mycology Research Unit of the Plant Protection Research Institute, Pretoria, as *Stemphylium vesicarium*. A culture was

deposited in the National Collection of Fungi, Plant Protection Research Institute, Private Bag X134, Pretoria 0001, South Africa (PREM 50634). Isolates also corresponded to those of *S. vesicarium* obtained from the International Mycological Institute (IMI 135457) and the Centraalbureau Voor Schimmelcultures (CBS 715.68).

Lesions are at first, small and pale yellow but enlarge becoming ovate-elongate and dark brown to black when sporulation occurs. Infection often starts at the leaf tips, which wither prematurely, resulting in a reduction in yield.

Conidia of *S. vesicarium* from cultures and leaf pieces were a dark brown colour, oblong to ovoid, densely verrucose with 1-5 transverse and a series of longitudinal septa, 13-21 X 25-40 µm (Figs 1 & 2). Pseudothecia were immersed, dark brown to black, globose and erumpent on host tissue, 200-360 µm in diam. Asci were hyaline, bitunicate, clavate and contained 8 ascospores that were light to dark brown, ellipsoidal, verrucose with 5-7 transverse and several longitudinal septa, usually in incomplete series, 9-17 X 17-46 µm (Fig. 3).

Excised diseased leaf pieces were placed on moist filter papers in sealed petri dishes and maintained at room temperature (23-25 °C) to induce formation of the teleomorph. After 7 d the petri dishes were opened, the filter paper allowed to dry and the dishes resealed. After a further 10 d the filter papers were moistened again and resealed. One to four weeks later, dark brown to black globose pseudothecia, with asci and ascospores, formed immersed in the leaf pieces and filter paper. Pseudothecia also formed immersed in PDA in 3-mo-old cultures but

failed to differentiate into asci and ascospores. No pseudothecia were present on leaf samples collected from diseased fields. Conidia and pseudothecia, formed in culture and on incubated infected leaf pieces, were examined with a light microscope.

Garlic plants (cv. Large Egyptian White) were grown from bulbs and maintained in a greenhouse at 20 °C. The leaves of 4-to 8-wk-old healthy, sprouted bulbs were used for inoculation. Inoculum was prepared aseptically by pouring sterile distilled water onto sporulating colonies in petri dishes, agitating with a glass rod and filtering the suspension through two layers of sterile muslin cloth. Garlic leaves were spray-inoculated to run-off with conidia at a concentration of 5×10^4 conidia ml^{-1}. Ten inoculated plants were placed in a mist chamber for 6, 12, 24 or 48 h and then returned to the greenhouse. After 8 d, leaves from plants that had been in the mist chamber for 24 and 48 h were severely diseased, with lesions similar to those seen in the field. Lesions did not develop on plants that were in the mist chamber for only 6 or 12 h. Cultures of *S. vesicarium* were reisolated from inoculated plants using the technique described above and were identical to the original cultures, proving Koch's postulates.

Field and laboratory observations indicate that severe foliage damage by *S. vesicarium* and subsequent yield loss occurs only when continuous leaf-wetness periods exceed 24 h and that warm, humid summers are conducive to the development of severe epidemics.

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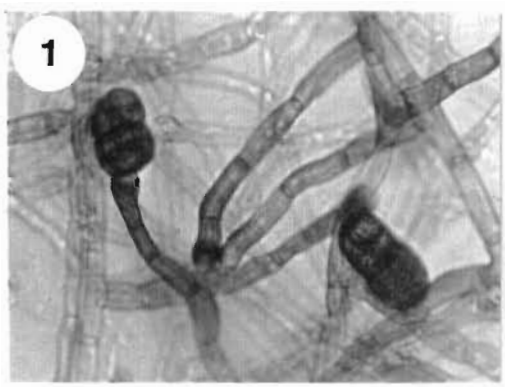
CAPTIONS TO FIGURES

Fig. 1 Conidiophores and conidia of *Stemphylium vesicarium* in culture (X 600)

Fig. 2 Mature conidia of *Stemphylium vesicarium* from a diseased garlic leaf (X 950)

Fig. 3 Bitunicate octosporus asci of *Pleospora allii* (X 550)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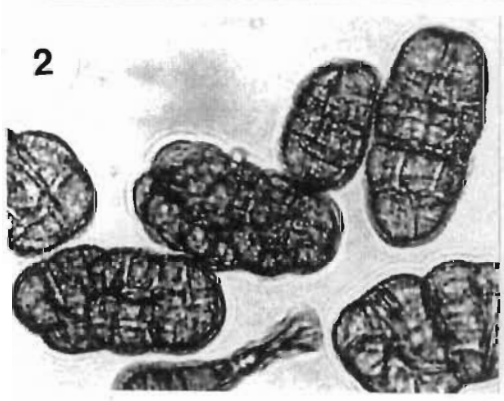
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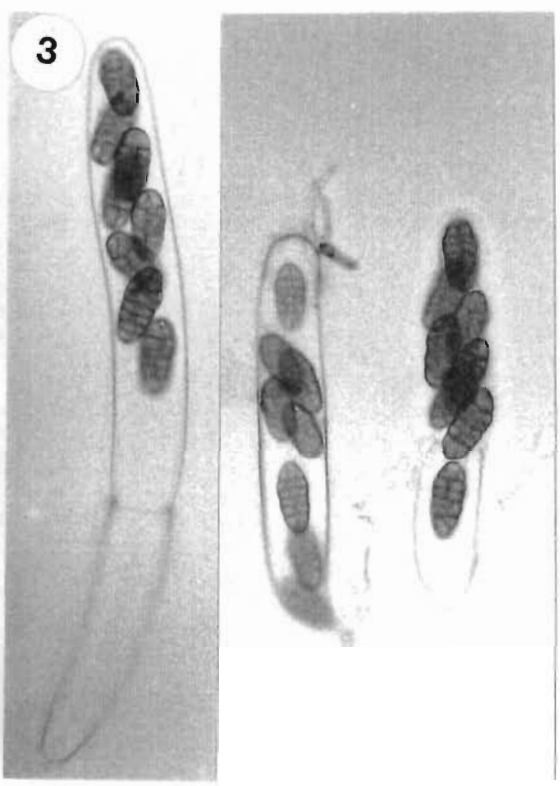
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CHAPTER 7

GENERAL DISCUSSION

In the first chapter of this thesis, literature pertaining to the topic of the investigation was reviewed, and several gaps in our knowledge were highlighted. The research presented in this report attempts to fill in some of these gaps. As the various chapters consist of manuscripts submitted for publication in scientific journals, each with its own discussion, there is no need to reiterate these deliberations here. Instead, the author will attempt to point out the similarities and differences between the two pathogens, *Alternaria porri* and *Stemphylium vesicarium*, and between their interactions with the *Allium* host.

CONIDIUM FORMATION BY *STEMPHYLIUM VESICARIUM* AND *ALTERNARIA PORRI* ON ONION LEAVES

Occasionally solitary, but more usually fasciculate conidiophores of *S. vesicarium* and *A. porri* emerged through the onion leaf epidermis. Conidiophores of both pathogens were straight or flexuous, simple, smooth (and/or verrucose in the case of *S. vesicarium*) and cylindrical, but enlarged apically at the site of conidium production. Smooth, round, bud-like conidial initials were produced singly at the apex of conidiophores. As conidia of *S. vesicarium* matured, they became oblong to ovoid and densely verrucose. Those of *A. porri* showed pronounced elongation but only slight growth in width, and event-

ually became obclavate with a simple, tapering beak. Once the mature conidium had been seceded, a small pore was clearly visible at the apex of the conidiogenous cell in both pathogens. The presence of these pores confirms that conidia of both these fungi are porospores, as suggested by Simmons (1967), i.e. conidia which develop through a pore in the wall of the conidiophore (Hughes, 1953). Both pathogens produced a single conidium at the apex of a conidiophore. However, *S. vesicarium* differed from *A. porri* in one important aspect, i.e. conidiophores of the former species proliferated percurrently at the distal region, forming secondary conidiophores and conidia. Production of a single conidium at the apex of a conidiophore which has a percurrent method of proliferation is the unique character which assigns isolates to *Stemphylium*, and distinguishes the genus from otherwise superficially similiar taxa (Simmons, 1967, 1969).

The author realizes that transmission electron microscope techniques are required to study the conidiogenesis of *S. vesicarium* and *A. porri*. Nevertheless, scanning electron microscopy used in this thesis has provided useful information on the formation of conidiophores and conidia by these fungi, and will supplement future studies.

INFECTION STUDIES OF *STEMPHYLIUM VESICARIUM* AND *ALTERNARIA PORRI* ON ONION LEAVES

There are several similarities in the pre-penetration behaviour of these two pathogens on the onion leaf. In both pathogens, each conidium formed several germ-tubes which were

initiated at random positions on the conidium and grew in any direction across the leaf surface. These germ-tubes usually terminated in bulbous appressoria, but occasionally extensive growth formed a hyphal network. Appressoria were formed directly on the epidermal cells or on stomata. No evidence of long-distance attraction to stomata was detected in either pathogen, and germ tubes often passed close to stomata showing no apparent trophic response.

The pre-penetration behaviour of *S. vesicarium* differed from that of *A. porri* in that single germ tubes of the former pathogen often formed more than one appressorium on the same stoma. Less frequently observed were compound appressoria produced by several hyphae.

The manner in which these two pathogens penetrate the onion leaf can also be compared. Penetration of the leaf surface by both pathogens, either directly through the epidermis or through a stoma, occurred only after the formation of an appressorium. This indicates that appressoria may be obligatory for infection by these pathogens. The percentage of successful penetrations by *S. vesicarium* via stomata was higher than penetration through the epidermis, whilst penetration by *A. porri* through stomata or the epidermis occurred with about equal frequency. Whether cuticular penetration was by mechanical force, or involved enzymic hydrolysis, was not determined in either study. However, the apparent discolouration of the cell wall at the infection site below appressoria suggests that enzymes play a role in direct penetration by both pathogens.

Following penetration of a stoma or direct penetration of the

outer epidermal cell wall, one, or occasionally more, bulbous, septate primary hyphae developed below the appressorium within the stoma or the epidermal cell, or between the cell walls of adjacent epidermal cells. The formation of these primary hyphae within the host, as found in both studies, has also been reported by other researchers studying different host-*Stemphylium* spp. interactions (Higgins and Lazarovits, 1978; Pierre and Millar, 1965) and the bean-*Alternaria tenuis* interaction (Saad and Hagedorn, 1969).

The epidermal cell walls and guard cell walls became swollen and discoloured in the vicinity of the invading primary hyphae of *A. porri* or *S. vesicarium*. Plasmolysis and cytoplasmic disruption of epidermal cells adjacent to, and mesophyll cells in the near vicinity of, penetrating hyphae suggest a diffusion of toxic metabolites from the pathogens. *S. vesicarium* has been found to produce a toxin, stemtoxin, but its enzymatic activity has not yet been determined (Heiny and Gilchrist, 1991). Studies on pathogenic species of *Alternaria* have shown that toxins produced by these fungi are responsible for much of the disease syndrome on the host (Gilchrist and Grogan, 1976; Lansdorf *et al.*, 1990). *A. porri* has been found to produce the metabolic pigments macrosporin, altersolanol, alterporriol A, B, C, D and E, and the phytotoxins tentoxin and porritoxin (Ohnishi *et al.*, 1991; Suemitsu *et al.*, 1990a, 1991, 1992), but their role in disease development has not yet been determined.

In both studies, severe disruption of host cells was characterised by chloroplast damage. The membranes surrounding the chloroplasts in these cells usually disintegrated and the

remains of the stroma and disrupted granum membranes were released into the cytoplasm.

SEED TREATMENTS FOR REDUCING *ALTERNARIA PORRI* AND *STEMPHYLIUM VESICARIUM* ON ONION SEED

A. porri and *S. vesicarium* are seed-borne pathogens. Preventing or reducing their occurrence in/on infested seed would also curtail their introduction into the vegetative onion crop. Consequently, losses at the seedling stage and disease expression in the mature plant should be less severe. With fewer primary foci, reduced seed yields and infection of developing seed in a seed production crop can be minimised.

Of the various seed treatments evaluated here, soaking in hot water proved to be the most effective for reducing *A. porri* and *S. vesicarium* on onion seed, even though it impeded seed germination and seedling emergence. Further manipulation of water temperature and/or the submergence time could possibly allow for improvement in seed germination and emergence, while still controlling the two pathogens.

In considering the usefulness of the hot-water soak, however, a critical appraisal must be made of the limitations of this method and of the precautions necessary in applying it. Only small quantities of seed can be treated at one time and the temperature and submergence time must be strictly monitored and adhered to.

Another aspect warranting investigation is the possibility that seed treatment using biological control agents could provide

an alternative to the use of chemical fungicides, or even to physical methods such as the hot-water soak.

FIRST REPORTS ON *STEMPHYLIUM VESICARIUM* AND *ALTERNARIA PORRI* ON GARLIC IN SOUTH AFRICA

Although leaf blight and purple blotch of garlic, caused by *S. vesicarium* and *A. porri* respectively, have been recorded in other parts of the world (Black *et al.*, 1985; Rao and Pavgi, 1975), this thesis constitutes the first report of the two diseases in South Africa. Locally, *A. porri* produces two types of lesions on leaves of onion and garlic, viz. the characteristic purple blotch, as well as a small, light-yellow lesion resembling that of *S. vesicarium*. These findings thus ensure that South African agricultural extention officers and plant pathologists will be aware of the diseases, and their associated symptoms. Recording of the diseases also paves the way for registration of fungicides to combat them.

Perhaps it is apt to conclude this thesis with the words of Sutton (1986): "Conidial fungi have been improvising for much longer than mycologists and for most of us trying to understand them is like the untutored instrumentalist attempting the comprehension of an orchestral score, or, the classical virtuoso trying to master the impulsive approach of free expression".

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