Studies on the etiology, epidemiology and control of Synchytrium psophocarpi (Rac) Gaumann on winged bean in Papua New Guinea.

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STUDIES ON THE ETIOLOGY, EPIDEMIOLOGY AND CONTROL OF SYNCHYTRIUM PSOPHOCARPI (RAC) GÄUMANN ON WINGED BEAN IN PAPUA NEW GUINEA.

Submitted by M.J. Drinkall

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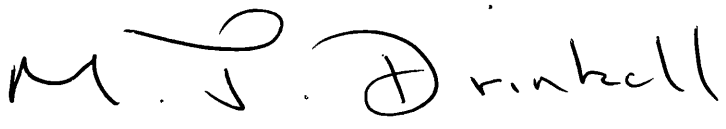
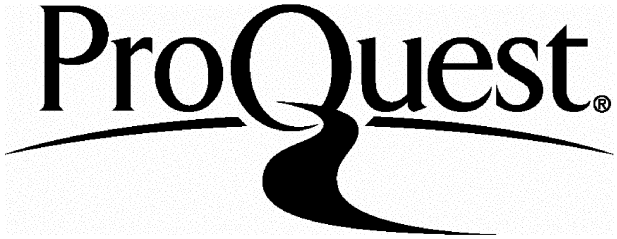
the University of Bath

1981





To my wife Barbara

SUMMARY

Surveys in 1976 and 1977 showed that false rust, caused by Synchytrium psophocarpi (Rac) Gäumann occurred widely throughout Papua New Guinea (P.N.G.) on winged bean (Psophocarpus tetragonolobus (L.) DC.). During this period disease severity was slight in most localities. Descriptions of the pathogen based on material collected in P.N.G., and the Philippines are given.

Sporangia of S. psophocarpi germinated over a temperature range of 5 - 30°C in the presence of free moisture. When exposed to air sporangia lost viability in 4 days. Zoospores became stationary 30 min after emergence and encysted 2 - 3 h later. A minimum period of 12 h of leaf surface wetness was required for infection to occur. Following inoculation disease symptoms were recorded after 7 days, mature sori rupturing after 22 days. Attempts to infect winged bean seeds and other legume species were unsuccessful. Resting spores were not found.

Sporangia were dispersed by wind or water splash. Spore trap counts of airborne sporangia showed a diurnal periodicity. Maximum catches occurred between 16.00 and 18.00 h, lowest counts being recorded between 06.00 and 07.00 h. Concentration of airborne sporangia increased during the wet season and subsequently declined two months

after the onset of the dry season. Trapping sporangia from an infection source revealed a concentration gradient, with the highest concentration recorded at 1m and the lowest at 20m. Observations of disease development in the field showed that infection occurred at random.

Of the fungicides evaluated *in vitro* tridemorph plus maneb was the most effective in inhibiting germination of sporangia. Fentinacetate plus maneb gave the best control in a preliminary field trial.

All 125 lines of winged bean screened from the P.N.G., germplasm collection were susceptible to *S. psophocarpi*. In regional trials of 10 of these lines, however, variation in susceptibility was apparent. Two lines from Thailand showed resistance following artificial inoculation.

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INTRODUCTION

The winged bean (Psophocarpus tetragonolobus (L.) DC.) is a tropical legume belonging to the family Leguminosae, sub-family Papilionoideae (Purseglove, 1968). Rumf (cf. Purseglove, 1968) first recorded the plant in the Moluccas although its origin is uncertain (Masefield, 1973; Khan, 1976). Cultivation is practiced throughout South east Asia, the Indian sub-continent, parts of Oceania and Africa and now in the New World (Erskine, 1980). It has not been found growing wild (Masefield, 1973) but has been noted as an escape plant in Burma (Burkill, 1906), the Philippines (Agcaoili, 1929) and Indonesia (Claydon, personal communication).

Until recently the winged bean has been considered as a legume of minor importance.Masefield (1961, 1967), however, drew attention to the agricultural potential of the plant and was later supported by Pospisil, Karikari and Boamah-Mensah (1971). Winged bean is ideally suited for subsistence agriculture as the seeds, pods, flowers, leaves and roots, all of which are edible, have a high protein content. Soil fertility is improved where the plant is grown through symbiotic nitrogen fixation (Masefield, 1973). Recently it was listed in a survey of underexploited tropical food crops (Anon., 1975a).

This led to a further study by an international panel of scientists who published information on the crop (Anon,, 1975b) to stimulate interest and research. In response to this initiative representatives from 26 countries attended the first international workshop/seminar on the crop in January 1978 (Anon.,1980).

In Papua New Guinea (P.N.G.) protein malnutrition is widespread (Korte, 1974) and the need to expand legume production and consumption was recognised (Khan, personal communication). In 1973 a full scale research programme was started on the winged bean by the University of Papua New Guinea (U.P.N.G.) and the Department of Primary Industry. Khan (1973, 1976) acknowledging that P.N.G., had become a centre of a genetic diversity of a winged bean, initiated the first germplasm collection of the species there.

Information on pests and diseases of the winged bean is scant, fragmented and often unpublished. False rust or orange gall, caused by the obligate fungus Synchytrium psophocarpi (Rac.) Gäumann, is one of the major diseases of the crop in P.N.G., (Price, 1976). Raciborski (1898) first recorded the disease in Java and identified the causal agent. The parasite infects leaves, stems, sepals and pods causing severe galling and malformation of these parts. Although disease symptoms are well documented

(Raciborski, 1898, Reinking, 1918, 1919; Gäumann, 1927; Alicbusan, 1965; de Vera, 1973, 1977) the only studies on the biology of the fungus are those of Raciborski (1898), Gäumann (1927), Alicbusan (1965) and de Vera (1973, 1977). No work on the epidemiology or field control of the disease has been reported.

SECTION 1 REVIEW OF LITERATURE

1.1 THE HOST, WINGED BEAN (PSOPHOCARPUS TETRAGONOLOBUS (L.) DC.)

1.1.1 Description

Winged bean is a climbing herbaceous perennial (Anon., 1975b) although it is normally cultivated as an annual. The plant has an indeterminate growth habit and can reach 3-4m in height when supported (Anon., 1975b) (Fig. 1). Considerable variation in qualitative characters have been reported between pure lines (Sastrapradja, and Aminah-Lubis, 1975, Sastrapradja, Aminah-Lubis, Lubis and Sastrapradja, 1978; Khan, 1976; Chomchalow, Suputtitada and Peyachoknagul, 1980; Mamicpic and Movillon, 1980) (Table 1).

The leaves are trifoliate, although occasional tetrafoliate and pentafoliate types do occur, (Khan, 1976) and are deltoid, ovate and lanceolate in shape (Sastrapradja et al., 1975; Chomchalow et al., 1980). The basic colour of the stem is purple green (Khan, 1976; Mamicpic et al., 1980). Khan (1976) reported flower colour as blue or purple although the intensity of flower colour varied greatly. A few almost white flowers which were observed are considered to be an extreme variation of the light blue types. Chomchalow et al. (1980) and Sastrapradja et al. (1978), however, reported the occurrence of pure white

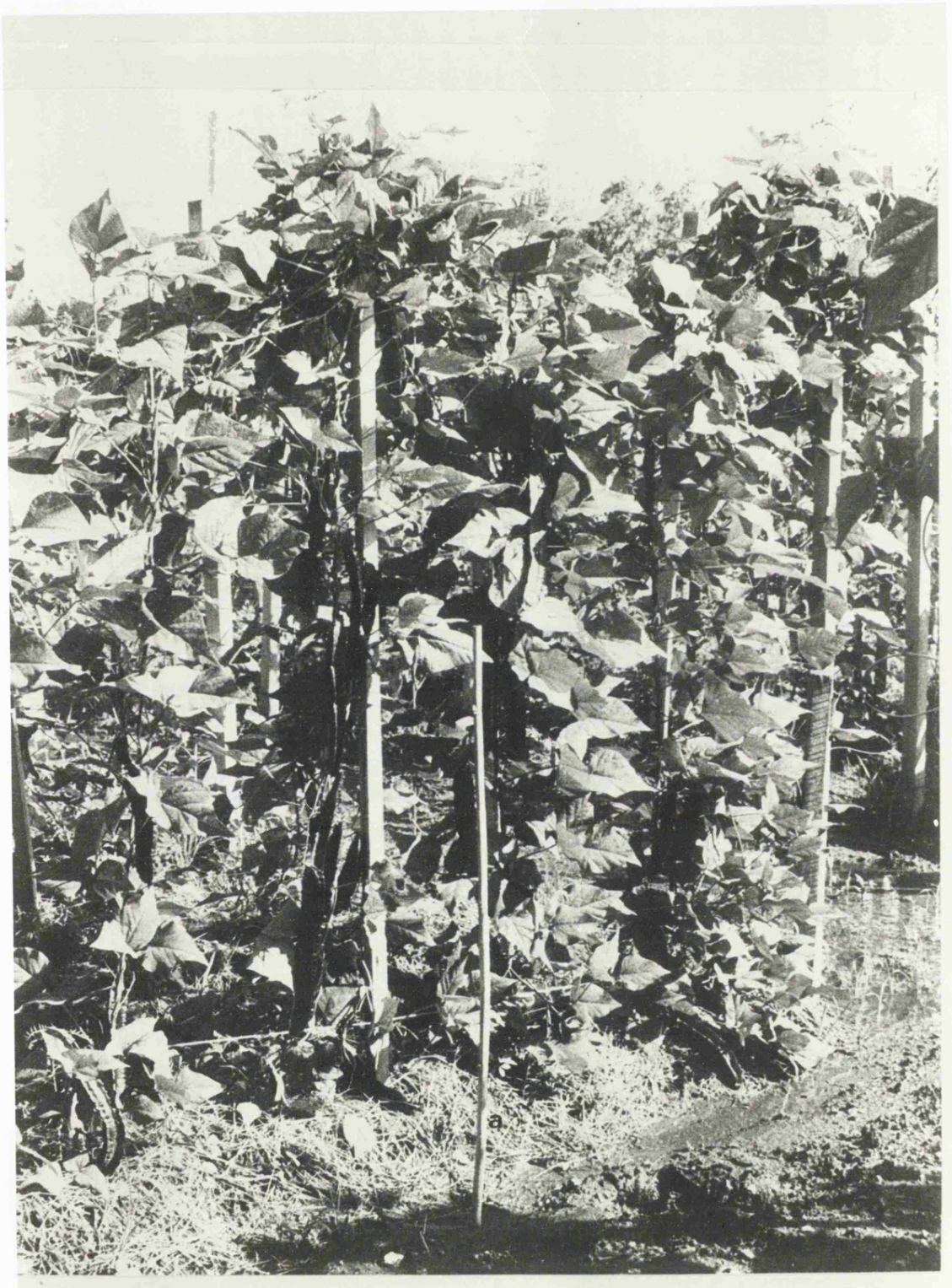

Fig.1. Winged beans growing in the Experimental Agriculture Garden at the University of Papua New Guinea, a) 1 m cane to give scale.

Table 1 Comparison of the variation in qualitative characters of winged bean lines recorded in Indonesia, Papua New Guinea, Philippines and Thailand

Country	Author	Flower colour	Leaf shape	Pod colour	Pod wing margin shape	Pod wing margin colour	Pod shape (viewed in cross section)	Pod surface texture	Seedcoat colour	Stem colour
Indonesia	Sastrapradja et al (1975; 1978)	b,bv,v, w	deltoid, ovate, lanceolate	g,plg	undulate, serrate, lobed	-	rectangular,semi flat, flat on side, flat on suture	-	bg,bl,br,brt, cw,dkbr,gbr, ltbr,ltt, mdbr,v	-
Papua New Guinea	Khan(1976)	b,bp, ltp,p, vyltb	-	g,gp, pk,ply	-	dkp,gp, ltp,p	as above	rough, moderately rough, smooth	br,brt,dkbr, ltbr,ltt,t	dkp,g, ltp,p
Philippines	Mamicpic et al (1980)	bv,ltb, p,pv,v, vyltb	-	g,p	-	p	-	-	bi,bl,br, dkbr,ltbr, m	dkg,ltp, p,plg
Thailand	Chomchalow et al (1980)	b,bp,dkp, ltb,vyltb, w	deltoid, lanceolate, long lanceolate	dkg,g, plg	-	-	as above	contour, moderately rough, smooth	bl,br,brg, c,dkbr, dkbrg,dkp, ltbr,ltbrg, mmbr,rdbr, rdp,vyltbr	-

Colour code:

b = blue
bi = biege
bl = black
blgr = black/grey
bp = blue/purple
br = brown
brg = brownish green
brt = brown/tan
bv = blue/violet
c = cream
cw = creamy white
dkbr = dark brown
dkbrg = dark brownish green
dkg = dark green
dkp = dark purple
g = green
gp = greenish purple
gybr = greyish brown
ltb = light blue
ltbr = light brown
ltbrg = light brownish green
ltp = light purple
ltt = light tan
m = maroon
mdbr = mottled brown
mmbr = medium brown
p = purple
pk = pink
plg = pale green
ply = pale yellow
pv = purple/violet
rdbr = reddish brown
rdp = reddish purple
v = violet
vyltb = very light blue
vyltbr = very light brown
w = white

flowers. Pods have three background colours of green, pink and pale yellow with varying amounts of purple colouration (Sastrapradja et al., 1975; Khan, 1976; Mamicpic et al., 1980). Purseglove (1968) described four longitudinal wings with irregular margins on pods but Sastrapradja et al. (1975) considered the margins to be undulate, serrate or lobed. Khan (1976) suggested that wing colour was dependent on the background colour of the pod: light to deep purple on green pods, purple or mixture of green and purple on pink pods and green and purple on yellow pods. In cross section pod shape has been reported as rectangular, semi-flat, flat on side and flat on suture (Khan, 1976; Sastrapradja et al., 1978; Chomchalow et al., 1980) and with a smooth, rough or contoured surface (Khan, 1976; Chomchalow et al., 1980). Chomchalow et al. (1980) considered seed coat colour exhibited the greatest variation and reported 13 colours ranging from cream to dark brownish green whilst Khan (1976) suggested brown and tan were the two basic colours. Black seeds are also known to occur (Sastrapradja et al., 1978; Mamicpic, et al., 1980). Pod length varies from 6-36cm each pod containing between five to 20 globular seeds (Anon., 1975b). Variation of these and other characters is discussed further by Khan (1976), Sastrapradja et al. (1978) and Mamicpic et al. (1980). Purseglove (1968) and Masefield (1961, 1973) reported that roots become

thickened and tuberous a few months after planting with heavy nodulations. Nodules measured up to 12mm diameter (Masefield, 1961).

1.1.2 Origin

Rumphius in 1747 first recorded winged bean in the Moluccas and considered it had been brought there from Java (cf. Purseglove, 1968). Burkill (1906), however, suggested the plant was introduced to Asia from Mauritius, the Malagasy Republic or East Africa. Degener (1945) considered it originated in India or Mauritius but Vavilov (1951) favoured India whilst Hymowitz and Boyd (1977) maintained P.N.G., was the place of origin. According to Masefield (1973) all other wild species of the genus Psophocarpus Neck ex. DC. are native to Africa and this suggests that winged bean may also have originated in that continent.

The genus has been reported to contain 10 species (Willis, 1966; Gillett, Polhill and Verdcourt, 1971) but Ekpenyong and Borchers (1980) consider there are eight, Hymowitz and Boyd (1977) six, Purseglove (1968) five and Masefield (1973) four. Verdcourt and Halliday (1978) suggest there are nine species. Of these P. scandens (Endl.) Verdc., and P. palustris Desvaux are closely related to P. tetragonolobus but Pickersgill (1980) considers they are the same species. P. scandens is found

throughout tropical Africa and the Malagasy Republic (Verdcourt, 1968) whilst P. palustris, which is often confused with P. scandens, is restricted to the north west part of West Africa. P. scandens is found in Africa both under cultivation and in the wild (Khan, 1976). It also occurs in South America (Gillett, et al., 1971) and is grown as a cover crop under rubber in Indonesia (Saleh, 1975). The other species have only been found in Africa. P. grandiflorus Wilczek has been reported in Uganda, Zaire and Ethiopia whilst P. lancifolius Harms occurs in Uganda, Kenya, Tanzania, Nigeria, Zaire, Zambia and Malawi. (Gillett, et al., 1971). P. lecomtei Tisserant occurs in Zaire and the Central African Empire (Verdcourt and Halliday, 1978) and P. lukafuensis (de Wild) Wilczek (syn. Vignopsis lukafuensis de Wild) in Zaire (Wilczek, 1954) and Zambia (Masefield, 1961; Verdcourt and Halliday 1978). P. monophyllus Harms occurs in Mali, Guinea, Upper Volta and the Ivory Coast and P. obovalis Tisserant in the Central African Empire and the Sudan (Verdcourt and Halliday, 1978).

The confusion over the number of species is probably due to different synonyms being used. Thus P. longepedunculatus Hassk, P. golungensis Welw., P. mabala Welw., and Botor palustris Kuntze are reported as synonyms of P. palustris by Wilczek (1954). Verdcourt (personal communication)

however, lists P. longepedunculatus, P. golungensis, P. mabala and P. comorensis (Vatke) Baill., as synonyms of P. scandens. The confusion over species recently led to a revision of the genus by Verdcourt and Halliday (1978).

1.1.3 Ecology

The winged bean can be successfully grown in a wide range of soil types (Thompson and Sawyer 1914; Khan, Bohn and Stephenson, 1977) although it cannot withstand long periods of drought (Anon., 1975b) or water-logging (Burkill, 1906; Thomson et al., 1914). Masefield (1973) and Rachie (1974) suggested high rainfall is necessary whilst Purseglove (1968) recommended its production during the drier season in Nigeria and Trinidad. Khan (1976) stated the plant could be grown under irrigation during the dry season in P.N.G. Winged bean is a short day plant (Anon., 1975b; Khan, 1980) and flowering has been reported to occur 48-90 days after planting in P.N.G., (Khan, 1976) and after 38-57 days in the Philippines (Mamicpic et al., 1980).

1.1.4 Agricultural potential

The plant has an exceptional capacity for the formation of large and numerous root nodules under a wide range of tropical conditions (Thomson et al., 1914; Masefield, 1957,

1961; Wong, 1975; Elmes, 1976; Khan et al., 1977; Table 2) and can grow successfully in poor soils possibly through symbiotic nitrogen fixation (Anon., 1975b). This is of particular importance in the tropics where soils are often of low nutritional status and winged bean could provide a good restorative crop (Anon., 1975b). In Burma, Thomson et al., (1914) reported an increase of 50% of sugar cane yield following a crop of winged bean. However Khan (1980) stated nutritional problems occurred frequently in P.N.G., as a result of heavy soil leaching.

The composition of the winged bean was probably first analysed by Greshoff at the turn of the century who found the seed consisted of 29.8% protein and 15% oil (cf. Masefield, 1973). Detailed analysis of all the edible plant parts (Fig. 2) has since been reported (Table 3) which confirms the nutritional value of the plant. Cerny (1980) considered that for human nutrition dry mature seeds are the most valuable nutrient source of the winged bean although Claydon (1975, 1980) stated that young pods were most commonly consumed in Asia. The seed has been successfully used as a milk substitute in the treatment of children suffering from Kwashiorkor (Cerny and Addy, 1973) and compares favourably in nutritional value with soya bean

Table 2 Comparison of nodulation of Psophocarpus tetragonolobus with other legumes

Legume	Mean no., of nodules per plant	Mean fresh weight of nodules per plant (g)
Arachis hypogaea	396.0	1.03
Glycine max	256.3	3.29
Pachyrhizus erosus	1.0	0.023
Phaseolus aureus	29.8	0.42
P. vulgaris	113.0	0.93
Pisum sativum	16.1	0.03
Psophocarpus tetragonolobus	441.2	21.65
Vigna unguiculata	60.0	3.69

Data from Masefield (1957)

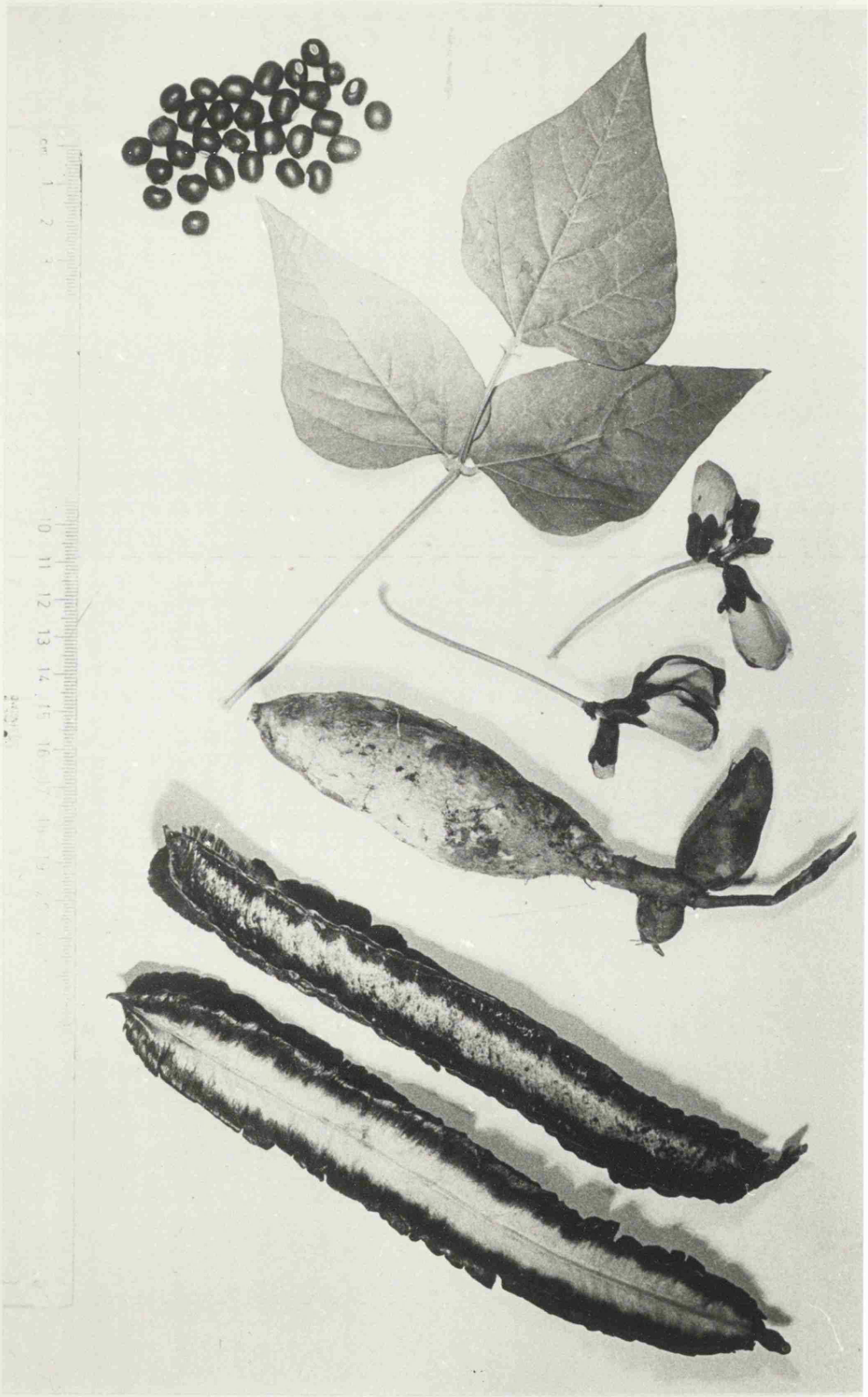

Fig. 2. Edible parts of the winged bean.

Table 3 Composition of the edible parts of the winged bean

Component	Flowers	Immature pods	Leaves	Tubers	Seed
Major constituents (g/100 g fresh weight)	84.2	76.0 - 93.0	64.2 - 77.7	54.9 - 65.2	6.7 - 24.6
Water					
Crude protein	5.6	1.9 - 3.0	5.7 - 15.0	12.2 - 15.0	29.8 - 37.4
Fat	0.9	0.1 - 3.4	0.5 - 1.1	0.5 - 1.1	15.0 - 20.4
Carbohydrate	3.0	1.1 - 7.9	3.0 - 8.5	27.2 - 30.5	31.6 - 28.0
Fibre	-	0.9 - 2.6	-	17.0	5.0 - 12.5
Ash	-	0.4 - 1.9	1.0 - 2.9	0.9 - 1.7	3.6 - 4.0
Minerals (mg/100g fresh weight)	-	53.0 - 236.0	113.0 - 260.0	25.0 - 40.0	204.0 - 370.0
Calcium					
Magnesium	-	-	-	-	120.0 - 204.0
Potassium	-	205.0	-	-	110.0 - 120.0
Sodium	-	3.0	-	-	20.0 - 56.0
Phosphorous	-	26.0 - 60.0	52.0 - 81.0	30.0 - 64.0	320.0 - 276.0
Iron	-	0.2 - 12.0	6.2	0.5 - 3.0	9.6 - 11.8
Vitamins (mg/100 g fresh weight; Carotene given in International Units, Folic acid in μg)	-	340 - 595	3145 - 20833	-	250
Carotene					
Thiamine	-	0.06 - 0.24	-	-	0.08 - 1.39
Riboflavine	-	0.08 - 0.12	-	-	0.18 - 0.33
Pyridoxine	-	-	-	-	0.11
Folic acid	-	-	-	-	25.6
Ascorbic acid	-	21.0 - 37.0	14.5 - 29.0	-	Trace
Niacin	-	0.5 - 1.2	-	-	3.09
Tocopherol	-		-	-	22.8 - 126.0

Range of values reported by Brown (1954), Bailey (1968), Kapsiotis (1968), Purseglove (1968), Cerny et al. (1971) Cerny (1980), Pospisil et al., (1971), Watson (1971), Anon (1972), Rachie (1974), Anon (1975b), Wong (1975), Claydon (1975, 1978, 1980), Jaffe and Korte (1976).

(Glycine max (L.) Merr.), peanut (Arachis hypogaea L.) and cowpea (Vigna unguiculata (L) Walp.) (Cerny, Kordylas, Pospisil, Svabensky and Zajic, 1971; Claydon, 1975, 1980) (Table 4). The leaves are also a good source of proteins, minerals and vitamins especially carotene precursor (Cerny, 1980). The latter is of particular importance since carotene deficiency is common in tropical countries (Anon., 1975b). The use of tubers as a food appears to be restricted to Burma and P.N.G., although they are rich in protein (Claydon, 1975, 1980). Flowers are used for food colouring as well as their nutritional value in P.N.G. (Claydon, 1975, 1980)

Table 4 Composition of mature winged bean seeds compared with soyabean, peanut and cowpea

Major constituents (g/100g fresh weight)	[1] Winged bean	[2] Soyabean	[2] Peanut	[2] Cowpea
Moisture	8.7	10.0	5.4	10.5
Fat	15.3	17.7	48.4	1.5
Crude protein	36.6	34.1	26.0	22.8
Carbohydrate (total)	35.6	33.5	17.6	61.7
Fibre	3.7	4.9	1.9	4.4
Ash	3.8	4.7	2.3	3.5
Minerals (mg/100g fresh weight)				
Potassium	1100	1677	674	1024
Phosphorus	450	554	409	426
Magnesium	255	265	206	230
Calcium	230	226	59	74
Sodium	64	5	5	35
Iron	10.8	8.4	2.0	5.8
Vitamins (mg/100g fresh weight)				
Thiamine	1.05	0.66	1.00	0.59
Riboflavine	0.33	0.22	0.13	0.22
Niacin	3.09	2.2	16.8	2.3

Data from [1]Claydon (1975, 1978); [1]Jaffee *et al.* (1976); [2]Anon (1972)

1.2 THE DISEASE - FALSE RUST

1.2.1 Nomenclature

The disease caused by Synchytrium psophocarpi (Rac.) Gäumann is most commonly known as false rust although in the Philippines it is known as orange gall (Alicbusan, 1965; de Vera, 1973, 1977).

1.2.2 Distribution

False rust was first recorded in Java (Raciborski, 1898, 1900). This was later confirmed by Gäumann (1927), Sastrapradja et al. (1975) and Aminah-Lubis (1980). It has also been recorded in the Philippines (Baker, 1916, 1931; Reinking, 1918, 1919; Sydow and Petrak, 1928; Teodoro, 1937; Alicbusan, 1965; de Vera, 1973, 1977; Dogma, 1975), Malaya Peninsular (Thompson and Johnson, 1953) and P.N.G., (Shaw, 1963, Price, 1976). Bandara (personal communication) reported the disease in Sri Lanka but this has not been confirmed. Sydow (1914) (cf. Baker, 1931) and Pospisil, et al. (1971) state false rust occurs in West Africa and Karikari (personal communication) noted its occurrence on winged beans in Ghana. Allen (personal communication), however, considered it was absent from Africa (Fig. 3).

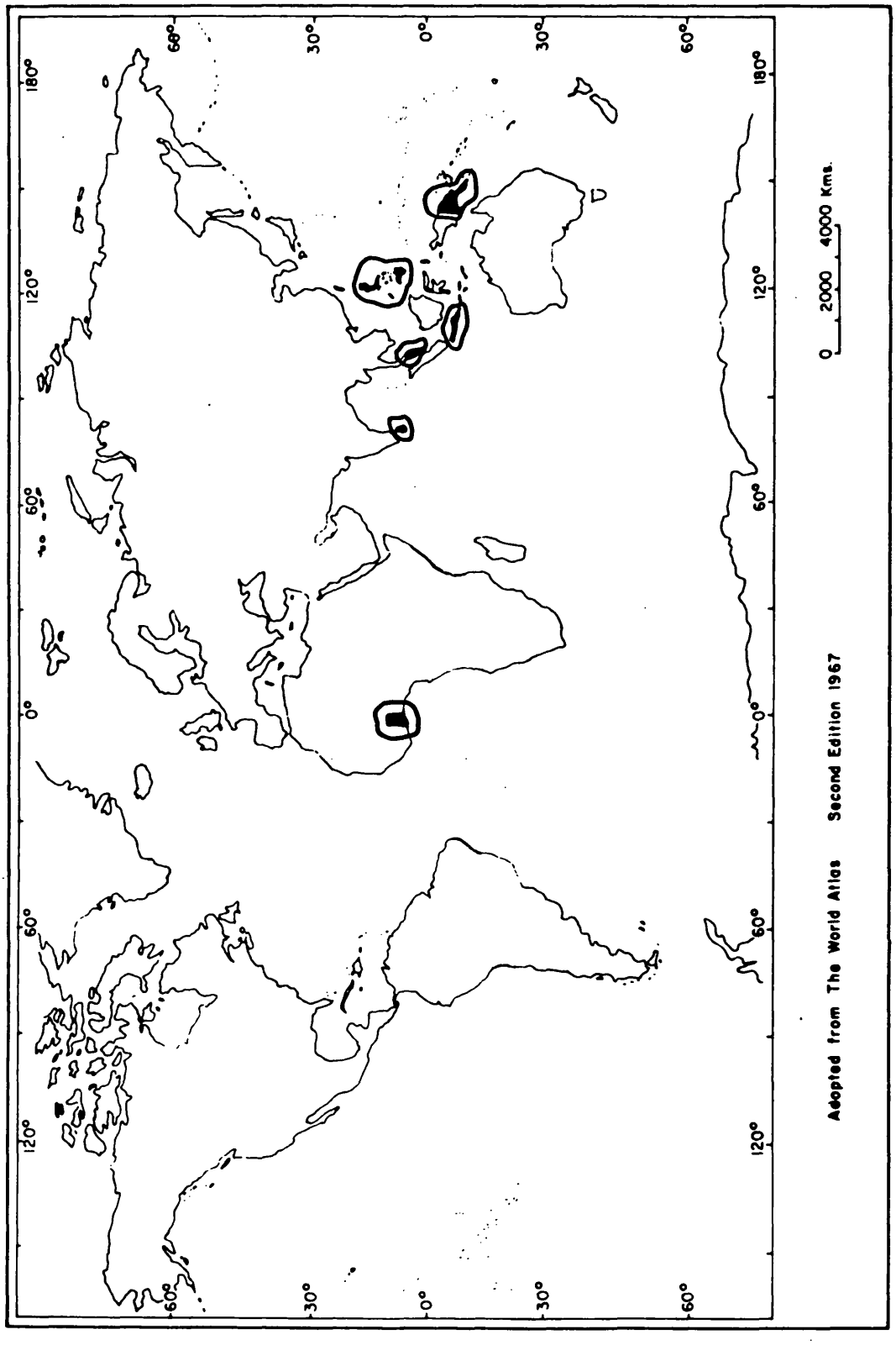

Fig.3. World wide distribution of false rust.

1.2.3 Economic importance

Some workers (Pospisil, et al., 1971; Masefield, 1973) have suggested that there are no serious pests or diseases of winged beans. Reinking (1918, 1919), however, considered false rust to be a disease of economic importance in the Philippines whilst Price (1976) reported false rust as one of the major diseases of the crop in P.N.G. De Vera (1973, 1977) considered that false rust caused a severe reduction of both tubers and pods although no quantitative data was given. According to Reinking (1919) infected plants in the Philippines were undesirable for use and were unsaleable while de Vera (1973,1977) also stated that heavily infected pods were bitter. In Java, however, it is traditional to infect winged beans with false rust and following disease development young infected parts of the plant are steamed and eaten as a delicacy (Degener, 1945; Sastrapradja, et al., 1975). Winged beans naturally infected with false rust are also preferred in parts of the Highlands of P.N.G., (Claydon, 1980; Min, personal communication). There is no information available, however, on what chemical alterations, if any occur, within the plant as a result of infection.

1.2.4 Etiology

1.2.4.1 Identification and classification

Raciborski (1898) first described the causal agent as an obligate fungal parasite belonging to the Chytridiales and erected a new genus Woroninella with the type species W. psophocarpi. The zoospores were described as being biflagellate and therefore differed from Synchytrium de Bary and Woron. The fungus was considered to occupy an intermediate taxonomic position between Woronina Cornu and Synchytrium (Karling, 1964). Dietel (1900), however, considered the crater-shaped sori to be a new form of fructification within the Uredinales and Sydow and Sydow (1903) renamed the fungus Uromyces psophocarpi. Sydow (1914) later agreed with Raciborski's classification and transferred all those species of Synchytrium with aecidium-like fructifications devoid of resting spores to Woroninella. Kusano (1909), however, reported that W. puerariae (Henning) Sydow had uniflagellate zoospores and this was confirmed by Ito and Homma (1926) who found similar flagellation in W. aecidioides (Peck) Sydow. They therefore erected a new genus Miyabella based on these two species. Gäumann (1927) found the zoospores of W. psophocarpi to be uniflagellate which invalidated the erection of both Woroninella and Miyabella. Woroninella was subsequently relegated to form a new sub-genus of Synchytrium. This nomenclature was later recognised by Fitzpatrick (1930). The causal agent of false rust of winged bean was finally

designated Synchytrium psophocarpi (Rac.) Gäumann sub-genus Woroninella.

1.2.4.2 Morphology

Mycelium in S. psophocarpi is absent and the fungus simply consists of sori, sporangia and zoospores (Karling, 1964).

1.2.4.2.1 Sori

The sori are ovoid to subspherical in shape, lie solitary in the epidermal cells (Karling, 1964) and are yellow/ orange in colour. De Vera (1973, 1977) recognised oblong shapes and considered their formation was dependent on the density of infection as sori became angular on two sides in galls which occurred close together.

The sori vary considerably in size and range from 140.0 - 230.0 µm (Karling, 1964). De Vera (1973, 1977) found the size depended on the plant organs infected: sori on sepals (304.0 x 222.0 µm) > sori on stems (286.0 x 228.0 µm) > sori on young leaflets (284.0 x 212.0 µm) > sori on old leaflets (267.0 x 232.0 µm) > sori on pods (227.0 x 163.0 µm).

1.2.4.2.2 Sporangia

The shape and size of sporangia vary from globose, 20.0 - 25.0 µm

diam., sub-globose, ovate, 19.0 - 34.0 x 17.0 - 26.0 µm diam., elongate, 16.0 x 50.0 µm diam., to angular, polyhedral and irregular (Karling, 1964). Alicbusan (1965) reported a size range of 34.0 - 49.5 µm diam., but did not distinguish between the different shapes.

Karling (1964) considered the sporangia possessed fairly thick walls. Alicbusan (1965), however, described immature sporangia as having thin hyaline walls enclosing finely granulated yellow protoplasm. Mature sporangia also had thin walls but the protoplasm was more coarsely granulated and orange in colour and did not completely fill the sporangium.

Increased granulation of the protoplasm and bulging of the sporangial wall occurred prior to germination (Alicbusan, 1965). De Vera (1973, 1977) stated oscillation of the sporangial contents occurred prior to emergence of the zoospores. These escaped singly through openings in the sporangia wall. Four equidistant apertures were visible after staining with crystal violet. In contrast Alicbusan (1965) reported the presence of only a single orifice. Sporangia that failed to germinate had either distinct dark coloured granulation (Alicbusan, 1965) or showed a loss of colour (de Vera, 1973, 1977). These conclusions were apparently based on visual observations. There is no

quantitative data on germination of either type of sporangia; neither is there any information on the factors affecting maturation of sporangia.

1.2.4.2.3 Zoospores

Zoospores are hyaline (Alicbusan, 1965) and pyriform in shape (Karling, 1964) with an orange fat globule and a basal dot-like blepharoplast where the whiplash-type flagellum is inserted (de Vera, 1973, 1977). A single nucleus is positioned at the tapering anterior end of the zoospore body (de Vera, 1973, 1977). Raciborski (1898) reported the size of zoospores ranged from 3.0 - 3.5 x 6.0 - 8.0 μm diam. Zoospores fixed in osmic acid become spherical (de Vera, 1973, 1977) and range in size from 3.4 - 7.4 μm diam., (Alicbusan, 1965) and 4.5 - 8.0 μm diam., (de Vera, 1973, 1977).

Raciborski (1898) erroneously described the zoospores as being biflagellate but Gäumann (1927) showed they were uniflagellate. Occasional biflagellate types, however, do occur (Gäumann, 1927; de Vera, 1973, 1977). Kusano (1930) considered these may have been the results of sexual fusion. De Vera (1973, 1977) claimed to have observed bi, tri and quadriflagellate zoospores and concluded that unequal cleavage in the sporangium during zoosporogenesis may have taken place. The flagellum length ranged from 5.0 - 8.0 μm

(Raciborski, 1898), 4.1 - 10.9 μm (Alicbusan, 1965) and three to four times the diameter of the body length (de Vera, 1973, 1977). De Vera (1973, 1977) reported the flagellum was withdrawn 2h after active swimming. She did not, however, state the conditions under which this occurred.

1.2.4.2.4 Resting spores

S. psophocarpi does not produce resting spores (Fitzpatrick, 1930; Karling, 1964). Alicbusan (1965), however, claimed resting spores were produced on diseased tissue of winged beans that were buried in the soil and described them as spherical, thick-walled and light yellow in colour with well granulated protoplasm. He therefore renamed the fungus Exosynchytrium psophocarpi. De Vera (1973, 1977), however, did not obtain any resting spores when she repeated this work and considered the report questionable. In her view the use of non-sterile soil did not preclude the possibility that the spores described were contaminants. It is still uncertain therefore whether the fungus does or does not produce resting spores.

1.2.5 Symptomatology

S. psophocarpi can attack all aerial parts of the winged bean, i.e., leaves, stems, flower buds and pods (Gäumann, 1927;

de Vera, 1973, 1977) at any stage of growth (de Vera, 1973, 1977). The symptoms were first described by Raciborski (1898) and later by Reinking (1918, 1919), Gäumann (1927), Alicbusan (1965) and de Vera (1973, 1977).

Infection by Synchytrium species leads to hypertrophy, hyperplasia, cell differentiation and the formation of galls on the host (Karling, 1964). These galls may be either simple or composite (Karling, 1962). Simple galls consist of a single enlarged infected cell whilst composite galls consist of a number of infected and non-infected cells; both types are primarily histoid in that they usually involve only certain tissues (Karling, 1964). In S. psophocarpi composite galls are formed (Karling, 1964; de Vera, 1973, 1977). These are usually closely aggregated, slightly raised above the host surface, semi-globular in shape and measure 180.0 - 460.0 µm diam., (Karling, 1964). Young galls are green and change colour to yellow, orange (Alicbusan, 1965) and orange/brown (de Vera, 1973, 1977) as they mature. Open galls are crater-shaped and were often mistaken by the early mycologists for various stages of the rust fungi (loc. cit.) - hence the name 'false rust'.

1.2.5.1 Leaf Symptoms

Alicbusan (1965) reported that the first symptom to appear on the leaves after infection were minute water soaked spots.

This was followed by leaf malformation as gall formation progressed. Light green galls then became visible and these later changed colour to yellow. He observed that galling would occur anywhere on the upper and lower leaf surface and on the petiole and that young leaves were very susceptible to infection. The lower surface was usually more heavily infected especially along the primary and secondary veins. There is, however, no experimental evidence to indicate what effect leaf age has on the susceptibility to infection nor is there any information available on the factors that influence the predominance of infection along the veins. Young infected leaves rarely attain normal size (Reinking, 1919; de Vera, 1973, 1977) but assume abnormal shapes and become thickened (Reinking, 1919) with cupping and curling in severe cases (de Vera, 1973, 1977).

1.2.5.2 Stem symptoms

De Vera (1973, 1977) reported that the entire stem may become covered with galls and growth of apical buds was often arrested leading to the formation of side shoots. She observed that these gnarled, twisted side shoots became bunched together at the nodes and the internodes were longer and thinner than healthy ones. Growth was frequently checked completely (Reinking, 1919).

1.2.5.3 Flower symptoms

Alicbusan (1965) and de Vera (1973, 1977) stated the calyx was the only part of the flower to be attacked. De Vera (1973, 1977) suggested that the corolla and the reproductive parts of the flower escaped infection because they are fast growing and short lived. She did not, however, test whether calyx infection is due to the presence of photosynthetic tissue. She observed that flowers frequently aborted especially when attacked secondarily by a species of Fusarium Link ex Fr.

1.2.5.4 Pod symptoms

Young pods are readily infected and the entire pod can become completely covered with galls giving a 'freckled' appearance (Alicbusan, 1965). De Vera (1973, 1977) reported the common site of infection was the pod wings. Young infected pods remain stunted or become malformed (Reinking, 1919; de Vera, 1973, 1977). Mature pods can also be infected although malformation does not occur (Alicbusan, 1965).

1.2.6 Epidemiology

1.2.6.1 Germination of sporangia

The effect of temperature, pH and aeration on the germination of sporangia *in vitro* was investigated by Alicbusan (1965). He found germination occurred within a temperature range of 24 - 36°C. There was no optimum pH value; germination occurred over a range of pH 5 - 10. He suggested exposure to the air was necessary for rapid and high percentage of germination; sporangia which floated on the surface of a water film germinated whilst those which sank to the bottom failed to germinate. Given the necessary conditions, mature sporangia suspended in sterile distilled water germinate in 1 - 2 h (Alicbusan, 1965; de Vera, 1973, 1977). The effect of relative humidity on germination has not been investigated.

Alicbusan (1965) reported that 30 - 50 zoospores emerged from a single sporangium whilst 200 were reported by de Vera (1973, 1977). The number or size of sporangia used in their estimations was not given and it is difficult, therefore, to make valid conclusions from their reports.

1.2.6.2 Host parasite interaction

1.2.6.2.1 Parasite penetration

The process of parasite penetration was studied by de Vera

(1973, 1977). Zoospores released from the sporangium were mobile in water for 2 - 3h. Most die and disintegrate after this period although a few come to rest on the host surface. A slight flattening of the penetrating zoospores occurs entry into the epidermal cell being achieved within 1 h. Although penetration appears to be direct the exact mechanism of entry is unknown.

1.2.6.2.2 Sorus development

Sorus development has also been studied by de Vera (1973, 1977). After breaking out of the epidermal cell the parasite thallus travels intracellularly by a process resembling protoplasmic migration and penetrates four to six cells deep in the parenchyma tissue. It is thought more likely to enter the cell wall by pressure than enzymatic action. After four days the parasite almost fills the infected cell and in most cases invades two or more adjacent cells as it enlarges. Although there is no evidence it is thought the parasite either produces a growth promoting substance directly or induces hormone production in the plant as infected cells become hypertrophied. As the parasite develops its single nucleus disappears leaving behind uniformly granular protoplasm. The initials of the secondary nuclei are visible in the central portion of the parasite 11 or 12 days after initial penetration of the

epidermis. The formation of these nuclei is followed by cleavage of the protoplasm which forms the sporangia. The entire protoplast of each sporangium becomes segmented into as many parts as there are nuclei. These parts develop into uninucleate zoospores. There is no information on the number of sporangia produced per sorus.

After cleavage the sorus expands and eruption of the gall is brought about by the combined turgor pressure of the fungus and the sheath cells which surround the gall.

1.2.6.3 Incubation and latent periods

Incubation and latent periods have not been studied specifically although Alicbusan (1965) found that yellow galls appeared 10 days after infection on leaves and 15 - 30 days on pods whilst de Vera (1973, 1977) reported sorus rupture occurred about 2 wk after infection. Neither, however, stated the conditions under which these occurred.

1.2.6.4 Dispersal

The eruption of the galls is followed by rupture of the sorus membrane and the powdery mass of sporangia are reported to be disseminated by wind (Karling, 1964; Alicbusan, 1965). Dispersal was also considered to occur through insect and other natural agencies (Alicbusan, 1965)

although this has not been confirmed.

1.2.6.5 Viability

Alicbusan (1965) stated that sporangia stored under natural environmental conditions and in the laboratory remained viable for 4 - 6 months respectively. His conclusions are questionable since infected material was placed in a covered glass container prior to being placed in the field; germination tests were not carried out and viability was only based on sporangia colour and shape. De vera (1973, 1977) reported that sporangia failed to germinate after one week's storage at - 10°C.

1.2.6.6 Host range

Repeated attempts by Gäumann (1927) to infect Phaseolus lunatus L., and Vigna sinensis with S. psophocarpi by dusting sporangia on leaves failed. He therefore came to the conclusion the fungus was host specific. This was supported by Alicbusan (1965) who failed to infect the following leguminous and non-leguminous species using zoospore suspensions: Cajanus cajan (L.) Millsp., Colopogonium munconoides Desv., Lablab niger Medik., Phaseolus lunatus, P. mungo L., P. vulgaris L., Vigna sinensis, V. sesquipedalis (L.) Fruw., (Leguminosae);

Capsicum frutescens L., Lycopersicum esculentum Mill. (Solanaceae); Amaranthus spinosus L., (Amaranthaceae); Cyperus rotundus L., (Cyperaceae); and Paspalum conjugatum Berg., (Poaceae).

1.2.7 Control

1.2.7.1 Cultural methods

False rust is reported to be favoured by high rainfall, being less serious in the dry season (Alicbusan, 1965; de Vera, 1973, 1977). Khan et al. (1977) reported that winged beans are mainly cultivated in the dry season in the Western and Eastern Highlands of P.N.G. They considered this practice may have evolved so as to avoid losses by pests and diseases. Alicbusan (1965) suggested the development of the disease within the crop may be restricted by the early removal and destruction of infected leaves, stems and pods.

1.2.7.2 Fungicides

Alicbusan (1965) reported that the following fungicides inhibited sporangia germination in vitro at the manufacturer's recommended rate: Dithane Z-78, Duphar-Zineb, Ferbam, Manzate, Orthocide 50, Ortho-cop 53, Parzate, Shell Bordeaux, Tersan 75 and Zerlate. Tindall (1968) suggested copper based fungicides will reduce damage in the

field and Khan (1977) recommended weekly sprays of Mancozeb. Neither worker, however, carried out any field evaluation trials of these fungicides.

1.2.7.3 Resistant varieties

All lines screened from the P.N.G.,winged bean germplasm collection were susceptible to S. psophocarpi (Price, 1980) However resistance has been reported by Thompson and Haryono (1979) in some of the Indonesian lines and may also be present in lines from Thailand (Chomchalow, personal communication).

SECTION 2 FIELD OBSERVATIONS OF WINGED BEANS AND FALSE RUST IN PAPUA NEW GUINEA

2.1 TRADITIONAL METHODS OF WINGED BEAN CULTIVATION

2.1.1 Introduction

The time of introduction of winged bean into P.N.G., is uncertain although Powell (1974) considered it may have been introduced in late 17th century. Khan (1976) suggested it may have first reached the Sepik Provinces, being introduced later into Highland areas through the neolithic trade system. The crop is traditionally cultivated in these areas (Khan, et al., 1977). However cultivation occurs elsewhere in P.N.G., due to the migration of the Highlanders and the recent promotion of the crop by the Department of Primary Industry and U.P.N.G. Khan et al. (1977) reported winged beans being extensively grown in the Central, Western and Eastern Highlands where plantings of 0.3 - 0.5 ha were not uncommon. The crop is not so widespread in the Southern Highlands (Paia, 1975). In the East Sepik only a small amount of winged beans are grown by subsistence growers. The crop is not grown to any extent above 2,200m altitude (Powell, 1974) and is most commonly found between 1,500 - 1,800 m in the Highlands and from 0 - 500 m in the Lowlands of the Sepik (Khan, et al., 1977).

In the Western Highlands the crop is mostly grown during the dry season (June - November) although limited cultivation does take place in the wet season (December - May). In the Eastern Highlands and in the East Sepik winged beans are grown throughout the year.

2.1.2 Varieties

Many different varieties of winged beans are grown in the Highlands; 43 being listed by Powell (1974). Some of these are grown solely for pods whilst others are grown for tubers. The distinction between varieties, however, has led to confusion since one variety may have several different local names. Khan (1976) also reported that seed identified by growers as a single variety may contain more than one genotype.

2.1.3 Soil preparation and sowing

The preparation of the seed bed in the Highlands depends on rainfall. In high rainfall areas deep (15-30cm) drainage ditches are dug which divide the bed approximately into $2.5m^2$ blocks (Khan, et al., 1977) or sowing is done on ridges (30-50cm high and 0.5 - 1m apart) (Fig. 4). In lower rainfall areas level seed beds are prepared. The sowing pattern varies from garden to garden. In some the seeds are sown in rows 0.5 - 0.75m apart with 30-50cm

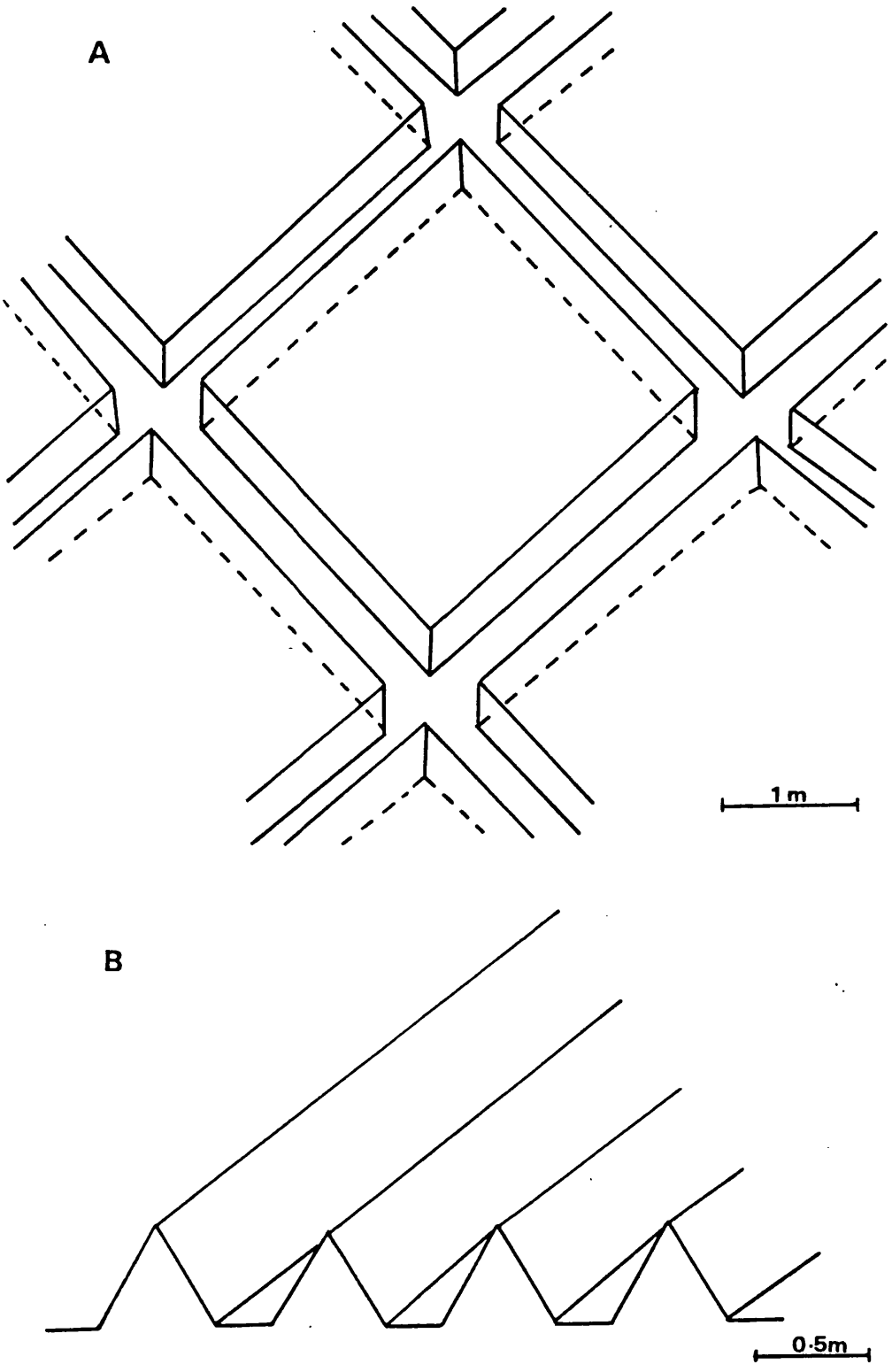

Fig. 4. Soil preparation for winged cultivation in the Highlands A) chocolate block pattern B) ridges.

spacing within the row whilst in others the density is much greater, eg., seventy plants per m^2, with no distinct pattern. Subsistence growers obtain seed from the local market (Khan et al., 1977) or more usually use their own stored from a previous crop.

Winged beans are grown in pure stands (Fig. 5) or interplanted (Fig. 6). In the Highlands they are frequently grown with sweet potato (Ipomoea batatas (L.) Lam.), maize (Zea mays L.), sugar cane (Saccharum officinarum L.), Amaranthus spp., and banana (Musa spp.). In the Sepik taro (Colocasia esculenta (L.) Schott), cassava (Manihot esculenta Crantz), cowpea (Vigna unguiculata (L.) Walp) and banana are the most common intercrops.

2.1.4 Crop maintenance

2.1.4.1 Staking

Winged beans are weak stemmed climbing plants with an indeterminate growth habit and are traditionally staked. In a few gardens, however, staking is not practiced and the plants are left to ramble over the ground. The crop is normally staked after 6-8 wk with a single stake providing support for one to four plants; these stakes ranged in height from 1 - 1.5m for plants grown for tubers

Fig. 5. Pure stand of winged beans in the Western Highlands.

Fig. 6. Winged beans interplanted with maize in the Eastern Highlands.

and 2 - 3m for those grown for pods (Khan, et al., 1977). Most stakes were derived from stems of pit pit (Saccharum spp.) although branches of bushes and trees adjacent to the winged bean garden are sometimes used. Dead maize stalks from a previous crop, interplanted maize and sugar cane are also used to provide support.

2.1.4.2 Pruning

In the Highlands periodic removal of flowers and young pods to enhance tuber formation is widely practiced. Bala and Stephenson (1980) showed experimentally this was the most effective pruning method.

2.1.5 Pest and diseases[1]

Weeds, insect pests, nematode and fungal diseases can cause problems in the cultivation of the crop especially during the wet season. A check list of insect pests on winged beans has been compiled (Lamb, 1980) although the amount of damage they cause is unknown. The root knot nematode, Meloidogyne incognita Chitwood, has been recorded by Khan et al. (1977), Price and Linge (1979) and Shaw (1963). The nematode appears to be widely distributed and may cause considerable damage in some areas (Khan, et al., 1977).

[1]Accession numbers of fungi and nematodes recorded attacking winged bean in P.N.G., are given in Appendix 1.

Another nematode species M. javanica (Treub.) Chitwood, is also known to attack winged bean (Price, et al., 1979). The following fungal diseases have been reported: false rust (Synchytrium psophocarpi - Shaw, 1963; Price, 1976; Khan, et al., 1977), leaf spot (Pseudocercospora psophocarpi (Yen) Deighton - Shaw, 1963; Price and Munro 1978a), powdery mildew (Oidium spp. - Shaw, 1963; Price, 1977), collar rot (causal agent(s) not established - Price and Munro, 1978b), leaf blight (Thanatephorus cucumeris (Frank) Donk - Price, 1980) and flower blight (Choanephora cucurbitarum (Beck and Rae) Thaxt - Price, 1980). Sooty mould caused by the non-parasitic Meliola spp., occurs on leaves especially in the East Sepik. Of these fungal diseases false rust and leaf spot appear to be the most widespread and cause the most serious problems.

Most growers grow winged beans in rotation, eg., with sweet potato in the Highlands and taro in the East Sepik, or interplanted (loc. cit.) and it is possible that these cultural practices may reduce losses caused by pest and diseases. Single plots of winged beans often consist of a number of different varieties sown randomly. In most areas of cultivation weeds are periodically removed by hand or hoes especially during the establishment phase of

the crop. In the Western Highlands winged beans are mainly grown in the dry season when pests and diseases are not a major problem.

2.1.6 Harvesting and marketing

Young pods are harvested after 4 - 6 months and tubers after 8 - 9 months (Powell, 1974). The crop is well suited for subsistence growers as several harvests of young pods can be made during the growing season. Some pods are allowed to mature to provide seed for the next season's crop. Estimates of dry seed yield of local varieties range from 800-1000 kg/ha and tubers from 5,533-11,754 kg/ha (Khan, et al., 1977). Although winged beans are a subsistence crop some are marketed locally. Pods, tubers, flowers and leaves are commonly seen in markets in Mt Hagen (Western Highlands) and Goroka (Eastern Highlands) (Figs 7 and 8).

Fig.7. Winged bean leaves (arrowed) for sale in Goroka market, Eastern Highlands.

Fig.8. Winged bean pods (arrowed) for sale in Goroka market, Eastern Highlands.

2.2 SYMPTOMS OF FALSE RUST

An easily recognised diagnostic symptom of the disease is the appearance of small (c. 200 - 450 μm diam.) yellow to orange galls (Figs 9 and 10) which occur on the upper and lower leaf surfaces (Figs 11 and 12). The galls occur separately or in clusters. Non-ruptured galls are semi-globular and slightly raised above the host surface whilst ruptured empty galls show marked depressions (Fig. 12). Infection is usually greater on the lower leaf surface with galls concentrated along the veins (Fig. 13). Galling of the stem (Fig. 14), pods (Fig. 15) and calyx (Fig. 16) also occurs. The corolla and reproductive organs of the flower are not attacked and seed from infected pods appears free of infection. The tissue surrounding galls on pods is often black (Fig. 17). This is most noticeable on pods which have a green background colour. Blackening also occurs along the mid-rib and main veins of the lower leaf surface (Fig. 18) and on the petiole and stem. Infection of young expanding leaves results in malformation with cupping and curling of leaflets (Fig. 19), thickening of the petiole and lamina and dwarfing. Severe or moderate infection on young pods leads to abnormal growth and malformation. Infection at the node stimulates the production of numerous lateral shoots which are closely aggregated (Fig. 20).

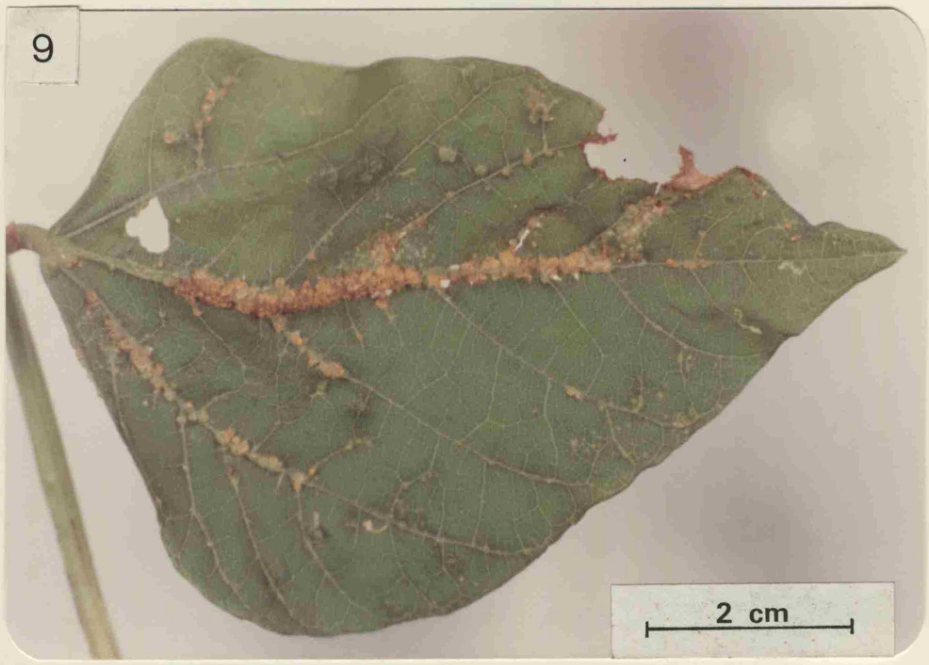

Fig.9. Orange galls on lower leaf surface.

Fig.10. Orange galls on stem.

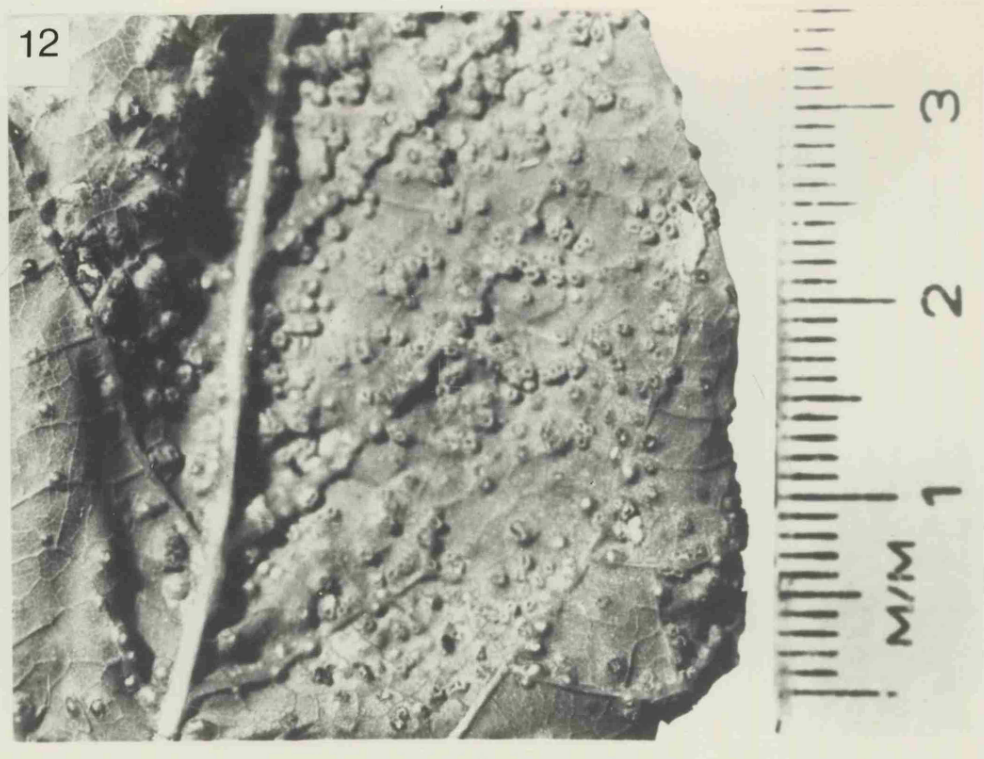

Fig.11. Infected upper leaf surface.

Fig.12. Infected lower leaf surface.

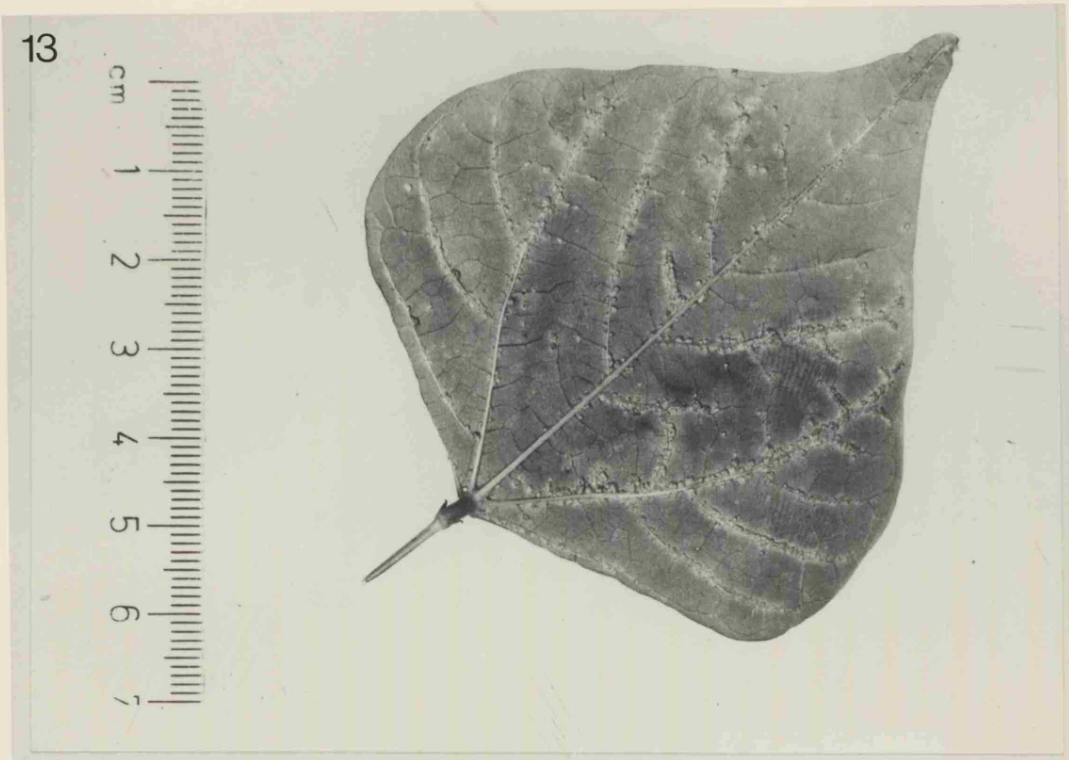

Fig.13. Galls along primary and secondary veins of lower leaf surface.

Fig.14. Infected stem.

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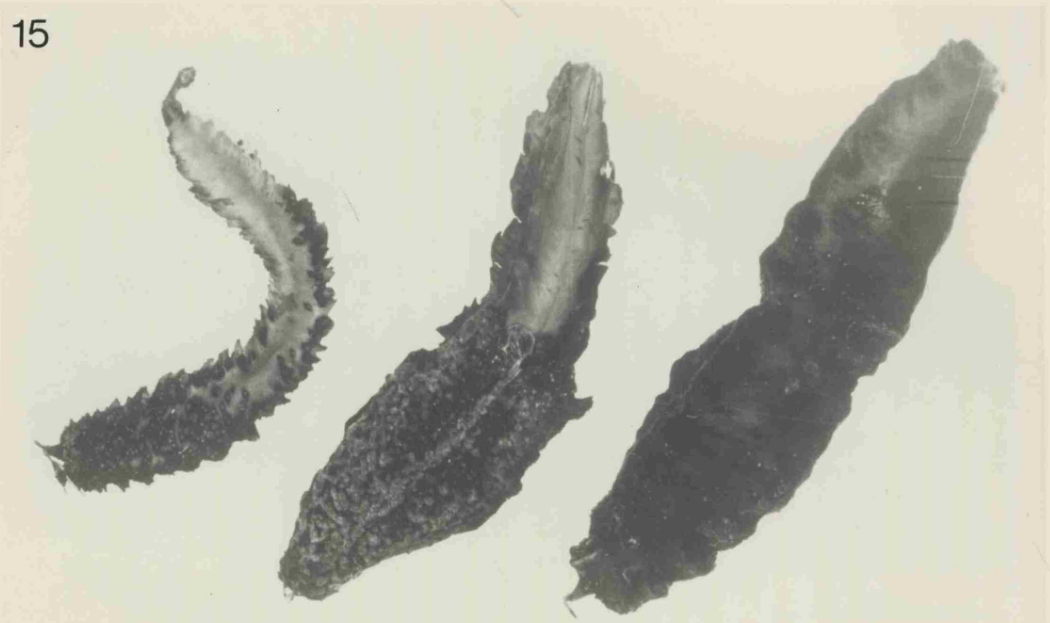

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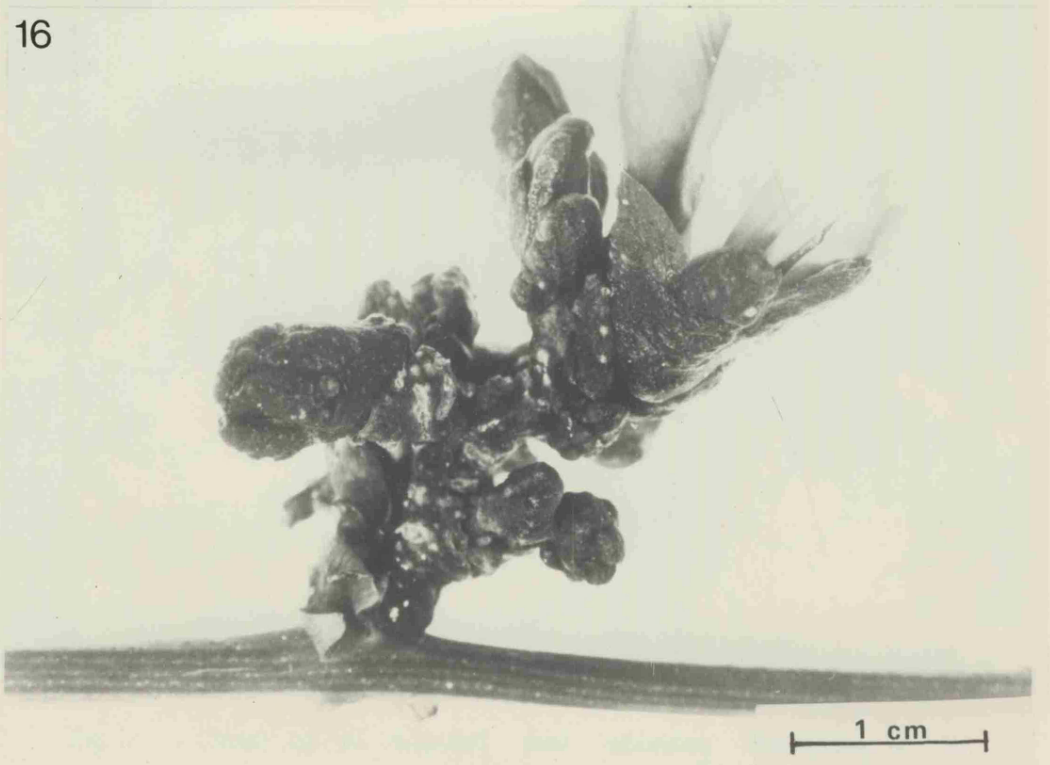
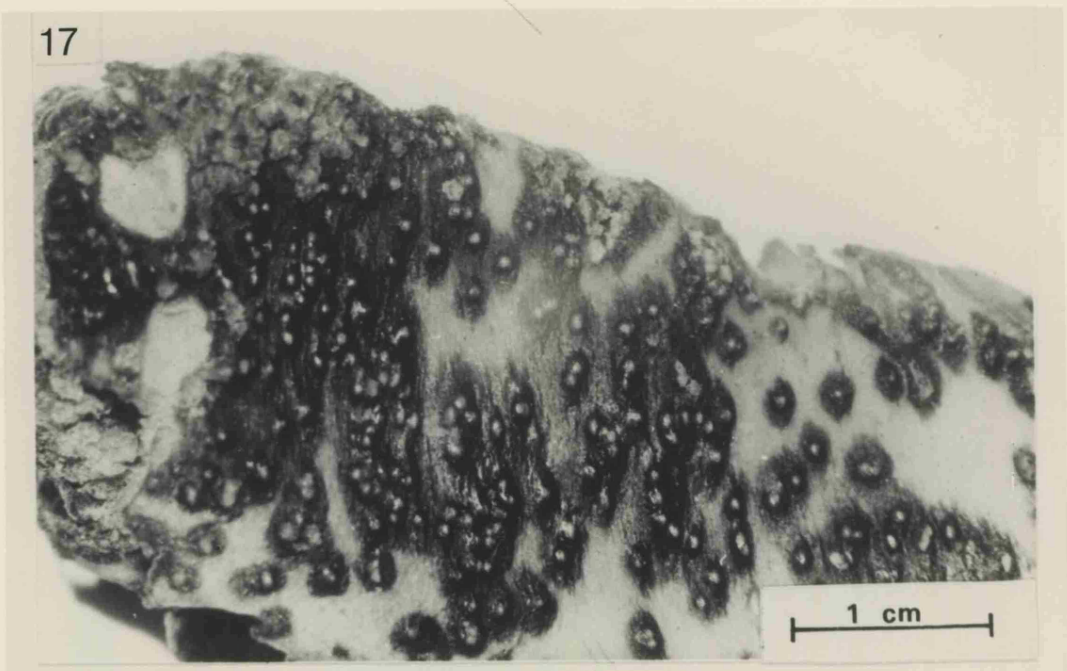


Fig.15. Infected pods.

Fig.16. Infected calyx.



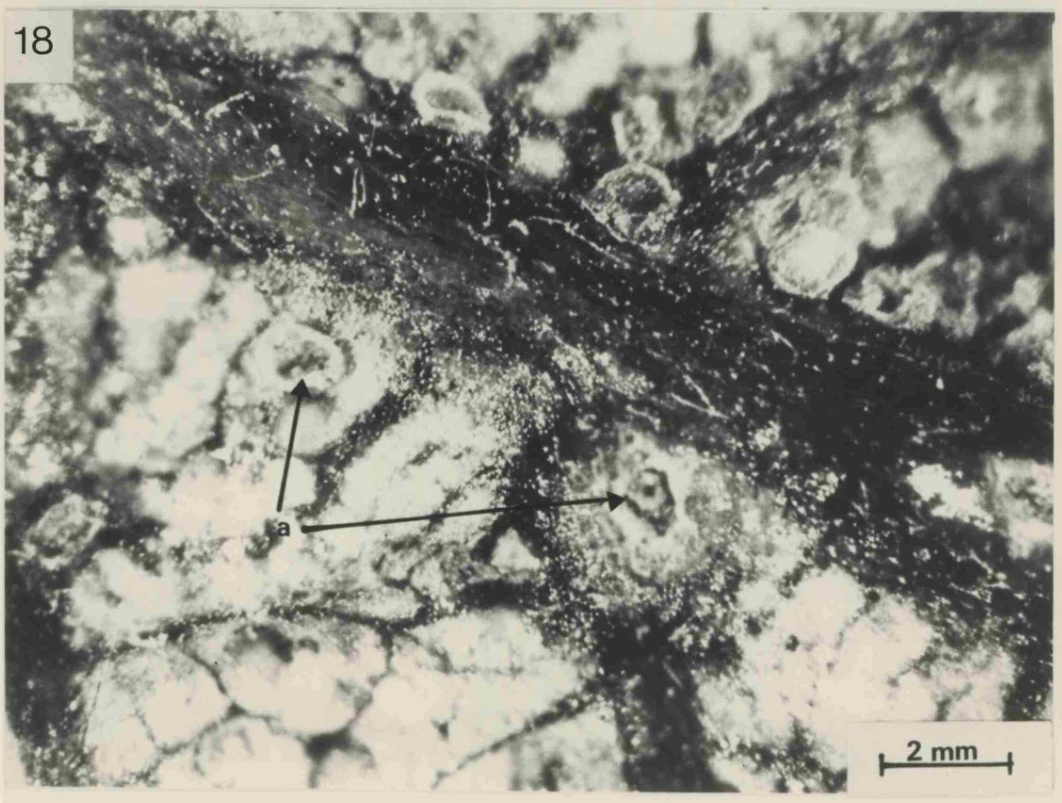


Fig. 17. Close up of infected pod showing blackening of tissue around galls.

Fig. 18. Close up of infected lower leaf surface showing blackening along veins, a) empty galls surrounded by dry powdery mass of sporangia.

Fig. 19. Curling of infected leaflets.

Fig. 20. Aggregation of lateral shoots at the node.

2.3 INCIDENCE, SEVERITY AND DISTRIBUTION OF FALSE RUST

2.3.1 Introduction

False rust was first recorded in P.N.G., in 1959 by Shaw (personal communication). Since this time, although surveys of winged bean cultivation and consumption have been made (Powell, 1974; Paia, 1975; Khan, et al., 1977; Strathern, 1980), little additional information on the disease has emerged. Surveys were therefore conducted during the wet and dry seasons to record the incidence, severity and distribution of false rust in both the traditional and other winged bean growing areas. Attempts were made to design a simple key for disease assessment and to detect local varieties which showed field resistance.

2.3.2 Methods

2.3.2.1 Disease assessment

The incidence and severity of false rust were both recorded. Disease incidence was expressed as the percentage of infected plants in the sample. Disease severity was estimated based on a descriptive key (Table 5) using the entire plant. This was subsequently replaced by a standard area diagram disease key (Fig. 21) to give more precision in quantifying the area of plant tissue diseased. The diagram was prepared by tracing leaf outlines onto graph paper and areas of 1,5,10,25 and

50% were blacked in according to the infection pattern. In this method 10 leaves were selected at random from each plant and given a percentile rating according to the diagram and the mean percentage leaf area diseased per plant calculated. The growth stages of the crop were divided into vegetative, flowering, young pod and mature (dry) pod for each assessment. The use of the percentage scale in assessing disease severity is discussed further by James (1974).

Table 5 Descriptive key for assessment of false rust severity

Description	Severity category
No infection	Nil
>0, <20% of plant infected, galls on leaves, stems, pods and calyx	Slight
20-50% of plant infected, galls on leaves, stems, pods and calyx	Moderate
51-100% of plant infected, malformed shoots bunched together at nodes, galls on leaves, stems, pods and calyx	Severe

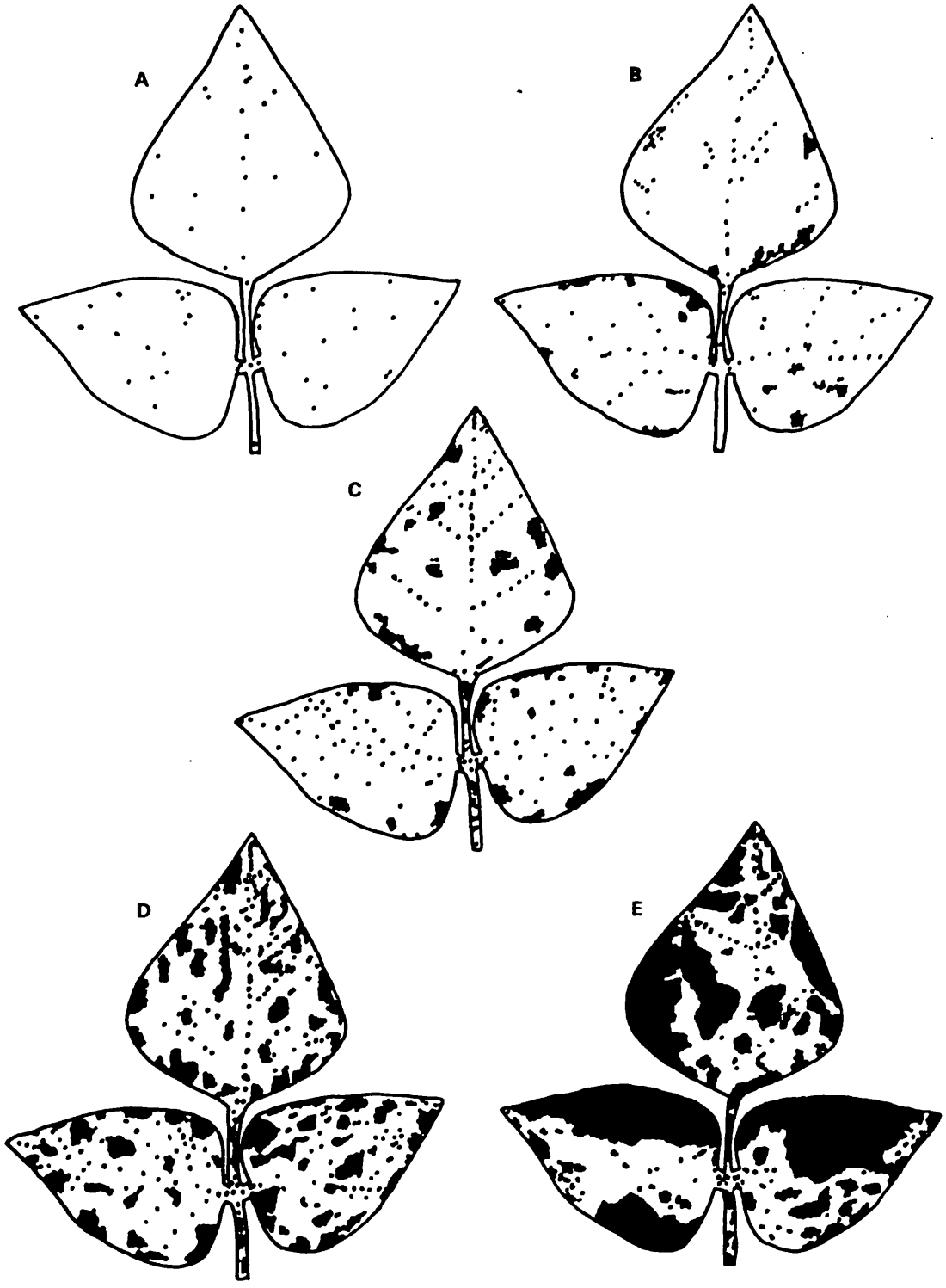


Fig. 21. Disease key for false rust; leaf area infection A. 1% B. 5% C. 10% D. 25% E. 50%

2.3.2.2 Sampling

Assessments were made at a number of sites, mainly subsistence gardens, in different localities. Plants were selected at random at each site and assessed for false rust. Sample size ranged from one to 540 plants depending on plot size.

Whilst conducting the sampling a number of problems became apparent:

1. Access to the traditional areas of winged bean cultivation from Port Moresby was only possible by air which made the surveys expensive.

2. Roads were mainly unsealed, except in the larger towns, and some of the minor roads were inaccessible without a four-wheel drive vehicle.

3. A local guide was necessary who was familiar with the area and could speak both English and Pidgin English and even then, although these are two of the main languages spoken in P.N.G., there are numerous local dialects. A few kilometres may separate people who speak an entirely different language and this resulted in communication problems with subsistence growers.

4. Government maps were incomplete for some of the areas

visited and sometimes inaccurate. Cartographers are hampered by the fact that villages and agricultural sites often change names. In some areas, therefore, it was necessary to rely on the knowledge of the Agriculture Advisory Field Officers of the Department of Primary Industry and the local people for location of the sampling sites.

5. The majority of subsistence growers enthusiastically assisted in the surveys and showed great interest on occasions, however, some areas were unsafe to survey as a result of inter-tribal disputes.

2.3.2.3 Areas surveyed

Surveys of false rust were conducted in the Western and Eastern Highland Provinces during June - November 1976 (dry season), December 1976 - May 1977 (wet season) and in the East Sepik and Madang Provinces during June - November 1978 (dry season). These Provinces were selected because they form part of the traditional areas of cultivation of the winged bean (Khan, et al., 1977). Surveys were also made in the Morobe Province in April 1977 and the East New Britain Provinces in September 1977 where limited cultivation is practiced.

[1]Assessments of severity were also made on experimental trials of pure lines of winged beans grown at Port Moresby (Central Province) in May, October and November 1977; Kuk (Western Highlands Province) in November 1976, April, July 1977, October 1978; Aiyura (Eastern Highlands Province) and Lae (Morobe Province) in October 1978.

2.3.3 Results

The distribution of false rust in P.N.G., is shown in Fig. 22. The disease occurred throughout the Western and Eastern Highlands (Figs 23 and 24) in both the wet and dry seasons. It also occurred in the East Sepik and Madang Provinces, sites in Port Moresby, Lae and [2]Wau (Morobe Province) but it was not recorded at Keravat (Nr. Rabaul, East New Britain Province). The incidence and severity of the disease in the Provinces included in the survey are given in Tables 6, 7 and 8. In the majority of the localities visited disease severity was slight.

Frequently some plants in a sample were severely infected whilst others were only slightly infected. The disease even on mature plants, was often restricted to a few leaves.

[1]See Section 6 Control.

[2]Although Wau was not visited in the surveys it is included since the disease was identified on winged bean leaves sent from the Wau Ecological Institute by Gagné in August 1978.

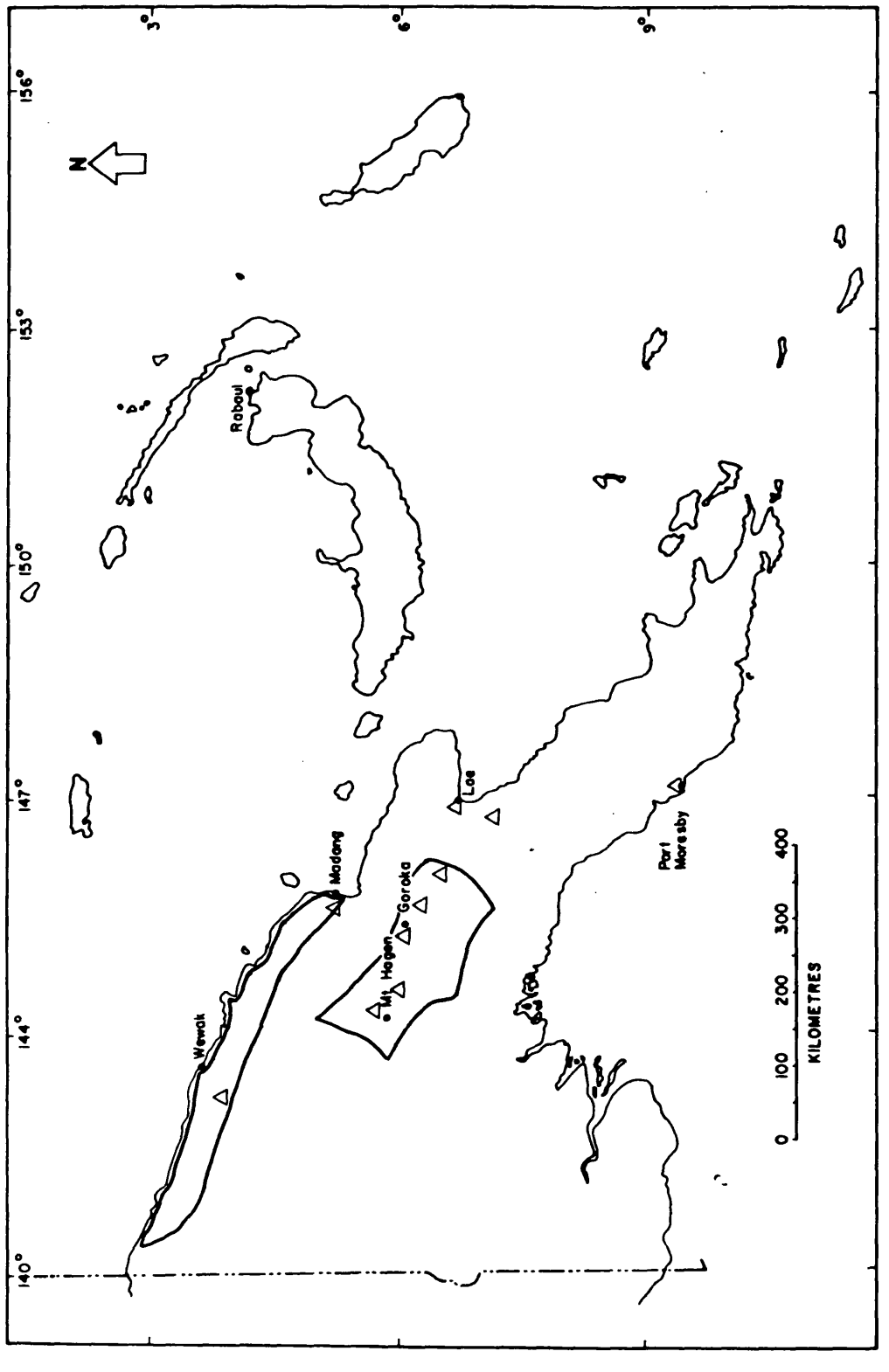


Fig. 22. △ Distribution of false rust in Papua New Guinea, ⬭ traditional winged bean growing areas after Khan (1976).

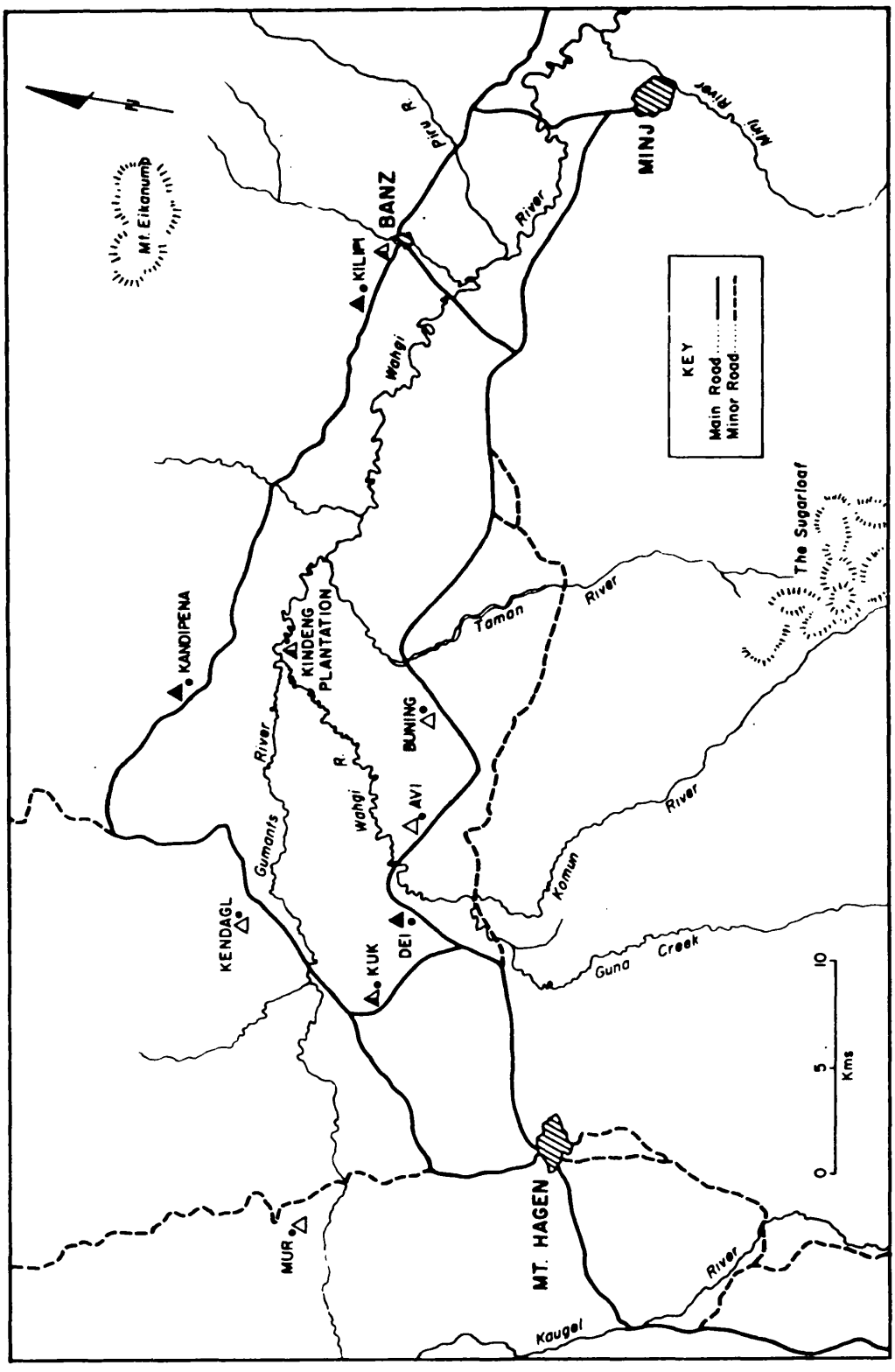


Fig. 23. Occurrence of false rust in the Western Highlands recorded in △ November 1976 and ▲ April 1977 surveys.

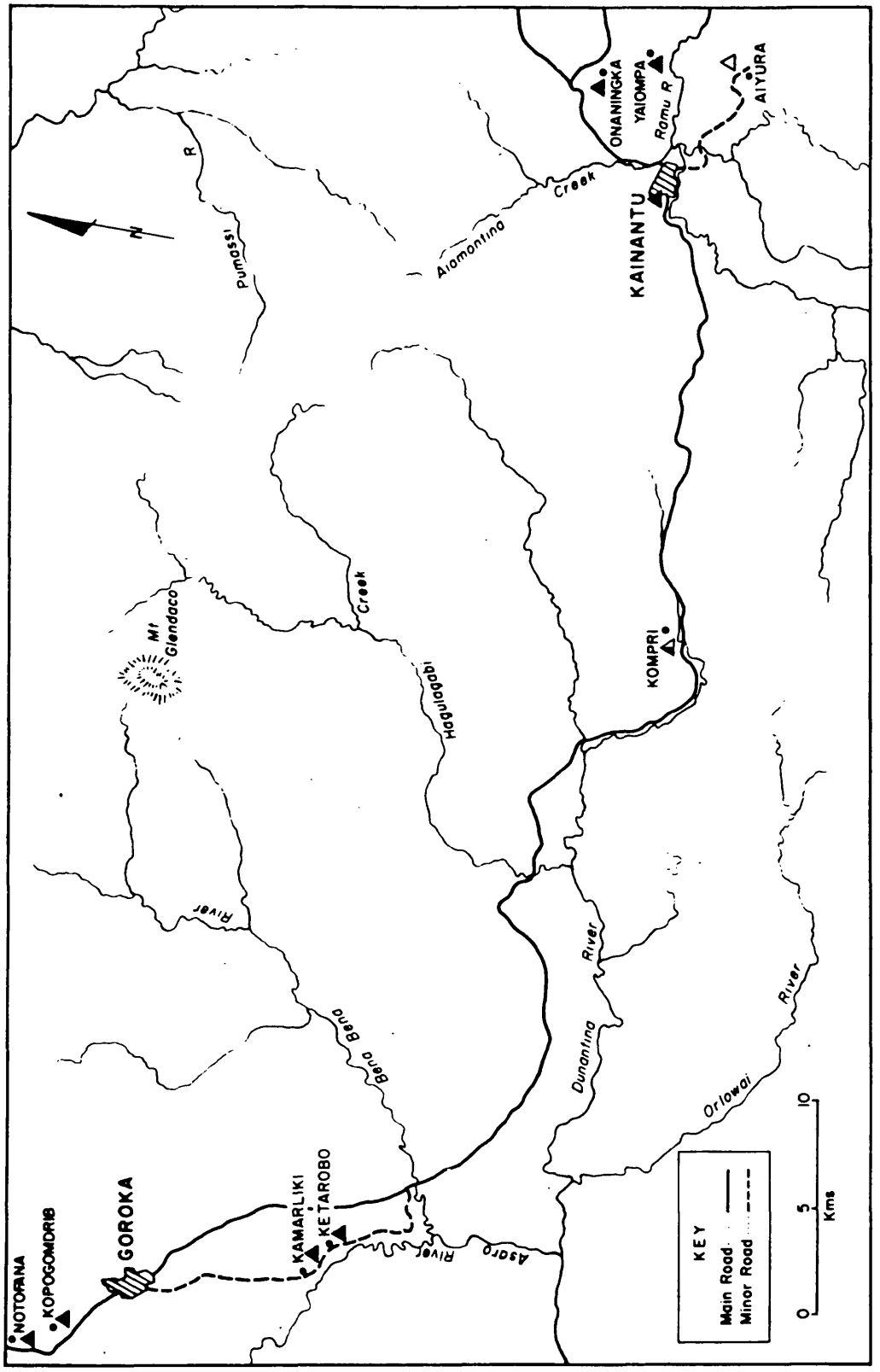


Fig. 24. Occurrence of false rust in the Eastern Highlands recorded in △ November 1976 and ▲ April 1977 surveys.

Table 6 Incidence and severity of false rust in the Western Highlands Province

Locality	Site	Date assessed	Season	No.plants sampled	[1]Growth stage	Incidence (%)	[2]Severity [A]Category	[B]Mean % leaf area diseased
Banz	Agric.Coll.	2nd Nov 1976	Dry	10	YP	100	Moderate	-
	" "	"	"	10	V	80	Slight	-
	" "	24th April 1977	Wet	10	YP	100	"	-
	" "	"	"	5	MP	60	"	-
	" "	"	"	5	V	20	"	-
	Kilipi	"	"	15	F	100	"	-
	"	"	"	5	MP	100	"	-
Mount Hagen	Avi	1st Nov 1976	Dry	30	MP	60	"	-
	Buning	"	"	10	V	0	Nil	-
	"	"	"	20	YP	5	Slight	-
	Dei	28th April 1977	Wet	10	F	30	"	-
	Kandipena	"	"	10	F	90	"	-
	Kendagl	2nd Nov 1976	Dry	20	YP	70	"	-
	Kindeng	1st Nov 1976	"	20	V	15	"	-
	"	28th April 1977	Wet	25	V	0	Nil	-
	Kuk Agric.St.	1st Nov 1976	Dry	100	V	30	Slight	-
	" " "	28th April 1977	Wet	200	V	2	"	-
	" " "	7th July 1977	"	200	YP	80	"	-
	" " "	3rd Oct 1978	Dry	200	MP	74	-	0.82
	Mur	2nd Nov 1976	"	20	YP	5	Slight	-
Minj	Vocational Sch.	3rd Nov 1976	"	10	V	0	Nil	-

[1]Growth Stage V = Vegetative, F = Flowering, YP = Young pod, MP = Mature (dry) pod

[2]Severity A. based on descriptive disease key.
" B. " " standard area diagram disease key.

Table 7 Incidence and severity of false rust in the Eastern Highlands Province

Locality	Site	Date assessed	Season	No.plants sampled	[1]Growth stage	Incidence (%)	[2]Severity [A]Category	[B]Mean % leaf area diseased
Aiyura	Agric. St.	4th Oct 1978	Dry	200	MP	100	-	4.49
	Gard. nr.Agric.St.	4th Nov 1976	"	10	YP	40	Slight	-
	" " " "	"	"	20	YP	25	"	-
	" " " "	"	"	10	YP	100	"	-
	" " " "	"	"	10	V	0	Nil	-
	" " " "	"	"	10	YP	0	Nil	-
	" " " "	"	"	10	YP	0	Nil	-
	" " " "	"	"	10	YP	90	Slight	-
Goroka	Kamarliki	25th April 1977	Wet	20	MP	25	"	-
	Kopogomdrib	"	"	10	MP	60	"	-
	Ketarobo	"	"	5	MP	40	"	-
	Notofana	"	"	20	MP	50	"	-
Kainantu	Gard. in town centre	26th April 1977	"	10	YP	80	"	-
	Kompri	5th Nov 1976	Dry	10	YP	0	Nil	-
	"	"	"	10	MF	0	"	-
	"	"	"	10	YP	0	"	-
	"	26th April 1977	Wet	10	MP	100	Slight	-
	"	"	"	10	YP	100	"	-
	Onaningka	25th April 1977	"	10	MP	100	"	-
	Yaiompa	"	"	10	YP	80	"	-

[1]Growth Stage V = Vegetative, F = Flowering, YP = Young Pod, MP = Mature (dry) pod

[2]Severity A. based on descriptive disease key.
" B. " " standard area diagram disease key.

Table 8 Incidence and severity of false rust in the Central, East New Britain, Madang and Morobe Provinces

	Locality	Site	Date assessed	Season	No.plants sampled	[1]Growth stage	Incidence (%)	[2]Severity [A]Category	[B]Mean % leaf area diseased
A. Central Province	Port Moresby	Agric.Gard.U.P.N.G.	27th May 1977	Wet	50	YP	100	Slight	-
		" " "	21st Oct 1977	Dry	540	Mixed	100	-	0.12
			17th Nov 1977	Dry	493	"	99.8	-	3.49
B. East New Britain	Rabaul	Keravat Agric. St.	16th Sept 1977	Dry	20	YP	0	-	0
C. East Sepik	Wewak	Kubalia Agric. St.	12th July 1978	Dry	10	V	0	-	0
		" " "	"	"	10	F	30	-	0.43
		" " "	"	"	4	MP	50	-	3.19
		Gardens nr.Agric.St.	"	"	3	MP	0	-	0
		" " "	"	"	2	YP	0	-	0
		" " "	"	"	1	YP	100	-	1.15
		Nagumarum	"	"	8	MP	75	-	2.08
		Vamindugum	"	"	4	MP	75	-	8.33
D.	Madang	Gard.nr.town centre	10th July 1978	"	10	V	0	-	0
	Madang	Omura Agric. St.	"	"	20	YP	90	-	2.50
		" " "	"	"	20	YP	70	-	0.13
E.	Morobe	Agric.St.University	28th April 1977	Wet	10	V	0	Nil	-
	Lae	" " "	3rd Oct 1978	Dry	200	MP	100	-	8.56

[1]Growth Stage V = Vegetative, F = Flowering, YP = Young Pod, MP = Mature (dry) pod

[2]Severity A. based on descriptive disease key.
" B. " " standard area diagram disease key.

The indeterminate growth habit of the winged bean and the numerous lateral shoots which twist round each other and the supporting stake made assessment difficult. In some gardens, especially in the East Sepik, only a few plants were grown and rapid assessment could be made whereas in others extensive cultivation was practiced and assessment took much longer.

2.3.4 Discussion

False rust was present at sites isolated (c. 1-5 km) from others suggesting that infection was either from infected planting material or wind borne sporangia. The disease incidence at some sites was high (50-100%) whilst at others in close proximity (c. 50-200m) it was nil. It is possible that winged bean plantings that escaped infection were due to the wind carrying sporangia from the infection source, away from these sites. This is considered a more plausible explanation than host resistance since winged beans in adjacent subsistence gardens were often grown from the same batch of seed. The disease was not recorded in the East New Britain Province which was the only area visited outside the mainland of P.N.G. Insufficient samples were taken to state conclusively that the disease was absent from the Province. Since sporangia lose viability rapidly (loc. cit.) it is possible they are not able to travel long distances and cause successful infection. False rust was found in the

Sepik the Province considered by Khan (1976) where winged beans were first introduced. It is possible that the disease may have been introduced at the same time and followed the same pathway as the crop as it moved inland. Since the winged bean is not a native plant to P.N.G., (Khan, 1976) and false rust is host specific (Alicbusan, 1965; loc. cit.) the disease was probably introduced into the country on propagative material. Movement of this material possibly by migrating Highlanders and plant collectors may also explain the movement of the disease to Provinces outside those where the crop is traditionally cultivated. This could account for the appearance of false rust in experimental winged bean trials at Port Moresby in May 1976 (Price, personal communication) after pods of local varieties were brought back from the Highlands. It later spread to winged beans grown at the nearby settlements of Gerehu and Laloki and became established in the area. Khan, et al., (1977) reported winged beans were grown in the dry season to avoid loses by pests and diseases, however, during the surveys plantings were also observed in the wet season especially in the Eastern Highlands and the East Sepik Provinces. False rust occurred on winged bean plants grown in the dry as well as the wet season. In some sites visited during both seasons, however, eg. Kompri, greater

disease incidence was recorded. Greater severity was also recorded on plants at the young and mature pod growth stages. In areas where winged beans are grown in both seasons infection can occur throughout the year and may ensure the maintenance of continuous inoculum. Seasonal effects on losses caused by the disease, however, are not known.

Although the standard area diagram was a useful guide in assessing disease severity there was a problem with its use. The infection distribution on leaves often did not follow a regular pattern, eg, one leaflet occasionally was completely covered with galls whilst the other two comparatively few were present. On occasions therefore it was necessary to use subjective judgements in the assessment of individual leaves. Further surveys using the standard area diagram keys to measure disease are required in areas not previously covered and to attempt to find local varieties which are less susceptible to the disease.

SECTION 3 ETIOLOGY

3.1 INTRODUCTION

In previous studies of S. psophocarpi (Raciborski, 1898; Gäumann, 1927; Alicbusan, 1965; de Vera, 1973, 1977) the fungal thallus was described as holocarpic which at reproduction segmented into sporangia giving rise to motile zoospores. Descriptions and measurements of these structures, however, were not always in agreement. Information on the physiology of S. psophocarpi is limited to one unpublished report (Alicbusan, 1965).

No details of the biology of the fungus have been reported from P.N.G. This afforded an opportunity for comparisons of the structure and behaviour of sporangia and zoospores with previously published accounts. In addition the present study attempts to provide information on the biology of the fungus required for an understanding of the epidemiology of the disease and the development of control strategies.

3.2 MATERIALS AND METHODS

3.2.1 Slides and coverslips

Microscope cavity slides (75 x 25 x 3 mm) and coverslips (22 mm^2) were made grease free by washing with 'Pyroneg'

(Diversey Pty. Ltd.), rinsing three times in tap water, followed by a final rinse in distilled water. They were then transferred to 95% ethanol and stored until required.

3.2.2 Fixing, staining and mounting of sori

Pieces of leaves (c. 1cm diam.) infected with S. psophocarpi were cut and fixed in 'Navashin's Fluid' for 24 h (de Vera, 1973, 1977). These were transferred to a water bath and washed in running tap water for 24 h before dehydrating in an alcohol series culminating in 95% ethanol and tertiary butyl alcohol and clearing in cedar wood oil for 48 h (Johansen, 1940). After embedding in 'Paraplast' blocks, 10-15 μm sections were cut, dewaxed in xylene for 10 min, washed in 100 and then 90% ethanol for 5 min and stained in 1% phloxine B (Searle Diagnostics, Yorkshire, England) for 12 h. The sections were then washed twice in 90% ethanol prior to and following counterstaining with 1% aniline blue (Searle Diagnostics, Yorkshire, England) for 30-60 sec, cleared in xylene for 10 min and mounted in Canada Balsam (Searle Diagnostics, Yorkshire, England).

3.2.3 Collection of sporangia

Sporangia were removed from erupted sori on infected leaves with a mounting needle whilst viewing under a stereomicroscope (x20).

3.2.4 Size of sporangia

Sporangia were transferred to a small drop of tap water which had been placed in the centre of a coverslip by means of an Arnold Hand Microapplicator (Arnold, 1967), and a hanging drop preparation made according to the method of Iino and Enomoto (1971). One hundred sporangia were measured at a magnification of x 600. This was repeated three times on sporangia from different sori.

3.2.5 Germination of sporangia

Sporangia were considered to have germinated if they were empty, partially empty or showed zoospores actively emerging. Germination was tested by placing sporangia from erupted sori in cavity slides which were partially filled with sterile, distilled water. These slides were then placed in slide racks and incubated at 100% r.h., in sealed plastic boxes which had been partially filled with tap water and equilibrated for 24 h at 25^{o}C.

3.2.6 Effect of temperature on germination

The effect of temperature was measured by placing sporangia from a single sorus in seven cavity slides. A mark was made on the edge of each cavity and fifty sporangia were assessed for germination in each slide prior to incubation. This was

repeated five times using sporangia from different sori. Each box of replicates was placed in incubators (Model No. 1H 330 DMA, Gallenkamp, London, England) held at 5, 10, 15, 20, 25, 30 and 37°C and germination was assessed after 24, 48 and 72h.

3.2.7 Effect of relative humidity and surface wetness on sporangia germination

The effect of relative humidity was investigated by placing saturated salt solutions in the plastic boxes to maintain the following relative humidities: $NaNO_2$, 66%; KBr, 84%; $ZnSO_4$, $7H_2O$, 90% $PbNO_3$, 98%; H_2O, 100% (O'Brien, 1948). The effect of surface wetness was investigated by placing one set of sporangia in cavity slides that did not contain water. Germination was assessed before and after 24 h incubation at 20°C. Four replicates were used for each treatment and fifty sporangia were counted in each replicate.

3.2.8 Germination of sporangia and zoospore production

Sporangia were placed in a hanging drop of 1% carboxy methyl cellulose (Marsland, 1971; Stiles and Hawkins, 1971) and incubated at 25°C. The sequence of germination was observed microscopically and the number of zoospores emerging from a single sporangium was counted on five

separate occasions. These sporangia were measured prior to germination and selected on a uniform diameter basis.

3.2.9 Rates of germination of sporangia

One hundred sporangia in each of four replicates were assessed for germination at 10 min intervals for the first 2 h and at 4, 6 and 24 h after incubation at 25°C.

3.2.10 Effect of sporangial age on viability

One hundred sporangia from an unopened sorus, a recently erupted sorus and one that had been opened for longer periods were assessed for germination before and after 24 h incubation at 25°C. This was repeated eight times using sporangia from different sori.

3.2.11 Effect of storage on viability of sporangia

Infected leaves and stems were removed from plants growing in the University Experimental Agriculture Garden and placed in terylene net bags (1mm^2 mesh) and air-tight plastic boxes. One bag was placed in the field at U.P.N.G., 2 m above ground level, another bag was held at room temperature (25 - 28°C), one bag and one box were placed in a refrigerator held at 5°C and one bag and one box in a deep freeze held at -85°C. Outdoor measurements of temperature

relative humidity and rainfall were obtained from recording instruments at the University Meterological Station. Room temperature and relative humidity were recorded with a 7-day bimetallic thermograph and hair hydrograph (Ota Keiki, Seisakusho Co. Ltd., Japan).

Sporangia from erupted sori were removed from each treatment on the day of collection (day 0) and 1,2,3,4,12, 16,20 and 24 days later and assessed for germination after 24 h incubation at 20°C. Three replicates, each from different sori, were prepared from each treatment and 100 sporangia were counted in each replicate.

3.2.12 Number and viability of sporangia in a single sorus

A single unopened sorus was removed from a leaf surface with mounting needles whilst viewing under a stereo-microscope (x20) and the sporangia were teased out under distilled water. Germination of all the sporangia was assessed after 24 h incubation at 20°C. This was repeated twice using sori of the same diameter.

3.2.13 Size of fixed zoospores and flagella length

Sporangia were germinated in a drop of water and the emerged zoospores were fixed by exposure to 2% osmic acid for 1 min, stained in a 0.04% solution of crystal violet,

dried in a desiccator for 2 h, cleared in clove oil, washed in xylene and mounted in euparal (McLean and Ivimey Cook, 1965). Measurements of 100 zoospores and 200 flagella were made at a magnification of x600.

3.2.14 Size of viable zoospores

Sporangia were germinated in a hanging drop. Zoospores which had ceased active swarming were measured under a magnification of x600 using phase contrast microscopy. The time between emergence from the sporangium and cessation of motility was recorded.

3.2.15 Encystment of zoospores and observations on the flagellum

Sporangia were germinated in a hanging drop incubated at 25°C. Zoospores were fixed as previously described immediately after emergence and 1,2,3,4 and 5 h after germination. Two hundred zoospores were counted for each time interval and the number of zoospores with and without flagella recorded. Measurements of 100 encysted zoospores were made at a magnification of x600. Observations were also made on viable and fixed zoospores to determine whether the flagellum was retracted or shed.

3.2.16 Scanning electron microscopy

Sporangia were allowed to germinate in sterile water on microscopic slides; samples were removed at intervals of 1,2,4,6,12 and 24 h after incubation at 25°C and fixed in 2% osmic acid for 1 min. The fixed material was then transferred into 70% alcohol and despatched to Australia where the samples were rehydrated and allowed to settle on coverslip glass pieces previously coated with poly-L-lysine for 15 min. The glass pieces were then washed and dehydrated in an ethanol series before critical point drying from amyl acetate and coating with gold (Tsutsui, Hiromi, Hiroyuki and Jutaro, 1976). They were then examined under a JEOL Stereoscan Electron Microscope at 15 kV.

3.3 RESULTS

3.3.1 Colour, shape and size of sporangia

Sori (Fig. 25) contained sporangia which had pentagonal and hexagonal walls (Fig. 26) with regular surfaces (Fig. 27). Immature sporangia had hyaline walls enclosing finely granulated pale yellow protoplasm which became darker and more coarsely granulated at maturity. The protoplasm of non-viable sporangia was detached from the walls (Fig. 28). Sporangia from P.N.G., were either globose in shape

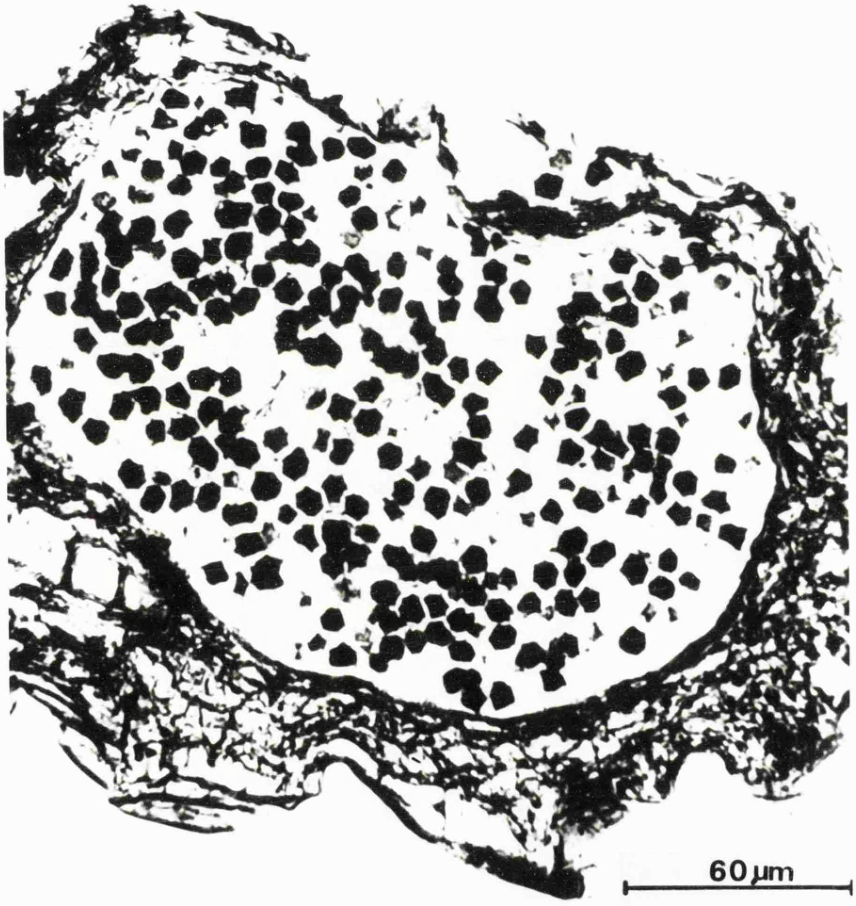
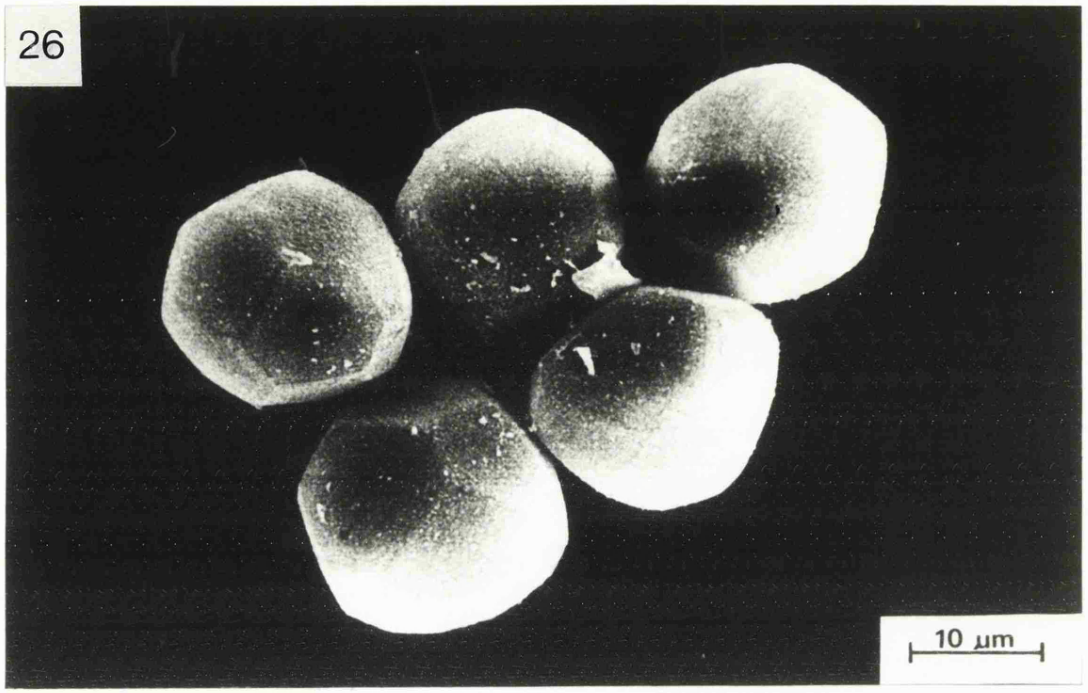


Fig. 25. Transverse section of sorus.



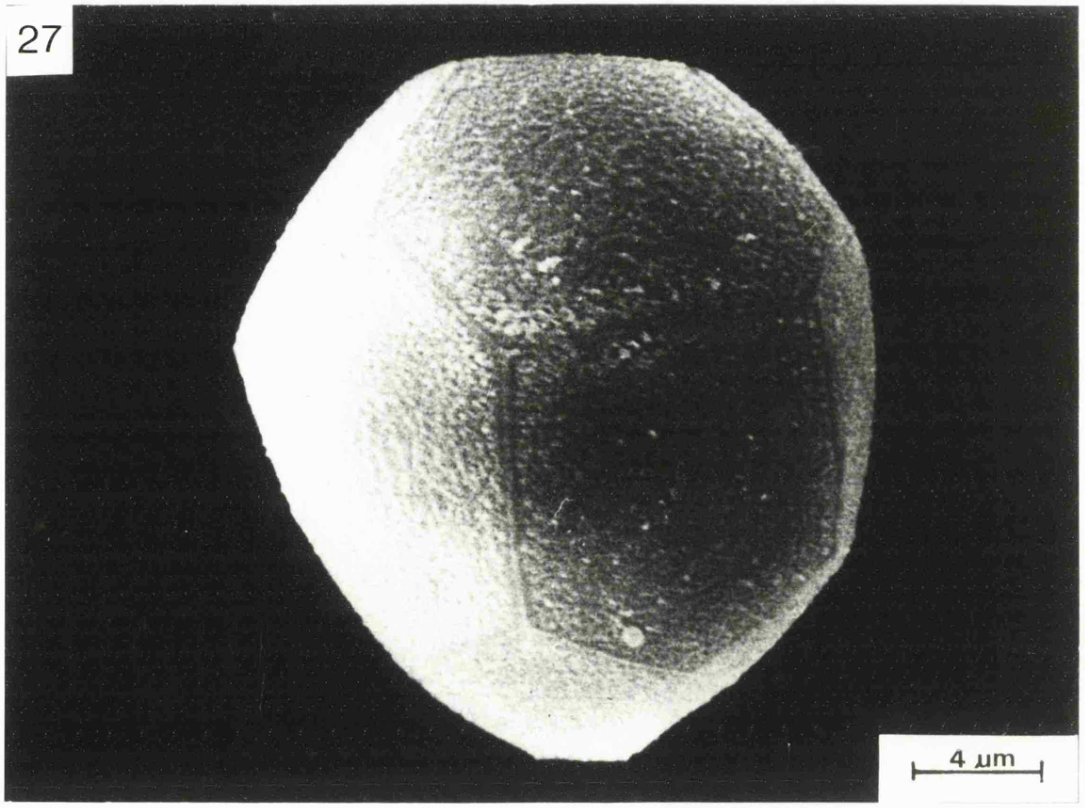
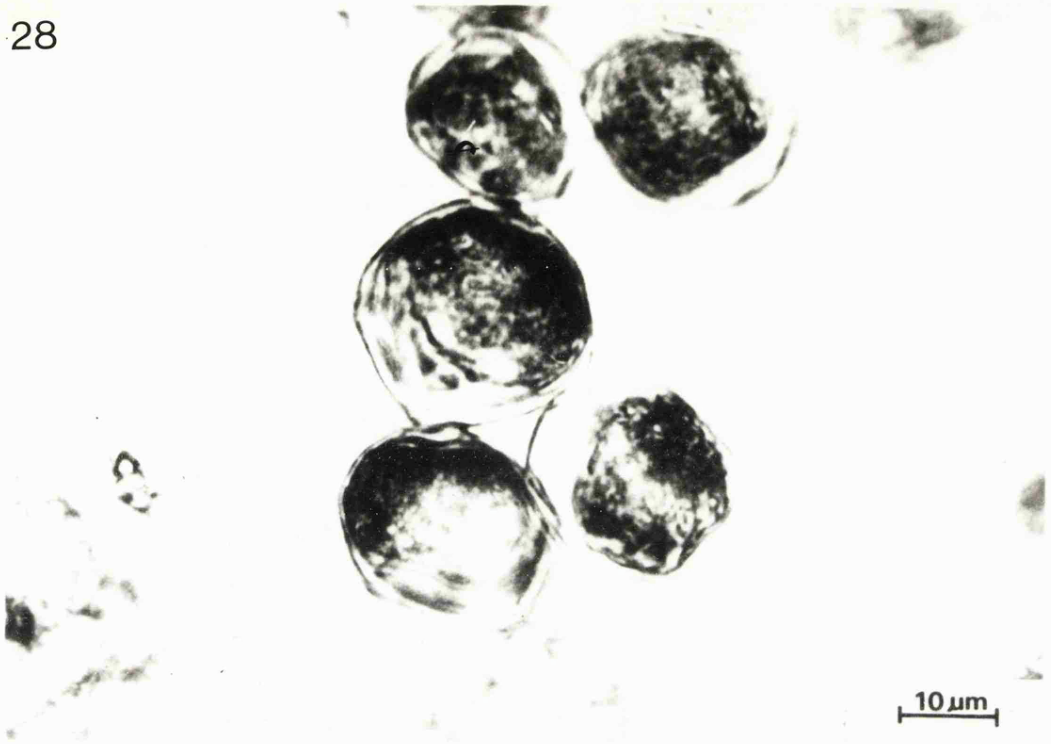


Fig. 26. Scanning electron micrograph of sporangia showing pentagonal and hexagonal walls.

Fig. 27. Scanning electron micrograph of sporangium showing the texture of wall surface.



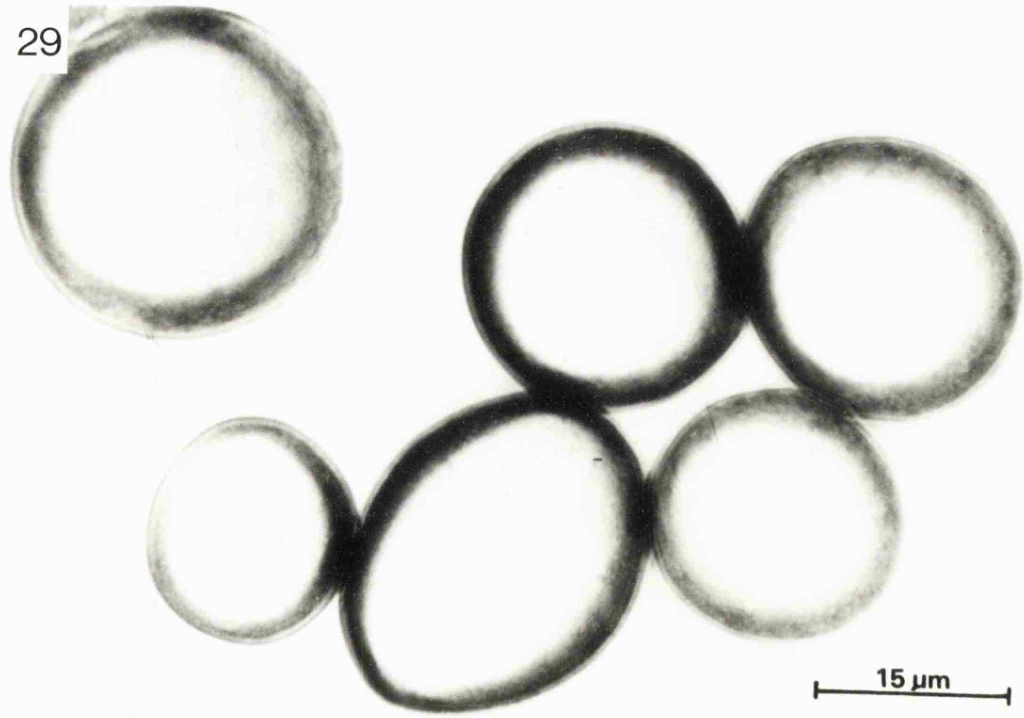


Fig. 28. Non-viable sporangia, protoplasm detached from sporangial wall.

Fig. 29. Globose and ovate sporangia.

measuring 12.5 - 27.0 (21.4) μm or ovate measuring 20.0 x 15.0 - 35.0 x 22.5 (25.2 x 19.4) μm (Fig 29). Sporangia from the Philippines (examined from dried infected leaves collected by Price in 1978 from Palawan Island) were also globose or ovate in shape and measured 12.5 - 30.0 (22.1) μm and 20.0 x 15.0 - 32.5 x 22.5 (25.7 x 20.2) μm respectively. Comparison of measurements between P.N.G., and Philippine sporangia revealed no significant differences (P = 0.1), (Table 9 and 10).

Table 9 Size of globose sporangia from P.N.G. and the Philippines

Diameter μm	Frequency	
	PNG	Philippines
12.5	3	2
15.0	1	3
17.5	9	13
20.0	26	18
22.5	48	29
25.0	12	26
27.5	1	7
30.0	0	2

95% Confidence interval for sporangia from P.N.G., 21.4 $\pm$ 5.2 and from the Philippines 22.1 $\pm$ 6.3
Not significantly different at P=0.1

Table 10 Size of ovate sporangia from P.N.G. and the Philippines

Length µm	Width µm	Frequency PNG	Philippines
20.0	15.0	7	5
22.5	17.5	26	14
25.0	20.0	39	45
27.5	20.0	16	22
30.0	22.5	7	13
32.5	25.0	4	1
35.0	22.5	1	0

95% Confidence interval for sporangia from P.N.G., length 25.2 ± 6.0, width 19.4 ± 4.2 and from the Philippines, length 25.7 ± 5.2, width 19.8 ± 3.4
Not significantly different at P=0.1

3.3.2 Effect of temperature on germination of sporangia

The mean percentage germination after 24 and 48h incubation is shown in Table 11. Germination did not occur at 37°C and was significantly greater (P=0.05) at 20 and 25°C than at 5 and 30°C. Germination increased after 48h incubation for all temperatures except 37°C and no further increase occurred after 72h. Sporangia which failed to germinate at 37°C after 72h did not germinate after subsequent transfer to 20°C for 24h.

Table 11 Effect of temperature on germination of sporangia

Temperature (°C)	Mean germination (angular transformed percentages)	
	24h	48h
5	61.36 b	64.38
10	63.54 a b	70.19
15	70.41 a b	81.33
20	71.77 a	76.35
25	71.97 a	74.96
30	62.5 b	66.81
37	0 b	0

Values for 24h followed by the same letter are not significantly different (P=0.05 Duncan's Multiple Range Test)

3.3.3 Effect of relative humidity and surface wetness on germination of sporangia

A small number of sporangia germinated at 100% r.h., but a reduction of 2% completely prevented germination. Free water is obviously necessary for germination, which however did not exceed 81% in these experiments (Table 12).

Table 12 Effect of relative humidity and surface wetness on germination of sporangia

Relative humidity (%)	Mean germination (angular transformed percentages)	
	+ water	- water
100	80.11 a	15.34
98	75.66 a	0
90	77.78 a	0
84	73.39 a	0
66	0 b	0

Values for + water followed by the same letter are not significantly different(P=0.05 Duncan's Multiple Range Test)

3.3.4 Germination process and number of zoospores per sporangium

Immediately prior to germination the contents of sporangia first became coarsely granular; this was followed by bulging of the sporangial wall (Fig.30). Zoospores were then differentiated and began to oscillate within the sporangium. Rupture of the wall occurred at the bulges and the zoospores located the opening by moving in a random circular motion within the sporangium. It was difficult to determine if there was more than one exit point or pore in the sporangial wall using light microscopy (Fig. 31). Scanning electron micrographs do, however, indicate that the walls are ruptured at one or more places (Figs 32 and 33). The number of zoospores produced by five sporangia, all of 22.5 μm diam, were 102,122,134,134 and 156 respectively. Discharged zoospores swam around erratically, often stopping temporarily. The germination process is shown diagramatically in Fig. 39.

3.3.5 Rate of germination

Zoospores first began to emerge from a few sporangia after 40 min incubation in water at 25^{o}C. This was followed by rapid germination of the majority over the next 30 min. A few sporangia required a longer time period for germination. A similar pattern occurred with all the replicates (Fig.41).

3.3.6 Effect of sporangial age on viability

The mean percentage germination of sporangia from unopened and recently erupted sori was significantly higher (P=0.05) than sporangia taken from old sori (Table 13). Zoospores from sporangia taken from old sori were less active than those taken from recently opened and unopened sori.

Table 13 Viability of sporangia in relation to age of sorus

Sorus	Mean germination (angular transformed percentages)
Unopened	68.31 a
Recently opened	62.70 a
Opened for some time	14.67 b

Values followed by the same letter are not significantly different (P=0.05 Duncan's Multiple Range Test)

3.3.7 Effect of storage on viability of sporangia

The viability of sporangia from bags stored in the field, at room temperature and 5°C decreased to zero after 4 days; those held in a sealed plastic box at 5°C remained viable for 13 days before germination declined. Sporangia stored in bag and plastic boxes at -85°C were non-viable after 24h (Fig.42).

3.3.8 Number and viability of sporangia in a single sorus

The number of sporangia counted from two unopened sori both of 160 μm diam., was 4272 and 4890; a mean of 83% germinated.

3.3.9 Dimensions of viable and fixed zoospores; flagella and swarming period

Viable zoospores were pyriform in shape and varied in size from 5.0 x 2.5 - 10.0 x 5.0 (6.9 x 4.6) μm (Table 14). Fixed zoospores assumed a spherical shape (Figs 34 and 35) and ranged in size from 2.5 x 2.5 - 5.0 x 3.0 (3.34 x 2.5) μm (Table 15) which differed significantly (P = 0.05) from measurements of live zoospores. The flagellum length ranged from 7.5 - 16.25 (12.98) μm (Table 16). Some zoospores had ceased swarming 30 min after emergence at 25°C.

Table 14 Dimensions of viable zoospores

Length μm	Width μm	Frequency
5.0	2.5	2
5.0	3.75	17
6.25	3.75	19
7.5	5.0	53
8.75	5.0	7
10.0	5.0	2

95% Confidence interval for live zoospore length 6.9 ± 2.3 and width 4.6 ± 1.2

Table 15 Dimensions of fixed zoospores

Length µm	Width µm	Frequency
2.5	2.5	17
3.0	2.5	40
3.75	2.5	31
4.5	2.5	9
5.0	2.5	2
5.0	3.0	1

95% Confidence interval for fixed zoospore length 3.34 ± 1.2 and width 2.5 ± 0.1

Table 16 Length of flagellum

Length µm	Frequency
7.5	4
10.0	12
12.5	90
13.75	83
16.25	11

95% Confidence interval for flagella length 12.98 ± 2.6

3.3.10 Observations on flagella and the encystment of zoospores

Zoospores retracted their flagella by a process of vesicular retraction. The process is completed within 10 min. Prior to retraction the flagellum vibrates and undulates and the zoospores swim intermittently. A vesicle then appears at the base of the flagellum (Fig.36) and moves up towards the zoospore (Fig.37) and is finally

absorbed into the main body of the zoospore. This process is shown diagramatically in Fig.40. Encysted zoospores were first observed 3h after emergence but flagellate zoospores were still present after 5h (Table 17). Encysted zoospores varied in size from 3.0 - 6.0 (4.39) ~m diam., (Table 18) (Fig.38).

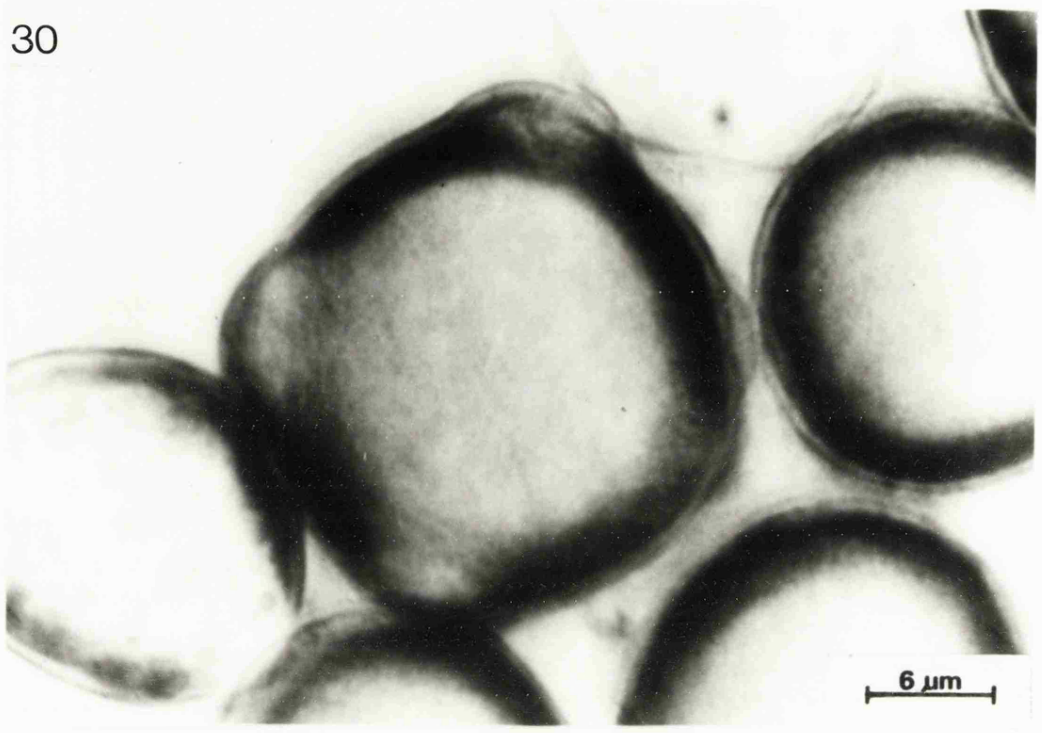
Table 17 Time of encystment of zoospores

Time after germination (h)	Flagellate zoospores	Encysted zoospores
0	200	0
1	200	0
2	200	0
3	163	37
4	126	74
5	83	117

Table 18 Dimensions of encysted zoospores

Diameter ~m	Frequency
3.0	12
4.0	47
5.0	31
6.0	10

95% Confidence interval for encysted zoospores 4.39 $\pm$ 1.6



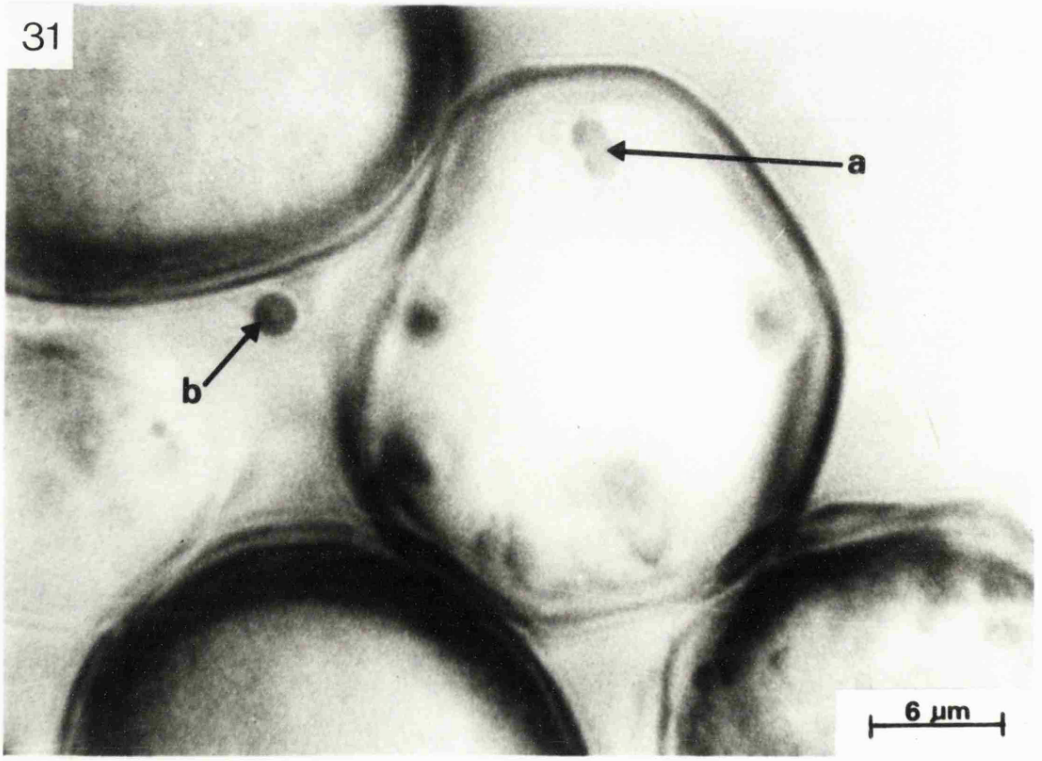
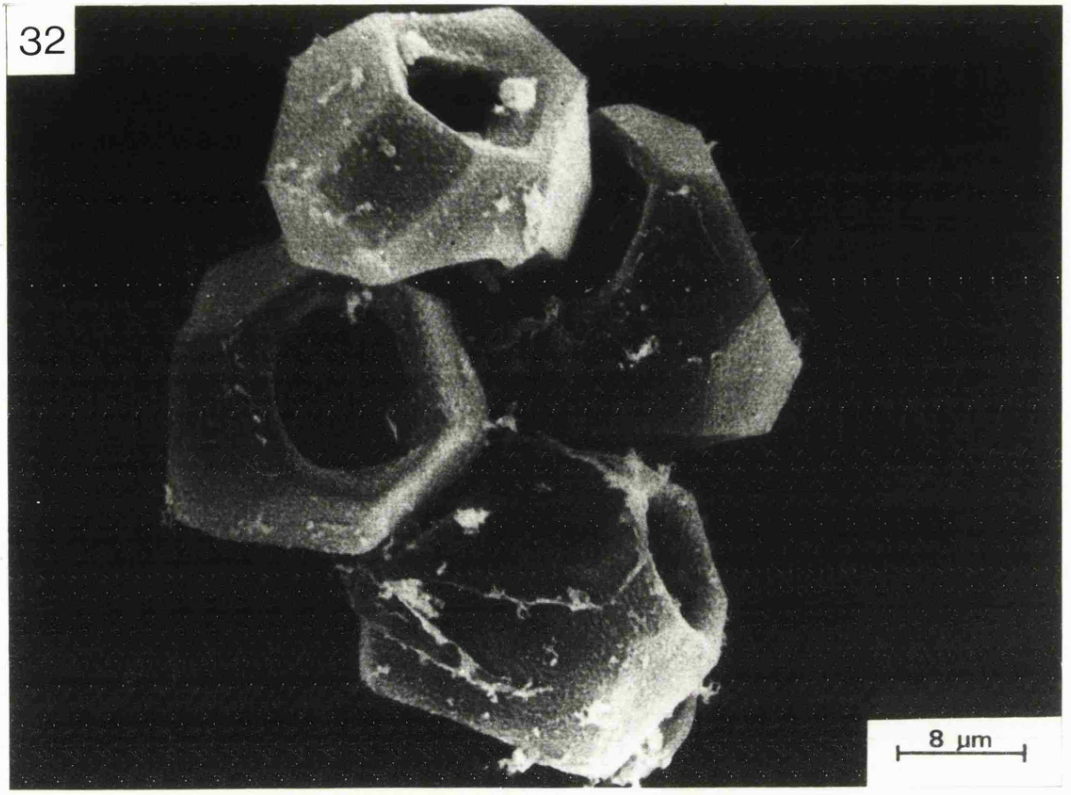


Fig. 30. Sporangium showing bulging of the wall prior to germination.

Fig. 31. Sporangium in process of germination showing zoospores a) inside and b) outside sporangial wall.



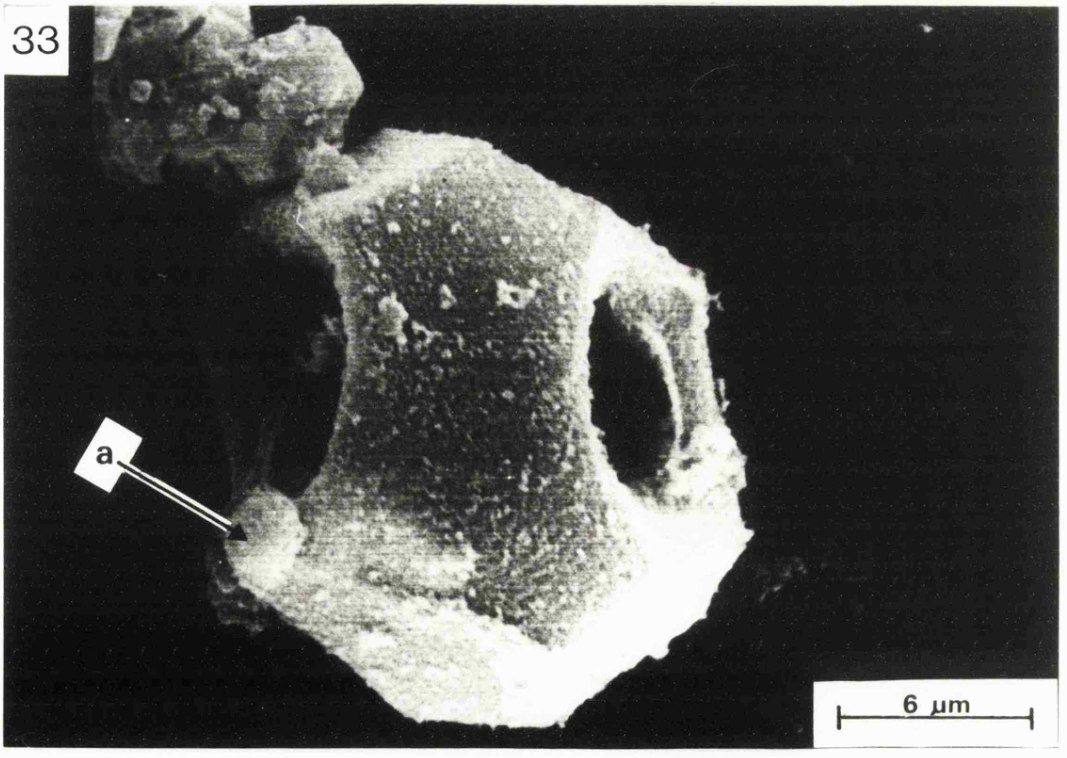
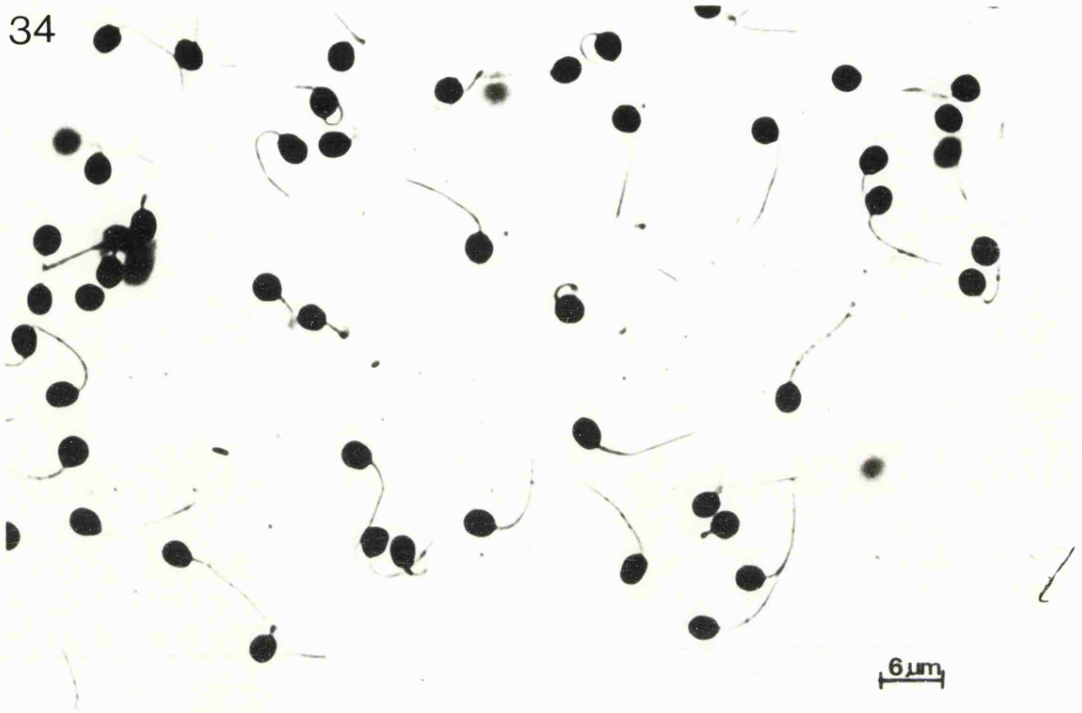


Fig. 32. Scanning electron micrograph of germinated sporangia.

Fig. 33. Scanning electron micrograph of germinated sporangium with a) emerging zoospore.



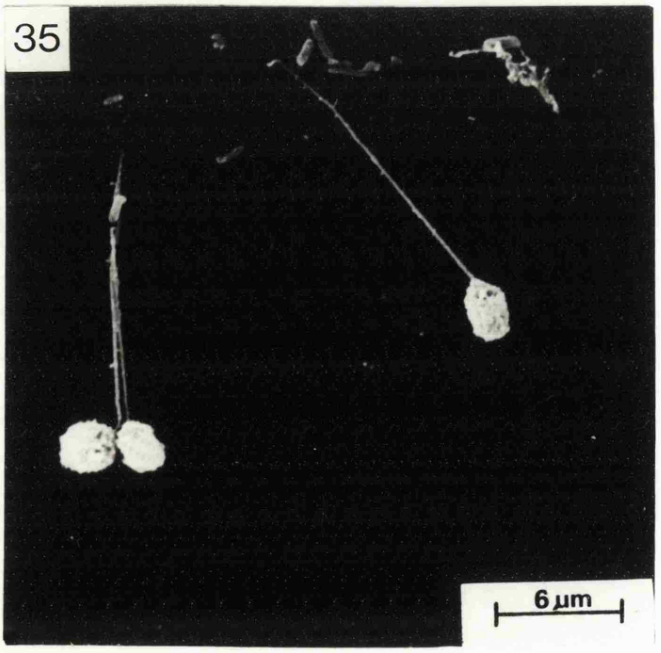
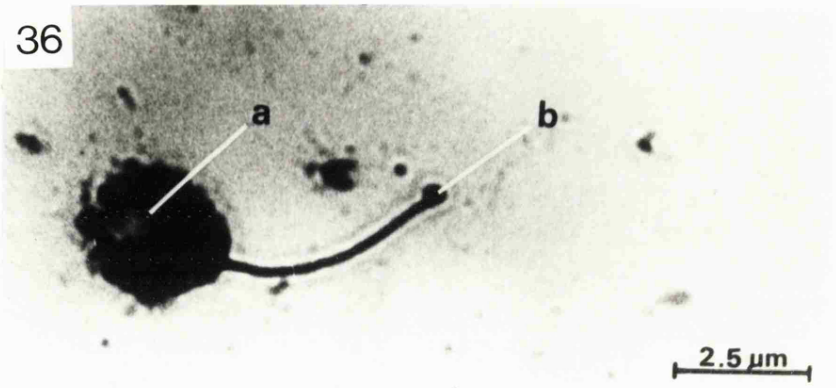
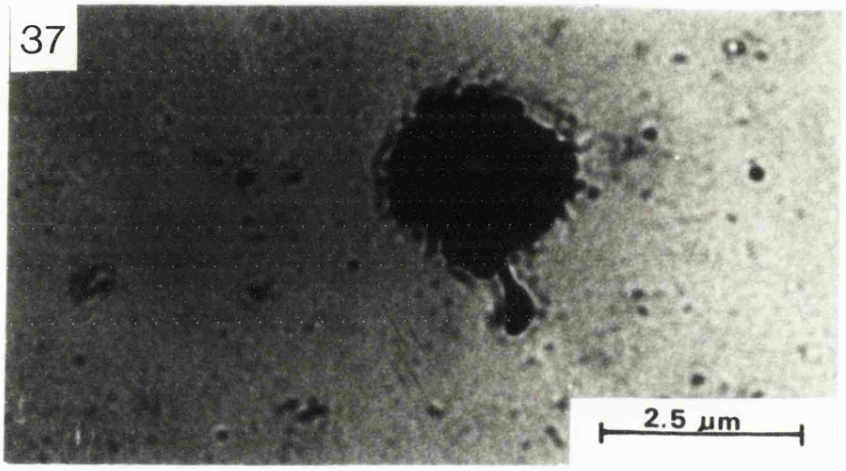


Fig. 34. Fixed zoospores stained with crystal violet and viewed under the light microscope.

Fig. 35. Scanning electron micrograph of fixed zoospores.





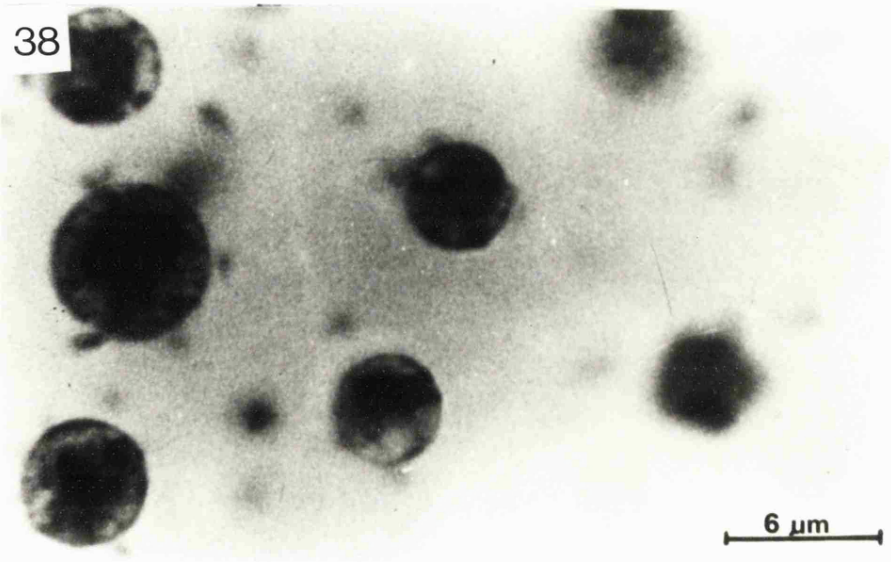


Fig. 36. Zoospore in the process of vesicular flagella retraction; a) oil globule, b) vesicle.

Fig. 37. Zoospore with flagellum almost completely retracted.

Fig. 38. Encysted zoospores.

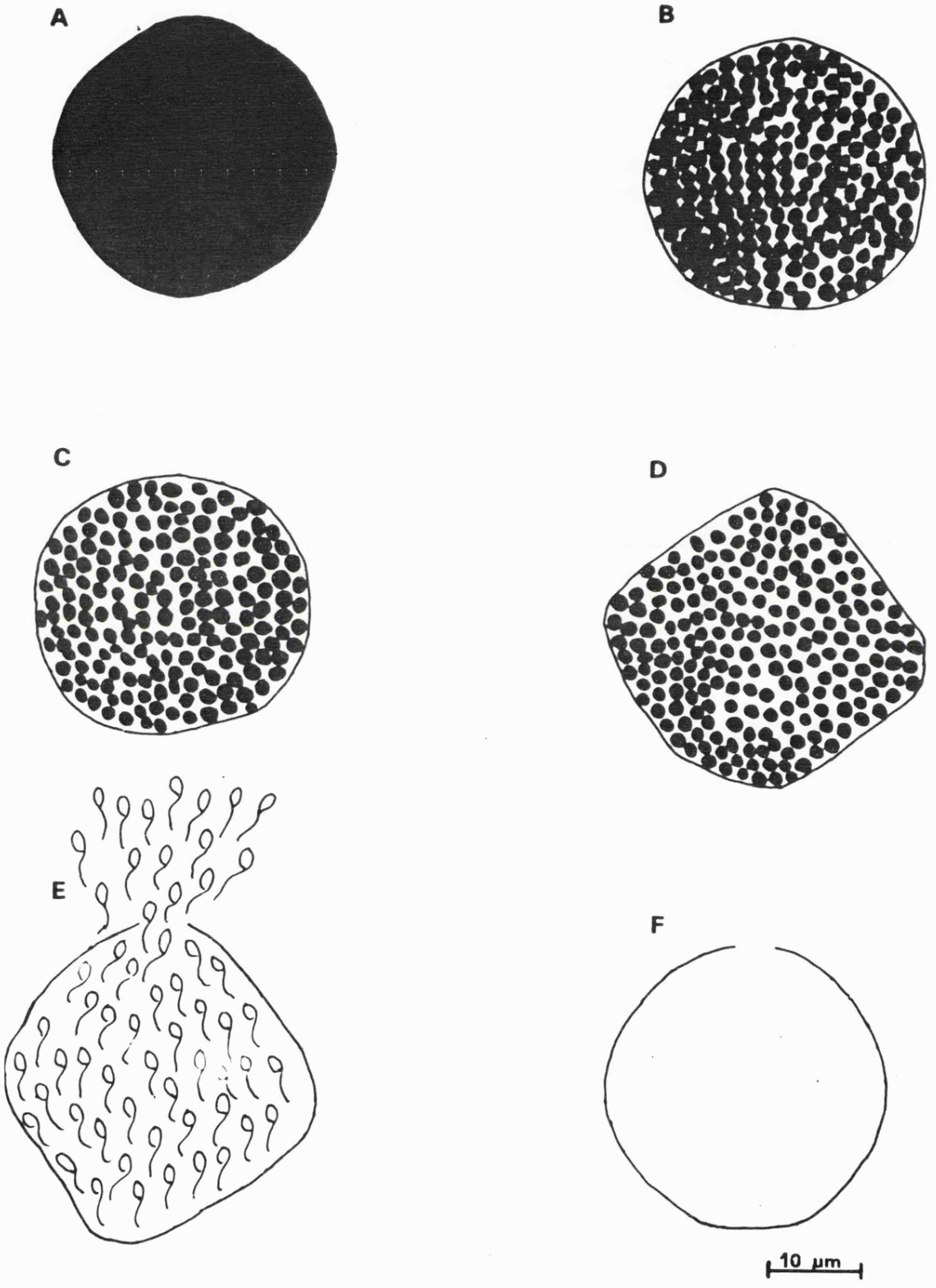


Fig. 39. Stages in sporangia germination, A) uniformly granular protoplasm, B) differentiation of zoospores, C) oscillation of zoospores visible, D) bulging of sporangial wall, E) wall rupture and discharge of zoospores, F) empty sporangium.

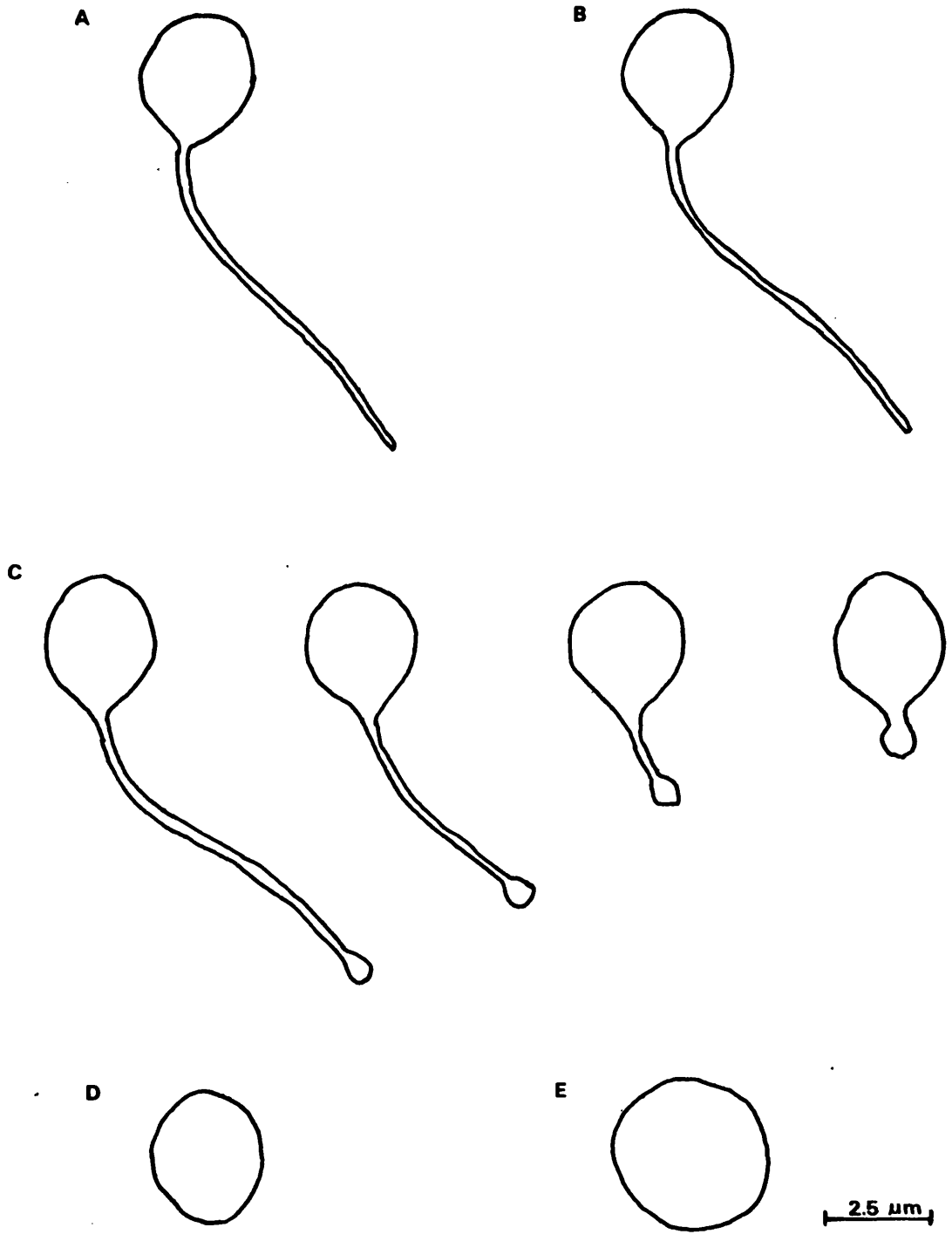


Fig. 40. The fate of zoospores following their discharge from the sporangium, A) swim actively (c. 30 min at 25°C), B) loss of motility, C) flagellum withdrawn by vesicular retraction, D) flagellum completely retracted, E) encysted zoospore increases in size and becomes spherical.

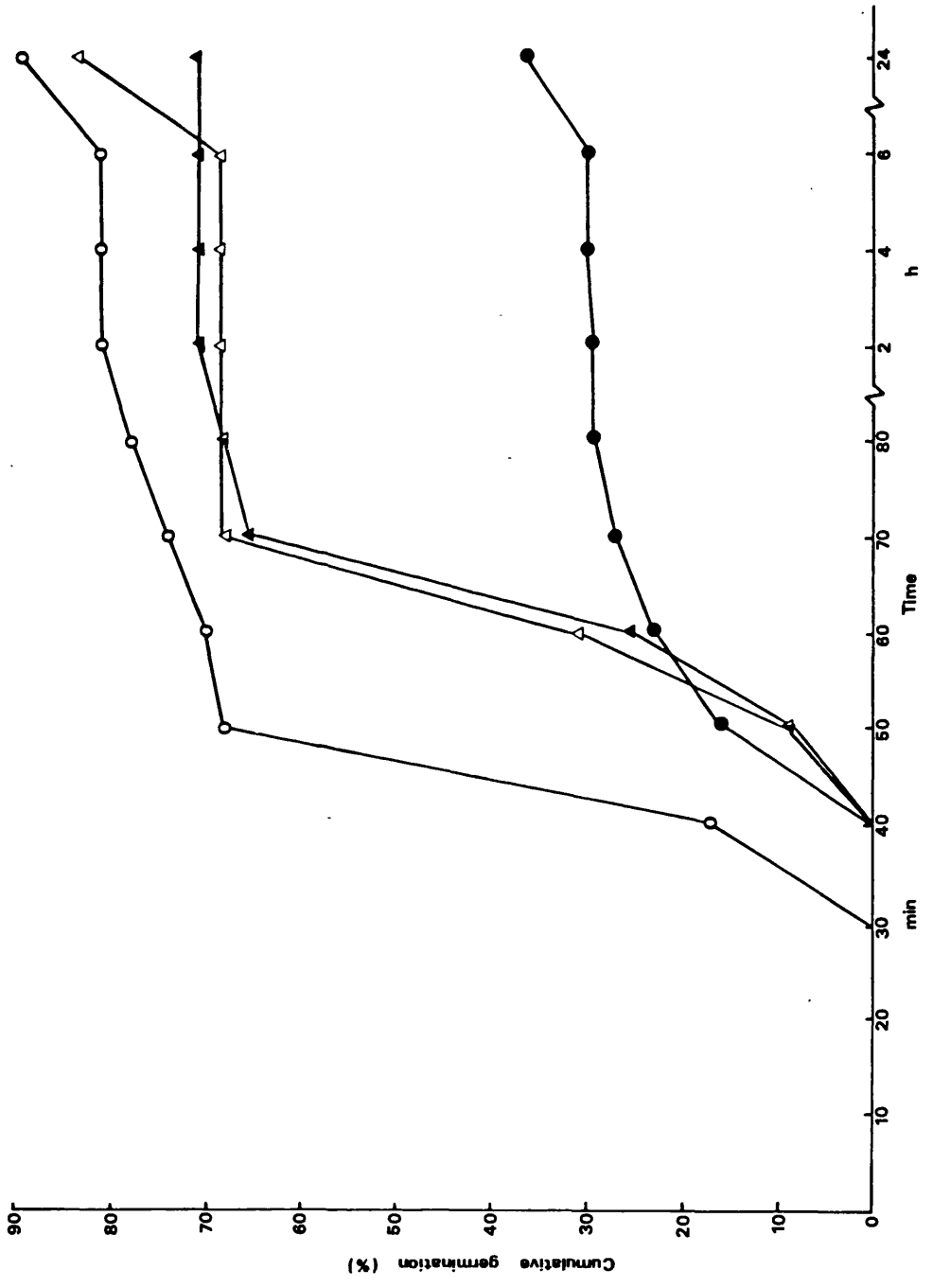


Fig. 41. Rate of sporangia germination at 25°C. ○●△▲ replicates

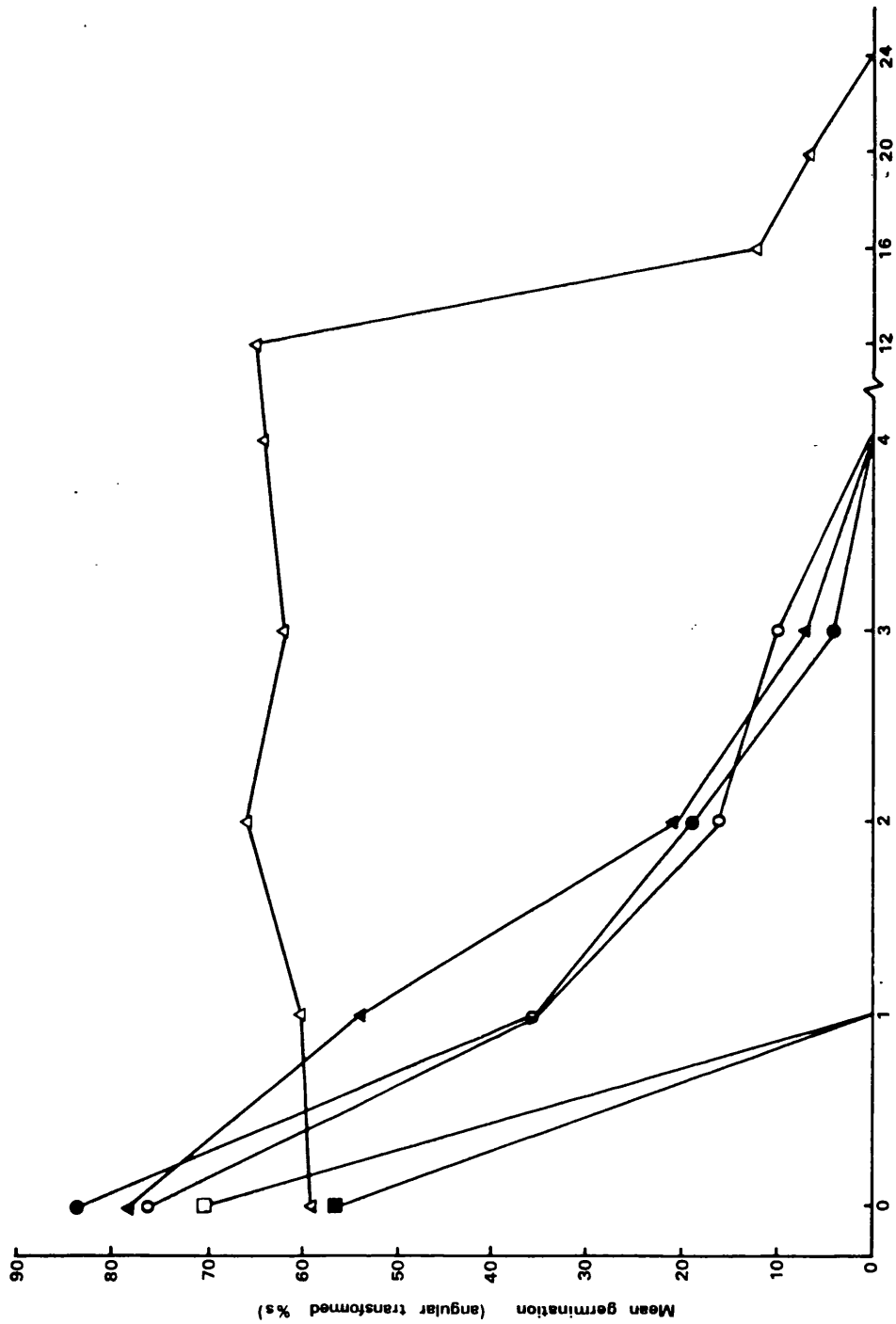


Fig. 42. Effect of different storage conditions on sporangia viability; standard errors of germination means (from storage day 0) ○ field, terylene bag (t max. 30.9 min. 20.9°C r.h.max. 84 min. 55% rain 1.9 mm) ± 11. 6. 9. 9 ● room temperature, terylene bag (t max. 28 min. 26°C r.h. max. 88 min 70%) ± 6.19. 2. 8 △ 5°C, plastic box ± 16. 8.10. 2. 2. 6. 6. 8 ▲ 5°C, terylene bag ± 5. 25. 21.7 □ – 85°C, plastic box ± 13 ■ – 85°C, terylene bag ± 23

3.4 DISCUSSION

The majority of sporangia were either globose or ovate although a few irregular shapes were also observed. The measurements of sporangia from P.N.G., and the Philippines are in agreement with those reported in Karling (1964) but are smaller than those quoted by Alicbusan (1965). However Alicbusan did not indicate the number of sporangia measured, which if small could account for the differences noted.

Temperatures between 10-25°C were more favourable for germination which was reduced at 5 and 30°C. No germination was recorded at 37°C. S. brownii Karling also germinates over a wide temperature range of 3-30°C (Lingappa, 1958) although no optimum was given. Germination of S. psophocarpi required the presence of free water although a little germination occurred at 100% r.h., which is probably associated with the formation of some water condensation.

The increased granulation and bulging of the sporangial wall prior to germination has also been observed by Alicbusan (1965). Similar observations have been made on S. endobioticum (Schilberszky) Percival (Curtis, 1921), S. fulgens Schroeter (Kusano, 1930) and S. brownii (Lingappa, 1958). Protrusion of the sporangial wall in S. endobioticum and S. fulgens was considered by Curtis (1921) and Kusano (1930) respectively to be due to water

absorption. Both considered that rupture of the sporangia occurred at one of the projected bulges. However, in S. psophocarpi, scanning electron microscopy revealed one or more exit pores. These pores occurred in the centre of the pentagonal and hexagonal sporangial walls and possibly represented those areas least able to withstand internal physical pressures exerted during germination. The possibility that different structural strengths exist in the wall was also reported in S. fulgens by Kusano (1930) who stated the bulges were the sites of least resistance.

The demonstration of one or more exit pores in S. psophocarpi is not in accord with the conclusion of de Vera (1973, 1977) who reported four equidistant escape apertures. This may be due to the difficulty associated with observing the pores using light microscopy. Zoospores were observed to emerge rapidly either a few at a time or in large numbers. Kusano (1930) made similar observations in S. fulgens and thought the number released was dependent on the size of the exit pore. It is possible in S. psophocarpi the number of pores, as well as their size, determines the rate of zoospore emergence.

The erratic movement of zoospores of S. psophocarpi following emergence from sporangia has previously been described in other species of chytrid fungi by Couch (1941).

Koch (1959) stated this irregular motion was due to frequent motile and non-motile phases associated with a change in direction. He suggested the change in direction was a result of stopping abruptly as this usually caused a violent movement of the flagellum which pivoted the zoospore around.

It is not known whether the decreased vigour of zoospores from old sporangia is related to their capacity to infect. Further studies are required to elucidate this. Curtis (1921) observed that the behaviour of S. endobioticum zoospores at discharge was dependent on their age although she did not distinguish between types. She also noted that zoospore movement was reduced when incubated at 5°C.

The number of zoospores that emerge from sporangia ranged from 102 to 156. A previous estimate of 50 was given by Alicbusan (1965). It is possible that the number of zoospores is dependent on the size of the sporangium.

The minimum period for germination in the presence of free water at 25°C was 40 min, most sporangia germinating within 60 - 120 min. These results are in agreement with those of Alicbusan (1965) and de Vera (1973, 1977). Germination of S. fulgens at 20°C commenced after 1.5h, the rate being almost identical within the temperature range of 18-22°C

(Kusano, 1930). However in S. brownii germination occurred within 30 min at 20-25°C and after 10h at 10°C (Lingappa, 1958). The proportion of S. psophocarpi sporangia that germinated after further incubation was low and indicate that most viable sporangia will germinate within 2h provided conditions such as temperature and moisture are favourable.

The results indicate that sporangia from unopened sorus and recently erupted sorus were more viable than sporangia taken from old sori. The loss of viability in sporangia from an old sorus may be caused by a loss of moisture after exposure to the atmosphere. The viability of sporangia stored in porous bags declined rapidly to zero after 4 days whereas sporangia stored in a sealed container at 5°C remained viable for a maximum of 20 days. However Alicbusan (1965) reported that sporangia remained viable for 6 months in the laboratory and 4 months in the field based on the colour and shape of sporangia. Germination tests were not made in Alicbusan's experiments and this may explain the differences noted. A rapid loss of viability does mean that sporangia must be dispersed to reach an infection court within a few days after eruption of the sorus membrane. Kusano (1930) reported that the viability of S. fulgens sporangia was dependent on temperature; those remaining outside the sorus losing viability, due to

desiccation, more rapidly than those protected by the sorus.

The shape and size of viable zoospores are in agreement with those given by Raciborski (1898). Zoospores fixed in osmic acid became spherical and shrank in size; this effect of osmic acid has also been reported by Koch (1958). The flagella were longer than those reported by Raciborski (1898). It is not known whether his measurements were made on newly emerged zoospores; these retract their flagella prior to encystment and it is possible that this may account for the differences noted. It is also possible that strains of the pathogen exist since Raciborski's material was from Java.

The swarming of zoospores was observed to last for at least 30 min at 25°C. Kusano (1930) reported the swarming period in S. fulgens to be 30 - 40 min at 20°C. His observations indicated that the duration of swarming increased at temperatures below or exceeding 20°C. In S. brownii zoospores remain active for over 72h at 3°C and 2 - 5h at 24-28°C (Lingappa, 1958), whereas in S. endobioticum Curtis (1921) reported a much shorter period of 10-20 min. Unfortunately she did not state the temperature at which this took place. The effect of different temperatures on the duration of swarming of S. psophocarpi zoospores was not studied and further work is needed since this may be important in host infection.

The swarming of zoospores and vesicular retraction of flagella have also been noted by de Vera (1973, 1977). Zoospores of S. endobioticum also retract their flagellum by this method (Curtis, 1921) whilst those of S. fulgens emply lash around retraction (Kusano, 1930). Koch (1968) lists more species and genera of the chytrids as retracting their flagella by vesicular retraction than any other method and considered this may be due to the vesicles being easily seen. The encysted zoospore of S. psophocarpi assumes a spherical shape and increased in size. This also occurs in S. fulgens (1930) and S. brownii (Lingappa, 1958) and was thought to be caused by the absorption of water.

Some pairing of zoospores was observed. Similar observations have also been made by Gäumann (1927) and de Vera (1973, 1977); pairing in other species have also been reported by Curtis (1921) and Karling (1955).

SECTION 4 INFECTION

4.1 INTRODUCTION

Synchytrium psophocarpi is an obligate parasite and at present cannot be cultured in the laboratory. Previous studies on infection of winged beans by S. psophocarpi were done in Java (Gäumann, 1927) and the Philippines (Alicbusan, 1965; de Vera, 1973, 1977). Gäumann (1927) successfully inoculated winged bean with S. psophocarpi and Alicbusan, (1965) reported the incubation period to be 10 days, with older leaves showing no symptoms. An account of parasite penetration and development is given by de Vera (1973, 1977).

Inoculation techniques have been described using sporangia (Gäumann, 1927) and zoospores (Alicbusan, 1965; de Vera, 1973, 1977). The winged bean cultivars used and the experimental conditions, however, were not indicated in these studies. The aim of the present investigation was to develop simple inoculation techniques for use in leaf infection studies and host range experiments. Attempts were also made to determine if the pathogen was seed borne and produced resting spores.

4.2 MATERIALS AND METHODS

4.2.1 Winged bean plants

Winged bean seeds cv UPS-122 were soaked in tap water for 24h to enhance germination and sown singly, c. 2cm deep, in 'Jiffy 7' peat blocks. After 2 wk the seedlings were transplanted into 12cm diam., plastic pots containing peat moss (Hauraki Peat Ltd., New Zealand) and staked with a single bamboo cane. Any abnormal seedlings which had developed were discarded. The pots were placed on benches in a wire screened shade house under natural conditions and were drenched weekly with a balanced nutrient solution ('Aquasol' rate - as per manufacturers:- Hortico Ltd., Australia).

4.2.2 Inoculation of plants

4.2.2.1 Inoculum

Inoculum was obtained from winged beans naturally infected with S. psophocarpi growing in the Experimental Agriculture Garden at U.P.N.G., infected leaves and pods were removed from plants and placed in plastic bags to prevent drying out. Most inoculum was used immediately or stored at 5^{o}C and used within 24 h of collection.

4.2.2.2 Effect of different inoculation methods on infection of winged bean by S. psophocarpi

1. Inoculation with sporangia

A rectangular framed polyethylene chamber (1.0 x 0.5 x 0.5m) was constructed and placed on a bench in a wire screened shade house. Temperature and humidity inside the chamber were recorded with a 7-day bimetallic thermograph and hair hydrograph (Ota Keiki, Seisakusho Co. Ltd., Japan). Sporangia from infected leaves and pods were dusted onto ten 2 wk old winged bean plants using a camel hair brush; the plants were then incubated for 48h inside the chamber and thereafter on a bench in the shade house. Sporangia were also dusted onto microscope cavity slides containing sterile distilled water and germination assessed as previously described (loc. cit.). Plants were examined daily for gall symptoms. This was repeated twice.

2. Inoculation with zoospores

(a) Spraying

Sporangia were removed from erupted sori on infected leaves with a mounting needle whilst viewing under a stereoscopic microscope(x20) and placed in a watch glass containing sterile distilled water. This was incubated at room temperature (25-28^{o}C) and examined microscopically (x150) for germination.

The suspension was transferred to a hand operated spray gun as soon as emergence of zoospores was recorded, usually 1-2h later and sprayed onto twenty 2 wk old winged bean plants. These were then covered individually with a polyethylene bag held in place with a rubber band, and incubated in the shade house. The bags were removed after 48h and the plants examined daily for gall symptoms. This was repeated once.

Twenty 4 wk old plants were placed inside a polyethylene mist chamber (1.8 x 1.8 x 1.2m) held at 100% r.h., with an electrically operated humidifier ('Defensor' British Engineering Pty. Ltd., Australia) for 1h so as to allow a film of water to develop on the leaves. The plants were then sprayed with a zoospore suspension, held in the chamber for a further 48h and then transferred to a bench in the shade house. Plants were examined daily for gall symptoms. The temperature and humidity inside the chamber was monitored with a 7-day bimetallic thermograph and hair hydrograph.

Ten leaves selected at random from ten 4 wk old plants in the shade house were sprayed with a zoospore suspension. Each leaf was then enclosed

in a non-vented 9cm plastic petri dish. The petiole of each leaf was inserted through a $5mm^2$ hole made in the rims of upper and lower lids plugged with cotton wool and the leaf laid flat on filter paper inside the petri dish moistened with sterile distilled water. The petri dish was then held in a horizontal position by attaching the base with plasticine to a bamboo cane (Fig.43), and the lids sealed with cellulose adhesive tape. The petri dish was removed after 48h and the leaves examined daily for gall symptoms. This was repeated once.

Twenty 1cm diam., discs were cut from randomly selected winged bean leaves; these were then surface sterilized by immersion in 10% sodium hypochlorite for 3 min, washed three times in sterile distilled water and placed onto the surface of sterile tap water agar (Difco Bacto Agar 1.5%) in petri dishes in a laminar flow sterile cabinet. The discs were then sprayed with a zoospore suspension and the plates incubated at 20°C. The discs were examined microscopically (x40) for signs of galls 48h later and daily thereafter.

(b) Moist tissue paper

Pieces of 'Kleenex' tissues were dipped in a

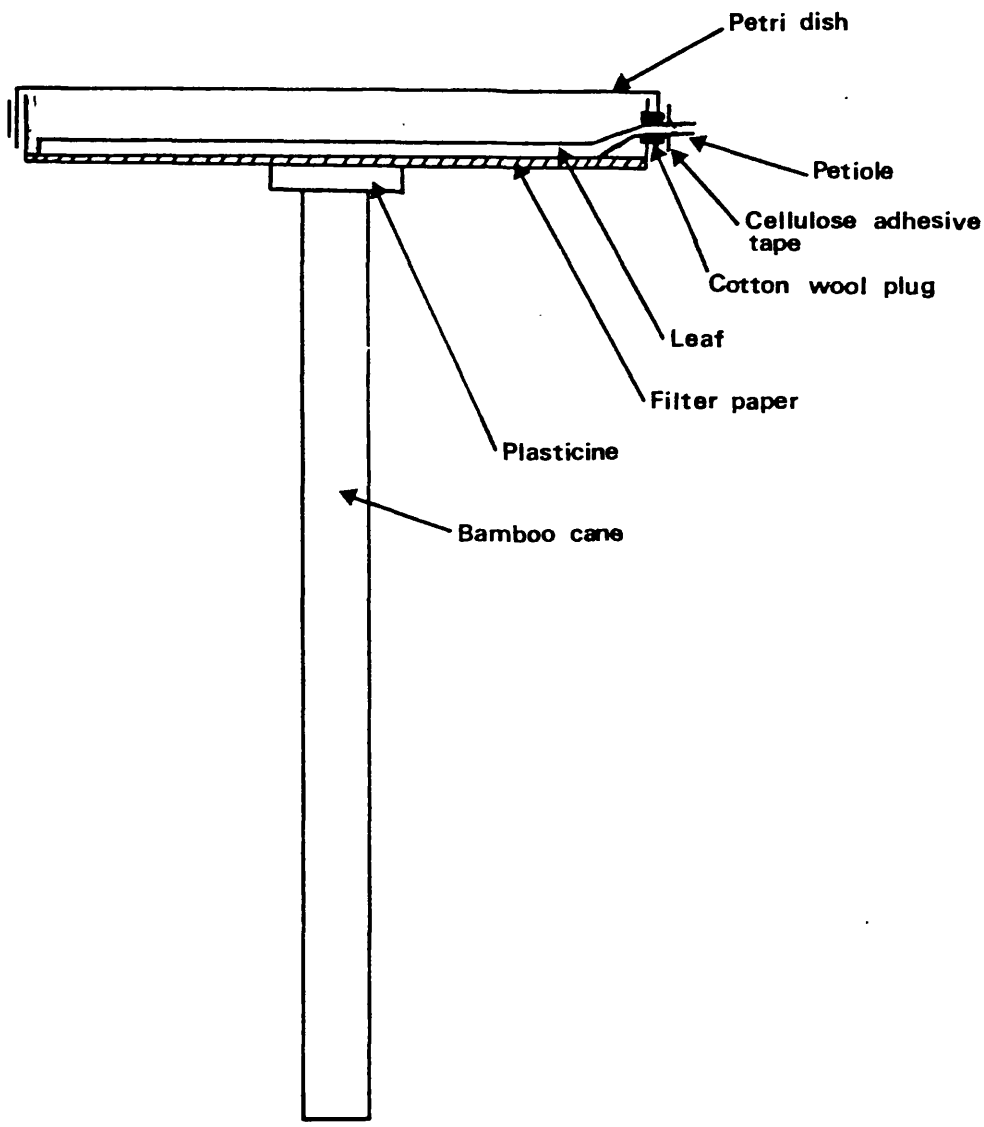


Fig. 43. Incubation chamber used for single attached leaves.

suspension containing freshly emerged zoospores and wrapped around ten leaflets selected at randon from ten 4 wk old plants in the shade house. The tissues were held in place with hair spring clips, kept moist with sterile distilled water and removed after 48h. The leaflets were then examined daily for gall symptoms. This was repeated once.

4.2.3 Incubation and generation times

The incubation period was defined as the period needed between inoculation and appearance of visible symptoms; the generation time was defined as the average number of days that elapse between inoculation and rupture of sori. Estimates of these were made by daily observations on all plants that were sprayed with zoospores and incubated under a plastic bag and in a mist chamber.

4.2.4 Effect of leaf age on infection

Leaves on forty plants in the shade house were tagged on the day of emergence from the leaf sheath (day 1, Fig.44) until a range of 1-20 day old leaves was present. The plants were then transferred to the moist chamber, where they were sprayed with zoospores and incubated for 48h. Plants were then removed to benches in the shade house

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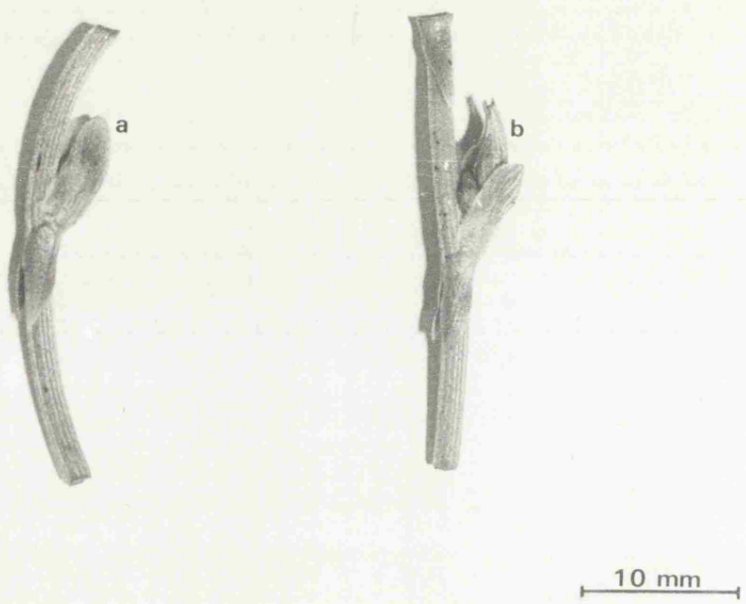


Fig. 44. a) Leaf not emerged from leaf sheath (day 0),
b) leaf on day of emergence (day 1).

and the number of galls that developed on each leaf was counted after 3 and subsequent weeks.

4.2.5 Effect of leaf wax removal on infection

Leaves on fifteen plants were tagged as previously described; the leaves on ten of these were gently rubbed with cotton wool soaked in carbon tetrachloride to remove surface wax. The plants were transferred to the mist chamber 24h later where they were sprayed with zoospores and incubated for 48h. Plants were then transferred to benches in the shade house and the number of galls that developed on each leaf were counted after 3 wk.

4.2.6 Effect of duration of leaf surface wetness on infection

Sixty 4 wk old plants were placed in the mist chamber and inoculated with zoospores. Ten plants were removed from the chamber after 1,2,4,6,12 and 24h, placed in direct sunlight to dry surface moisture and then placed on benches in the shade house. Plants were examined for galls after 3 and subsequent weeks.

4.2.7 Seed infection

Winged bean seed CV UPS-122 was used in all tests for seed infection.

(a) Twenty seeds from naturally infected pods were sown at the rate of four seeds per pot in plastic pots containing peat moss as previously described (loc. cit.).

(b) Freshly harvested infected leaves were cut into pieces and mixed with peat moss. The top 4cm of each of five pots containing peat moss was filled with this mixture and each pot was sown with four seeds from uninfected pods.

(c) Batches of forty seeds were

(i) Surface sterilized in 10% sodium hypochlorite for 3 min followed by three washes in distilled water.

(ii) Scarified with emery paper to damage the seed coat.

(iii) Soaked in sterile distilled water for 24h.

Twenty seeds from each of these treatments were then placed in a zoospore suspension for 24h at 25^{o}C prior to sowing.

A sample of sporangia was tested for viability before dilution with talc (B.P.) and the remaining twenty seeds from each treatment were coated with this mixture prior to sowing. Twenty untreated, uninoculated seeds were sown as a control. All pots were watered to field capacity immediately after sowing with sterile distilled water

followed by tap water when required. All plants were examined daily for symptoms.

4.2.8 Host range

Ten 4 wk old plants of Arachis hypogaea L., Glycine max (L.) Merr., Phaseolus aureus Roxb., P. coccineus L., P. vulgaris, Pisum sativum L., Psophocarpus scandens, P. tetragonolobus (cv UPS-122), Vicia faba L., Vigna sesquipedalis and V. unguiculata (L.) Walp., were placed in the mist chamber, sprayed with zoospores, incubated for 48h and transferred to the shade house. Plants were examined for gall symptoms after 3 and subsequent weeks.

Pueraria lobata (Wild) Ohwi infected by Synchytrium minutum (Pat.) Gäumann has been reported in P.N.G., by Shaw (1962). It produces symptoms which closely resembles infection by winged beans by S. psophocarpi. Morphologically the two fungi are very similar. To determine the relationship between S. psophocarpi and S. minutum dried herbarium specimens were despatched to the Commonwealth Mycological Institute for identification. A preliminary attempt was also made to inoculate winged beans with S. minutum. Leaves of P. lobata infected by S. minutum were obtained from the Eastern Highlands Agriculture Research Station and ten 4 wk old plants of S. psophocarpi

were inoculated with zoospores and incubated in a mist chamber as previously described (loc. cit.).

4.2.9 Resting spores

Infected mature pods were removed from winged bean plants and, after shelling, the husks were placed in paper bags and stored in a wire screened shade house. After 12 wk sporangia were removed from ruptured and non-ruptured sori, placed in hanging drops and examined under the microscope (x400) for any spore type that differed in shape, size or structure from normal sporangia. Sporangia from plant debris remaining after harvest of a winged bean crop was also examined.

4.3 RESULTS

4.3.1 Effect of different inoculation methods on infection of winged bean by S. psophocarpi

Successful infections were recorded only on plants sprayed with zoospores and incubated either in polyethylene bags or inside the mist chamber (Table 19).

Table 19 Effect of different inoculation methods on infection of winged bean by S. psophocarpi

Inoculum	Inoculation		Incubation (48h)			No. inoculated	No. infected
	Method	Part inoculated	Method	Temp °C	r.h.%		
Sporangia	Dusting	Whole plant	Humidity chamber	28±5	95-100	30	0
Zoospores	Spraying	" "	Polyethelene bags	-	-	20	20
	"	" "	Mist chamber	26±4	100	20	20
	"	Leaves	Petri dishes in shade house	-	-	20	0
	"	Leaf discs	Petri dishes in incubator	20	-	20	0
	Tissue paper soaked in distilled water containing motile zoospores	Leaflets	Shade house	26.5±3.5	68-96	10	0

4.3.2 Incubation and generation times

Seven days after inoculation and incubation under natural conditions (t. max 31, min 24°C; r.h., max 90 min 70%) small discrete dark green spots were observed on the young leaves on all inoculated plants. These occurred more frequently on the lower leaf surface being clearly visible if the infected leaves were held up against the light. Tissue around the infection site was convoluted. Semi-globular yellow galls slightly raised above the host surface were observed after a further 3 days when distortion of the tissues surrounding the gall was more pronounced. Rupture of the sori first occurred 22 days after inoculation on some plants although this took up to 28 days on others.

4.3.3 Effect of leaf age and leaf wax removal on infection

Young leaves, 1-2 days old, were the most susceptible. The degree of infection decreased with leaf age and was not recorded on leaves more than 10 days old. Leaves covered by a leaf sheath at the time of inoculation were also infected (Table 20). Removal of wax had no significant effect on infection ($P = 0.05$). Rubbing with carbon tetrachloride caused slight necrosis on some leaves.

Table 20 Effect of age on the susceptibility of leaves to infection by S. psophocarpi

Leaf age (days)	No. sampled	Mean no. of galls/leaf
'0	45	28.8 a c
1 - 2	22	42.2 a
3 - 4	16	12.9 b c
5 - 6	22	11.28 b c
7 - 8	28	3.4 b
9 - 10	14	3.0 b
10	80	0 d

'Day 0 leaf not emerged from leaf sheath. Values followed by the same letter are not significantly different at P = 0.01 (Student's t-test)

4.3.4 Effect of the duration of leaf surface wetness on infection

Infection only occurred on plants that retained free water on the leaf surfaces for a minimum of 12h. For the 12 and 24h periods 70 and 80% infection was recorded respectively.

4.3.5 Seed infection

No infection developed in any of the treatments 6 wk after inoculation in spite of the presence of motile zoospores in the suspension and the high viability of sporangia

(73%) used in the talc mixture.

4.3.6 Host range

All legumes, apart from winged bean, inoculated with S. psophocarpi remained healthy 6 wk after inoculation. Typical yellow gall symptoms developed on 80% of winged bean plants 10 days after spraying with zoospores. Winged beans inoculated with S. minutum were not infected after 6 wk. Stamps (personal communication) indicated that S. psophocarpi and S. minutum were recognised as distinct species in spite of similarities in their morphology.

4.3.7 Resting sporangia

Spores from ruptured or intact sori on dried winged bean husks or on plant debris were similar in shape, size and structure to sporangia previously described (loc. cit.). This suggests that resting spores did not exist in the material examined. Sporangia observed showed a distinct loss of colour and were desiccated. Shrinkage and detachment of protoplasm from the cell wall was frequently observed suggesting the sporangia were non-viable.

4.4 DISCUSSION

In all the inoculation procedures sporangia were taken from ruptured sori on infected leaves and pods collected from the field and their age, i.e., the time from sorus rupture, could not be determined. From previous experiments (loc. cit.) the viability of sporangia taken from old sori was significantly (P=0.05) lower than that of sporangia taken from recently ruptured sori. Therefore estimates of sporangia concentration prior to inoculation would have had little value without a check on viability. The number of sporangia or zoospores given to each plant or plant part was unknown. However, viability of the inoculum was monitored by germination tests prior to inoculation and by the use of a known susceptible cv UPS-122.

Inoculation using ungerminated sporangia, as described by Gäumann (1927), failed to produce any infection, but two techniques using zoospores were successful. Free water has been shown to be essential for germination (loc. cit.) which suggests that condensation must have occurred on the inoculated plants used by Gäumann (1927). This did not occur in the current experiments although the relative humidity was high (95-100%). Failure to infect single leaves, leaflets and leaf discs with zoospores cannot be explained by the absence of free water since this was maintained throughout the incubation period. Young leaves,

1 - 2 days old, were shown to be the most susceptible to infection whilst leaves older than 10 days were apparently resistant. Since the age of single leaves, leaflets and leaf discs were unknown they may have aged sufficiently to escape infection. More information is required to determine whether non-infection of leaf discs is attributed to an alteration in their physiology compared with attached leaves. Alicbusan (1965) also found older leaves to be immune although he did not give the age of the leaves. Other species of Synchytrium, however, can infect older leaves. Synchytrium fulgens Schroeter infects both young and old leaves of Oenothera grandiflora Ait., and O. biennis (Kusano, 1930). Rubbing the surface of winged bean leaves with carbon tetrachloride prior to inoculation did not increase susceptibility. No studies have been made on winged bean leaves to establish any variation in the presence of leaf wax and its thickness with leaf age. Neither is there any information on the development of the cuticle. It is possible that the resistance of older leaves is due to factors other than a physical barrier. Further studies on host-parasite interaction are required to elucidate this. Small immature leaves within the leaf sheath were less susceptible than 1 - 2 day old leaves. This may have been due to the leaf sheath providing some measure of protection by excluding water droplets containing the inoculum. However, once opened they provide a catchment area for such droplets. The leaf sheath may also provide a favourable microclimate for infection since water evaporation will be reduced

compared with exposed leaves. Other infection sites away from direct sunlight where water may be retained for longer periods include the nodes and the primary and secondary veins of the lower leaf surface. Comparisons of incubation periods are of no value unless the first symptoms which appear after inoculation and the type of observation, i.e., macro or mircoscopic, are stated. Thus the minimum incubation periods for the appearance of dark green spots and the appearance of yellow galls on leaves was 7 and 10 days respectively; these were observed macroscopically. The period of 10 days for the appearance of yellow galls is in agreement with Alicbusan (1965). The period between inoculation and sorus rupture, i.e., the generation time, was first recorded after 22 days. These times are longer than those of de Vera (1973, 1977) who reported between 13 - 15 days. Leonard (1969) stated that the generation time may be determined in part by factors inherent in the fungus but is also related to the physiological conditions of the host tissue in which the pustule is located and this may explain the differences noted.

No infection was obtained in seed inoculation experiments. This suggests S. psophocarpi is not seed borne. Karling (1936) also reported that seeds taken from pods of Amphicarpa bracteata (L.) Fernald heavily infected with deep seated sori of S. decipiens Farlow were not infected

and considered the possibility of the parasite being seed borne remote.

S. psophocarpi did not infect any legumes tested which suggests the parasite is host specific. The conclusion has previously been made by other workers (Gäumann, 1927; Alicbusan, 1965). Similar results have been reported for other Synchytrium species. Gäumann (1927) considered S. atylosiae (Petch) Gäumann was confined to the genus Atylosia Wight and Arn., after failing to infect four other legumes. Karling (1954) concluded that S. decipiens was restricted to A. bracteata and A. bracteata var. Comosa after failing to infect nineteen leguminous and twelve non-leguminous species. All species of the sub-genus Woroninella, which S. psophocarpi, S. atylosiae and S. decipiens are members, appear to be limited to the family Leguminosae. It is possible that S. psophocarpi is restricted to a single species since P. scandens was not infected following artificial inoculation. This species also remained healthy when grown in the Experimental Agriculture Garden at U.P.N.G., alongside naturally infected winged beans.

SECTION 5 EPIDEMIOLOGY

5.1 INTRODUCTION

Sporangia of S. psophocarpi have been reported to be dispersed by wind (Karling, 1964; Alicbusan, 1965), insect and other natural agencies (Alicbusan, 1965). There is, however, no quantitative information relating to these observations. An investigation was therefore made of the numbers of sporangia present in the air and the effect of weather factors on these numbers; the distance sporangia were dispersed from an infection source, the spread of disease within a crop and the dispersal of sporangia by water splash.

S. psophocarpi has been classified as having a short life cycle (Karling, 1964) the duration of which has not been established. Additional information on this was sought in view of its importance in epidemiological analysis.

5.2 MATERIALS AND METHODS

5.2.1 Experimental field site

All field experiments were carried out in the Experimental Agricultural Garden at U.P.N.G., (147° 9' 25" East and 9° 24' 25" South).

5.2.2 Wind dispersal

5.2.2.1 Air sampling

The number of sporangia present in the air was estimated by means of a Hirst volumetric spore trap (Hirst, 1952). This was installed between plots of winged beans grown in the Experimental Garden, with the orifice 1.5m above ground level (Figs 45 and 46). The sampling rate was adjusted to 10 l/min and prepared slides (Hirst and Stedman, 1961) were changed daily at 0800h. Sampling commenced on the 1st of December 1976 and ceased on the 30th of November 1977.

Exposed slides were mounted in Hydromount (Searle Diagnostics, Yorkshire, England) and examined under a compound microscope (x150) for sporangia of S. psophocarpi. These are large, yellow in colour and easily recognised (Fig. 47). The number of sporangia trapped in each hour

for thirty consecutive sampling days from 12 Feb - 13 March 1977 was estimated from 14mm wide traverses across the slide. Sporangia trapped on the remaining slides were estimated from a central traverse 2mm wide along the length of the slide.

Counts of sporangia were converted to give an estimated number per m^3 of air (Appendix 2). Corrections for wind speed were not made. Since trapping efficiency varies with wind speed and never reaches 100% (Hirst, 1953), the counts were probably underestimates.

Geometric and arithmetic means were calculated for the number of sporangia trapped each hour over the 30 days and for the weekly count over the rest of the sampling period.

5.2.2.2 Meteorological measurements

Weather data was collected from the University Meteorological station c. 200 m from the trapping site. Temperature and relative humidity was recorded with a 7-day bimetallic thermograph and hair hydrograph (Ota Keiki, Seisakusho Co. Ltd., Tokyo, Japan) in a Stevenson Screen (Standard Australian Bureau of Meteorology design). Rainfall was measured with a Natural Siphon Rainfall Recorder (No. WS386, C.F. Casella and Co. Ltd., London, England), solar radiation with a Pyranometer

(Model PSP, Eppley Laboratory Inc., Newport R.I., 02840, U.S.A.) and wind speed with a continuously recording anemometer (Type 1M-146, R.W. Munro Ltd., London, England). The hours of dew were monitored using a weighing type dew gauge designed at U.P.N.G., (Kruijshoop, 1973).

5.2.2.3 Computation

Spore catch data were transformed and expressed as percentages of the peak geometric (Chee, 1976) and arithmetic (Filan, personal communication) means. The relationships between spore counts and meteorological data were analysed on computers at U.P.N.G., the University of New South Wales, Australia and the University of Bath, U.K.

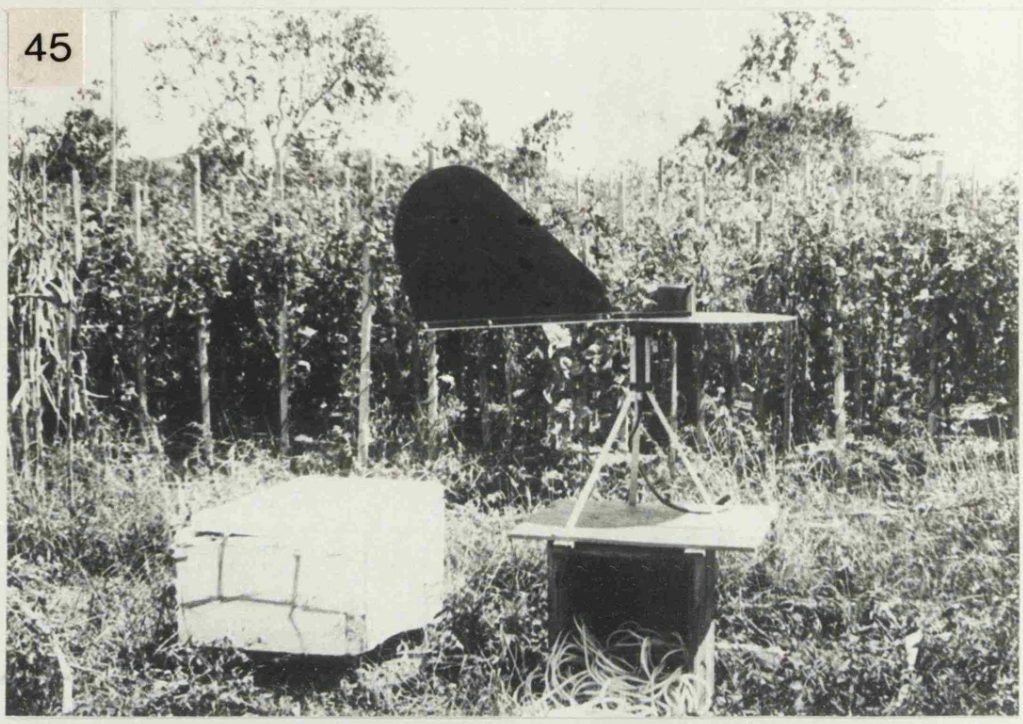
5.2.3 Splash dispersal

A hypodermic syringe clamped into a retort stand was positioned so that its orifice was 150cm above the surface of a sheet of glass plate. A leaf bearing ruptured sori of S. psophocarpi was placed on the sheet in direct line with the orifice. Coated microscope slides as used for spore trapping were placed in a circle of 15cm radius from the sorus. The syringe was filled with tap water and drops (4mm diam.) (Holliday, 1969) were allowed to fall onto the

leaf. The resulting splash droplets that fell onto the slides were examined under a compound microscope (x25). The diameter of the droplets were measured and the number of sporangia per droplet counted. A maximum of 10 droplets were examined on each slide, rapid evaporation preventing further counts.

5.2.4 Dispersal gradients and the maximum distance of sporangia travel

A rotorod air sampler modelled on those of Perkins (1957) and built by Keane (1972) (Fig. 48) was used to sample the number of sporangia in the air at known distances from an infection source. The sampler was prepared as described by Keane (1972) except that pure Vaseline was used as the adhesive. This was gently heated and applied to Sellotape strips which had previously been stuck onto the leading surface of the two vertical brass rod sampling arms (6.0 cm high, 0.167 cm diam., and 8.0 cm apart). The rod revolution was estimated using a stroboscope and the volume of air sampled calculated (Appendix 3). The sampler was placed 2m above the ground level and a single air sample taken at 14.00 h on 28 Feb 1977. Air was sampled for 10 min at distances of 1,5,10,15 and 20m downwind from winged beans naturally infected with S. psophocarpi. An interval of 15 min was allowed to lapse between each sampling period in order to avoid



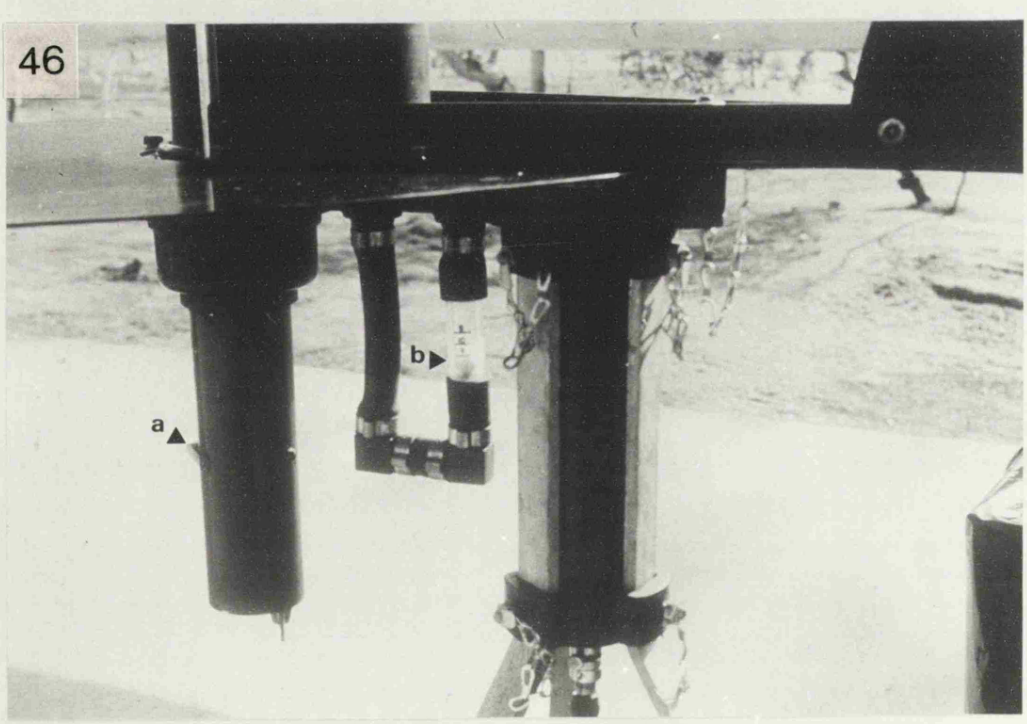


Fig. 45. Hirst volumetric spore trap sited near plot of winged beans.

Fig. 46. Close up of Hirst volumetric spore trap showing a) orifice and b) suction rate gauge (l/min).

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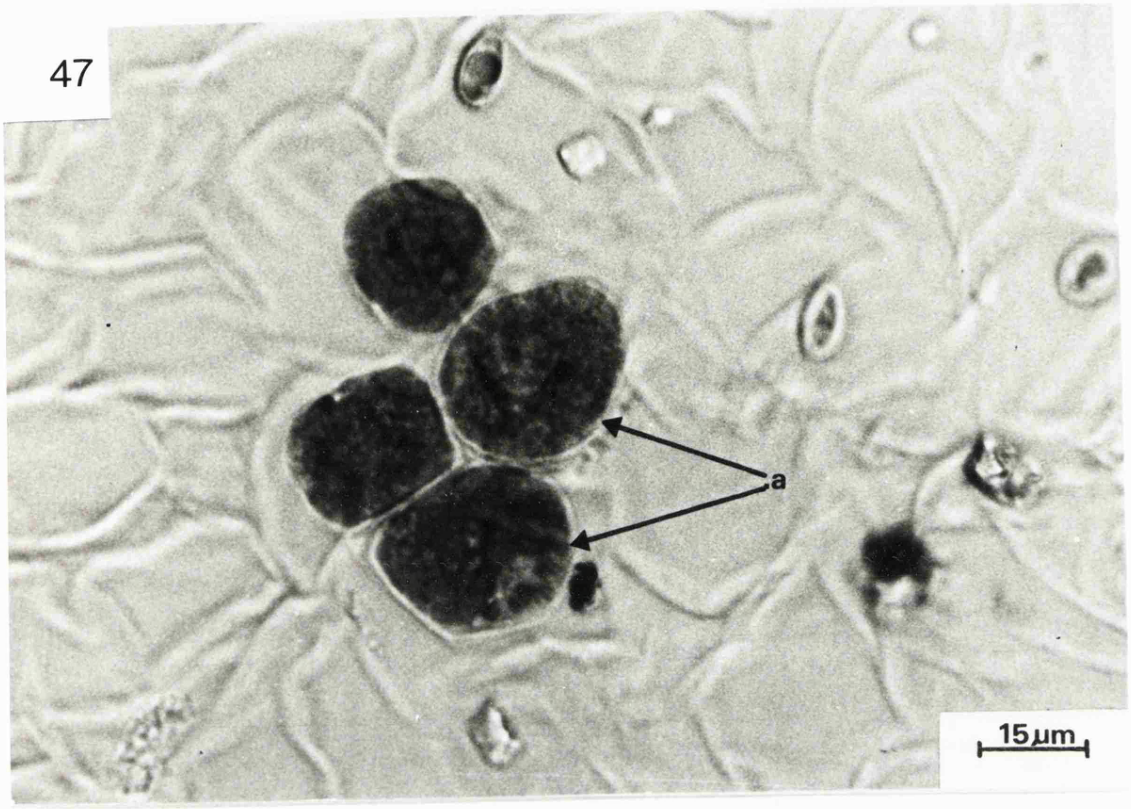

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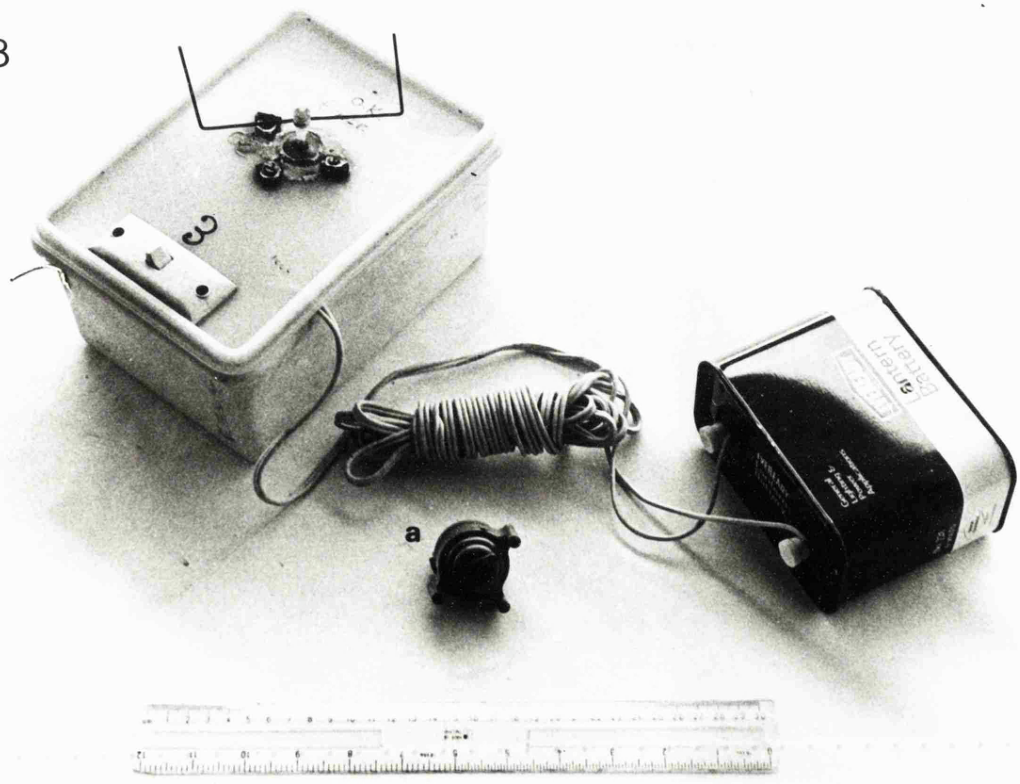

Fig. 47. a) Sporangia of S. psophocarpi on spore trap slide.

Fig. 48. Rotorod air sampler a) gramophone electric motor.

excessive battery discharge.

Exposed Sellotape strips were removed, placed on microscope slides, mounted in Hydromount and examined for sporangia under the microscope (x150). These were counted and the concentration per m^3 of air estimated for each site. No corrections were made for windspeed.

The maximum distance sporangia could travel were claculated from the terminal velocity of the spore and the prevailing wind speed (Gregory, 1961).

5.2.5 Disease development in the field

The rate of new infections of winged beans by S. psophocarpi was monitored under field conditions during the 1977 wet season in a plot of winged beans growing at the Experimental Agriculture Garden at U.P.N.G. Plot size was 10 x 17m and consisted of rows with inter and intra-row spacing of 1m and 0.5m respectively. Sowing, which was carried out in mid December 1976, staking, crop maintenance and pest and disease control was according to the methods described by Khan and Erskine (1977). Fifty plants at the vegetative growth stage were selected at random from a plot of winged beans free from false rust on the 31st of January 1977. Each plant was tagged and examined weekly for gall symptoms.

5.3 RESULTS

5.3.1 Life cycle of S. psophocarpi

The life cycle of S. psophocarpi based on previous infection studies (loc. cit.) is shown in Fig. 49. Since no resting spores were found it is assumed the fungus remains haploid throughout the cycle. The generation time was estimated as 22-28 days.

5.3.2 Wind dispersal

5.3.2.1 Diurnal periodicity

The numbers of sporangia trapped showed a diurnal periodicity (Fig. 50). The numbers increased during the afternoon, reached a maximum between 16.00 and 18.00h, and then decreased rapidly reaching a minimum in the early morning between 06.00 and 07.00h. A similar pattern occurred on days of high and low sporangia catch (Fig. 51).

The gross effect of each weather factor (independent variable) on sporangia concentration (dependent variable) is shown in the scatter diagrams (Butt and Royle, 1974) (Fig. 52). Correlation of sporangia concentration with all weather factors, except rainfall, (Table 21) increased when these were lagged 1h; the highest significant

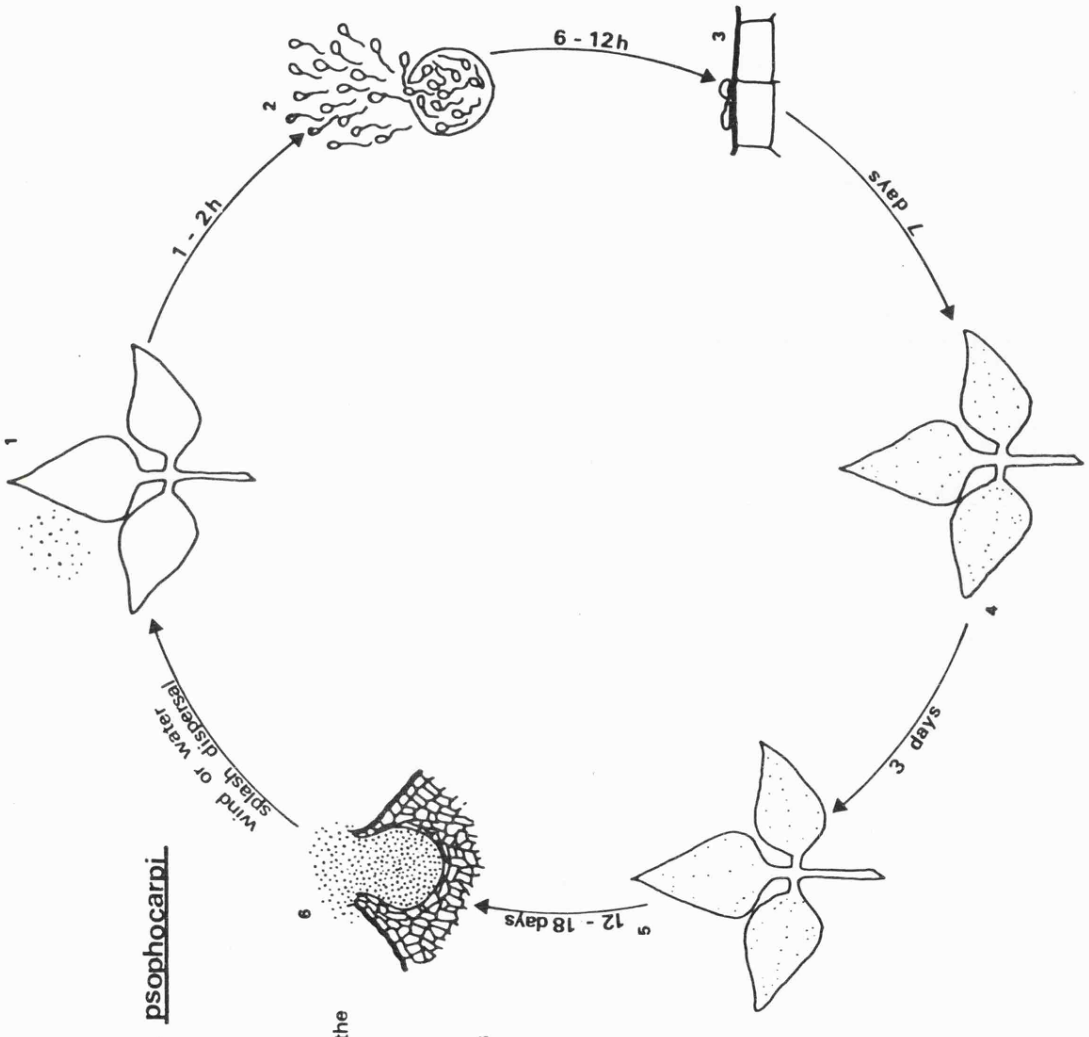

Fig. 49. Life cycle of S. psophocarpi

1 Sporangia arrive on host

2 Germination of sporangia in the presence of free water releasing motile zoospores

3 Penetration of epidermal cells

4 Dark green spots appear

5 Yellow pustules visible

6 Sorus membrane rupture releasing dry powdery mass of sporangia

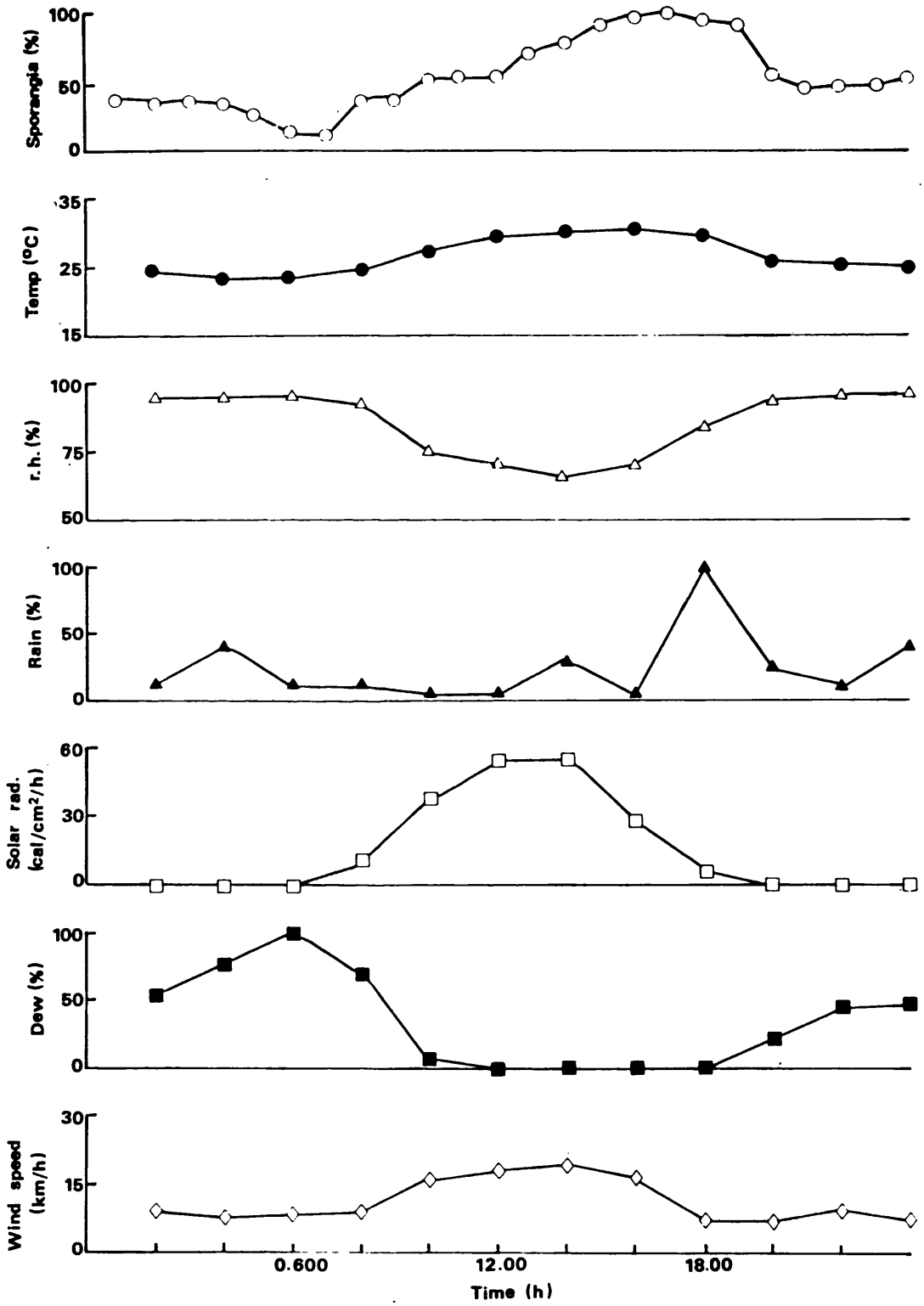

Fig. 50. ○ 30 day mean diurnal periodicity curve of S. psophocarpi expressed as a percentage of the peak geometric mean concentration at each h. ● mean temperature, △ relative humidity and ◇ wind speed every 2h with □ solar radiation, ■ dew and ▲ rain expressed as a percentage of the peak total every 2 h

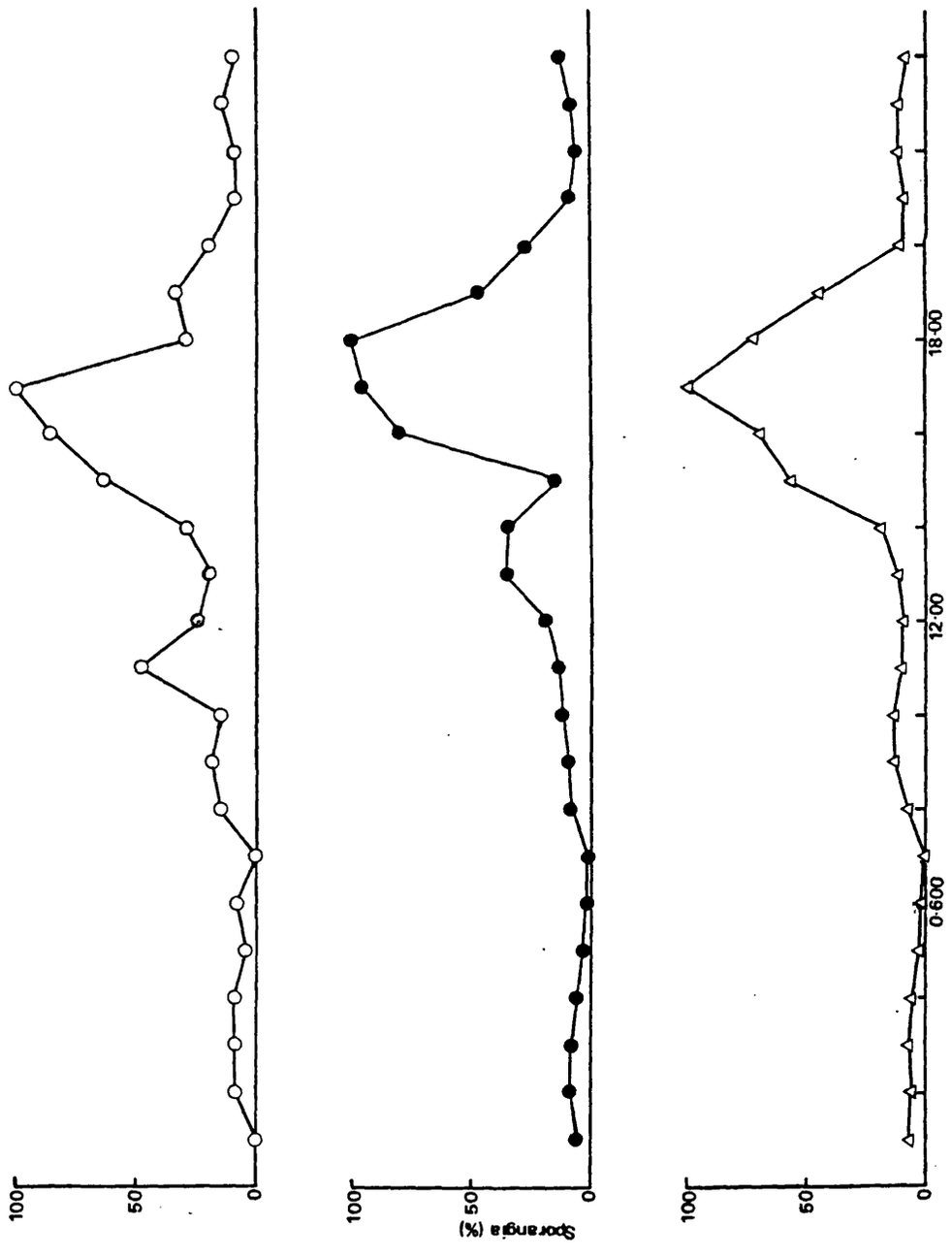

Fig.51. Comparison of diurnal periodicity curves of S. psophocarpi each expressed as a percentage of the peak arithmetic mean concentration at each h. Each curve represents 6 consecutive days of low ○● (12th - 17th February 1977), (9th - 14th March 1977) and high △ (26th February 1977 - 3rd March 1977) sporangia catch.

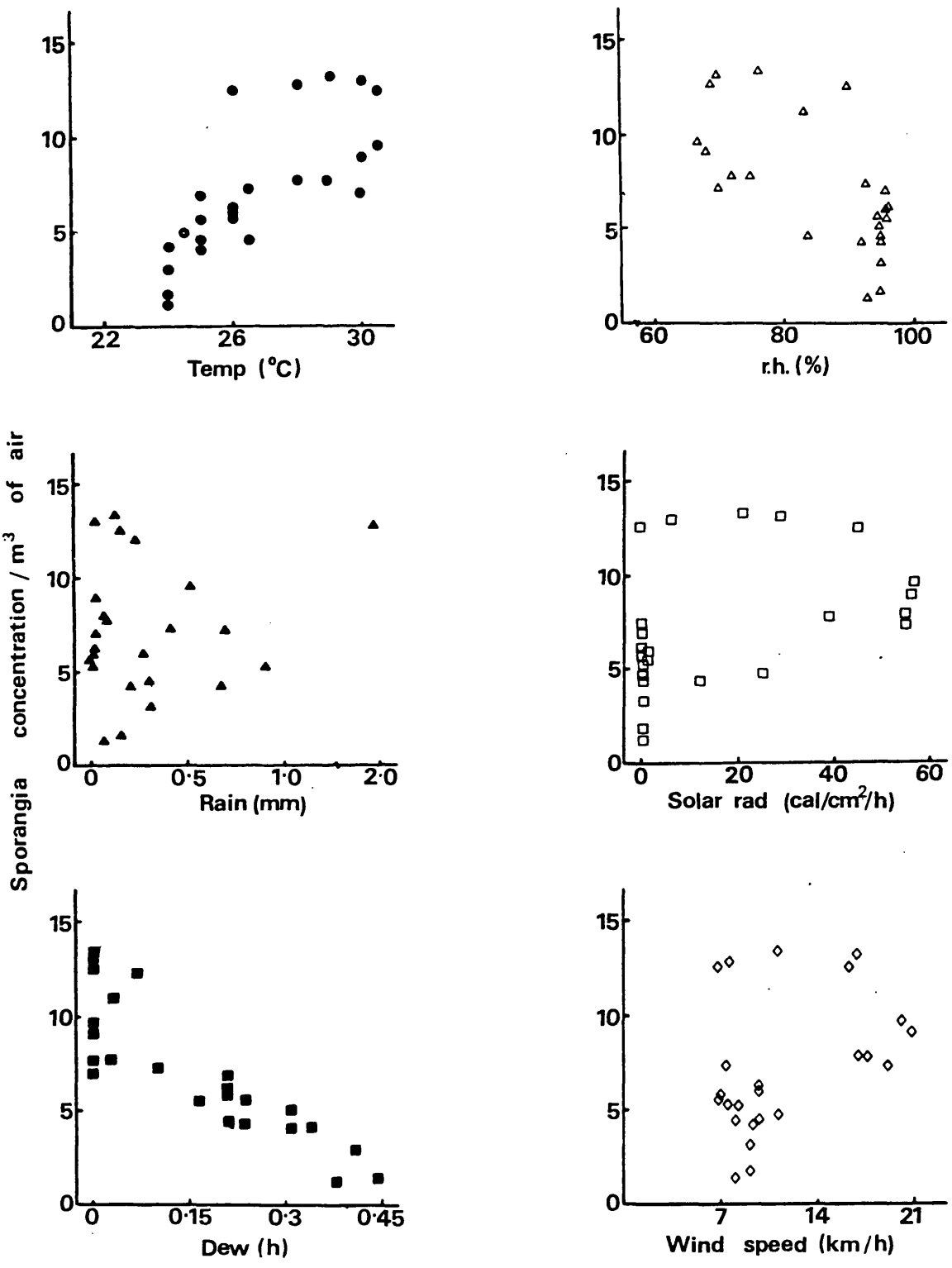

Fig. 52. Relationship between the concentration of S. psophocarpi sporangia (30 day geometric means) and weather factors (30 day arithemetic means).

correlation (P=0.01) being recorded with temperature, relative humidity and dew. Further lags of all weather factors were not examined since, apart from rainfall, they were all highly intercorrelated (Table 22). Since temperature appeared to be more highly correlated with sporangia concentration (r = 0.72, lag 1 h) regression equations were developed to determine the closest fit to the relationship. There was a significant (P=0.01) departure from a linear regression (Table 23) when lagged 1 h and a curvilinear relationship was established. Regression equations were therefore calculated for sporangia concentration as a quadratic function of temperature lagged 1,2 and 3h. Cubic and quatric functions for the 3h time lag were also calculated since a greater proportion of the variation in sporangia concentration could be explained for this time (R^2 = 0.77) (Equations 1-4). The cubic function gave the best description of the diurnal pattern (Fig. 53). Means for sporangia concentration and weather factors are given in Appendix 4.

Table 21 Correlation of sporangia concentration with weather factors recorded at the same hour and lagged one hour

Independent variable	Same hour	Lagged one hour
Temperature	0.598**	0.72**
Relative humidity	-0.495**	-0.67**
Rainfall	0.239	-0.068
Solar radiation	0.206	0.451*
Dew	-0.604**	-0.625**
Windspeed	0.175	0.484*

* Significant at P=0.05

** " " P=0.01

Table 22 Intercorrelations amongst weather factors recorded at the same hour

	Temp.	Rel.Humid.	Rain	Solar rad.	Dew	Windspeed
Temp.	1.0	-0.955**	-0.022	0.862**	-0.926**	0.828**
Rel.Humid.		1.0	0.155	-0.944**	0.819**	-0.91**
Rain			1.0	-0.265	-0.059	-0.341
Solar rad.				1.0	-0.71**	0.96**
Dew					1.0	-0.656**
Windspeed						1.0

* Significant at P=0.05

** " " P=0.01

Table 23 Test of significance of departure from linear regression of sporangia concentration as a function of temperature lagged 1h

Source of variation	df	S.S.	Mean Sq	F
Deviation from linear regression	21	8774.54	417.83	
Deviation from curved regression	21	4930.64	234.79	
Reduction in sum of squares		3843.9		16.37**

** Significant at P=0.01

Equation 1 'Quadratic function, temperature lagged 2h

$$Y = 1470.38-113.81x_1+2.21x_1^2$$

$R^2 = 0.746$

$F = 30.86$**

Equation 2 Quadratic function, temperature lagged 3h

$$Y = 1633.7-125.82x_1+2.43x_1^2$$

$R^2 = 0.77$

$F = 35.1$**

Equation 3 Cubic function, temperature lagged 3h

$$Y = -20042.9+2281.92x_1-86.4x_1^2+1.09\ x_1^3$$

$R^2 = 0.874$

$F = 46.16$**

Equation 4 Quatric function, temperature lagged 3h

$$Y = 105946.0-16310.52\ x_1+939.95x_1^2 - 24.03x_1^3 + 0.23x_1^4$$

$R^2 = 0.898$

$F = 41.97$**

** Significant at P=0.01

' Quadratic function of temperature lagged 1h not significant

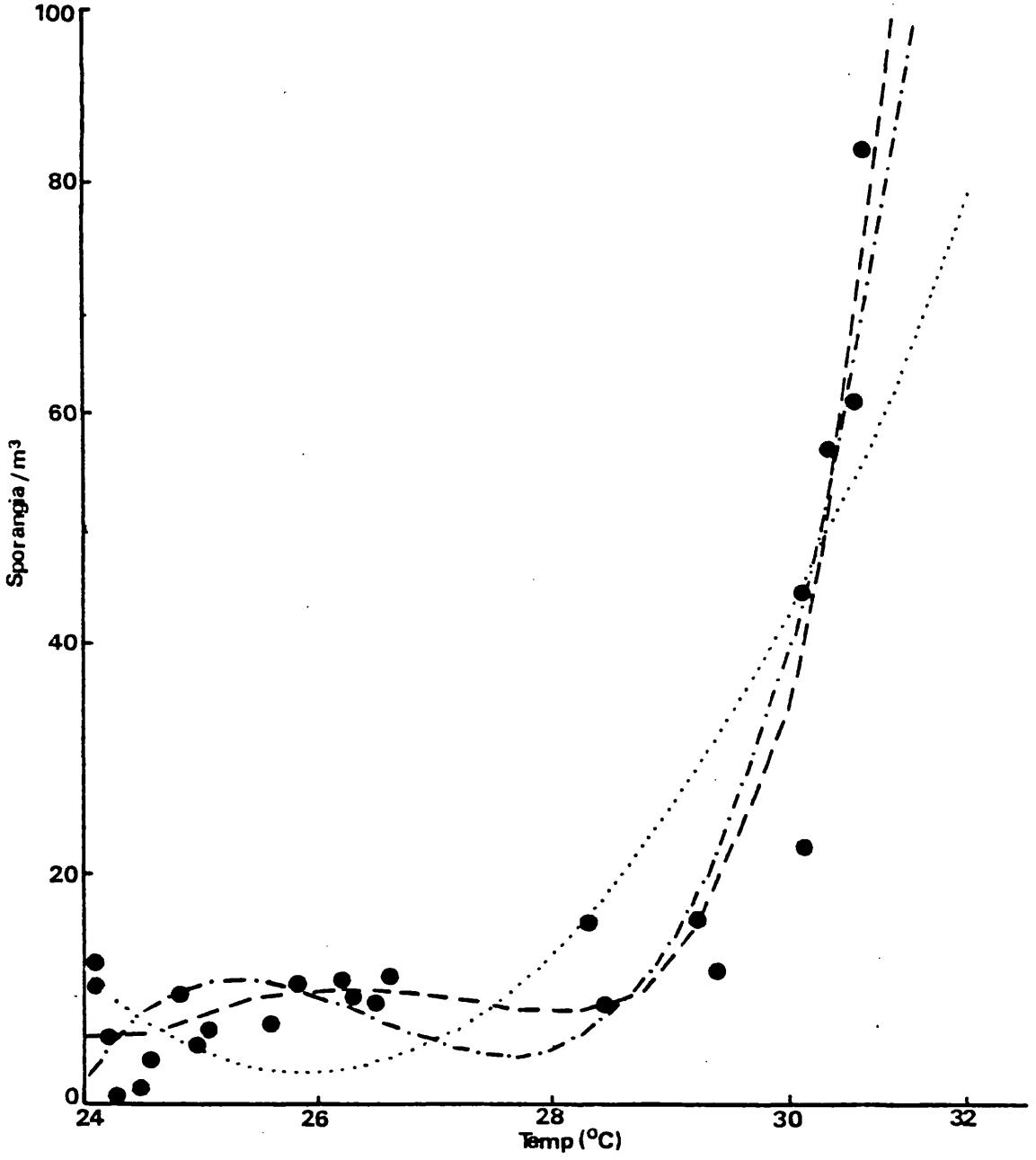

Fig. 53. Relationship between the concentration of S. psophocarpi sporangia (30 day arithmetic means) and the .— cubic –– quatric and .. quadratic functions of temperature lagged 3h.

5.3.2.2 Seasonal fluctuation

The mean weekly sporangia concentration for the wet (November - April) and dry (May - October) seasons together with weather factors is shown in Fig. 54. Sporangia concentration was low at the beginning of the wet season and a zero daily catch was often recorded. The mean concentration, however, increased during February and although weekly fluctuations occurred after this period the concentration remained high for the duration of the wet season and for two months after the onset of the dry season. The numbers subsequently declined at the end of June and low concentrations were recorded for the remainder of the dry season.

No significant relationship was obtained between the sporangial count per week and associated weather factors. A significant correlation ($r = 0.77$, $P = 0.01$) was only established between the mean spore catch per week and rain that fell three months previously (Table 24). Weekly means for sporangia concentration and weather factors are given in Appendix 5.

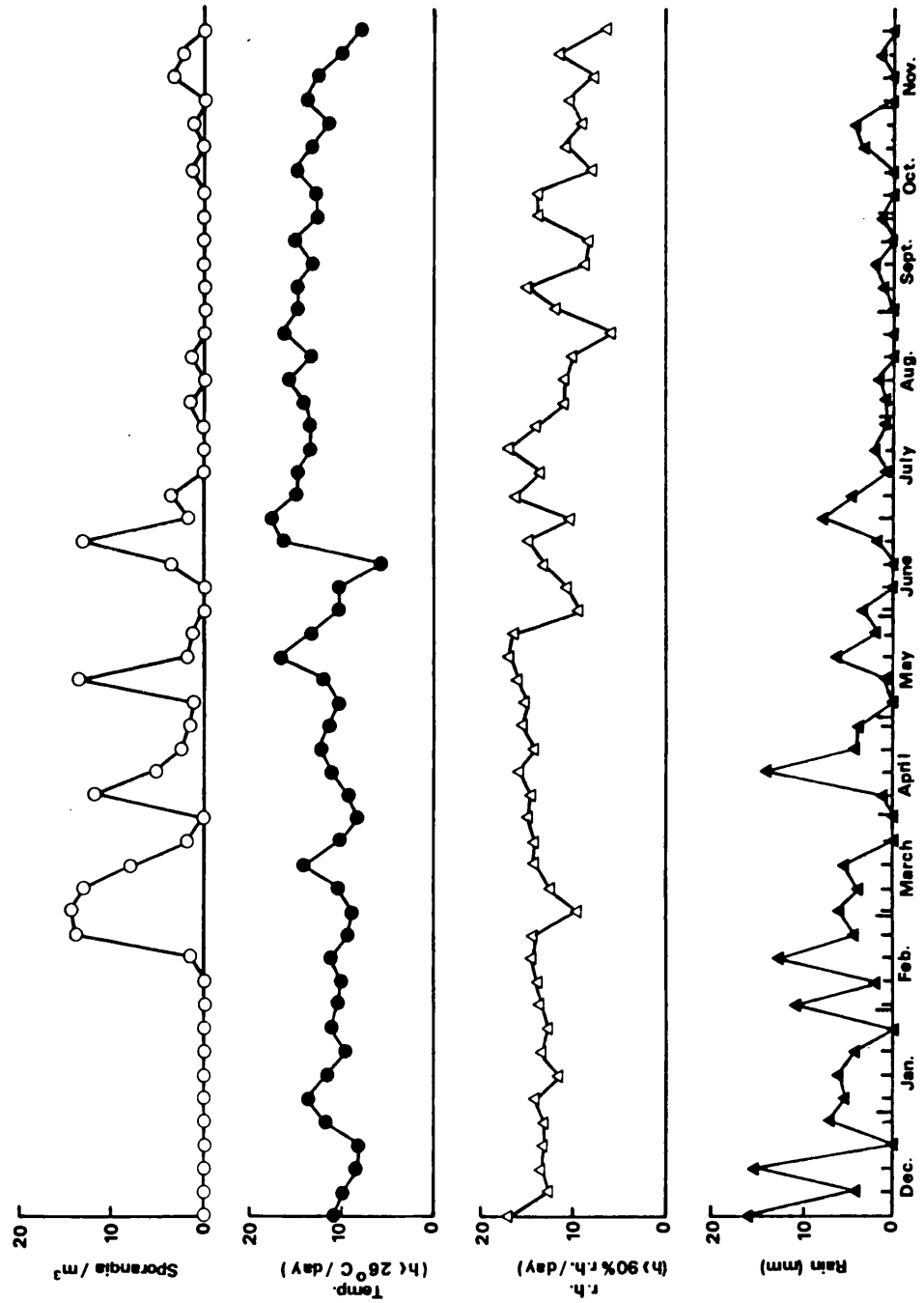

Fig.54. ○ 7-day geometric means for sporangia of S.psohocarpi with arithmetic means for
● temperature, △ relative humidity and ▲ rain from 1st December 1976 - 30th November 1977

Table 24 Correlation of sporangia concentration with rainfall recorded during the same month and lagged 1, 2, 3 and 4 months

Independent variable	Same month	Lagged 1 month	Lagged 2 months	Lagged 3 months	Lagged 4 months
Rainfall	0.007	0.411	0.533	0.768**	0.275

** Significant at P=0.01

5.3.3 Splash dispersal

Sporangia were dispersed by water splash in large ($>1000\mu m$ diam.) and small ($<200\mu m$ diam.)droplets (Table 25).

Table 25 Size of splash droplets and the proportion containing sporangia following the fall of incident drops onto leaves bearing ruptured sori of S. psophocarpi

Droplet diam(μm)	No. of droplets	% containing sporangia	Mean no. of sporangia/droplet
> 1000	90	99	61.71
601-1000	106	92	17.95
401-600	108	82	7.3
200-400	83	68	3.02
< 200	13	15	0.46

5.3.4 Dispersal gradients and maximum distance of dispersal of sporangia

The highest concentration of sporangia was recorded at a distance of 1m from the infection source. The concentration gradient decreased rapidly after this distance and the lowest values were recorded at 15 and 20m (Fig. 55).

The mean radius of globose sporangia of S. psophocarpi was estimated as 10.7 μm (loc. cit.). The terminal velocity $VS = 0.0121\ R^2 = 1.39$ cm/sec. Sporangia released from a sorus 1.5m above ground will therefore be airborne for an average of 150/1.39 = 107.9 sec. The highest mean wind windspeeds (20.5 km/h $\cong$ 5.69m/sec) over any one hour during the period from the 13th of February to 13th March 1977 were recorded between 13.00 and 14.00h. Under those conditions sporangia could theoretically be dispersed a maximum distance of 107.9 x 5.69 = 613.9m.

5.3.5 Disease development in the field

Symptoms of false rust were first recorded on the 24th February 1977. Forty four percent of the plants examined were infected. This subsequently increased to 90% by the 25th March 1977. A random infection pattern occurred (Fig. 56) which indicates sporangia were carried by wind probably from infected winged beans growing adjacent to the plot.

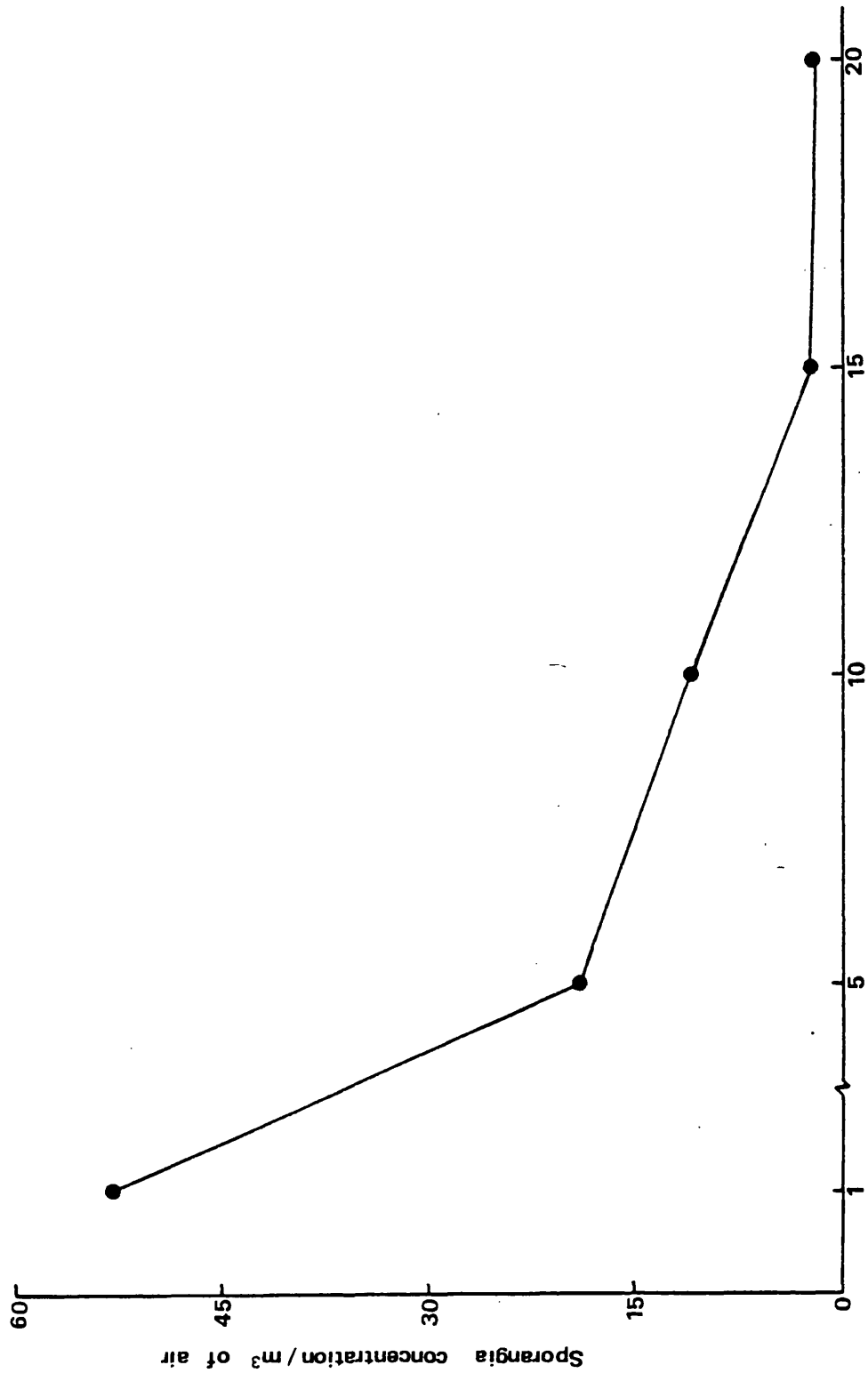

Fig.55. Concentration of sporangia of S. psophocarpi at different distances downwind from infected plants.

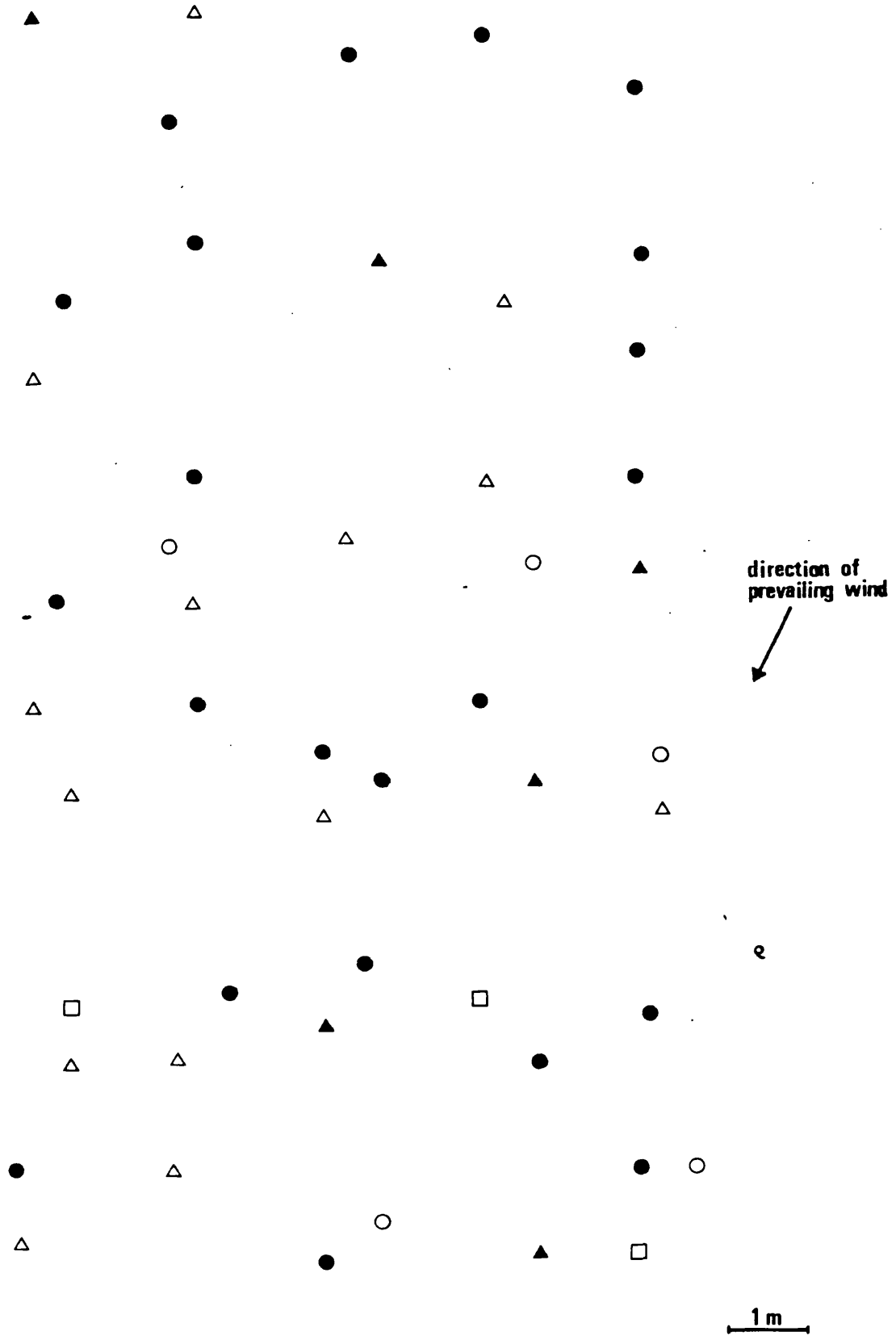

Fig.56. Observation on the development of false rust in winged bean plot from 31st Jan 1977 - 25th March 1977; ○ no infection, first infection recorded on ● 24th Feb, △ 3rd March, ▲ 11th March and □ 25th March.

5.4 DISCUSSION

The results indicate that sporangia of S. psophocarpi were present in the atmosphere with a diurnal periodicity in concentration. Maximum numbers were recorded in the afternoons, lowest counts being recorded in the mornings. Similar fluctuations in spore concentrations have been reported for other fungi such as Erysiphe spp. (Hirst, 1953; Cole, 1966), Alternaria spp., Cladosporium spp., and Ustilago spp., (Hirst, 1953). High concentrations during the day was attributed to decreases in relative humidity and increases in temperature prior to spore capture. The observations support the suggestion of Karling (1964) that the sporangia are dry spores. Low concentration recorded at night could be related to high relative humidity and dew formation causing the sporangia to adhere more closely together in the sorus. Higher windspeeds recorded in the afternoon also contributed to the increase in the numbers of airborne sporangia. Similar observations have been made for uredospores of Puccinia graminis (Hirst, 1961; Smith, 1966) and dry mould spores (Zoberi, 1961). Frequent changes in wind direction were more common during the early afternoon. These directional changes must have caused air turbulence which is considered by Grace and Collins (1975) to be an important factor in the liberation of passively dispersed phylloplane fungi. In addition to being liberated

from the sorus by wind, sporangia were probably kept airborne for longer periods during the day because of the higher wind speeds and air turbulence whereas the low wind speeds and relatively calm conditions which occurred mainly at night would lead to sedimentation. The results indicate this would be rapid in still air because of the high terminal velocity of the sporangia of 1·39 cm/sec.

All weather factors, except rainfall, were highly intercorrelated. This multicollinearity means that the variation in sporangia concentration attributed to each factor could not be separated in a satisfactory statistical sense. Complete freedom from multicollinearity can only be assumed where experimental control of all the independent variables is feasible which was not possible for the present study. The problems of multicollinearity in statistical analysis are discussed further by Heady and Dillon (1961) and Koutsoyiannis (1973). Although it was not possible to quantify the separate effects of each factor the cubic function of temperature when lagged 3h gave a good description of the diurnal pattern of sporangia concentration and also showed that the relationship was curvilinear. Temperature was adopted as the explanatory variable since it was more highly correlated with sporangia concentration compared with other weather factors. This approach using multiple regression analysis has been used in previous epidemiological studies (Cole, 1966; Royle and Thomas, 1972).

Although geometric means of sporangia concentration were used as the dependent variable in regression analysis Filan (personal communication) points out the limitations of this approach. Compared to the arithmetic mean the geometric mean would tend to place a lower value on sets of observations with varying spore numbers, i.e., periods of build-up or decline, relative to periods of constant spore numbers. The geometric mean is, however, useful in expressing spore concentration because of the high numbers which are often involved and in the recognition of trends in the data.

The high concentrations of sporangia which were recorded during the wet season are related to increased germination and infection during this period. Since the generation time is in the order of 3-4 wk the numbers of sporangia trapped are out of phase with rainfall. There was a significant correlation ($P=0.01$) between sporangia concentration and rainfall when lagged 3 months. Although leaf surface wetness was not monitored during the dry or wet seasons it is possible that the number of hours the leaves remained wet may more accurately account for the variation in sporangia concentration that the amount of rain. Although seasonal variation in sporangia concentration has been shown in this preliminary study further work is required before predictive equations can be developed that will allow forecasts to be made of epidemics.

Sporangia can also be liberated by water splash resulting in primary or secondary infections during rainfall or overhead irrigation. This type of dispersal would become more effective with increasing plant density. The maximum distance of travel of splash droplets was not investigated but is is possible that smaller droplets are likely to travel much further in association with air currents (Stedman, 1979). The size of the falling drop, its angle of inclination and distance of fall all have significant effects on the volume of the resultant splash droplets. It is possible that complete evaporation of small splash droplets may occur before deposition and sporangia may subsequently be able to travel long distances in the air as dry spores as suggested for Venturia inaequalis (Cke) Wint., (Hirst, et al., 1961). Downward water movement along stems could influence infection along their length and at the lower nodes which are common infection sites.

While calculations suggest that sporangia could travel at least 613m away from an infection source if the windspeed was high, the distance is likely to be much greater in practice since no correction was made for upward air turbulence. The concentration of sporangia decreased rapidly with distance from the infection source. These results are in agreement with other studies of dispersal gradients (Keane, 1972; Legg and Powell, 1979). It is

possible that upward air currents may account for the decrease but further work is required possibly using wind tunnels. Since sporangia are short lived they may not be able to travel very long distances in dry conditions and maintain viability.

SECTION 6 CONTROL

6.1 INTRODUCTION

The use of fungicides to control S. psophocarpi was first investigated by Alicbusan (1965) who listed 10 fungicides causing inhibition of sporangia germination in vitro. Although fungicide control is not recognised as being economically or technically feasible for subsistence growers it may have an important role in control of the disease in experimental trials and in commercial winged bean production. The efficacy of 20 fungicides in inhibiting sporangia germination was therefore compared in vitro and the most effective compounds were selected for further evaluation in a preliminary field trial.

The most promising method of control of this disease in subsistance agriculture is by the use of host plant resistance. Price (1980 and unpublished data) reported that all winged bean lines screened from the P.N.G., germplasm collection were susceptible. It is not known, however, whether differences in susceptibility exist between these lines. A number of new additions have been made since the initial screening (Kesavan, personal communication) and a further screening of the germplasm collection was therefore undertaken. Selected winged bean lines were also screened for susceptibility in

regional trials.

6.2 MATERIALS AND METHODS

6.2.1 Fungicides

6.2.1.1 In vitro assays

Ten systemic (Table 26) and ten protectant (Table 27) fungicides were tested for their efficiency in inhibiting germination of sporangia. Concentrations of 10-1000 ppm a.i., of each fungicide were prepared in sterile distilled water and 100 µl aliquots from each concentration transferred to grease-free microscope cavity slides using a Finnpipette (Jencons Scientific Equipment, Ltd., UK.). Samples of sporangia were removed from a single erupted sorus on an infected leaf with a mounting needle whilst viewing under the stereomicroscope (x20) and transferred to each slide beginning with the lowest fungicide concentration. The mounting needle was washed twice in sterile distilled water and air dried between each transfer. A sample of sporangia was also added to sterile distilled water as a control. The slides were incubated at 25°C at 100% r.h., and germination assessed after 24h as previously described (loc. cit.). This was repeated three times for each concentration using sporangia from different sori.

Table 26 Systemic fungicides used in in vitro assay

Trade name	Common name	Chemical name	*Formulation	Company
Afugan	Pyrazaphos	2-(O,O-diethylthionophosphoryl)-5-methyl-6-carbethoxyphyrazolo-1.5.a)-pyrimidine	29.5% w/v e.c.	Hoechst
Bavistin	Bavistin	Methyl-2-benzimidazole carbamate	50% w/w w.p.	B.A.S.F.
Benlate	Benomyl	Methyl-(butylcarbamoyl)-2-benzimidazolecarbamate	50% w/w w.p.	Du Pont
Calixin M	Tridemorph + Maneb	N-Tridecyl-2, 6-dimethymorpholine Manganese bisdithiocarbamate	11% w/v e.c. 36% w/v e.c.	B.A.S.F.
EL-222	E.L. - 222	-(2-chlorophenyl)- -(4-chlorophenyl) 5-pyrimidine methanol	12.5% w/v e.c.	Elanco
Plantvax	Oxycarboxin	5,6-Dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide	75% w/w w.p.	Uniroyal
Sabimin	Thiophanate Methyl + Oxycarboxin	Dimethyl 4,4-O-phenylenebis (3-thioallophanate) 5,6-Dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide-4, 4-dioxide	55% w/w w.p. 15% w/w w.p.	Nippon
Saprol	Triforine	N,N-(piperazinediylbis (2,2,2-trichloroethylidene)) bis (formamide)	20% w/v e.c.	Shell
Storite	Thiabendazole	2-(4-Thiazoyl)benzimidazole	45% w/v e.c.	Merck Sharp & Dohme
Topsin M	Thiophanate Methyl	Dimethyl 4, 4-O-phenylenebis (3-thioallophanate)	70% w/w w.p.	Nippon

* % = active ingredient, w/v = weight by volume, w/w = weight by weight, e.c. = emulsifible concentrate, w.p. = wettable powder

Table 27 Protectant fungicides used in in vitro assay

	Trade name	Common name	Chemical name	*Formulation	Company
A. Dithiocarb-amates	Brestan 60	Fentinacetate 54% Maneb 16%	Triphenyltin acetate Manganese bisdithiocarbamate	60% w/w w.p.	Hoechst
	Dithane M45	Mancozeb	16% Manganese, 2% Zinc and 62% ethylenebisdithiocarbamate ion/ manganese ethylenebisdithiocarbamate plus zinc ion	80% w/w w.p.	I.C.I.
	Manzate 200	Mancozeb	Manganous ethylenebisdithiocarbamate	80% w/w w.p.	Du Pont
	Polyram M	Maneb	Manganese bisdithiocarbamate	80% w/w w.p.	B.A.S.F.
	Zebtox	Zineb	Zinc ethylenebisdithiocarbamate	65% w/w w.p.	I.C.I.
B. Inorganics	Bordeaux mix.	-	Copper sulphate Calcium hydroxide	50% w/w 50% w/w	-
	Cuprox	-	Copper oxychloride	50% w/w w.p.	I.C.I.
	Kocide 101	-	Copper hydroxide	83% w/w w.p.	Shell
C. Organics	Captan	Captan	N-(Trichloromethlthio)-4-cyclohexene-1, 2-dicarboximide	83% w/w w.p.	Consolidated Fertilisers
	Panoctine	-	9-aza-1, 17-diguanidinoheptadecane	40% w/v e.c.	Murphy

* As for Table 26

6.2.1.2 Field trials

Six fungicides, fentinacetate plus maneb, captan, triforine, pyrazaphos, mancozeb and tridemorph plus maneb, selected from the *in vitro* assays were evaluated further in a preliminary field trial set up in the Experimental Agriculture Garden at U.P.N.G., during the 1978 wet season (November - April).

6.2.1.2.1 Experimental design

The design (Fig. 57) consisted of three completely randomised blocks. Each block had eight 5.0 x 2.5m plots consisting of six treatments and two controls with 2m pathways between plots. Guard plants were placed around the perimeter of the experimental area but none were placed around individual treatment plots as this caused intertwining problems (Stephenson, personal communication). Each plot consisted of six rows with six plants in each row. Inter and intra-row spacing was 1 and 0.5m respectively.

6.2.1.2.2 Sowing

Winged bean seeds cv. UPS-122 were soaked in tap water for 24h to enhance germination. Two seeds were sown *c.* 3 cm deep and 50 cm apart on ridges (*c.* 20 cm high and 1 m apart) on the 7th February 1978. The seedlings were thinned to a single plant 4 wk later and replanting was carried out where seeds had failed to germinate.

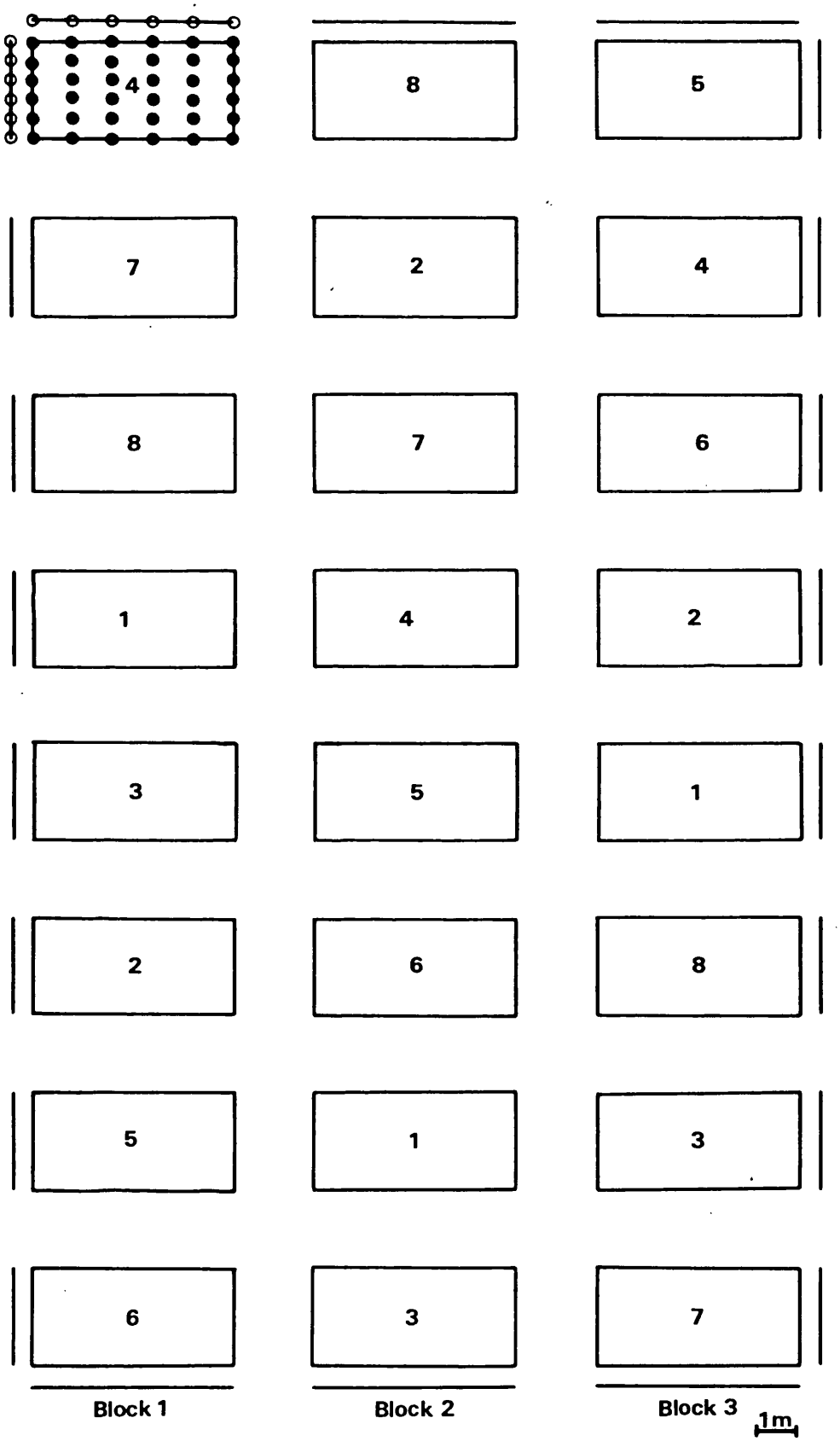

Fig.57. Experimental design of fungicide trial, ○ guard row and ● treated plants. Treatment (1) Fentinacetate+Maneb, (2) Captan, (3) Triforine, (4) Pyrazaphos, (5) Mancozeb, (6) Tridemorph + Maneb (all fungicides applied with Yates fixer); Control (7) Fixer only, (8) Unsprayed.

6.2.1.2.3 Crop maintenance

A single basal dressing of a complete fertilizer at the rate of 50 kg N: 50 kg P: 50 kg K per ha was applied to all plants after thinning and replanting. Weeds were removed by hand or hand hoes. No insecticide was applied and plants were watered by channel flooding as required.

6.2.1.2.4 Staking

Plants were supported using 'A' frame trellises constructed from wooden stakes 3.8 x 3.8 x 180 cm. These trellises have proved to be the most successful method of supporting winged beans in previous trials at U.P.N.G., since intertwining between adjacent plots is minimised, the trellis is stable and few stakes are required (Stephenson, personal communication).

6.2.1.2.5 Spraying

The fungicides were mixed with 'Yates Fixer' (Yates and Co. Ltd., New Zealand, 1ml/l) and applied to run-off with a pressurised knapsack sprayer (I.C.I. Ltd., U.K.) at the following rates of application: fentinacetate 0.54 g a.i./l plus maneb 0.16 g a.i./l, captan 1.08 g a.i./l, triforine 0.29 g a.i./l, pyrazaphos 0.3 g a.i./l, mancozeb 1.47 g a.i./l and tridemorph 0.55 g a.i./l plus maneb 1.8 g a.i./l.

Spraying commenced 4 wk after sowing and every 2 wk thereafter and was discontinued 2 wk before the first harvest. Controls were sprayed with mixtures of fixer and water or were unsprayed.

6.2.1.2.6 Disease assessment

Twenty plants at the young pod growth stage were selected at random from each plot. Ten leaves from each plant were assessed for false rust using the standard area diagram disease key (loc. cit.) and a severity percentage calculated for each treatment.

6.2.1.2.7 Yield parameters

Pods were harvested 20, 25 and 27 wk after sowing. The dry weight of pods (husk + seed) and the dry weight of seed after threshing was recorded.

6.2.2 Screening of germplasm lines for resistance

6.2.2.1 Artificial inoculation

Six to 10 plants of winged bean lines from P.N.G., (U.P.S. 62, 122 and 126) and Thailand (Accessions 1602/1, 1611/2) were grown singly in 12 cm diam., plastic pots containing peat moss in a wire screened shade house at U.P.N.G. One leaf at 1 - day old (Fig. 44) was selected and tagged from

each plant 4 wk after sowing and inoculated with a small drop (c. 25 µl) of suspension (14.0 x 10^5) of viable zoospores of S. psophocarpi in sterile distilled water using a Finnpipette. Viability was assessed by germinating sporangia and assessing germination (loc. cit.) 24h prior to making up the zoospore suspension.

All plants were incubated in a mist chamber (26 ± 4°C, 100% r.h.) for 48h and then transferred to the shade house. The leaves were detached 3 wk after inoculation, examined under the stereomicroscope (x20) and the number of galls counted.

6.2.2.2 Field trials

6.2.2.2.1 University of Papua New Guinea collection and accessions from Thailand

The susceptibility of 125 P.N.G., winged bean lines and two from Thailand to infection by S. psophocarpi was assessed using the standard area diagram disease key (loc. cit.) in field trials. The lines were grown out by V. Kesavan (U.P.N.G.) during the 1977 and 1978 dry seasons (May - October) in the Experimental Agriculture Garden at U.P.N.G., according to the methods used by Khan (1976) and Khan and Erskine (1977), to maintain the viability of the germplasm. From each P.N.G., line one to five plants, depending on the number that survived, were selected at random and assessed twice at different growth stages. Ten plants from each Thailand line were assessed once at the young pod growth stage. A disease index (loc. cit.) was calculated for each line following the assessments.

6.2.2.2.2 Regional winged bean variety trials

Ten winged bean lines (U.P.S. 31, 32, 45, 47, 53, 62, 99, 102, 121 and 122), grown by R.A. Stephenson (U.P.N.G.) to compare their agronomic performance under different conditions, were assessed for their susceptibility to infection by S. psophocarpi in regional trials carried out during 1977 and 1978. The experimental design consisted of five completely randomised blocks, each block consisting of 10 varieties (treatments). Guard rows were placed around the perimeter of the experimental area previously described (loc. cit.). Each plot consisted of two adjacent rows 5m long and 50 cm apart separated by 1m pathways. Sowing, staking and crop maintenance was as previously described (loc. cit.) except the plants were spaced at 25 cm within the row. Pest and disease control was according to the methods described by Khan and Erskine (1977). In the 1977 trial held at Kuk (Western Highlands Agriculture Station, 5^{o} 52' S 144^{o} 14' E) and Port Moresby (Experimental Agriculture Garden, U.P.N.G.) disease assessments were made using the descriptive key (loc. cit.). The standard area diagram disease key was used in assessments of the 1978 trial at Kuk, Aiyura (Eastern Highlands Agriculture Station, 6^{o} 19' S, 145^{o} 55' E) and Lae (Experimental Agriculture Station, University of Technology, 6^{o} 44' S, 146^{o} 59' E). Twenty plants from each line at the mature pod growth stage were selected at random at each site and assessed for false rust. Disease severity was calculated for each line as previously described (loc. cit.) using assessments from both individual and combined sites.

6.3 RESULTS

6.3.1 Fungicides

6.3.1.1 In vitro assays

Greater inhibition of germination of sporangia was achieved using tridemorph plus maneb, triforine followed by mancozeb, Captan and fentinacetate plus maneb (Table 28). Benzimidazole fungicides showed little inhibition at 1000 ppm.

6.3.1.2 Field trial

Disease was recorded in all sprayed plots. The lowest disease severity was recorded in plots sprayed with fentinacetate plus maneb (Table 29). Significant differences (P=0.01) were recorded between plots sprayed with fungicide and unsprayed plots, but there were no significant differences between fungicides. The fixer also reduced the severity of the disease. The highest yield of dry pods and seeds were harvested from plots sprayed with tridemorph plus maneb. However neither pod or seed yield was correlated with disease severity.

Table 28 Effect of fungicides on germination of sporangia of S. psophocarpi

Fungicide	* Germination means (angular transformed percentages) Fungicide concentration (ppm)					
	10	50	100	250	500	1000
Tridemorph + maneb	56.34	0	0	0	0	0
Triforine	72.15	0	0	0	0	0
Mancozeb	87.92	67.17	0	0	0	0
Captan	54.67	25.79	11.23	0	0	0
Fentinacetate + maneb	86.24	78.17	30.80	0	0	0
Mancozeb + zinc ion	77.57	67.30	48.64	0	0	0
E.L. - 222	87.0	45.38	22.62	18.60	0	0
Pyrazaphos	65.41	59.28	33.74	24.89	0	0
Panoctine	91.59	74.49	65.11	23.57	0	0
Copper oxychloride	81.57	72.34	60.25	53.75	16.50	0
Zineb	82.12	75.79	66.46	50.77	33.44	0
Copper hydroxide	55.21	42.05	42.30	38.22	33.04	19.46
Bordeaux mixture	82.72	76.94	70.73	46.0	29.26	23.33
Thiabendazole	93.11	91.61	88.21	62.89	51.11	29.74
Maneb	81.01	80.30	87.54	86.52	76.31	57.93
Benomyl	82.56	83.54	84.25	80.45	70.97	59.80
Bavistin	85.38	92.13	79.73	82.38	71.12	70.96
Oxycarboxin	91.89	87.72	95.77	79.95	84.40	81.67
Thiophanate methyl + oxycarboxin	78.16	85.67	83.75	83.9	84.38	83.11
Thiophanate methyl	84.74	83.85	92.54	90.93	90.26	89.11

* Sporangia germination was calculated as a percentage of control.

L.S.D. (P=0.01) = 9.16 (s.e. concentration/fungicide interaction mean $\pm$ 2.51, fungicide mean $\pm$ 1.02, concentration mean $\pm$ 0.56)

Table 29 Effect of fungicide on the severity of false rust and yield of winged bean

Fungicide	Mean % leaf area diseased/ plant	Dry pod(husk + seed) weight (kg/ha)	Dry seed weight (kg/ha)
Fentinacetate + Maneb	0.01	1097.0	398.2
Tridemorph + Maneb	0.02	1326.5	556.8
Triforine	0.13	852.1	347.5
Mancozeb	0.05	1037.4	324.0
Captan	0.04	998.8	419.6
Pyrazaphos	0.20	794.9	334.7
Fixer + Water	0.26	1166.1	469.0
Unsprayed	0.41	691.1	277.3
L.S.D. (P=0.05)	0.20	533.8	199.5
(P=0.01)	0.34	740.9	276.9

6.3.2 Screening of germplasm lines for resistance

6.3.2.1 Susceptibility following inoculation

All three lines from P.N.G., were susceptible to *S. psophocarpi* whilst the two from Thailand appeared to be resistant. The number of galls which developed on leaves within the same susceptible line, however, varied greatly (Table 30).

6.3.2.2 Field trials

6.3.2.2.1 University of Papua New Guinea collection and accessions from Thailand

All 125 lines from P.N.G., and Acc. No., 1602/1 from Thailand were susceptible to *S. psophocarpi* but no infection was recorded for Acc. No., 1611/2 from Thailand. A comparison of disease severity between lines is given in Table 31A. The rate of increase of disease severity differed between lines (Table 31B).

Table 30 Susceptibility of three winged bean lines from P.N.G. and two accessions from Thailand to S. psophocarpi following inoculation

Line	No.plants inoculated	No.plants infected	Total no. of galls	Range of gall no/leaf	Median gall no/leaf	Category
UPS No 122	8	6	90	0 - 33	11.5a	Susceptible
121	10	10	325	6 - 177	12.5a	"
62	10	7	162	0 - 74	1.5a	"
Thailand Accession No 1602/1	8	0	0	0	0b	Resistant
1611/2	6	1	9	0 - 9	0b	"

Values followed by the same letter are not significantly different at P=0.01 (Mood's median test)

Table 31A Susceptibility of winged bean lines from Papua New Guinea and Thailand to S. psophocarpi at the University of Papua New Guinea

Line	No.plants assessed on 21 Oct 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ±.S.E.	No.plants assessed on 17 Nov 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ±.S.E.
UPS No.						
1	5	YP	0.83 ± 0.29	3	MP	1.77 ± 1.07
3	5	MP	0.4 ± 0.12	5	MP	4.58 ± 4.48
4	3	F	0.1 ± 0.06	3	YP	8.12 ± 2.48
5	5	YP	0.67 ± 0.35	2	MP	0.03 ± 0.02
7	5	YP	0.72 ± 0.35	3	MP	1.8 ± 0.87
8	4	YP	1.95 ± 1.69	3	MP	6.78 ± 4.44
9	4	YP	1.64 ± 0.75	3	MP	5.22 ± 5.14
10	5	YP	0.22 ± 0.13	5	MP	2.49 ± 1.64
11	3	YP	0.07 ± 0.02	1	MP	0.05 ± 0.0
12	5	YP	0.17 ± 0.04	5	MP	0.33 ± 0.69
13	5	MP	0.11 ± 0.04	5	MP	0.73 ± 0.69
14	4	YP	0.14 ± 0.05	3	MP	0.23 ± 0.18
15	4	YP	0.23 ± 0.16	4	MP	0.38 ± 0.38
16	5	YP	0.52 ± 0.37	4	MP	0.34 ± 0.15
17	4	F	0.03 ± 0.01	3	YP	16.71 ±10.53
18	4	YP	0.18 ± 0.02	4	MP	1.06 ± 0.82
19	4	YP	0.05 ± 0.04	4	MP	3.29 ± 3.07
20	5	YP	0.59 ± 0.18	5	MP	4.16 ± 4.09
21	3	YP	0.10 ± 0.03	4	MP	3.7 ± 3.50
22	5	F	0.06 ± 0.04	5	YP	17.17 ± 4.94
25	4	V	0.15 ± 0.13	3	V	3.77 ± 1.88
26	4	YP	0.06 ± 0.03	4	MP	7.3 ± 6.6
27	3	YP	0.27 ± 0.14	3	MP	6.55 ± 3.45
29	5	YP	1.20 ± 1.09	4	MP	7.53 ± 2.15
30	5	YP	0.82 ± 0.26	5	MP	10.92 ± 7.12
31	5	YP	0.17 ± 0.09	5	MP	20.14 ± 7.88

Table 31A Cont:

Line	No.plants assessed on 21 Oct 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.	No.plants assessed on 17 Nov 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.
UPS No.						
32	4	YP	0.21 ± 0.12	4	MP	8.03 ± 6.35
34	5	YP	0.43 ± 0.19	4	MP	4.15 ± 1.78
35	4	YP	0.19 ± 0.03	4	MP	8.09 ± 4.09
37	5	YP	1.14 ± 0.54	4	MP	3.66 ± 2.33
38	4	YP	0.39 ± 0.16	3	MP	5.42 ± 5.32
39	5	YP	0.22 ± 0.11	4	MP	0.14 ± 0.12
40	5	YP	0.89 ± 0.45	5	MP	11.33 ± 5.88
41	5	YP	1.05 ± 0.40	5	MP	5.92 ± 4.28
42	5	YP	0.14 ± 0.05	5	MP	3.51 ± 3.49
43	3	YP	0.63 ± 0.02	2	MP	21.25 ±21.25
44	5	YP	0.18 ± 0.12	4	MP	5.9 ± 4.01
45	5	YP	0.16 ± 0.09	5	MP	3.44 ± 2.12
46	5	YP	0.04 ± 0.02	3	MP	3.9 ± 2.0
47	5	YP	0.34 ± 0.05	5	MP——	0.06 ± 0.04
48	4	YP	0.15 ± 0.01	3	MP	9.72 ± 5.78
51	5	YP	0.21 ± 0.12	5	MP	0.14 ± 0.13
52	2	YP	0.05 ± 0.05	2	MP	0.05 ± 0.0
53	5	YP	0.03 ± 0.02	5	MP	3.11 ± 1.21
55	3	F	0.02 ± 0.02	3	YP	3.92 ± 2.28
57	4	YP	0.05 ± 0.03	4	MP	8.53 ± 3.74
58	4	YP	0.35 ± 0.12	2	MP	3.43 ± 3.23
59	5	YP	0.11 ± 0.03	4	MP	1.01 ± 0.99
60	5	YP	0.07 ± 0.05	4	MP	5.43 ± 2.53
61	5	YP	0.26 ± 0.13	5	MP	1.33 + 1.05
62	4	YP	0.08 ± 0.02	4	MP	0.26 ± 0.15
63	5	YP	0.02 ± 0.19	5	MP	3.22 ± 2.10
64	3	YP	0.08 ± 0.02	3	MP	5.8 ± 3.64
65	5	YP	0.19 ± 0.11	4	MP	4.64 ± 4.58

Table 31A Cont:

Line	No.plants assessed on 21 Oct 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.	No.plants assessed on 17 Nov 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.
UPS No.						
66	5	YP	0.33 ± 0.16	5	MP	6.4 ± 2.65
67	5	MP	1.1 ± 0.64	4	MP	2.03 ± 1.21
68	5	YP	0.34 ± 0.09	5	MP	7.15 ± 5.48
70	5	YP	0.51 ± 0.23	3	MP	0.07 ± 0.04
72	5	YP	0.33 ± 0.11	4	MP	2.05 ± 0.89
73	4	YP	3.93 ± 2.41	4	MP	5.95 ± 2.26
74	4	YP	1.01 ± 0.55	4	MP	1.89 ± 1.02
76	4	YP	0.50 ± 0.14	3	MP	4.25 ± 3.25
77	5	YP	1.41 ± 1.18	4	MP	4.42 ± 2.17
78	4	YP	1.03 ± 0.49	4	MP	2.81 ± 1.62
79	5	YP	0.47 ± 0.31	4	MP	15.67 ±10.09
80	5	YP	0.29 ± 0.12	5	MP	10.05 ± 4.18
81	3	YP	0.57 ± 0.18	5	MP	7.06 ± 3.69
82	5	YP	0.13 ± 0.09	5	MP	1.21 ± 0.68
83	3	YP	0.52 ± 0.16	2	MP	1.55 ± 1.55
84	4	YP	0.25 ± 0.14	4	MP	1.18 ± 0.37
86	5	YP	0.71 ± 0.43	5	MP	17.43 ± 8.88
87	4	YP	0.43 ± 0.18	4	MP	1.11 ± 0.96
89	4	YP	0.06 ± 0.01	4	MP	4.43 ± 2.85
90	3	YP	0.07 ± 0.02	3	MP	5.04 ± 3.19
92	4	YP	0.09 ± 0.03	3	MP	5.5 ± 2.78
93	5	YP	0.14 ± 0.09	5	MP	2.93 ± 1.33
94	5	YP	0.12 ± 0.11	5	MP	16.33 ± 4.62
95	5	YP	0.08 ± 0.05	5	MP	10.11 ± 4.70
96	5	YP	1.86 ± 1.78	4	MP	16.05 ± 2.17
97	5	YP	0.03 ± 0.01	5	MP	13.59 ± 8.32
98	5	YP	0.92 ± 0.66	5	MP	7.33 ± 4.20
99	5	YP	0.44 ± 0.39	4	MP	7.04 ± 3.84

Table 31A Cont:

Line	No.plants assessed on 21 Oct 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.	No.plants assessed on 17 Nov 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.
UPS No.						
100	5	YP	0.16 ± 0.10	5	MP	11.77 ± 8.33
101	5	YP	0.31 ± 0.11	4	MP	7.4 ± 1.98
102	5	YP	0.56 ± 0.27	5	MP	2.9 ± 1.29
103	5	YP	0.67 ± 0.27	5	MP	24.81 ± 6.35
104	-	-	-	2	F	0.65 ± 0.55
105	5	YP	0.57 ± 0.39	5	MP	6.3 ± 2.37
107	4	YP	5.89 ± 3.90	3	MP	5.72 ± 2.56
109	5	YP	0.8 ± 0.4	4	MP	4.6 ± 3.33
110	5	YP	0.61 ± 0.31	5	MP	6.64 ± 5.22
111	5	YP	0.4 ± 0.2	5	MP	8.65 ± 4.89
112	5	YP	1.31 ± 0.96	4	MP	11.75 ± 3.25
113	5	YP	0.97 ± 0.25	5	MP	2.99 ± 1.89
114	4	YP	0.24 ± 0.11	4	MP	10.96 ± 5.99
115	5	YP	0.15 ± 0.04	4	MP	3.0 ± 1.85
116	3	YP	0.35 ± 0.10	3	MP	1.58 ± 1.3
119	4	YP	0.30 ± 0.23	4	MP	7.8 ± 4.31
120	5	YP	0.05 ± 0.02	5	MP	0.88 ± 0.54
121	4	YP	0.19 ± 0.12	3	MP	4.42 ± 0.59
122	5	YP	0.09 ± 0.04	5	MP	3.52 ± 1.36
124	4	YP	0.43 ± 0.26	4	MP	7.94 ± 3.56
128	5	YP	0.04 ± 0.02	5	MP	10.06 ± 2.54
129	4	F	0.03 ± 0.01	4	YP	0.01 ± 0.01
130	5	F	0.01 ± 0.01	4	YP	0
131	4	F	0.05 ± 0.5	4	YP	1.26 ± 1.19
132	4	F	0.04 ± 0.01	4	YP	9.18 ± 2.90
133	5	F	0.66 ± 0.47	5	YP	4.76 ± 2.58
137	5	YP	1.33 ± 0.36	5	MP	2.98 ± 1.67
138	4	F	0.06 ± 0.04	2	YP	3.85 ± 0.77

Table 31A Cont:

Line	No.plants assessed on 21 Oct 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.	No.plants assessed on 17 Nov 1977	[1]Growth stage	[2]Mean % leaf area diseased/ plant ± S.E.
UPS No.						
139	4	V	0.04 ± 0.02	4	F	3.31 ± 1.26
140	5	YP	1.6 ± 0.67	4	MP	0.9 ± 0.26
141	5	V	0.04 ± 0.03	5	F	3.91 ± 2.41
142	4	V	0.61 ± 0.36	4	F	10.75 ± 2.76
143	5	F	0.67 ± 0.04	5	YP	6.3 ± 2.26
144	5	V	0.94 ± 0.53	4	V	5.11 ± 2.26
146	5	V	0.32 ± 0.32	5	V	2.42 ± 0.48
147	4	V	0.71 ± 0.51	4	V	2.06 ± 1.03
148	4	V	0.54 ± 0.31	4	V	0.99 ± 0.59
149	5	V	1.81 ± 0.89	5	V	1.98 ± 0.88
150	2	V	0.08 ± 0.07	2	V	0.75 ± 0.67
152	-	-	-	2	F	0.55 ± 0.45
154	-	-	-	1	YP	1.55
156	2	V	0.1 ± 0.07	2	F	6.33 ± 1.49
157	3	V	0.05 ± 0.05	-	-	0.75 ± 0.35

Thailand 7 July 1978

1611/2	10	YP	0
1602/2	10	YP	0.01 ± 0.0

[1]V = vegetables, F = flowering, YP = young pod, MP = mature (dry) pod

[2]Based on standard area diagram disease key

Table 31B Comparison of the increase in severity of false rust on winged bean lines from Papua New Guinea from 21 Oct - 17 Nov 1977

Increase of mean % leaf area diseased/ plant	Line UPS No.
< 0.5	5, 11, 12, 14, 15, 16, 39, 47, 51, 52, 62, 70, 105, 129, 130, 140, 148, 149
0.5 - 3.0	1, 7, 10, 13, 18, 37, 59, 61, 67, 72, 73, 74, 78, 82, 83, 84, 87, 93, 102, 113, 115, 116, 120, 131, 137, 146, 147, 150, 157
> 3.0	3, 4, 8, 9, 17, 19, 20, 21, 22, 25, 26, 27, 29, 30, 31, 32, 34, 35, 38, 40, 41, 42, 43, 44, 45, 46, 48, 53, 55, 57, 58, 60, 63, 64, 65, 66, 68, 76, 77, 79, 80, 81, 86, 89, 90, 92, 94, 95, 96, 97, 98, 99, 100, 101, 103, 104, 107, 109, 110, 111, 112, 114, 119, 121, 122, 124, 128, 132, 133, 138, 139, 141, 142, 143, 144, 156

6.3.2.2.2 Regional winged bean variety trials

Disease was slight in the 1977 trial at Kuk and Port Moresby and differences between susceptibility of lines to S. psophocarpi were not apparent (Table 32A). Disease was more severe in the 1978 trials, especially at Aiyura and Lae. Comparison of disease indices at individual and combined sites (Table 32B) show that lines differ in susceptibility. The highest mean disease index was recorded for U.P.S., 99 and 122 whilst the lowest was U.P.S., 32 and 47.

Table 32 Susceptibility of winged bean lines to S. psophocarpi in regional trials

A. Assessment based on descriptive disease key

UPS No	Kuk				Port Moresby	
	Growth stage on 28 April 1978	Severity category	Growth stage on 7 July 1978	Severity category	Growth stage on 27 May 1977	Severity category
31	V	Nil	YP	Slight	YP	Slight
32	V	"	YP	"	YP	"
45	V	Slight	YP	"	YP	"
47	V	Nil	YP	"	YP	Moderate
53	V	Slight	YP	"	YP	Slight
62	V	"	YP	"	YP	"
99	V	Nil	YP	"	YP	"
102	V	"	YP	"	YP	"
121	V	"	YP	"	YP	"
122	V	Slight	YP	"	YP	"

B [1]Assessment based on standard area diagram disease key

UPS No	Mean % leaf area diseased/plant ± S.E.		
	Aiyura	Kuk	Lae
31	2.40 ± 0.83	1.18 ± 0.52	5.85 ± 1.29
32	2.13 ± 0.82	0.06 ± 0.02	5.07 ± 1.84
45	3.18 ± 0.94	0.07 ± 0.03	9.87 ± 4.66
47	0.58 ± 0.26	0.04 ± 0.02	13.93 ± 2.57
53	3.52 ± 1.12	1.75 ± 0.55	12.68 ± 1.75
62	3.15 ± 0.92	0.18 ± 0.08	5.96 ± 1.84
99	7.39 ± 2.09	2.92 ± 1.10	6.97 ± 2.31
102	1.31 ± 0.33	0.12 ± 0.06	9.91 ± 4.0
121	10.45 ± 1.43	0.69 ± 0.24	5.46 ± 3.13
122	10.81 ± 1.59	1.19 ± 0.09	10.09 ± 1.62

[1]Assessment at mature pod growth stage at all sites

6.4 DISCUSSION

Of the 10 systemic fungicides evaluated *in vitro* for inhibiting germination of sporangia of *S. psophocarpi* only tridemorph plus maneb, triforine, E.L. 222 and pyrazaphos proved effective. Although tridemorph was assayed in combination with maneb it is considered to have been the major inhibiting compound since maneb alone failed to inhibit sporangia germination completely even at 1000 ppm. The benzimidazole fungicides (bavistin, benomyl, thiobendazole) had little effect. Oxycarboxin and thiophanate methyl also failed to inhibit sporangia germination. Of the dithiocarbamate fungicides mancozeb and fentinacetate plus maneb were the most effective. Alicbusan (1965) also found mancozeb prevented sporangia germination *in vitro* although the concentration was not given. He considered the effectiveness of the fungicide was due to the high permeability of the sporangial wall. The fungicides Captan and panoctine were more effective inhibitors compared with the copper compounds (Bordeaux mixture, copper oxychloride and copper hydroxide). Only copper oxychloride of the copper fungicides achieved 100% inhibition which was recorded at 1000 ppm.

Disease severity throughout the duration of the fungicide field trial in both the treatment and control plots was slight. Although the trial had taken place in the wet

season, prolonged dry periods and persisted and these may not have favoured disease development. The lowest disease severity was recorded in plots sprayed with fentinacetate plus maneb and tridemorph plus maneb. Further fungicide trials are necessary, however, in areas where the continuity of rainfall is assured or by using overhead misting units to enhance the development of the disease before recommendations can be made. The timing of fungicide application needs to be critically examined as it is doubtful whether fortnightly sprays can be justified even in commercial production of winged beans. Given favourable environmental conditions (loc. cit.) the fungus is capable of producing more than one generation of sporangia during the production cycle of the crop. It is suggested, therefore, that in further trials disease assessments should be made at all of the crop's major growth stages.

Comparisons between fungicide treatments revealed no significant (P=0.05) differences in yield. This was not unexpected because of the slight disease severity recorded in the control plots.

All lines from the P.N.G., winged bean germplasm collection and one accession from Thailand were found to be susceptible to the natural inoculum of S. psophocarpi. Similar results for the P.N.G., lines were reported by Price (1980). Variation between lines, however, was apparent in the differences between severity levels recorded on two occasions.

results for the P.N.G., lines were reported by Price (1980). Comparisons of the P.N.G., and Thailand lines following inoculation, however, revealed that the Thailand lines were less susceptible. This is supported by Chomchalow (personal communication) who considered they were resistant. Accession No. 1602/1 appears to be resistant since no infection was recorded following artificial inoculation and only slight severity was recorded in field trials.

Variation existed between the number of successful infections within both the P.N.G., and Thailand lines. It is possible the method used for assessing leaf age was not sufficiently accurate and may account for the differences noted. Regional trials are necessary to establish the stability of the possible resistance of the Thailand lines under different environmental conditions and inoculum levels.

In the regional variety trials variation in disease severity was recorded between sites. The highest recorded at Aiyura and Lae and the lowest at Kuk. It is possible that the different environmental conditions at each site and different inoculum levels may be responsible for such variation. Variation in the severity of false rust was also apparent within the 10 winged bean lines, eg. at Lae the highest mean disease index recorded for U.P.S. - 47 and the lowest for U.P.S. - 32. Since valid statistical comparisons of susceptibility of only a few lines can be made in field trials at any one time there is a need for a simple preliminary screening to detect any differences before further trials are undertaken. The

artificial inoculation technique described may be suitable although further refinement is needed to determine critically the inoculum concentration and also a continuity of viable inoculum must be assured. The inoculum could be obtained from naturally or experimentally infected plants. It is possible that host resistance may be stable since no sexual stages have been conclusively isolated (Fitzpatrick, 1930; Karling, 1964; loc. cit.). It is also possible, however, that different races of the fungus exist in other parts of the world where winged beans are grown.

DISCUSSION

The studies on false rust and its causal agent S. psophocarpi described in this thesis were undertaken because of the importance of the disease in relation to the selection and breeding programme of the winged bean in P.N.G. Plant disease surveys revealed that the disease is widely distributed throughout the country in both traditional and non-traditional areas of cultivation and that in the Highlands this was predominately practiced during the dry season. Survival of the fungus in the absence of the crop is not understood since no alternate hosts or resting spores were found. It is possible perennation of the fungus may depend on winged beans being present throughout the year. The perennial nature of the plant and the overlap of cropping, particularly in parts of the Highlands of P.N.G., may ensure its survival.

Although no resting spores were isolated occasional pairing of zoospores was observed to take place which in S. endobioticum and S. fulgens results in zygote formation and resting spore development (Curtis, 1921; Kusano, 1930). It is not known, however, whether the pairing of zoospores observed in S. psophocarpi was a result of sexual fusion or incomplete cleavage in the cytoplasm. Pairing of zoospores in S. endobioticum (Curtis, 1921; Kohler, 1930), S. fulgens

(Kusano, 1928, 1930) and S. macrosporum Karling (Karling, 1960) occurred frequently and was considered to be sexual fusion suggesting a well developed sexual mechanism in Synchytrium. Occasional pairing has also been reported in S. longispinosum (Couch) Karling (Couch, 1931, 1937), S. brownii (Karling, 1954; Lingappa, 1958) and S. callirrhoeae Karling (Karling, 1958). In contrast Kusano (1930) failed to observe pairing in S. decipiens (Farlow) and S. minutum (Patouillard) Gäumann (Syn. S. puerariae (Henning) Sydow). He considered different species exhibited varying degrees of sexuality and that sexual regression may have occurred within the genus from obligatory sexual fusion to the total loss of sexuality. He also suggested that the occasional fusion observed in S. psophocarpi and other species may be attributed to the sex character which has not been completely lost. Studies on the genetics of S. psophocarpi and other species possible involving nuclei staining, however, are required before sexual pairing can be conclusively stated to occur.

Although resting spores are unknown in all members of the subgenus Woroninella (Fitzpatrick, 1930; Karling, 1964; loc. cit.) some species have the ability to survive adverse conditions. Karling (1936) reported that sori and sporangia of S. decipiens may remain dormant on the coats of underground fruits of Amphicarpa bracteata during the winter and infect

young seedlings in the following spring. Kusano (1932), however, considered S. decipiens survived the winter by dormant sori in the dead and dried vines and that S. minutum, parasitic on Pueraria triloba, overwinters by sori lying deep in the cortical tissue of the lower and older part of the vine which survives the winter. Kusano suggested that the absence of resting spores may be correlated to the tropical habitat and that species such as S. decipiens and S. minutum have only been able to extend beyond the tropics because of their adaptation for hibernation during the winter and their survival is not dependent on resting spores. He considered as this adaptation is connected with the winter season those species which occur in the tropics would simply repeat their life cycle throughout the year uninterrupted by a dormant period. This does not explain, however, how S. psophocarpi and other tropical species of Woroninella survive in the dry season when conditions are unfavourable.

The low sporangia concentration recorded during the dry season indicates that false rust can remain endemic during that period provided susceptible tissue is present. The periodicity of sporangia dispersal indicates that liberation occurs mainly during the day and sedimentation at night when conditions often favour germination. In the dry season germination and infection, therefore, probably occurs

mainly at night following dew formation. The duration of wetness associated with dew is most important since c. 1h is required for sporangia germination and 6h for infection (loc. cit.). During the wet season in the Highlands of P.N.G., these moisture requirements would frequently occur over extended periods. Temperatures in the Highlands are also mostly within the optimal temperature range for germination. The life cycle of S. psophocarpi given these favourable weather conditions is in the order of 3-4 wk (loc. cit.). Since the fungus appears to remain haploid throughout the cycle, provided susceptible host tissue is present, continuous and rapid production of sporangia is possible. Epidemics therefore are likely to develop rapidly if no control measures are practiced.

Fungicide sprays of fentinacetate plus maneb applied at 2 wk intervals reduced the severity of false rust although there was no significant increase in either pod or seed yield. De Vera (1973, 1977) claimed that the disease could cause a reduction in both tubers and pods. However since no quantitative data was given to support this conclusion the relationship between yield loss and disease severity is yet to be established.

Although all lines screened from the P.N.G., germplasm were susceptible to false rust variation in this susceptibility was apparent in regional trials. The reason for this

variation is not known although Preston (1977) isolated several strongly antifungal phytoalexins from winged beans which were produced in relatively substantial quantities in response to the action of Botrytis fabae Sardina or B. cinerea Pers. ex. Fr., on pods and cupric chloride on leaves and pods. He suggested (personal communication) that these phytoalexins may be important in the susceptibility of winged beans to S. psophocarpi. Further work is needed to establish if S. psophocarpi also stimulates the production of these compounds and, if so, to compare their production between lines. Resistant winged bean lines were suspected amongst the Indonesian germplasm by Aminah-Lubis (personal communication) and was confirmed by Thompson and Haryono (1979). Chomchalow (personal communication) also considered some of the Thailand lines were less susceptible and, although this was supported following artificial inoculation of the fungus (loc. cit.), is yet to be confirmed in field trials. Two closely related species of the winged bean, P. palustris and P. scandens, are immune to S. psophocarpi (Price, 1980; loc. cit.) and it may be possible to transfer this resistance. Attempts to hybridize winged bean with P. scandens (Erskine, 1980), however, were unsuccessful and further investigations to explain this failure have recently been made (Pickersgill, 1980).

The movement of propagative material from infected areas is of significance since the fungus appears not to be seed borne (loc. cit.). Previously Highlanders migrating to the lowland areas of P.N.G., and plant collectors have been responsible for the movement of false rust probably by carrying infected parts. Therefore movement of pods and any other vegetative part of the plant should be restricted and only clean seed should be used. With the international exchange of germplasm this finding has even more importance.

In the short term for the subsistence grower since fungicides are inappropriate and resistant varieties are not widely available cultural methods offer the best means of control. Plantings should be made in the dry season and the practice of mixed cropping and the use of several varieties, as practiced in many of the traditional areas of winged bean cultivation in P.N.G. (Khan, 1976), should be encouraged.

Appendix 1

Accession numbers of fungi and nematodes recorded attacking winged bean in Papua New Guinea

Fungi	Accession No.
Oidium spp.	P.N.G. 10030
Pseudocercospora psophocarpi	P.N.G. 10030b
	P.N.G. 10050
	I.M.I. 199375
Synchytrium psophocarpi	P.N.G. 2334
	I.M.I. 77902
Thanatephorus cucumeris	I.M.I. 199337a
Nematodes	
Meloidogyne incognita	P.N.G. 2208
M. javanica	C.I.H. 42/76(3), 42/76(4)

Appendix 2

Calculation of sporangia counted on spore trap slide to give an estimated number per m^3 of air

Calculated from the rate of slide movement, dimensions of the scanning traverse and the suction rate.

1. Fraction scanned at each traverse

Rate of slide movement = 2mm/h

Length of 24h trace = 24 x 2mm

= 48mm

Dimensions of scanning traverse = 14 x 2mm

At each traverse fraction of deposit scanned =

$$\frac{\text{Area of 1 h deposit}}{\text{Area of total deposit}} = \frac{14 \times 2}{48 \times 14}$$

= 0.041

2. Volume of air sampled

Suction rate = 10l/min

Volume of air sampled per day = 10 x 60 x 24l/day

= $14.4 m^3$/day

3. Conversion factor

Volume of air sampled at each traverse $= 0.041 \times 14.4$

$= 0.59 m^3$

$\therefore$ to convert number of sporangia counted at each traverse to an estimated number per m^3 of air multiply by a factor of $\frac{1}{0.59}$.

Appendix 3

Calculation of the volume of air sampled using a rotorod air sampler

Calculated from the dimensions of the sampling rods and their revolution rate.

Rod dimensions:

Diameter (d) = 0.167 cm

Height (h) = 6.0 cm

Distance between rods = 8.0 cm

Circumference (c) = 25 cm

Volume of air sampled by:

(a) one rod per rev = d x h x c = 25 cc

(b) two rods per rev = d x h x c x 2 = 50 cc

Revolution rate of sampler = 2700 cc/min

$\therefore$ volume of air sampled = 50 x 2700 cc/min

= 135 l/min

Appendix 4

Thirty day means for sporangia concentration and weather factors from 12 Feb - 13 March 1977

Hour	Sporangia/ m^3 of air [1]	Sporangia/ m^3 of air [2]	Temp (°C)	R.H. (%)	Rain (mm)	Solar rad. (Cal/cm^2/h)	Dew (h)	Windspeed (km/h)
0800	6.4	4.3	24.8	92.0	0.22	11.9	0.31	9.2
0900	10.3	4.8	26.5	83.8	0.02	25.0	0.21	11.1
1000	12.4	8.0	28.3	75.2	0.08	39.0	0.03	16.7
1100	9.5	8.0	29.2	71.8	0.07	54.6	0	17.2
1200	8.9	7.4	30.1	70.0	0.03	55.4	0	18.9
1300	15.8	9.3	30.3	68.0	0.03	55.5	0	20.5
1400	21.1	10.0	30.6	67.0	0.53	56.2	0	19.7
1500	44.2	12.9	30.5	68.7	0.17	45.4	0	16.1
1600	56.9	13.5	30.1	70.3	0.02	29.2	0	16.6
1700	82.8	13.6	29.4	75.5	0.13	19.4	0	11.0
1800	60.1	13.1	28.4	82.9	1.82	5.9	0.03	7.4
1900	22.4	12.8	26.3	90.2	0.23	0	0.07	6.7
2000	11.8	7.6	26.6	93.1	0.43	0	0.1	7.3
2100	9.0	5.8	26.2	94.7	0.93	0	0.17	7.0
2200	9.8	6.1	25.8	95.2	0.27	0	0.21	9.7
2300	11.2	6.2	25.6	96.2	0.02	0	0.21	9.6
2400	11.1	7.2	25.1	96.4	0.73	0	0.21	7.4
0100	10.5	5.9	25.0	96.0	0	0	0.24	6.9
0200	7.3	5.0	24.6	95.0	0.2	0	0.24	9.6
0300	6.9	5.3	24.5	95.4	0.02	0	0.31	8.2
0400	5.7	4.5	24.3	95.1	0.72	0	0.34	8.0
0500	3.7	3.3	24.2	94.9	0.32	0	0.41	9.1
0600	1.2	1.7	24.1	95.2	0.17	0	0.45	8.7
0700	0.7	1.4	24.1	93.3	0.08	2.0	0.38	7.7

[1] Arithmetic means
[2] Geometric "

Appendix 5

Weekly means for sporangia concentration and weather factors

Week	Sporangia/ m^3 of air [1]	[2]	Hours $t < 26^{o}C$	Hours r.h. > 90%	Rain (mm)
1- 7 Dec 1976	0.5	0.01	10.86	17.0	16.24
8-14	0.71	0.13	10.0	12.86	4.91
15-21	0.79	0.13	8.71	13.71	15.59
22-28	0.5	0.01	8.57	13.29	0.64
29- 4 Jan 1977	0.21	0.1	12.0	13.29	7.39
5-11	0	0	14.0	14.0	5.76
12-18	0.07	0.1	11.57	11.43	3.79
19-25	0.71	0.13	9.86	13.42	4.67
26- 1 Feb	0.29	0.01	11.29	12.71	0.39
2- 8	0.64	0.01	10.47	13.57	11.27
9-15	1.33	0.1	10.29	13.71	2.17
16-22	1.82	1.7	11.43	14.86	12.54
23- 1 March	44.6	14.3	9.57	14.43	4.4
2- 8	66.3	14.6	9.29	9.86	6.41
9-15	17.6	13.1	11.57	12.71	4.04
16-22	16.9	8.2	14.43	14.43	5.7
23-29	3.2	2.1	10.43	14.14	0
30- 5 April	1.07	0.6	8.71	15.0	0
6-12	15.6	12.94	9.57	14.71	1.34
13-19	16.0	5.35	11.14	16.0	14.29
20-26	5.36	2.87	12.71	14.43	4.43
27- 3 May	2.29	1.89	11.14	15.71	4.04
4-10	1.5	1.43	10.57	15.29	0
11-17	90.93	14.11	12.0	16.0	0.54
18-24	7.29	2.25	17.14	17.29	6.96
25-31	1.57	1.68	13.57	16.57	1.36

Week	Sporangia/ m^3 of air		Hours $t < 26^{o}C$	Hours r.h. > 90%	Rain (mm)
1- 7 June	0.14	0	10.29	9.71	3.77
8-14	0	0	10.29	10.86	0
15-21	5.43	4.27	5.86	13.43	0
22-28	78.0	13.4	16.57	15.0	1.9
29- 5 July	3.14	1.97	18.14	10.14	7.67
6-12	4.93	3.15	14.86	16.29	4.73
13-19	0.57	0.48	14.71	13.57	0.76
20-26	0	0	13.67	17.33	2.2
27- 2 Aug	7.5	1.85	14.0	14.0	0.84
3- 9	0.5	0	16.0	11.14	1.1
10-16	10.79	0	13.7	11.71	1.93
17-23	0.14	0	16.86	10.29	0.07
24-30	0.71	0	15.0	6.0	0
31- 6 Sept	0	0	15.0	12.0	0
7-13	0	0	13.71	15.57	1.0
14-20	0	0	15.28	9.0	3.9
21-27	0.43	0	12.86	8.29	0
28- 4 Oct	0.29	0	13.0	13.86	1.63
5-11	0.36	0	15.25	13.5	0
12-18	0.79	0	13.71	8.14	0
19-25	2.07	1.32	11.57	11.14	3.43
26- 1 Nov	1.71	0.37	14.14	9.43	4.4
2- 8	3.57	3.47	13.0	10.29	0
9-15	5.36	2.57	10.14	7.86	0
16-22	0	0	8.14	11.57	1.23
23-29	0	0	7.42	6.86	0

[1] Arithmetic means

[2] Geometric means

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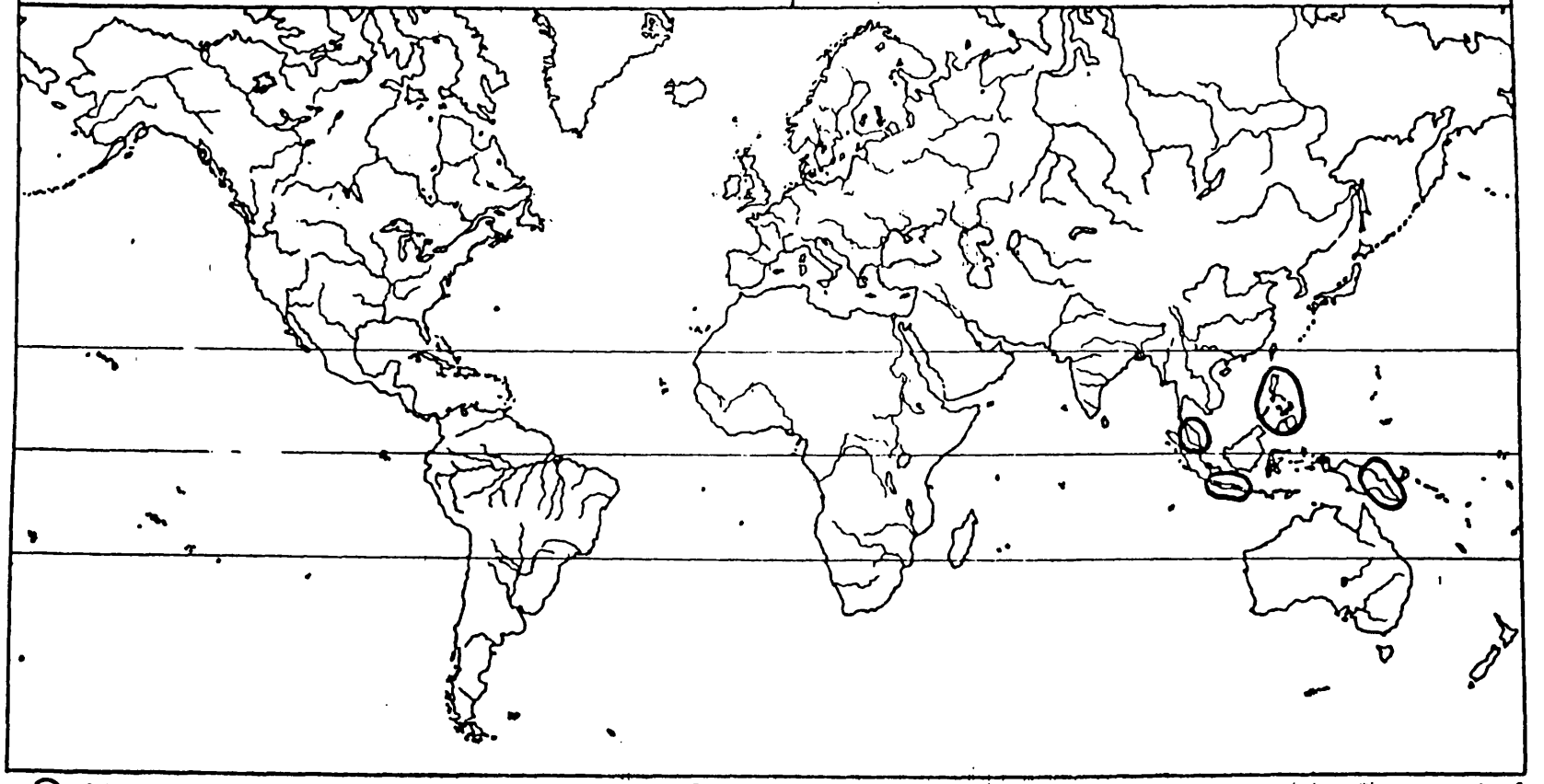
COMMONWEALTH MYCOLOGICAL INSTITUTE

DISTRIBUTION MAPS OF PLANT DISEASES

Map No.535 Edition 1 Issued 1.iv.1980

Pathogen: Synchytrium psophocarpi Racib.

Hosts: Winged bean (Psophocarpus tetragonolobus)



For list of countries in which this disease has been recorded, see overleaf

Map No. 535 Edition 1

ASIA
Indonesia (Java) (Raciborski, Parasitische Algen. und Bilze Java's I, 1900; 57, 5790)
*Malaysia (W.) (Thompson & Johnston, 34:548)
*Philippines (Reinking, Philipp. Agric. 8 (1-2), 1919 as Woroninella psophocarpi)

AUSTRALASIA & OCEANIA
*Papua New Guinea (43, 968)

NOTE: See M.J. Drinkall, PANS 24 (2): 160, 1978 (57, 5790)

* Specimens in Herb. IMI

Numbers in brackets, e.g. (54, 1234) refer to abstracts in the Review of Plant Pathology

PANS 24(2): 160–166

False Rust Disease of the Winged Bean

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Summary. The winged bean *(Psophocarpus tetragonolobus),* a little known tropical legume, is currently attracting international interest as a plant that may have considerable agricultural potential. The plant has, until recently, been considered as a vegetable of minor importance and information on pests and diseases which attack it is scant, fragmented and often unpublished. Current information on false rust, a major disease of the winged bean in Papua New Guinea, is reviewed. The disease is also known to occur in Java, the Philippines and Malaysia. Any of the above ground parts of the winged bean may be attacked, resulting in severe galling and malformation. The causal agent is an obligate fungal parasite *Synchytrium psophocarpi.* The parasite is simple in structure, consisting of sori, sporangia and zoospores and has a short life cycle. Sporangia are short lived and dispersed mainly by wind. The parasite is thought to be host specific and it is not known whether resting spores exist. Possible methods for field control of the disease have not been tested.

Introduction

The winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a tropical legume belonging to the family *Leguminosae* and sub-family *Papilionoideae* (Purseglove, 1974). It is almost exclusively grown in Papua New Guinea and southeast Asia (Anon., 1975) although its origin is uncertain. Rumpf first recorded winged beans growing in the Moluccas in the seventeenth century and considered that they had been brought there from Java (Purseglove, 1974). Burkill (1936), however, suggested that the plant was introduced to Asia from Madagascar or Mauritius, whilst Degener (1945) attributes its origin to India or Mauritius.

Masefield (1961, 1967) first drew attention to the agricultural potential of the winged bean. This was later supported by Pospisil *et al.* (1971). This potential is largely due to the high protein content of the seeds, pods, leaves and roots, which are all edible, and the increase in soil fertility brought about through symbiotic nitrogen fixation (Masefield, 1973). Khan (1976) reported that Papua New Guinea (PNG) was a centre of genetic diversity of winged bean and initiated the world's first germplasm collection of the species.

In this paper false rust, which is one of the major diseases of winged bean in PNG (Price, 1976), is reviewed. Disease distribution, economic importance and diagnostic symptoms are included with a description of the causal agent.

Distribution and economic importance of false rust

False rust was first recorded in Java (Raciborski, 1898). It has also been recorded in the Philippines (Reinking, 1918, 1919; Sydow and Petrak, 1928; Baker, 1931), Malaya (Thompson and Johnson, 1953) and PNG (Shaw, 1963). Surveys in 1976 and 1977 (Drinkall, M. J., and Price, T. V. (1977). *Survey of winged bean diseases in the Highlands.* Unpublished report of the Faculty of Agriculture, University of Papua New Guinea.) of subsistence gardens show the disease to be widespread in the Western and Eastern Highlands of PNG where winged beans are traditionally grown. Pospisil *et al.* (1971) mention the occurrence of the disease on winged beans in West Africa but this has not been confirmed.

Some workers (Pospisil *et al.*, 1971; Masefield, 1973) have reported that there are no serious pests or diseases on the crop. Reinking (1918, 1919) however stated that false rust was a disease of economic importance in the Philippines whilst De Vera (1973) reported that false rust caused a severe reduction of both tubers and pods. There is however no quantitative data available relating yield loss to disease severity. Both workers reported that infected pods were undesirable for use and were unsaleable in the market. However, in Java winged beans are traditionally artificially infected with the disease and subsequently steamed and eaten as a delicacy (Degener, 1945). Satrapradja *et al.* (1975) also reported that attacked pods are considered a delicacy in the Sundanese area of Java.

Symptomatology

An early diagnostic symptom of the disease is the appearance of yellow galls. These galls are semi-globular in shape and measure 180–460 μm in diameter; they are slightly raised above the surface of the host and are usually closely aggregated (Karling, 1964). Open galls are crater-shaped and were often mistaken by the early mycologists (Dietel, 1900; Sydow and Sydow, 1903) for various stages of rusts – hence the name 'false rust'. The open galls were frequently referred to as 'aecidia' or 'uredia' (Gaumann, 1927). Gall formation may occur on any of the above-ground parts of the winged bean, i.e., leaves, stems, flower buds and pods (Gaumann, 1927; De Vera, 1973) and at any stage of growth (De Vera, 1973).

Leaf symptoms

Alicbusan (1965) observed that infection could occur anywhere on the leaf epidermis, both on the upper (Fig. 1) and lower surfaces (Fig. 2). His observations indicated that the lower epidermis was usually more heavily infected especially along the primary and secondary veins (Fig. 3) and young leaves were very susceptible to infection. Young leaves rarely attained normal size (Reinking, 1919; De Vera, 1973) but assumed abnormal shapes and became thickened (Reinking, 1919) and in severe cases cupping and curling occurred (De Vera, 1973) (Fig. 4).

Stem symptoms

The entire stem may become covered with galls (Fig. 5) and growth of apical buds is often arrested, leading to the formation of side shoots. These gnarled, twisted side shoots become bunched together at the nodes (Fig. 6) and the internodes are longer and thinner than healthy ones (De Vera, 1973). Growth is frequently checked completely (Reinking, 1919).

Flower symptoms

The sepals are the only parts of the flower observed to be attacked (De Vera, 1973) (Fig. 7). De Vera suggested this may be because the petals and reproductive parts of the flower are fast growing and short lived.

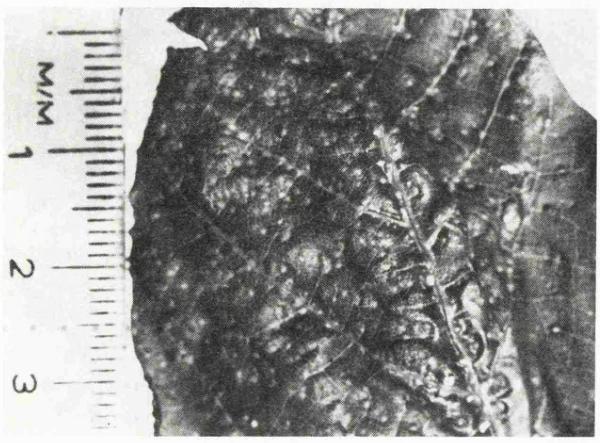

Fig. 1. Galling of upper leaf surface.

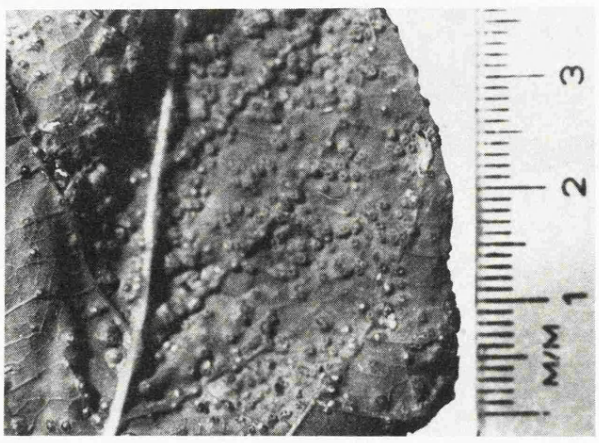

Fig. 2. Galling of lower leaf surface.

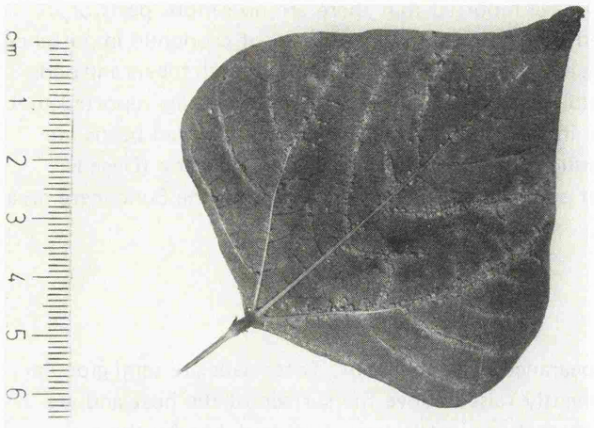

Fig. 3. Galls along primary and secondary veins of lower leaf surface.

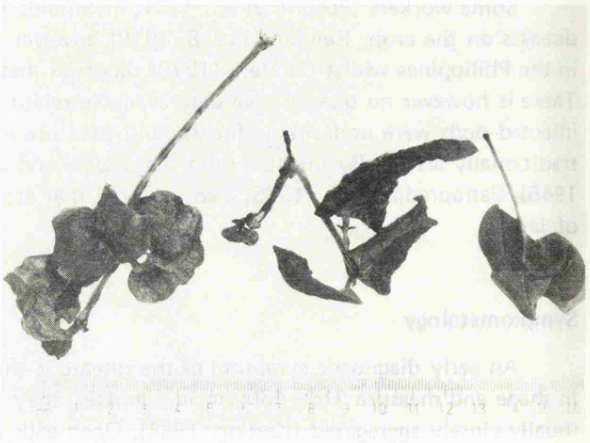

Fig. 4. Cupping and curling of young leaves.

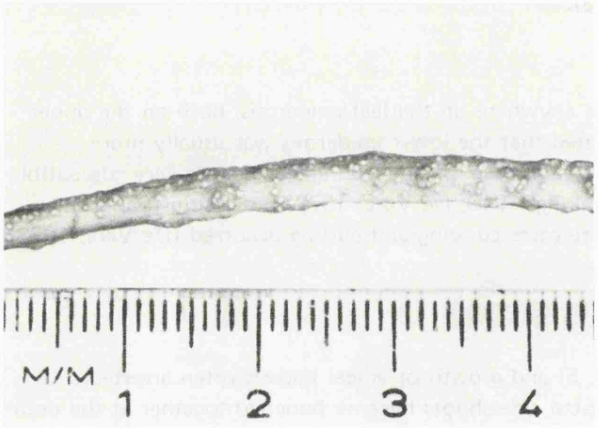

Fig. 5. Galling of young stem.

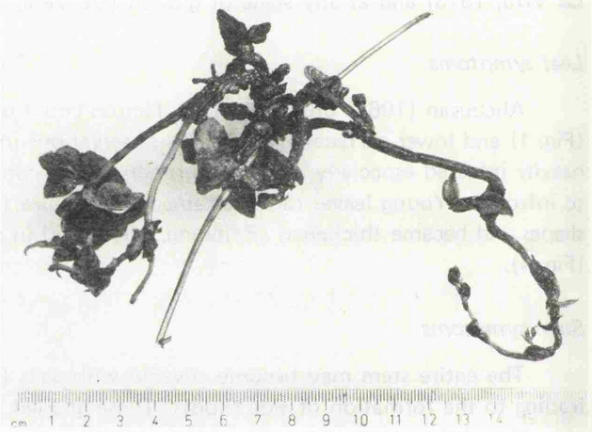

Fig. 6. Gnarled, twisted side shoots bunched together at node.

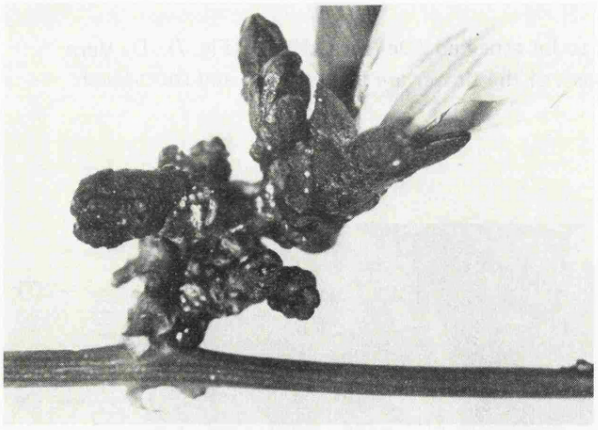

Fig. 7. Galling of sepals.

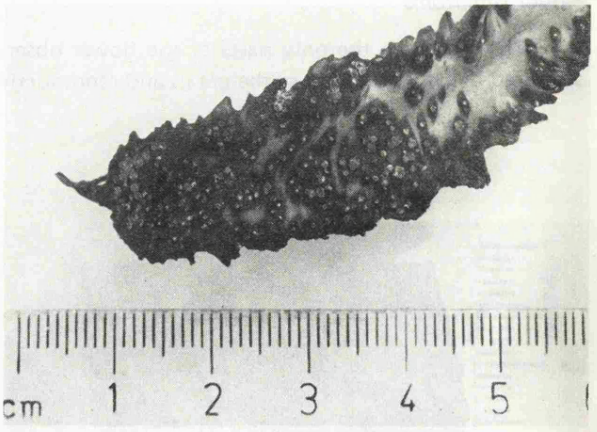

Fig. 8. Galling of pod giving a 'freckled' appearance. Note the blackening of the pod around each gall.

Pod symptoms

Pods may become completely covered with galls giving a 'freckled' appearance (Alicbusan, 1965) (Fig. 8). The growth of the young infected pods may be arrested or abnormal, the latter resulting in malformation (Reinking, 1919; De Vera, 1973). Mature pods can also be infected although malformation does not occur in this case (Alicbusan, 1965).

The causal agent

Classification

Raciborski (1898) first described the causal agent as an obligate fungal endoparasite belonging to the order Chrytridiales and erected the genus *Woroninella;* hence the fungus became known as *W. psophocarpi.* He described the zoospores as biflagellate and regarded the fungus as being intermediate between *Woronina* and *Synchytrium.* Sydow and Sydow (1903) thought that the organism was a rust and renamed it *Uromyces psophocarpi.* Sydow (1914) later agreed with Raciborski's classification. Further investigations by Gäumann (1927) however, revealed the zoospores to be uniflagellate and he reduced the genus *Woroninella* to form a new subgenus in *Synchytrium;* hence the fungus is known as *S. psophocarpi* (Rac.) Gaumann, subgenus *Woroninella.*

Description

The parasite is without mycelium and consists of sori, sporangia and zoospores and its life cycle is completed in a short time (in Karling, 1964).

Ovoid or subspherical sori are yellow-orange in colour and lie solitary in the epidermal cells and measure 140–230 μm in diameter, (Karling, 1964) (Fig. 9). Immature sporangia have a thin hyaline wall enclosing finely granulated yellow protoplasm; on maturation the wall thickens and the protoplasm becomes more coarsely granulated and orange in colour (Alicbusan, 1965). The shape and size of the sporangia vary from globose (20–25 μm diameter), sub-globose, ovate (19–34 x 17–26 μm diameter), elongate (16 x 50 μm diameter), to angular polyhedral and irregular (Karling, 1964) (Fig. 10). The zoospores are hyaline, pyriform in shape and measure 3–3.5 x 6–8 μm in diameter (Raciborski, 1898). They possess an orange fat globule and a basal dot-like blepharoplast, where the single whiplash-type flagellum is inserted (De Vera, 1973) (Fig. 11). Raciborski (1898) reported the flagella length to be 5–8 μm.

Epidemiology

Germination of sporangia

The effect of temperature, pH and aeration on the germination of sporangia *in vitro* was investigated by Alicbusan (1965). He found that germination occurred within a temperature range of 24–36°C. There was no optimum pH value; germination occurred over a range of pH 5–10. He suggested that exposure to the air was necessary for rapid and high percentage germination; sporangia which floated on the surface of a water film germinated whereas those which sunk to the bottom failed to germinate. Given the necessary conditions mature sporangia suspended in sterile distilled water germinated in 1 to 2 hours (Alicbusan, 1965; De Vera, 1973). The effect of relative humidity on germination has not been investigated.

Alicbusan (1965) stated that 30–50 zoospores were released from a sporangium and De Vera (1973), 200. The number and size of sporangia used in their estimations were not given and it is difficult therefore to make valid conclusions from their statements.

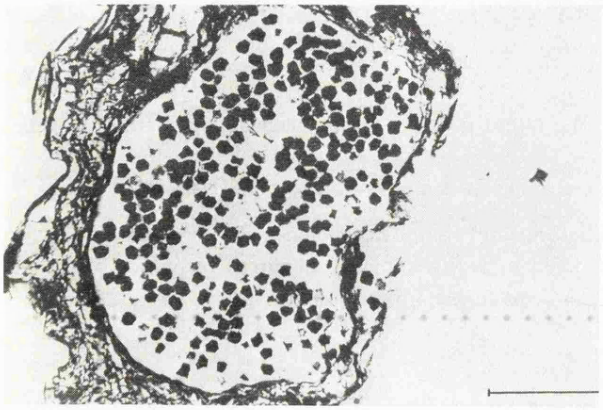

Fig. 9. Cross section of sorus of *Synchytrium psophocarpi.* Bar represents 60 μm.

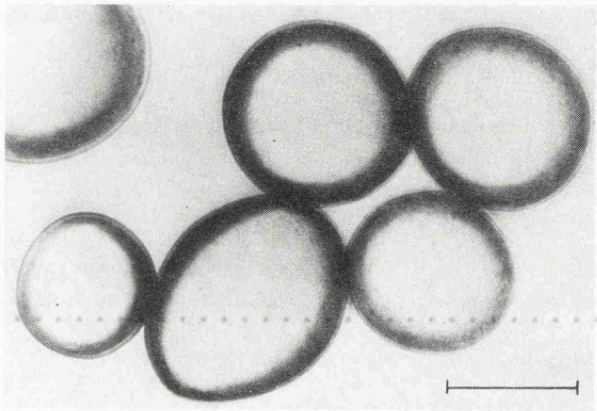

Fig. 10. Globose and ovate sporangia of *S. psophocarpi.* Bar represents 15 μm.

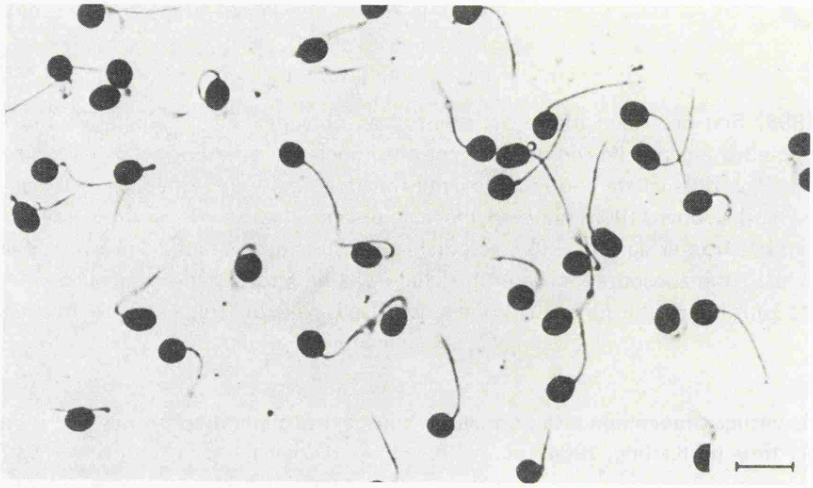

Fig. 11. Zoospores of *S. psophocarpi* fixed in 2% osmic acid and stained in 0.04% crystal violet. Bar represents 6 μm.

Parasite penetration

The process of parasite penetration was studied by De Vera (1973). Germinated zoospores are motile in water for 2–3 hours. During this period some come to rest on the host epidermis. A slight flattening of the penetrating zoospore occurs and the whole zoospore enters the epidermis directly. The mechanism of entry is unknown.

Host-parasite interaction and sorus development

Host-parasite interaction and sorus development have also been studied by De Vera (1973). The parasite travels intracellularly by a process resembling protoplasmic migration and penetrates four to six cells deep into the parenchyma tissue. It is thought to enter the host cell wall by either pressure or enzymatic action or a combination of these. After four days the parasite almost fills the infected cell and in most cases invades two or more cells as it enlarges. Although there is no evidence it is thought that the parasite either produces a growth promoting substance or induces hormone production in the plant since hypertrophy of infected cells occurs.

As the parasite develops its single nucleus disappers leaving behind uniformly granular protoplasm. The initials of the secondary nuclei are visible in the central portion of the parasite 11 or 12 days after penetration of the epidermis. The formation of these nuclei is followed by cleavage of the protoplasm which form the sporangia. The entire protoplast of each sporangium becomes segmented into as many parts as there are nuclei. These parts develop into the uninucleate zoospores. There is no information as to the number of sporangia produced per sorus.

After cleavage the sorus expands and eruption of the galls is brought about by the combined turgor pressure of the fungus and the sheath cells which surround the gall.

Incubation and latent periods

The incubation period is ten days (Alicbusan, 1965). The latent period has not been determined.

Dispersal

Following eruption of the galls the sorus membrane ruptures and the powdery mass of sporangia is disseminated by wind (Karling, 1964). Alicbusan (1965) reported that dissemination occurred by wind, insect and other natural agencies. However, there is no quantitative evidence to support these statements.

Viability

Alicbusan (1965) stated that sporangia kept under natural environmental conditions and in the laboratory remained viable for four to six months respectively. His conclusions are questionable since infected material was placed in a covered glass container prior to being placed in the field and so natural conditions did not prevail.

Germination tests were not carried out and viability was based only on sporangia colour and shape. De Vera (1973) reported that sporangia became dormant and failed to germinate after one week's storage at −10°C. This is supported by Drinkall (unpublished) who found sporangia lost their viability after four days under field conditions.

Host range and field survival

Gaumann (1927) failed to infect *Vigna sinensis* L. and *Phaseolus lunatus* L. with sporangia of the parasite and came to the conclusion that the fungus was host specific. This was supported by Alicbusan (1965) who failed to infect a range of legumes and other plants using zoospore suspensions.

It is not known how the parasite survives in the absence of the winged bean host, if indeed it does, since no alternate hosts have been found nor have any resting spores conclusively been isolated. Alicbusan (1965) claimed to have produced resting spores on infected winged bean tissue buried in the soil. De Vera (1973) repeated this work but was unable to produce any resting spores and considered the technique questionable. She stated that the use of non-sterile soil could not establish beyond doubt whether the spores described by Alicbusan (1965) were in fact contaminants.

Control

Possible methods for field control of the disease have not been tested although Tindall (1968) suggested that spraying with copper based fungicide will reduce damage. All lines currently being screened at the University of PNG are susceptible (Price, unpublished). Alicbusan (1965) reported that ten fungicides were effective in inhibiting the germination of sporangia *in vitro*. He suggested that the removal of infected plants at an early stage of infection could restrict the development of the disease within the crop.

Acknowledgements

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STUDIES OF *SYNCHYTRIUM PSOPHOCARPI* ON WINGED BEAN IN PAPUA NEW GUINEA

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Sporangia of *Synchytrium psophocarpi* (Rac.) Gäumann from Papua New Guinea were globose or ovate, had hexagonal walls and ranged from 12·5 to 27·0 (21·4) μm diam, and 20·0 × 15·0 to 35·0 × 22·5 (25·2 – 19·4) μm. Unopened sori contained between 4272 and 4890 sporangia. Germination occurred at temperatures of 5–30 °C and 84–100% r.h., in the presence of free water. Each sporangium contained between 102 and 156 zoospores; these emerged after 40 min incubation and maximum germination occurred after 70 min. The viability of sporangia from unopened and recently opened sori was greater than sporangia from old sori. Sporangia exposed to the air lost their viability after 4 days; this increased to 24 days when stored in a sealed plastic box at 5°. Live zoospores were pyriform and measured 5·0 × 2·5 to 10·0 × 5·0 (6·9 × 4·6) μm whilst fixed zoospores were spherical and measured 2·5 × 2.5 to 5·0 × 3·0 (3·34 × 2·5) μm. Flagellar length of recently emerged zoospores varied from 7·5 to 16·25 (12·98) μm. Zoospores lost their motility 30 min after emergence from the sporangium and encysted after a further 2–3 h; encysted zoospores were spherical and measured 3·0–6·0 (4·39) μm diam. The flagella were withdrawn by vesicular retraction.

The winged bean, *Psophocarpus tetragonolobus* (L.) DC. is a tropical legume that is almost exclusively grown in Papua New Guinea (PNG) and South East Asia (Anon., 1975). There is currently international interest in this crop since it has considerable agricultural potential (Anon., 1975). This is largely due to the high protein content of the seeds, pods, leaves, and roots, all of which are edible, and the increase in soil fertility through symbiotic nitrogen fixation (Masefield, 1973).

The potential of the winged bean to alleviate protein malnutrition in S.E. Asia resulted, in 1973, in a full scale research programme on the crop by the University of Papua New Guinea (U.P.N.G.) and the Department of Primary Industry. Khan (1976) reported that PNG was a centre of diversity of winged beans and initiated the first germplasm collection of the species.

The winged bean has, until recently, been considered as a vegetable of only minor importance and this has resulted in a lack of quantitative data on pests and diseases. False rust or orange gall caused by *Synchytrium psophocarpi* (Rac.) Gäumann is one of the major diseases of this crop in PNG (Price, 1976) and also occurs in Java (Raciborski, 1898), the Philippines (Reinking, 1918, 1919; Sydow & Petrak, 1928; Baker, 1931) and Malaya (Thompson & Johnston, 1953). The parasite infects the leaves, stems, flower buds and pods and causes severe galling and malformation of these parts. The symptoms are well-documented (Raciborski, 1898; Reinking, 1918, 1919; Alicbusan, 1965; De Vera, 1973) but the only studies on the biology of the organism are those of Raciborski (1898), Gäumann (1927), Alicbusan (1965) and De Vera (1973).

The importance of this disease to the selection and breeding programme of winged bean at the University of Papua New Guinea and the need for information on epidemiology and control of the disease resulted in further studies of the biology of the fungus in PNG which are reported here.

MATERIALS AND METHODS

Slides and coverslips

Microscope cavity slides (75 × 25 × 3 mm) and coverslips (22 mm²) were made grease free by washing with 'Pyroneg' (Diversey Pty Ltd), rinsing three times in tap water, followed by a final rinse in distilled water. They were then transferred to 95% ethanol and stored until required.

0007-1536/79/2828-4650 $01.00

Sporangia

Sporangia were removed from erupted sori on infected leaves with a mounting needle whilst viewing under a stereomicroscope (× 20).

Size of sporangia

Sporangia were transferred to a small drop of tap water which had been placed in the centre of a coverslip by means of an Arnold Hand Microapplicator (Arnold, 1967), and a hanging drop preparation made according to the method of Iino & Enomoto (1971). One hundred sporangia were measured at a magnification of × 600. This was repeated three times on sporangia from different sori.

Germination of sporangia

Sporangia were counted as germinated if they were empty, partially empty or showed zoospores actively emerging. Germination was tested by placing sporangia from erupted sori in cavity slides which were partially filled with sterile, distilled water. These slides were placed in slide racks and incubated at 100% r.h. in sealed plastic boxes which had been equilibrated for 24 h at 25 °.

Effect of temperature on germination

The effect of temperature was measured by placing sporangia from a single sorus in seven cavity slides. A mark was made on the edge of each cavity and fifty sporangia were assessed for germination in each slide prior to incubation. This was repeated five times using sporangia from different sori. Each box of replicates was placed in incubators held at 5, 10, 15, 20, 25, 30 and 37° and germination was assessed after 24, 48 and 72 h.

Effect of relative humidity and surface wetness on germination

The effect of relative humidity was investigated by placing saturated salt solutions in the plastic boxes to maintain the following relative humidities: $NaNO_2$, 66%; KBr, 84%; $ZnSO_4.7H_2O$, 90%; $PbNO_3$, 98%; H_2O, 100%. The effect of surface wetness was investigated by placing one set of sporangia in cavity slides that did not contain water. Germination was assessed before and after 24 h incubation at 20°. Four replicates were used for each treatment and fifty sporangia were counted in each replicate.

Germination process and number of zoospores per sporangium

Sporangia were placed in a hanging drop of 1% carboxy methyl cellulose and incubated at 25°. The sequence of germination was observed microscopically and the number of zoospores emerging from a single sporangium were counted on five separate occasions. These sporangia were all the same size.

Rate of germination

One hundred sporangia in each of four replicates were assessed for germination at 10 min intervals for the first 2 h and at 4, 6 and 24 h after incubation at 25°.

Effect of sporangial age on viability

One hundred sporangia from an unopened sorus, a recently erupted sorus and one that had been opened for some time were assessed for germination before and after 24 h incubation at 25°. This was repeated eight times using sporangia from different sori.

Effect of storage on viability of sporangia

Infected leaves and stems were removed from plants growing in the University Experimental Garden and placed in terylene net bags (1 mm^2 mesh) and air-tight plastic boxes. One bag was placed in the field at U.P.N.G. 2 m above ground level, another bag was held at room temperature (25–28°), one bag and one box were placed in a refrigerator held at 5° and one bag and one box in a deep freeze held at −85°. Outdoor measurements of temperature, relative humidity and rainfall were obtained from recording instruments at the University Meteorological Station. Room temperature and humidity were recorded on a thermohydrograph.

Sporangia from erupted sori were removed from each treatment on the day of collection (day 0) and 1, 2, 3, 4, 12, 16, 20 and 24 days later and assessed for germination after 24 h incubation at 20°. Three replicates, each from different sori, were prepared from each treatment and 100 sporangia were counted in each replicate.

Number and viability of sporangia in a single sorus

A single unopened sorus was removed from a leaf surface with mounting needles whilst viewing under a stereomicroscope (× 20) and the sporangia were teased out under distilled water. Germination of all the sporangia was assessed after 24 h incubation at 20°. This was repeated twice using sori of the same diameter.

Size of fixed zoospores and flagella length

Sporangia were germinated in a drop of water and the emerged zoospores were fixed by exposure to 2% osmic acid for 1 min, stained in a 0·04% solution of crystal violet, dried in a desiccator for 2 h, cleared in clove oil, washed in xylene and mounted in euparal (McLean & Ivimey Cook, 1965). Measurements of 100 zoospores and 200 flagella were made at a magnification of ×600.

Size of live zoospores

Sporangia were germinated in a hanging drop. Zoospores which had ceased active swarming were measured under a magnification of ×600 using phase contrast microscopy. The time between emergence from the sporangium and cessation of motility was recorded.

Encystment of zoospores and observations on the flagellum

Sporangia were germinated in a hanging drop incubated at 25° and the zoospores were fixed as previously described immediately after emergence and 1, 2, 3, 4 and 5 h after germination. Two hundred zoospores were counted for each time interval and the number of zoospores with and without flagella recorded. Measurements of 100 encysted zoospores were made at a magnification of ×600. Observations were also made on live and fixed zoospores to determine whether the flagellum was retracted or shed.

Scanning electron microscopy

Sporangia were allowed to germinate in sterile water on microscopic slides; samples were removed at intervals of 1, 2, 4, 6, 12 and 24 h after incubation at 25° and fixed in 2% osmic acid for 1 min. The fixed material was then transferred into 70% alcohol and despatched to Australia where the samples were rehydrated and allowed to settle on coverslip glass pieces previously coated with poly-L-lysine for 15 min. The glass pieces were washed and dehydrated in an ethanol series before critical point drying from amyl acetate and coating with gold (Tsutsui *et al.*, 1976). They were then examined under a JEOL Stereoscan Electron Microscope at 15 kV.

RESULTS

Colour, shape and size of sporangia

Immature sporangia had hyaline hexagonal and pentagonal walls (Fig. 1) enclosing finely granulated pale yellow protoplasm which became darker and more coarsely granulated with maturity.

Table 1. *Effect of temperature on germination of* Synchytrium psophocarpi *sporangia*

Temperature (°C)	Mean germination (angular transformed percentages) 24 h	48 h
5	61·36[b]	64·38
10	63·54[a,b]	70·19
15	70·41[a,b]	81·33
20	71·77[a]	76·35
25	71·97[a]	74·96
30	62·5[b]	66·81
37	0[b]	0

Values for 24 h followed by the same letter are not significantly different ($P = 0·05$, Duncan's multiple range test).

The sporangia were either globose in shape measuring 12·5–27·0 (21·4) μm or ovate measuring 20·0 × 15·0 to 35·0 × 22·5 (25·2–19·4) μm (Fig. 2).

Effect of temperature on germination of sporangia

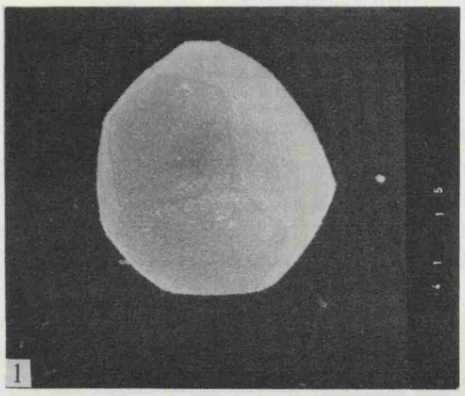
The mean percentage germination after 24 and 48 h incubation is shown in Table 1. Germination did not occur at 37°, and was significantly greater ($P = 0·05$) at 20 and 25° than at 5 and 30°. Germination increased after 48 h incubation for all temperatures except at 37° and no further increase occurred after 72 h. Sporangia which failed to germinate at 37° after 72 h did not germinate after subsequent transfer to 20° for 24 h.

Effect of relative humidity and surface wetness on germination of sporangia

Sporangia germinated equally well at relative humidities of 84–100% but not at 66% when incubated 'wet' and only at 100% when incubated 'dry' (Table 2).

Germination process and number of zoospores per sporangium

The contents of ungerminated sporangia first became coarsely granular; this was followed by bulging of the sporangial wall (Fig. 3). Zoospores were then differentiated and began to oscillate within the sporangium. The zoospores located their exit by moving in a random circular motion. It was difficult to determine whether there was more than one exit or pore in the sporangial wall whilst this process was being observed; scanning electron micrographs do, however, show that at least three of the hexagonal walls are ruptured (Fig. 4). The number of zoospores that



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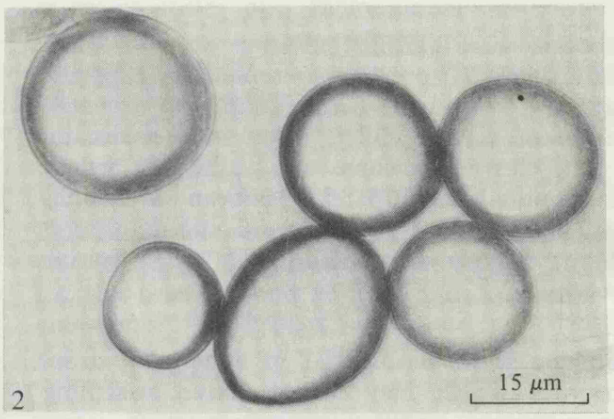

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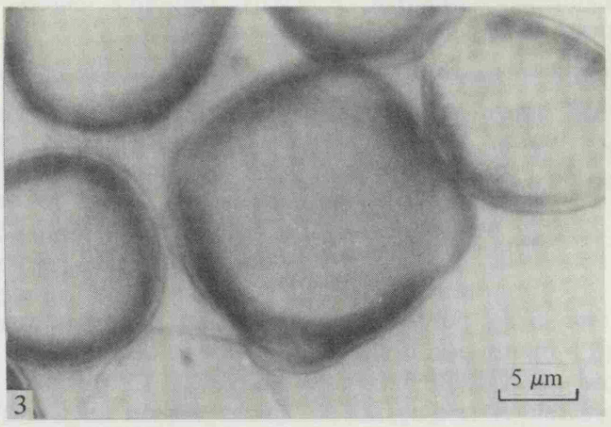

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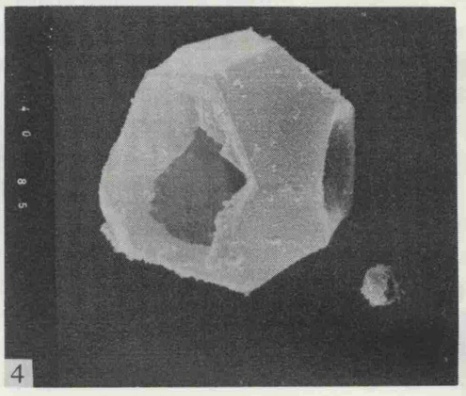

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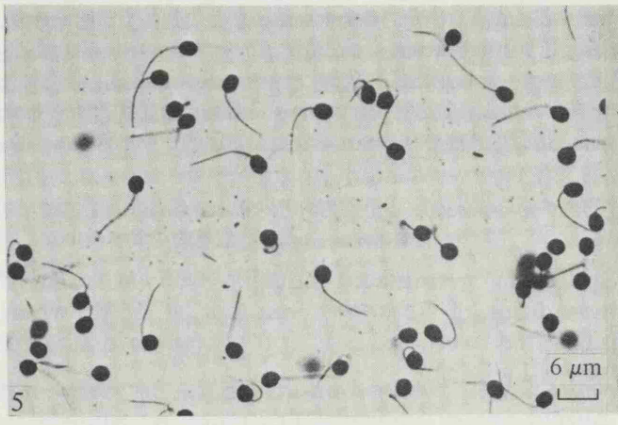

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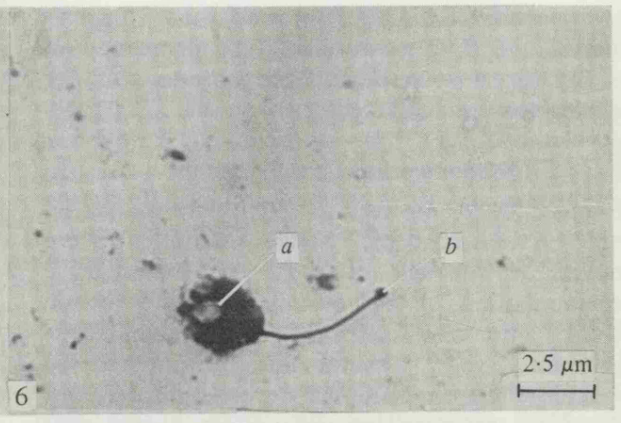

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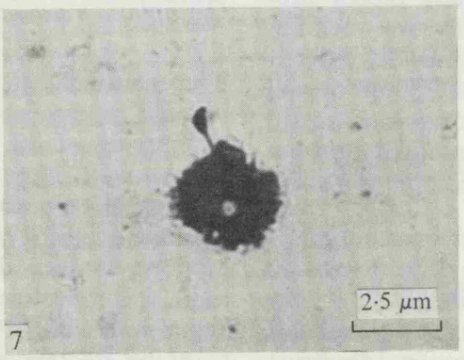

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Table 2. *Effect of relative humidity and surface wetness on germination of* Synchytrium psophocarpi *sporangia*

Relative humidity (%)	Mean germination (angular transformed percentages)	
	+Water	−Water
100	80·11[a]	15·34
98	75·66[a]	0
90	77·78[a]	0
84	73·39[a]	0
66	0[b]	0

Values for +water followed by the same letter are not significantly different ($P = 0{\cdot}05$, Duncan's multiple range test).

Table 3. *Effect of age on viability of* Synchytrium psophocarpi *sporangia*

Sorus	Mean germination (angular transformed percentages)
Unopened	68·31[a]
Recently opened	62·70[a]
Opened for some time	14·67[b]

Values followed by the same letter are not significantly different ($P = 0{\cdot}05$, Duncan's multiple range test).

emerged from five sporangia all of 22·5 μm diam, were 102, 122, 134, 134, and 156. The empty sporangial wall collapsed following emergence and the zoospores swam around erratically.

Rate of germination

Zoospores first began to emerge after 40 min incubation. This was followed by rapid germination for the next 30 min and only a few sporangia germinated with further incubation. A similar pattern occurred with all the replicates (Fig. 8).

Table 4. *Encystment of* Synchytrium psophocarpi *zoospores with time*

Time after germination (h)	Flagellate zoospores	Encysted zoospores
0	200	0
1	200	0
2	200	0
3	163	37
4	126	74
5	83	117

Effect of sporangial age on viability

The mean percentage germination of sporangia from unopened and recently erupted sori was significantly higher ($P = 0{\cdot}05$) than sporangia taken from old sori (Table 3).

Effect of storage on viability of sporangia

The viability of sporangia from bags stored in the field, at room temperature and 5° decreased to zero after 4 days; those held in a sealed plastic box at 5° remained viable for 13 days before declining, whereas sporangia stored both in a bag and plastic box at −85° lost viability after 24 h (Fig. 9).

Number and viability of sporangia in a single sorus

The number of sporangia counted from two unopened sori both of 160 μm diam, was 4272 and 4890; a mean of 83 % germinated.

Size of live and fixed zoospores, flagella length and swarming period

Live zoospores were pyriform in shape and varied in size from 5·0 × 2·5 to 10·0 × 5·0 (6·9 × 4·6) μm. Fixed zoospores assumed a spherical shape (Fig. 5) and ranged in size from 2·5 × 2·5 to 5·0 × 3·0 (3·34 × 2·5) μm which differed significantly ($P = 0{\cdot}05$) from measurements of live zoospores. The flagella length ranged from 7·5 to 16·25 (12·98) μm. Some zoospores had ceased swarming 30 min after emergence from the sporangium.

Fig. 1. SEM of sporangium of *Synchytrium psophocarpi*. (× 1260).

Fig. 2. Globose and ovate sporangia.

Fig. 3. Sporangium showing bulging of the wall prior to germination.

Fig. 4. SEM of germinated sporangium 2 h after incubation showing ruptured walls. (× 1350.)

Fig. 5. Fixed zoospores stained with crystal violet.

Fig. 6. Zoospore in the process of vesicular flagellar retraction. (*a*) Oil globule; (*b*) vesicle.

Fig. 7. Zoospore with flagellum almost completely retracted.

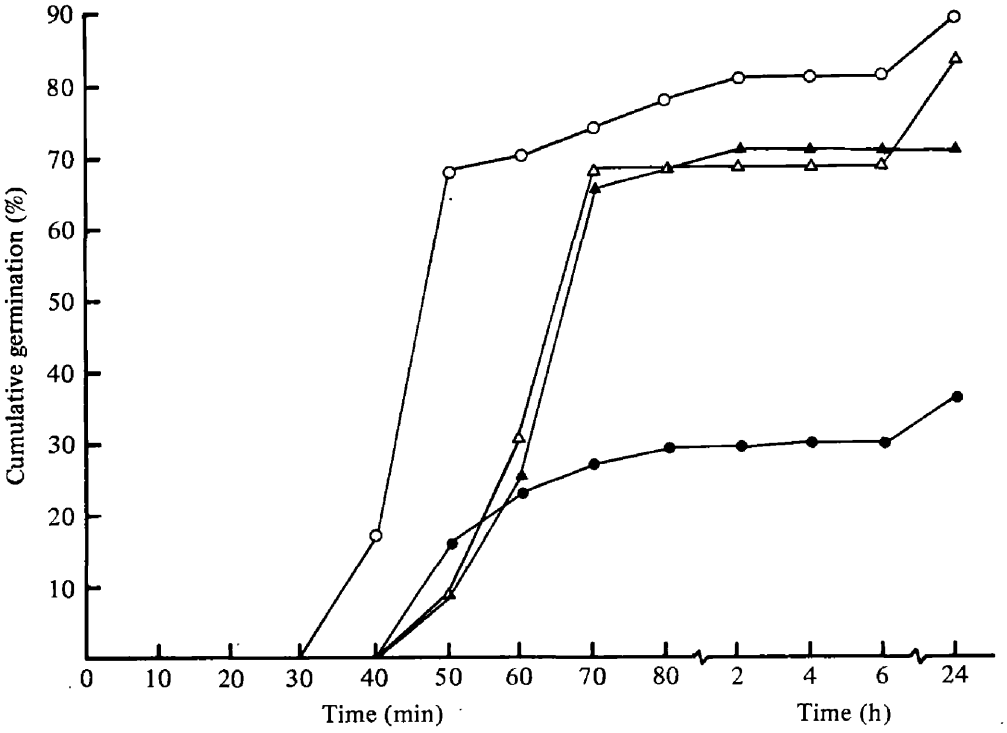

Fig. 8. Rate of sporangia germination at 25 °C. ○, ●, △, ▲, Replicates.

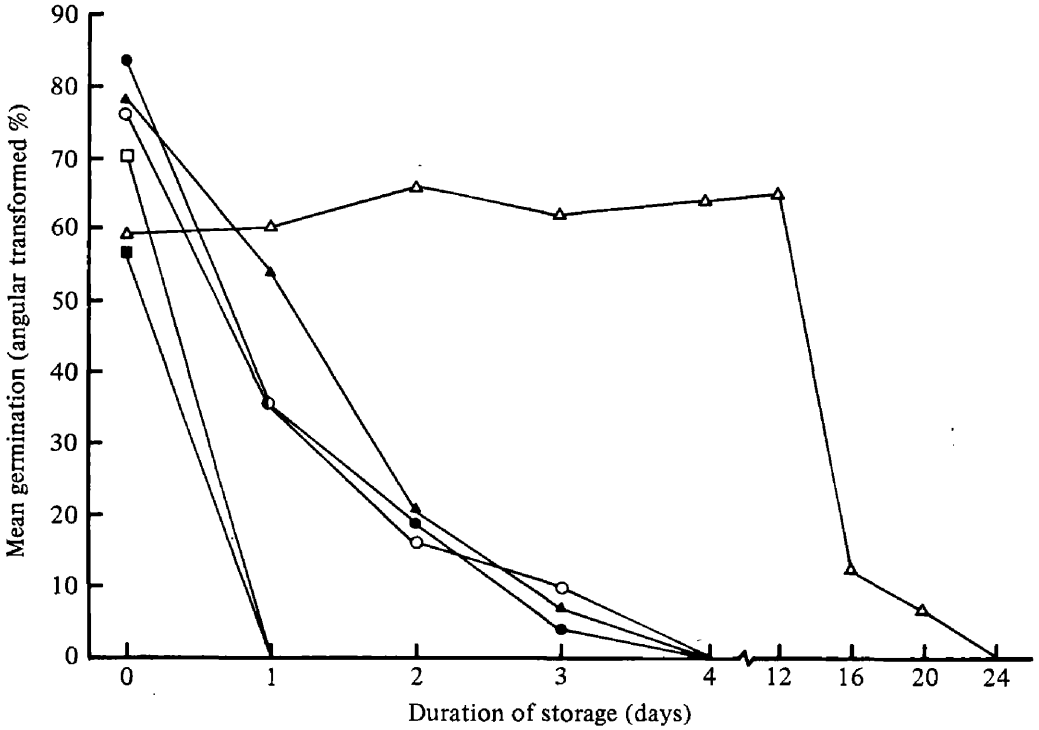

Fig. 9. Effect of different storage conditions on viability of sporangia. ○, field; ●, room temperature; △, 5° plastic box; ▲, 5° terylene bag; □, −85° plastic box; ■, terylene bag.

Encystment of zoospores and observations on the flagellum

Encysted zoospores were first observed 3 h after emergence but flagellate zoospores were still present after 5 h (Table 4). Encysted zoospores varied in size from 3·0 to 6·0 (4·39) μm diam.

The zoospores retracted their flagella by a process of vesicular retraction. The process is completed within 10 min, during which time the flagellum twitches and undulates and the zoospore swims intermittently. A vesicle appears at the base of the flagellum (Fig. 6) which moves up towards the zoospore (Fig. 7) and is finally taken in through the body wall.

DISCUSSION

The majority of sporangia were either globose or ovate, although a few irregular shapes were also observed. The measurements of sporangia agree with those reported in Karling (1964) but are significantly smaller than those recorded by Alicbusan (1965).

The results indicated that temperatures between 10 and 25° were more favourable for germination and that lower germination occurred at 5 and 30°. The results differed from those of Alicbusan (1965) in that germination was not recorded at 37°. Germination occurred equally well at relative humidities of 84–100% in the presence of a water film. The lack of germination in the absence of water except at 100% r.h. suggests that free water is essential for germination.

The minimum period for germination in the presence of water was 40 min and most sporangia germinated within 60–120 min. These results agree with those of De Vera (1973) and Alicbusan (1965). The proportion of sporangia that germinated after further incubation was low and indicated that most viable sporangia will germinate within 2 h provided conditions such as temperature and moisture are favourable. The range of temperatures in the Highlands of PNG, where winged beans are traditionally grown, are within these experimental temperatures and evidently sporangia will germinate if they are in contact with a film of water for at least 1 h.

The number of zoospores that emerged from each sporangium ranged from 102 to 156. These are less than the 200 reported by De Vera (1973) and significantly greater than the fifty reported by Alicbusan (1965). It is possible that the number of zoospores is dependent on the size of the sporangium.

The results indicated that sporangia from an unopened sorus, and a recently erupted sorus were more viable than sporangia taken from a sorus that had erupted for some time. It appears that some maturation of sporangia must occur prior to the rupture of the sorus membrane. The loss of viability in sporangia from an old sorus may also be caused by a loss of moisture after exposure to the atmosphere. This is supported by the fact that viability of sporangia stored in porous bags declined rapidly to zero after 4 days whereas sporangia stored in a sealed container at 5° remained viable for a maximum of 20 days. These results differ from those of Alicbusan (1965) who reported that sporangia remained viable for 6 months in the laboratory and 4 months in the field. These differences can be explained by the fact that he did not carry out any germination tests; his findings were only based on the colour and shape of sporangia. The rapid loss of viability does mean that sporangia are short-lived and to maintain the disease they must be dispersed to reach an infection court within a few days after eruption of the sorus membrane.

The shape and size of live zoospores are in agreement with those reported by Raciborski (1898). Zoospores fixed in osmic acid became spherical and shrank in size; this effect has also been reported by Koch (1958). The flagella were significantly ($P = 0{\cdot}01\%$) longer than those reported by Raciborski (1898). It is not known whether his measurements were made on recently emerged zoospores; these retract their flagella prior to encystment and it is possible that this may account for the differences noted. It is also possible that his material may be a different strain since it came from Java. The swarming of zoospores was observed to last for at least 30 min. Some pairing of zoospores was observed to take place but is not known whether this was a result of fusion or incomplete cleavage in the sporangium. Similar observations have been made by Gäumann (1927) and De Vera (1973); pairing in other species have also been reported by Curtis (1921) and Karling (1955).

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