ETIOLOGY AND EPIPHYTOLOGY OF STRAWBERRY FRUIT ROT CAUSED BY *BOTRYTIS CINEREA* PERS.

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1959

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Date thesis is presented May 15, 1959

Typed by Joyce Powelson

ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. Edward K. Vaughan for his assistance and counsel during the course of this investigation and to Dr. Frank H. Smith, Dr. Alfred N. Roberts and Dr. Roy A. Young for their constructive suggestions on preparation of the manuscript.

The author appreciates the cooperation of Walt B. Neuberg of Birds Eye Western Branch Laboratory, and Palmer S. Torvend, Washington County Extension Agent, in setting up spray plots and securing data. Thanks go especially to H. H. Millsap for his assistance with the photography.

The work was carried out with the aid of a fellowship supported by the Birds Eye Division of the General Foods Corporation.

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ETIOLOGY AND EPIPHYTOLOGY OF STRAWBERRY FRUIT ROT CAUSED BY BOTRYTIS CINEREA PERS.

INTRODUCTION

Strawberries are the most important of the small fruit crops grown in the Pacific Coast states. The three states of California, Oregon and Washington produce over half the nation's strawberries and rank first, second and third, respectively, in pounds produced (44, p. 47). Varieties and cultural practices vary in different areas. In Oregon and Washington, the two major varieties are Marshall and Northwest, and in California most of the acreage is in the varieties Shasta and Lassen.

In 1958, approximately 70 percent of California's strawberry production went into the fresh market, while in Oregon and Washington, over 90 percent of the berries went to processers for canning or freezing. Prior to preservation by freezing, the short shelf-life of the fresh fruit was a major factor in limiting the commercial production of strawberries. For this reason, most of the research on strawberry fruit rot has dealt with the study of conditions which predispose fruits to rot in storage and transit.

However, with rising costs of production and competition from other areas, profitable strawberry production is not possible when yields per acre are low. Growers who considered fruit rot in the field as a calculated risk

which must be assumed in strawberry culture, are now concerned about these frequently severe losses. The most serious losses are experienced when excessive rainfall occurs during the harvest period. For example, frequent rains during the 1958 harvest season in Oregon resulted in an estimated three million dollar loss to the growers as a direct result of strawberry fruit rot that occurred in the field. This estimate is based on a yield of marketable strawberries which grossed Oregon growers approximately ten million dollars for the 1958 season.

Sanitation and dultural practices which permit better air circulation and consequent rapid drying have been said to aid in control (3, p. 428). However, such recommendations have been based on observation and not on experimental data which have been reported in the literature. The use of fungicides for strawberry fruit rot control has not been generally practiced in the past, but since 1952 several investigators (29, p. 309), 30, p. 343), 56, p. 97), 57, p. 210), 41, pp. 230-233), 47, p. 216) and 36, p. 220) have reported a lower incidence of fruit rot brought about by preharvest applications of fungicidal dusts or sprays.

Although a number of fungi are known to cause strawberry fruit rot and the symptoms they produce have been described, not much is known about the etiology and epiphytology of these rots in the field. The initial work

on this investigation began in the summer of 1956. Isolations were made from rotting strawberries to determine the fungi responsible for fruit rot in the Pacific Northwest. Botrytis cinerea proved to be the main cause of field rot of strawberries in the Pacific Northwest. Experiments were then designed to determine: (1) the source and distribution of inoculum, (2) the time, place and method of infection and (3) the factors affecting disease development.

LITERATURE REVIEW

Organisms associated

A number of fungi are known to attack strawberries and their economic importance varies with the geographic areas where strawberries are grown. The two most common fungi causing extensive losses from fruit rot are *Botrytis cinerea* Pers. and *Rhizopus nigricans* Ehr. *Botrytis* rot (grey mold) has been reported (68, p. 8), 79, p. 12) and 3, p. 243) as being responsible for losses of 40-50 percent in the field. *Rhizopus* rot, commonly called leak, is primarily a market disease (67, pp. 1-2) and 61, p. 21) but it is not uncommon to find overripe strawberries rotting in the field as a result of *Rhizopus* infection (69, pp. 204-205). Most workers have considered that leak is caused by *Rhizopus* spp., but *Mucor* spp. may also be involved. Lowings (38, p. 87) found that 95.5-98 percent of the fungal contamination of processed Kentish strawberry fruits consisted of hyphae of *B. cinerea* and *Mucor piriformis* Fisch.

Rots caused by other fungi have been reported to be common in some areas. Rose (60, p. 357) considered that leather rot, caused by *Phytophthora cactorum* Leb. and Cohn was the most destructive disease of strawberries in the southern Mississippi valley in 1922 and 1923. In 1958, Felix (21, p. 841) reported that in some Tennessee

strawberry fields P. cactorum had affected an average of 50-65 percent of the pedicels, buds, flowers and berries. Where green or ripe berries touch the soil they may be attacked by Rhizoctonia spp. which cause a hard rot. This rot can be found in most strawberry fields but rarely results in serious loss. However, observations by Dodge and Stevens (19, p. 643) indicated that in 1923 and 1924 Rhizoctonia was responsible for half of the loss from field rots in the Plant City and Kissimmre areas in Florida.

Several fungi which cause foliage diseases of strawberries have also been reported to cause fruit rots. Dendrophoma obscurans (E. and E.) Ander., a well known cause of leaf blight, was reported by Alexopoulos and Cation (1948, p. 699) to cause a stem-end rot of strawberries in Michigan. D. obscurans has been found commonly associated with Gnomonia fructicola Arn., which has also been reported to cause strawberry fruit rot (8, p. 172), 23, p. 796), 1, p. 705) and 2, p. 221). Tan rot, caused by Pezizella lythri (Hainesia, imperfect stage) is considered an important fruit rot fungus in Southern United States (65, pp. 258-259) and 61, p. 23).

In addition to these organisms, Alexopoulos and Cation (1, p. 698) listed other fungi that have been reported to cause strawberry fruit rots. Mycosphaerella fragariae (Schw.) Lind. caused a blackseed disease of strawberries

in Maryland and North Carolina, a Septoria sp. caused a hard rot of strawberries in England, Sclerotinia sclerotiorum (Lib.) Mass. rotted fruits in the United States, Didymella lycopersici Kelb. attacked fruits in England, Sclerotium rolfsii Sacc. caused a soft rot of strawberries in Florida and a Sphaeronemella sp. was found on berries in the market at Urbana, Illinois. Sturgess (71, p.269) reported that over 50 percent of the waste of ripe fruit from strawberry fields in Queensland, Australia was caused by a species of Gleosporium.

Since this thesis is primarily concerned with the fruit rot of strawberries caused by Botrytis cinerea, further review of the literature will be confined to information pertinent to the etiology and epiphytology of Botrytis rot.

Source and distribution of inoculum

Botrytis cinerea occurs and fruits on almost any damp, decaying vegetation, and as a result, the spores are cosmopolitan in the air. Although this has been generally recognized by most workers (3, p. 242), 56, p. 97) and 36, pp. 220-224), only recently has any attempt been made to study the source and distribution of the spores which cause this disease (31, pp. 26-27), 32, pp. 38-39) and 45, p. 24).

In 1956, Jarvis (31, p. 26) used the Gregory portable spore trap (25, p. 475) to study the air spora of

strawberry plantations in Scotland. The conclusions were limited because of the few Botrytis spores trapped. However, there was some indication that Botrytis spores were most abundant during foggy or misty weather and that the concentration of spores decreased rapidly with increasing distance from the sporulation site. Miller and Wagner (45, p. 24) used the Hirst spore trap (27, pp. 258-263) in a similar study in Connecticut, and confirmed the observations of Jarvis. They used the Hirst spore trap with the orifice 18 inches above the ground and placed near a row which had about ten infected strawberries per foot of row. The highest concentration of Botrytis conidia they obtained was 112 per cubic meter of air. Miller and Wagner point out that this is very low when compared to the 21,000 ascospores of Venturia inaequalis obtained in a similar study on apple scab. From this they concluded that most infections of strawberries by B. cinerea originate from nearby primary inoculum. Jarvis (32, p. 38) reported the results of further studies where he used the Hirst spore trap. He found that peak concentrations of 600-1000 spores per cubic meter were trapped at midday when the relative humidity was at 65-85 percent and falling. He also noted that times of increase were at the end of dry, sunny days with rising humidity and during rain storms with high winds. The number of spores trapped was related to fruit ripening.

The low pre-harvest counts of 5-20 Botrytis spores per cubic meter rose rapidly as fruit ripened and fell after the crop was removed. Jarvis also observed that if temperatures were below 13°C for long periods at night, few spores were trapped in the morning.

Studies by Gregory (25, p. 475), Hirst (28, p. 375) and Last (37, p. 462) showed that spores of Cladosporium spp. were the main component of the general air spora but at times basidiospores (28, p. 375) were dominant. However, Botrytis spores were not recognized or listed as being a specific spore type trapped by the automatic, volumetric trapping devices used in these studies.

Time, place and method of infection

The literature contains no report of a well-planned experiment to determine when and where infection occurs in the Botrytis rot disease of strawberries and what little has been published has been based on the assumed behavior of B. cinerea. Most workers have noted that B. cinerea may attack the flowers, green fruit and ripe fruit (68, p. 8), 69, p. 210), 3, p. 242), 56, p. 97) et al).

Both Powell in Illinois (56, p. 98) and Horn (29, p. 309) in Louisiana obtained control of strawberry fruit rot with preharvest applications of fungicides. The two most effective fungicides tested were captan and ferbam. Horn (29, p. 310) also carried out fungicide screening

tests using fungicide-conidial suspensions incubated on glass slides and on apparently healthy, green to white strawberry fruits. None of the conidia germinated on the glass slides when a fungicide was present, whereas there was 99 percent germination in the control. Symptomless berries that were dipped in fungicide-conidial suspensions and incubated, developed 13.3 percent rot in the D H A-S (dehydroacetic acid salt) treatment, 35.8 percent with Orthocide 406 (captan), 84.2 percent in the inoculated control and 60.3 percent in the uninoculated control. It was concluded by Horn that some of the fruits must have been infected prior to treatment.

Field observations made by Wilkinson (79, p. 12) on Botrytis rot of strawberries in England indicated that much of the rot was originating in dead petals adhering to the calyx and at points of contact between the fruit and straw mulch. Kirby et al (36, pp. 220-221) observed that strawing does not prevent infection, especially if the petals have been subject to attack. Other English workers (41, p. 232) and 47, p. 213) have noted infection spreading from the sepals to the fruit.

In a 1956 paper, Stoddard and Miller (70, p. 443) stated that "In the field the causal fungus (B. cinerea) does not readily infect the ripe fruit but attacks more particularly the pedicels of the flower buds and the stems

and calyces of the small green fruit". They also stated that "Infection of these parts of the plant builds up a reservoir of inoculum which, in rainy weather, will infect the ripening crop with a massive spore load impossible to control with sprays during harvest". The above statements by Stoddard and Miller seem contradictory since, if direct infection of the ripe fruit does not readily occur, the build-up of a massive spore load during harvest would be of minor importance. However, in greenhouse experiments where they sprayed the buds, flowers and small green fruits once with thiram then inoculated with a heavy spore suspension of B. cinerea conidia, and with the plants receiving intermittent misting, 96 percent of the fruits receiving no fungicide had rotted 18 days after treatment and only 32 percent of the fruits sprayed with thiram.

The susceptibility of floral parts to attack by Botrytis spp. is well known. In 1888, Ward (78, pp. 319-382) found that a species of Botrytis was causing a serious flower blight of lilies in England. He found hyphae of the fungus in tissues of the calyx, corolla, anthers and ovary. More recently, Gould (24, pp. 1-33) has reviewed the literature on Botrytis diseases of gladiolus. The importance of petal infection in the development of blossom-end rot of tomatoes caused by Botrytis cinerea was clearly established by Newhook and Davison (51, pp. 166-183)

and 52, pp. 473-481). In an earlier paper, Newhook (50, p. 185) reported that saprophytic microorganisms established naturally on dead lettuce tissue in the field gave a considerable amount of protection against B. cinerea infection. The occurrence of similar natural protection against Botrytis on tomatoes is demonstrated in Newhook's 1957 paper (52, pp. 474-479). In experiments where Cladosporium herbarum and Penicillium sp. were established on dead petals adhering to fruits prior to inoculation with Botrytis conidia, blossom-end Botrytis infection was 1-3 percent compared with 46-80 percent in the checks. Newhook (52, p. 480) points out that although the nature of the antagonism to Botrytis by saprophytes, e.g. C. herbarum, is not understood, C. herbarum is able to colonize dead tissue under much drier conditions, which makes it one of the most common protectants of dead tissue against B. cinerea. Cox and Winfree (14, p. 758) reported that repeated application of bisdithiocarbamates, for control of Mycosphaerella fragariae leaf spot of strawberry, resulted in a higher incidence of fruit rot due to B. cinerea. They suggested that the bisdithiocarbamates either changed the susceptibility of the tissues or may have eliminated saprophytes that are antagonistic to Botrytis.

Nelson (48, p. 861), in studies on the infection of grapes by B. cinerea, found that the inoculated fruits were

most frequently invaded through the capstem area. He attributed this to the possibility that even after the drying of the fruits free water may have remained unobserved in crevices at the base of the capstem, in floral parts or in the lenticles of the capstem itself. Nelson did not use check lots of uninoculated fruits or consider the possibility of latent infection in the fruits he used. Harvey (26, p. 232) found that most of the decay in stored grapes results from incipient infection not evident at harvest. Emperor grapes from a Fresno, California vineyard which were surface-sterilized with sulfur dioxide, incubated ten days at room temperature and then stored at 31-32°F for 2½-4 months, developed 56.2 percent decay as a result of B. cinerea infection.

Further evidence of the occurrence of latent infection was found by Wade (77, pp. 504-515) during investigations on brown rot of apricots caused by Sclerotinia fructicola (Wint.) Rehm. He concluded that infection occurred early in the development of the fruit but remained latent until ripening commenced. Latent infections were confined to the epidermal layer and were not associated with any particular position on the surface. Recent studies by Jerome (33, pp. 132-140) on brown rot of peaches present evidence against Wade's (77, p. 504) hypothesis of latent infection. Jerome concluded that latent contamination of the fruit

surface occurs and the major limiting factor in infection and rot development, irrespective of the state of maturity of the fruit is the mechanical difficulties involved in penetration.

As far as this writer is aware, there is no published evidence to demonstrate how B. cinerea gains entrance into strawberry fruits. The only histological study on strawberries affected by Botrytis was made by Stevens (66, pp. 362-363) in 1916. His studies were confined to observations on the intercellular and intracellular arrangement of hyphae in rotting strawberry fruits. Blackman and Welsford (7, pp. 392-396), in studies of infection of broad bean (Vicia faba) leaves by B. cinerea, observed direct penetration with or without development of an appressorium and noted that the germ tube was held to the leaf by a mucilaginous sheath. Brown and Harvey (10, p. 649) demonstrated that the germ tube of B. cinerea can mechanically penetrate the epidermis of onion scales and Eucharis leaves.

Recent studies by Nelson (49, pp. 224-226) on the infection of grapes by B. cinerea have shown that penetration of the cuticle is by formation of an appressorium and infection peg. He found that the subcuticular mycelium was usually intercellular and largely restricted to the outermost 5-8 cell layers. The periclinal walls in the

3-5 outermost cell layers separate more easily than do the anticlinal walls. Wade (77, pp. 509-510) was unable to find any evidence of direct penetration of the cuticle of apricots by germ tubes of Sclerotinia fructicola spores. However, in a number of cases, conidia which had germinated were found lying within the stomatal cavity and a penetration tube had entered cells surrounding the cavity. Bolton (8, p. 179) has concluded that infection of strawberry fruits by Gnomonia fructicola takes place through stomata or wounds.

Although it is generally recognized that infection of strawberry floral organs by B. cinerea commonly occurs, most workers consider direct infection of the ripening fruit as being responsible for the major losses which occur (3, p. 240), 29, p. 309), 57, p. 209), 70, p. 443) and 47, p. 213).

Predisposition to infection and rot development

Severe epiphytotics of Botrytis rot in strawberries are always reported (68, p. 8), 3, p. 239), 43, p. 147). 79, p. 12) et al.) to follow rainfall and prolonged periods of high relative humidity. This same relationship to moisture was found to be important in the development of Botrytis rot of grapes in California (48, p. 859) and 26, p. 229) and brown rot of stone fruits (Sclerotinia spp.) in Australia (77, p. 524) and 5, pp. 294-295).

Various explanations for the effect of moisture on infection and rot development have been reported in the literature. Snow (64, p. 3) found that spores of B. cinerea have very high moisture requirements (93-100 percent r.h.) for successful germination and growth of germ tubes. Nelson (48, p. 863) found that consistent germination occurred when Botrytis conidia were dusted on either glass slides or grapes and incubated at 12°C for 48 hours at relative humidities of 92 percent or higher while at 90 percent r.h. only one spore on the grape skin had germinated. However, grapes which received a five day incubation at 90 percent r.h. showed as high as 53 percent infection. This may have been the result of latent infections which Nelson (48, p. 860) has not considered, since he failed to include uninoculated checks in his experiments. In recent studies by Jarvis (32, p. 39) on strawberries, there was 63 percent germination of spores dusted on ripe strawberries and incubated under humid conditions, against 91 percent where the spores were applied in a water suspension.

The importance of nutrients in the infection drop was demonstrated by Brown and Harvey (10, p. 647) when no penetration of a wax membrane occurred with Botrytis spores in pure water but penetration took place in all cases with spores in a nutrient solution. Chattopadhay (11, p. 39)

concluded that heat increased the susceptibility of certain vegetables to attack by B. cinerea because of the exosmosis of salts which stimulated germination of Botrytis spores. Studies by Tukey et al. (73, p. 12) on the leaching of carbohydrates and nutrients from plant tissues, indicate that during wet weather adequate nutrients would be available on the surface of plant tissue for germination and subsequent infection by B. cinerea.

Although there have been many published reports of the effect of moisture on germination of Botrytis spores and subsequent "infection" of the host, their conclusions have been based not on actual infection but on the appearance of disease symptoms. The effect of moisture on the host-parasite relationship between the time of actual infection and the expression of disease symptoms has been overlooked in most cases. Nelson (49, p. 226) was able to detect, microscopically, penetration of uninjured grapes by germ tubes of B. cinerea conidia within 18 hours after inoculation. Since only a relatively short time is required for actual infection to take place, the prolonged periods of rainy weather and high humidities required for severe epiphytotics of fruit rot must have an effect on the length of the incubation period and the rate of rot development.

The production of pectolytic enzymes which are

responsible for the actual breakdown of host tissue, has been well established for B. cinerea (9, pp. 313-348), 6, p. 253) and 13, pp. 15-30). Using enzymatic extracts from B. cinerea which would actively macerate discs of potato tuber tissue immersed in the extracts, Fernando (22, pp. 110-113) found little if any demonstrable action resulted if small quantities of the enzymatic preparation were laid on the surface of normal potato tissue. However, potato tissue which had been injected with water was actively attacked by the enzyme. Fernando's observations have been confirmed by Mishra (46, p. 339), who found that B. cinerea produced a typical soft rot of potato tubers in which the water content had been raised 6-10 percent by vacuum infiltration for two hours. Apparently no change in tissues during absorption was involved, as on desiccation (over fused calcium chloride) to the original water content, susceptibility was lost completely. The same results were produced by pectic enzymes in vitro where the water content directly conditions the attack. Recently, Dimarco and Davis (18, pp. 461-464) have reported that hydrocooling for prevention of post-harvest decay of strawberries increased the percentage of mold when no fungicide was added to the water in the hydrocooler.

Since the water content of the host tissue apparently affects the activity of pectolytic enzymes produced by

B. cinerea, the factors which influence the degree to which tissues become water-soaked must be important in rot development. Clayton (12, pp. 260-261) found that when water-soaked tobacco leaves are inoculated with *Pseudomonas tabaci* they develop large lesions involving an entire leaf, instead of the small, dead spots with a yellow halo typical of the normal wildfire disease. He was able to show that both high-nitrogen and low-potash increased the susceptibility of tobacco leaves to water-soaking, which was correlated with disease severity. In seven experiments conducted by Wade (77, p. 519) over a four year period, a negative regression between brown rot (*Sclerotinia fructicola*) incidence and potassium status of apricot trees was demonstrated in all cases.

There are numerous reports in the literature concerning the responses of strawberry plants to fertilizer application, but few include data on incidence of fruit rot. Darrow and Waldo (17, p. 323) noted that there was more fruit rot in plots fertilized with inorganic nitrogen than in unfertilized plots. They concluded that this was due to more shading from increased amount and density of foliage, as did Shoemaker (62, p. 28) in Ohio. An increase in size of fruit with application of nitrogen usually results in softer fruits (16, p. 232), and 55, p. 222). However, Culpepper *et al.* (15, p. 693) found that weather, especially

rainfall, affected the composition of strawberries more than did fertilizer. He found that, in general, as the moisture content of the fruit increased the sugar content decreased.

There appears to be a wide range of varietal resistance to decay but all known strawberry varieties are susceptible (43, p. 147), 79, p. 12) and 68, p. 8). Stevens (68, p. 8) did not note any differences in susceptibility among more than 300 varieties in test plots at the Arlington Experimental Farm in Virginia. As far as this writer is aware, no critical, comparative studies have been made on the susceptibility to fruit rot among the different strawberry varieties.

Also, no studies have been made on Botrytis rot of strawberries to determine the length of the incubation period between time of initial infection and the first appearance of symptoms. Dimarco and Davis (18, p. 464) found that rot appeared in 43 percent of marketable strawberries held for eight days at 42°F with 100 percent relative humidity, and in 97 percent rot of berries held only four days at 72°F with 85 percent relative humidity. Nelson (49, p. 227) observed that microscopically visible lesions were produced on grapes within 36 hours after inoculation with a conidial suspension of B. cinerea in a nutrient solution followed by incubation at 20°C. In the

field, where the temperature varied from 6-21°C, symptoms were observed four days after a rain.

GENERAL METHODS AND MATERIALS

The initial phase of this investigation consisted of a survey of strawberry fields to determine what fungi were causing fruit rot. Surveys were made of commercial strawberry fields in Oregon during 1956, 1957, and 1958. In 1957, the survey was expanded to include British Columbia (Abbotsford area), Washington (Mt. Vernon area) and California (Watsonville area).

All field experiments, other than fungicide testing trials, were carried out in an established planting at the Botany and Plant Pathology Farm, Corvallis, Oregon. The planting, established in 1954 for a varietal testing program, was approximately one acre in size and every other row was a Marshall "guard row". The strawberry varietal plots consisted of 14 foot sections of row replicated at random in alternate matted rows spaced 42 inches apart. Yield data were taken from this planting during the third and fourth seasons of production.

Culture media

Potato dextrose agar containing 50 ppm. of streptomycin-nitrate (strep-PDA) was used throughout this investigation for isolation work and also as a medium for trapping air spora. This medium effectively inhibited bacterial growth and facilitated the recovery of fungi from rotted

strawberry tissue. Pure cultures of fungi for inoculation studies, as well as stock cultures, were grown and maintained on this medium which was prepared according to the following formula:

Infusion from 200 grams of autoclaved potatoes
Dextrose 20 grams
Agar 20 grams
Phytomycin* 0.25 ml
Tap water to make one liter volume
Autoclaving for sterilization

Isolations

To make routine isolations the plant material was washed five to ten minutes in running tap water, surface-sterilized in 20 percent clorox for approximately one minute and small sections of tissue from the edge of the diseased part were transferred to petri plates containing strep-PDA. The plates were incubated at room temperature and fungal colonies growing from diseased tissue were identified after mature fruiting bodies had been produced. This method was modified when isolations were made to determine the distribution of latent infection in strawberry fruits and will be discussed in detail under that phase of the investigation.

*Phytomycin is a product of the Olin Mathieson Chemical Corporation and contains 20 percent streptomycin-nitrate.

Inoculations

Two methods of inoculation were used in pathogenicity trials. In one method inoculations were made with a spore suspension prepared in sterile, distilled water from eight to ten-day old cultures of Botrytis cinerea grown on strep-PDA. Spores were removed from the fungal mat by adding glass beads, along with a small quantity of water, to the flask and shaking. The spore suspension was then passed through several layers of cheese cloth to remove large mycelial pieces, and adjusted to one hundred thousand spores per ml. with the aid of a hemacytometer, by adding distilled water.

In greenhouse experiments, fruits were rinsed in sterile, distilled water, dipped in the spore suspension and then incubated in individual moisture chambers (Figure 1) while still attached to the plant. The chambers were small cups made with plastic-coated windowscreening and sealed with a cork in which the slit for the insertion of the pedicel was plugged with cotton. These chambers prevent the contamination of test fruits and the secondary spread of spores from diseased fruits. High humidity was produced in the chamber by placing absorbent paper discs saturated with sterile distilled water in the bottom of each chamber. The uninoculated checks were treated in the same manner except that fruits were dipped in sterile water.

A second method of inoculation was used in other greenhouse experiments. Preparation of inoculum consisted of growing pure cultures of B. cinerea on rolled oats in 10x12x4½ inch plastic containers. After ten to 14 days incubation at room temperature, the rolled oats were thoroughly infested and heavy sporulation appeared on the surface. The strawberry plants were grown in number 10 cans arranged in rows alternating with slightly higher inoculum platforms as diagrammed below:

o potted strawberry plant
x inoculum platform

o o o o
x x x
o o o o
x x x
o o o o

One petri dish-full of the rolled oat inoculum was dumped on each platform. Spore production by these inoculum centers produced a high density of air-borne spores capable of bringing about severe epiphytotics under certain environmental conditions.

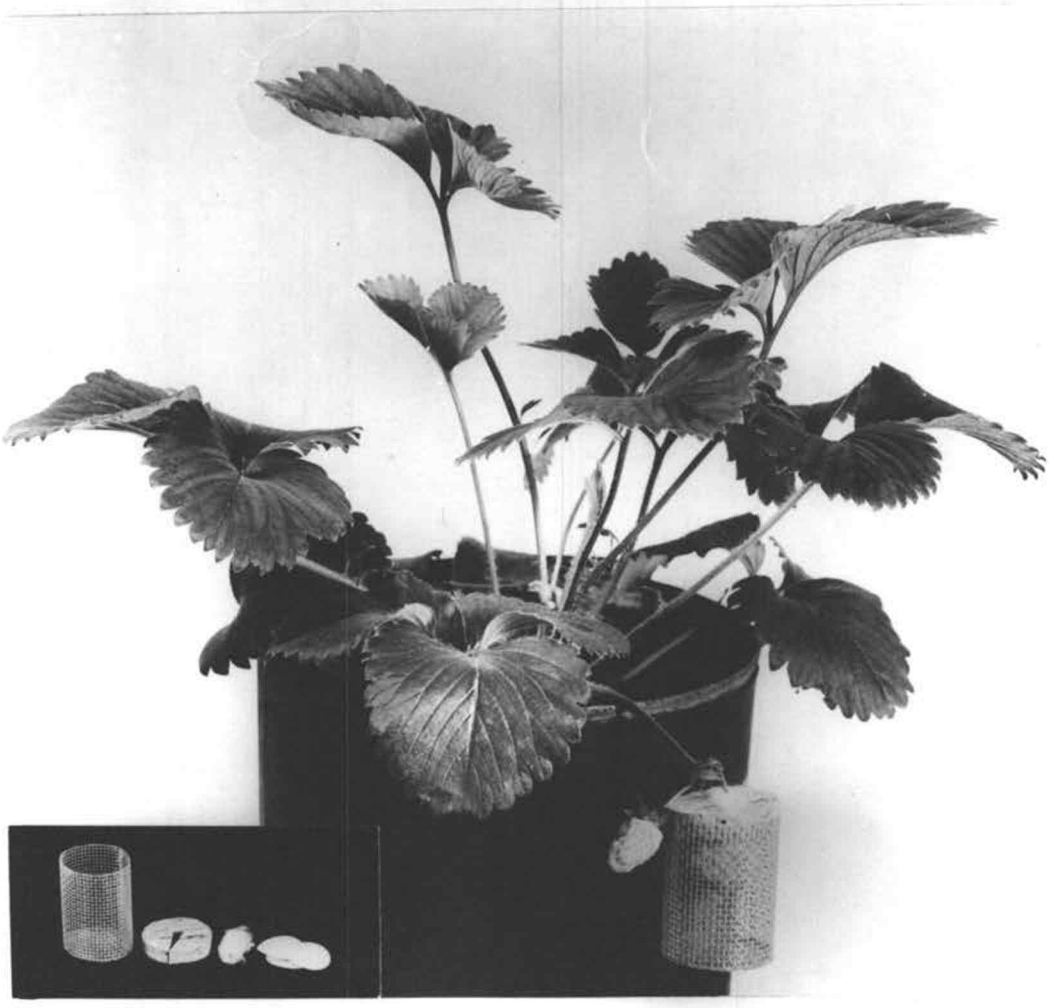


Figure 1. Individual moisture chamber used in greenhouse pathogenicity experiments. Insert (lower left) shows chamber made from plastic-coated window screening, cork with petiole insertion slit, cotton for plugging slit and absorbent paper discs for producing high humidity in chamber.

EXPERIMENTAL

Organisms associated with strawberry fruit rot

In order to determine the fungi responsible for fruit rot in Pacific Coast strawberry fields, isolations were made from 1310 fruits showing rot symptoms but no signs of the fungus causing the rot. These strawberries were collected from 22 fields surveyed in 1956, 1957 and 1958. Botrytis cinerea was by far the most frequently isolated fungus in all surveyed areas (Table 1). Of the other fungi reported by various workers to cause strawberry fruit rot, Rhizopus nigricans, Rhizoctonia spp., Dendrophoma obscurans and Gnomonia fructicola were also isolated. A species of Melanconium was commonly isolated from ripe fruits having rot symptoms.

The general symptoms of the different fruit rots caused by the above fungi have been adequately described in the literature, except for the rot caused by Melanconium sp. which has not been previously reported. Therefore, only a brief description of the writers concept of the various rots will be given.

Botrytis cinerea rot

The rot usually begins at the stem-end of the fruit as a light brown, somewhat soft, water-soaked area and under wet conditions the whole fruit is soon involved.

If damp weather prevails, the fungus will sporulate at the surface which gives the strawberry a grey, fuzzy appearance and accounts for the common name, grey mold rot. In dry weather, the infected fruit will shrivel into a dry, hard mummy. The fungus may cause a blasting of the blossoms as well as a rot of both green and ripe fruit.

Table 1. Frequency of isolation of fungi from strawberry fruits showing symptoms of rot but no signs of the associated fungus as determined by field surveys made of Pacific Coast strawberry fields.

	Percent frequency of isolation					
	Oregon			Cal.	Wash.	B. C.
Fungus	1956	1957	1958	1957	1957	1957
Botrytis cinerea	67.3	70.2	64.9	97.2	77.2	96.6
Rhizopus nigricans	5.0	10.6	13.9	2.0	10.9	1.8
Rhizoctonia sp.	1.3	3.8	5.3	0.4	6.5	1.6
Melanconium sp.	13.4	10.6	8.6	0.0	1.1	0.0
Dendrophoma obscurans	13.0	3.8	5.3	0.4	4.3	0.0
Gnomonia fructicola	0.0	0.0	2.0	0.0	0.0	0.0
Berries examined	446	180	240	228	120	96
Number of fields surveyed	3	3	5	3	3	3

Rhizopus nigricans rot

This fungus produces a rot, commonly called leak that is characterized by a collapse of the fruit with exudation of juices. In the field, only overripe fruits are attacked

which makes it of little importance as a field rot organism. In fresh market strawberries it frequently is of great importance during transport and storage.

Rhizoctonia rot

The rot produced by this fungus is a hard rot and occurs where green or ripe fruits are in contact with damp soil infested with Rhizoctonia spp. The rot advances very slowly and is characterized by having soil particles held to the diseased tissues when the fruit is lifted from the soil.

Dendrophoma obscurans rot

The rot typically begins at the stem-end of the fruit as a brownish discoloration of tissue under the calyx and eventually causes a soft rot of the whole fruit. Mummification of the fruit begins as a shriveling of the stem-end portion that causes the shoulders of the fruit to become depressed.

Gnomonia fructicola rot

This fungus apparently causes a stem-end rot similar to that caused by D. obscurans. However, because of its common association with D. obscurans and the small number of fruits from which isolations were made, no characteristic symptoms can be described.

Melanconium rot

Fruits from which this fungus was isolated were usually fully ripe and the flesh was a darker red than normal. An early symptom is the water-soaked appearance of the tissues at the stem-end of the fruit. The epidermis slips easily and there is a shallow maceration of the cells below. Sporulation of the fungus on the surface makes the fruit appear sooty. Limited attempts to determine the species of *Melanconium* were unsuccessful. However, it should be noted that *M. fuligineum* causes a bitter rot of muscadine grapes (39, p. 629) and *M. lycopersici* is responsible for a serious fruit rot of tomatoes in the Phillipines (54, p. 114). Alder (*Alnus rubra*) has been reported (80, p. 465) as a host for *M. candidum* in Oregon.

Pathogenicity of selected isolates of the fungi listed in Table 1 was proven by using the individual incubation chambers (Figure 1) and following the procedure of Koch's postulates.

During the 1957 and 1958 field surveys, an attempt was made to determine the production losses that resulted directly from fruit rot and also the amount of this rotting which could be attributed to *Botrytis cinerea* (Table 2). The average amount of rot per field, at the time the survey was made, ranged from 19.4 percent in California to 38.2 percent in British Columbia and, in all cases, over 90

percent of the rot was determined as having been caused by *B. cinerea*.

Thus, while several fungi have been isolated from rotting strawberries, the only fungus responsible for constant and extensive losses is *B. cinerea*.

Table 2. The incidence of strawberry fruit rot caused by *Botrytis cinerea* in Pacific Coast growing areas in 1957 and 1958.

Area surveyed	Number of fields surveyed	Rotted fruits for each 100 marketable fruits checked*		
		Number	Percent	Percent infected with *Botrytis***
Ore. 1957	3	42.2	29.7	91.5
Ore. 1958	5	43.0	30.1	93.9
Cal. 1957	2	24.1	19.4	99.6
Wash. 1957	2	54.5	35.3	95.4
B. C. 1957	3	61.7	38.2	97.9

*The average per area of three counts per field.
**Based on the number of fruits with *Botrytis* sporulating on the surface plus the number of fruits infected with *Botrytis* and showing symptoms of rot but no signs of the associated fungus as determined (Table 1) by isolation.

Source and distribution of inoculum

Botrytis cinerea was observed sporulating throughout the winter months on mummified fruits (Figure 2) remaining in the field from the previous season. Isolations made from the mummified fruit tissue consistently gave cultures of B. cinerea. Other plant debris was also found to be an effective source of overwintering inoculum (Figure 2). Pieces of plant debris were collected from the experimental plots at the Plant Pathology Farm in March of 1957 and 1958. The pieces were soaked in water containing a detergent, rinsed in running water and small sections were plated on strep-PDA. Out of 300 isolations attempted, B. cinerea was isolated from 113 pieces of plant debris. The most common saprophytic competitors isolated were species of Cladosporium, Rhizopus, Alternaria and Penicillium.

Various methods for trapping air-borne spores were tried in preliminary attempts to study the dispersal of Botrytis spores within a strawberry field. One device tested is similar to that used by Maddox (40, p. 286). This is a windvane type where the spores are blown through a funnel and are impacted on a glass slide coated with vaseline. The slides were exposed at various heights (6 inches to 2 feet) and for up to 24 hours duration Botrytis spores were not detected although a large number and variety of other spores were found on the slides.

The miscellany of air-borne particles found on the slides undoubtedly confounded the detection of Botrytis spores, which are very nondescript. Even when clouds of spores shaken from rotting fruit in front of the orifice could be seen entering the funnel, very few were identified on the vaseline-coated slide. Attempts were also made to collect spores in a water filter with the suction flask apparatus described by Kluyver and Visser (35, p. 310). Spores trapped in the water were transferred to dilution plates containing strep-PDA. At dilutions where the colonies growing on the plates could be accurately read, there were very few colonies of Botrytis found. The same difficulty was encountered when petri dishes containing mineral oil were exposed and dilution plates made after centrifuging the spores from the oil into water.

The method which proved best suited to this study was simply to expose petri dishes containing strep-PDA. Plates were exposed at ground level, at 21 inches, and at five feet above ground at each of three trapping stations within the strawberry field. Exposures were made at noon, six P.M., midnight, and six A.M. on each trapping date. Spores were trapped on ten different dates from postharvest 1957 to postharvest 1958. An exposure of two minutes was found to be the maximum that plates could be exposed and still make accurate counts of the colonies. Spores trapped on

exposed plates were incubated at 20°C for two to three days. During this time characteristic colonies of B. cinerea (Figure 3) five to ten mm. in diameter had developed which could be counted and distinguished from colonies of other fungi. The ability to distinguish Botrytis colonies from those of other fungi was checked throughout the course of the experiment by making hyphal transfers to plates containing sterile agar and making positive identification after the fungus had sporulated. Out of 137 colonies transferred, only four proved not to be B. cinerea.

The relative density of Botrytis spores followed a seasonal pattern similar to that of the other air spora trapped within the strawberry field (Figure 4). The number of Botrytis spores trapped was low throughout the winter months and did not begin to increase sharply until about the middle of the harvest period. The highest counts were made shortly after harvest and decreased rapidly after renovation of the planting the later part of July.

The relative vertical distribution and diurnal density of air spora trapped over and within the field from postharvest 1957 to postharvest 1958 are presented in Tables 3 and 4. The vertical density (Table 3) of Botrytis spores decreased rapidly with increasing height above the ground level, 14.9 percent at 21 inches, and 5.7 percent at five feet. The diurnal density of Botrytis spores was lowest

Figure 2. Sources of overwintering Botrytis cinerea inoculum. The fungus sporulates on plant debris and mummified strawberry fruit.

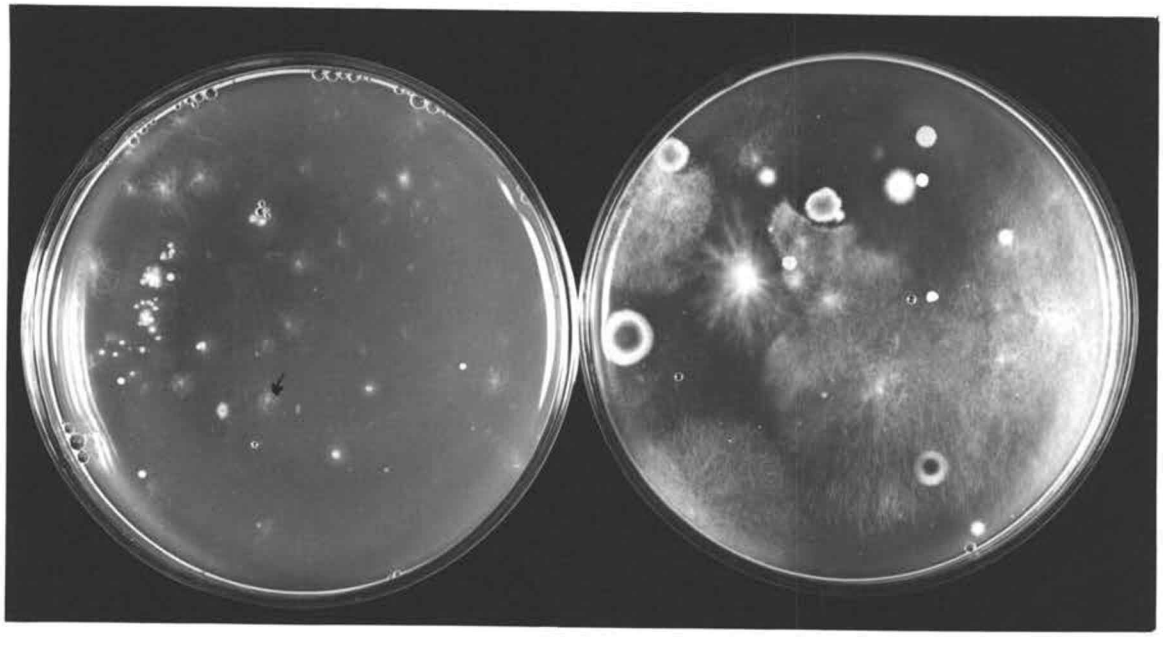
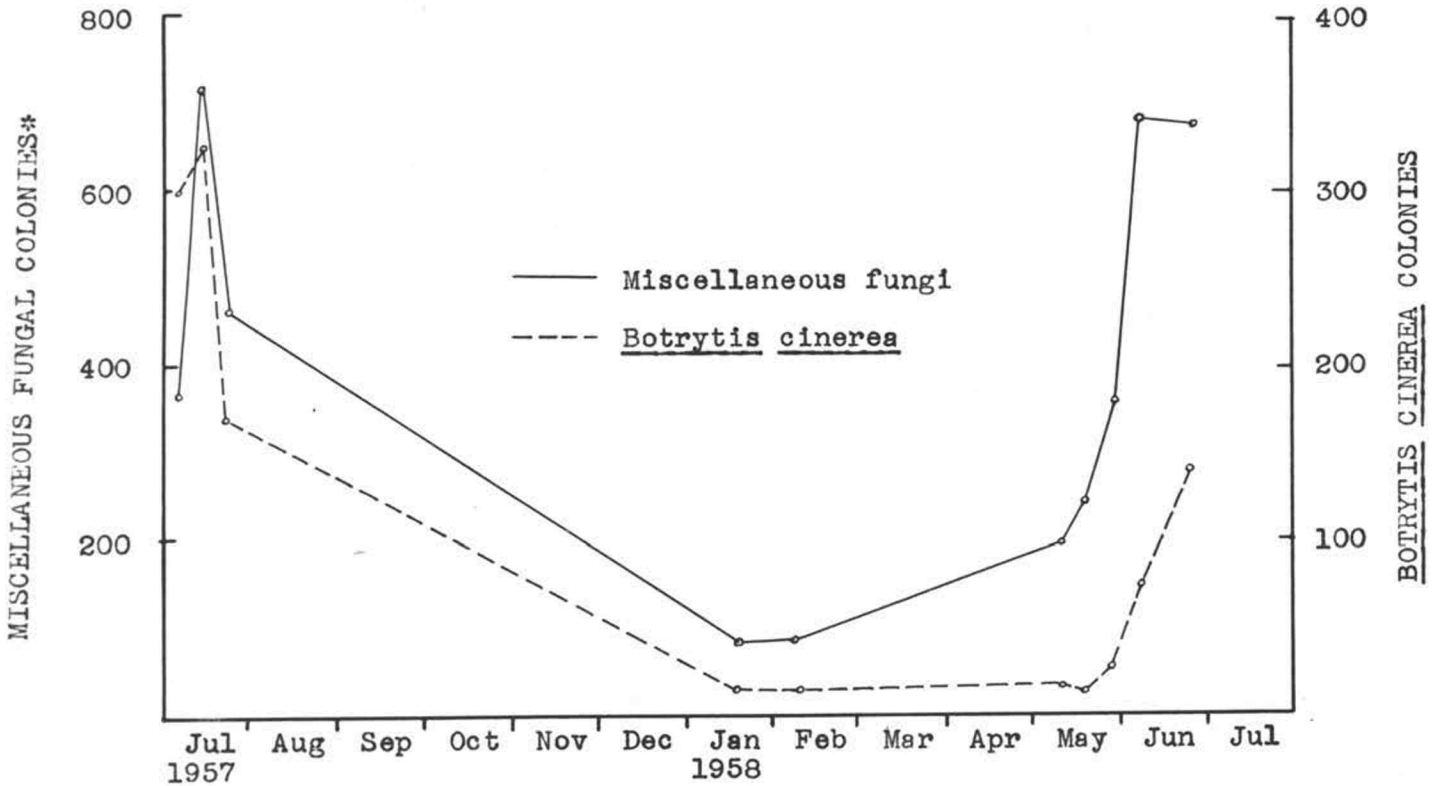


Figure 3. (left) Strep-PDA plate showing characteristic colonies of Botrytis cinerea (arrow) after 3 days incubation at 20°C. (right) Appearance of a similar plate after 5 days incubation at 20°C, showing overgrowth of colonies.



*Total colonies recovered on 36 plates exposed for 2 minutes each at three trapping stations and three heights (ground, 21 inches, and 5 ft.). The 36 plates were divided into lots of nine each and exposed at noon, 6 P.M., midnight, and 6 A.M.

Figure 4. Seasonal air spora over and within a strawberry field from post-harvest 1957 to post-harvest 1958.

at 6 A.M. but continued to increase, with most spores being trapped at midnight (Table 4).

Table 3. Vertical distribution of air spora over and within a strawberry field.

Colonies recovered	Height of plate exposure		
	Ground level	21 inches	5 feet
Botrytis	874*	164	62
Other fungi	2637	637	604
Plates exposed	120	120	120

*Total colonies recovered from 7/6/57 to 6/25/58.

Table 4. Diurnal density of air spora over and within a strawberry field.

Colonies recovered	Time of plate exposure			
	Noon	6 P.M.	Midnight	6 A.M.
Botrytis	254*	308	322	216
Other fungi	1503	1091	675	609
Plates exposed	90	90	90	90

*Total colonies recovered from 7/6/57 to 6/25/58.

When the data concerning the diurnal density of Botrytis spores at different heights is represented graphically (Figure 5) as a percentage of the total Botrytis spores trapped, the same general pattern described above is evident. However, the influence of time of day was greater

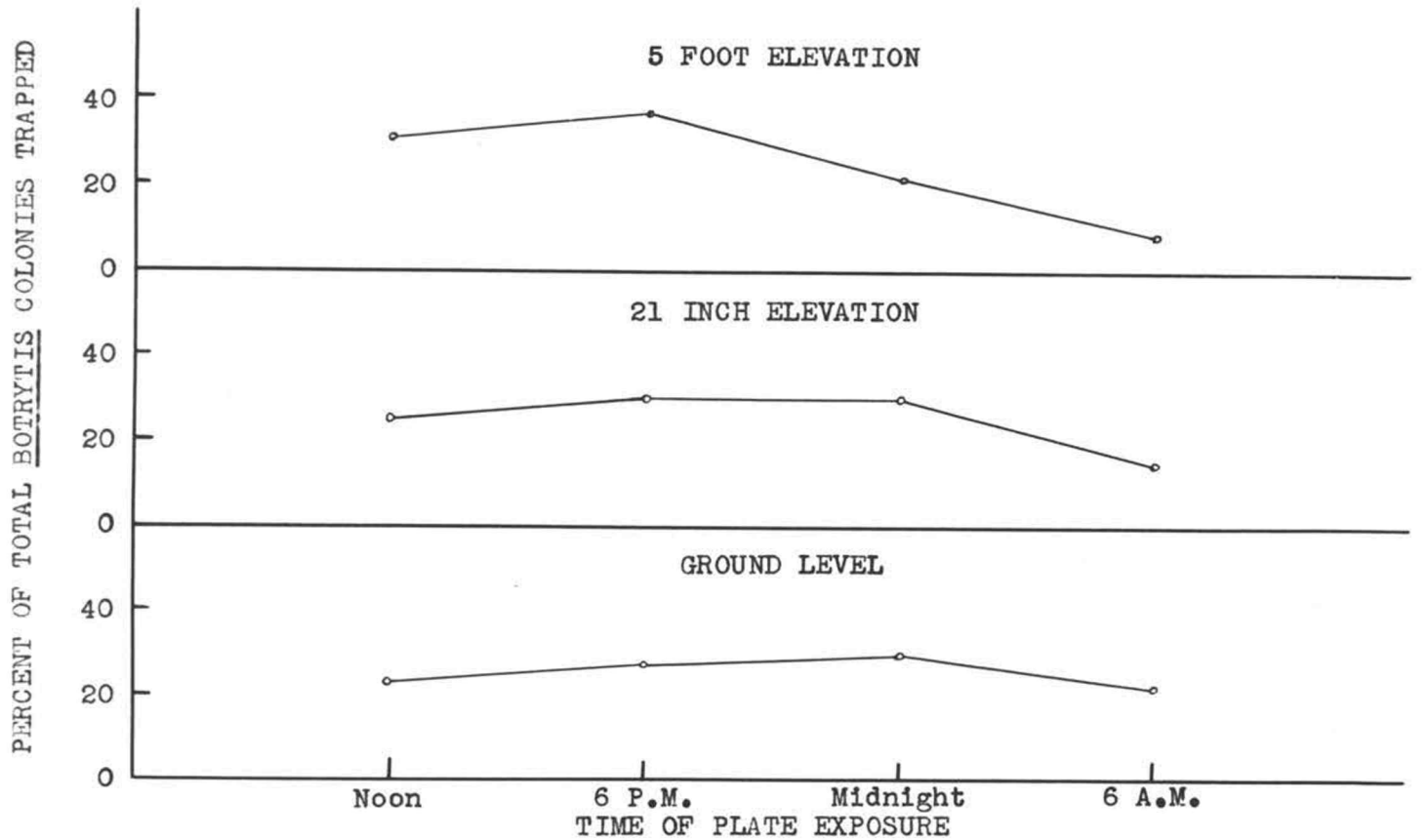


Figure 5. Diurnal density as related to vertical distribution of Botrytis cinerea spores over and within a strawberry field.

at the five foot elevation and at this elevation the highest number of spores were trapped at 6 P.M.

This method of exposing petri plates would not be practical for studying the dynamic factors of environment, e.g. temperature, humidity, and wind velocity in regard to their effect on spore dispersal. Therefore, although the macroclimatic conditions were recorded at the time of plate exposure, no attempt has been made to correlate this information with the data obtained.

These observations on source and distribution of inoculum demonstrate that, under Willamette Valley conditions, viable spores and mycelium of B. cinerea are present within and above strawberry fields throughout the year. This raises the question as to whether certain sanitation measures might not be effective in reducing the incidence of fruit rot. Many strawberry growers in the Willamette Valley use an IPC, dinitro and diesel oil combination as a winter weed control spray. Several growers felt that this combination was giving them some fruit rot control and the possibility existed that this spray might eradicate some of the overwintering inoculum in strawberry fields. Test plots were established in 1957 and 1958 at the Plant Pathology Farm to determine whether the above sanitation practice has an effect on the incidence of fruit rot. The entire field was sprayed with one

application of IPC and DN* in January of each year. The "non-sprayed" plots were covered during the spraying operation with polyethylene plastic sheets. Negative results were obtained both years. In 1957, both sprayed and non-sprayed plots had 21 percent rot and in 1958 there was 29 percent rot in the sprayed plots and 31 percent rot in the unsprayed plots. The frequent isolation of B. cinerea from mummified fruits and plant debris, two months after the application of the winter weed control spray, indicates that the IPC, dinitro and oil combination did not markedly reduce the amount of overwintering inoculum.

Another cultural practice that some Willamette Valley strawberry growers use is that of mowing the foliage shortly after harvest. During the post-harvest renovation of the strawberry field at the Plant Pathology Farm in 1957, plots were established to determine if mowing and debris removal would have any effect on the incidence of fruit rot. The entire field was mowed except for randomized, unmown check plots 40 feet long and replicated four times. Mowing was done with a power-driven sickle bar, elevated to prevent injury to the plant crowns and the debris within the mown experimental area (this included three rows on either side of the test plots) was removed with a hand

*IPC oil concentrate (2 gallons), dinitro general (2½ quarts), with diesel oil to make 10 gallons. Rate: 8 gallons per acre.

rake. Based on the number of marketable and rotted strawberries harvested in 1958, there was 31 percent rot in the mown plots and 26 percent rot in the unmown plots. Thus, foliage mowing and debris removal did not reduce the incidence of strawberry fruit rot.

Time, place, and method of infection

The disease, while regarded as a fruit rot, often causes a blossom blasting. In some cases only the petals (Figure 6) or sepals (Figure 7) may be blighted and in others the entire flower will show a blasted condition, usually with the infection extending part way down the pedicel. During the 1957 and 1958 disease surveys, an estimate was made of the amount of blossom blasting in the areas surveyed (Table 5). From conversation with growers whose fields were surveyed, it was noted that Oregon and Washington had a comparatively wet blossoming period in 1957, which is reflected in the estimated 20 percent blossom blasting in these two areas. Oregon had a comparatively dry blossoming period in 1958, as did California and British Columbia in 1957, which resulted in less than four percent blossom blasting.

Rotting of green fruits may occur during extended rainy periods and under conditions of high humidity. An estimate made of the amount of green fruit rot at the Plant Pathology Farm four days prior to the first picking of the

Table 5. Estimated amount of blossom blasting caused by B. cinerea in Pacific Coast growing areas during 1957 and 1958 seasons.

Area surveyed		Number of fields surveyed	*Number of blasted blossoms per ten feet of row	**Estimated percent blossom blasting per field
Ore.	1957	3	151	19.7
Ore.	1958	5	10	1.3
Cal.	1957	2	28	3.6
Wash.	1957	2	167	21.7
B. C.	1957	3	27	3.5

*Figures represent the average per area of three randomized counts per field surveyed.
**Based on a potential 10 T/A yield where the average marketable berry weighs 0.35 ounces and the rows are on a 42 inch spacing.

1957 harvest, indicated that 12.1 percent of the green fruits were rotting. Weather records show (Table 6) that during the prior ten day period rain fell on seven days for a total of 1.85 inches. During a comparable period in 1958, rain fell on two days for a total of 0.23 inches and there was only 1.2 percent of the green fruit rotting.

Close examination of rotting strawberry fruits revealed that, in the majority of cases, the rot had its origin at the stem-end of the fruit (Figure 8). In 1958, a survey was made of five strawberry fields in the Willamette Valley to determine the percentage of fruit rot that was of

Table 6. Daily precipitation recorded at the Corvallis Weather Station (74, pp. 56-113) and 75, pp. 54-111) during the strawberry bloom and harvest periods for 1957 and 1958.

Day of month	April 57	May 57	June 57	April 58	May 58	June 58
1	.07	--	--	.35	--	--
2	.02	.29	--	.42	--	.22
3	.01	.28	--	.42	--	.42
4	.01	--	--	.22	--	T*
5	.03	.01	--	T	--	--
6	.26	--	.20	--	--	.31
7	--	--	--	.07	--	.30
8	--	.04	--	.06	--	.12
9	--	.63	--	T	--	T
10	--	.04	--	.20	--	--
11	--	.05	--	--	.15	.18
12	.36	--	--	--	.08	--
13	.15	.08	.30	--	--	--
14	.25	.29	.40	.10	--	--
15	.34	.01	.22	T	--	--
16	--	--	.07	.37	--	--
17	.30	--	--	.36	--	--
18	.13	1.26	--	.38	--	--
19	--	.13	--	.03	.10	--
20	--	.03	--	.06	--	--
21	--	--	--	.03	--	--
22	--	.02	--	.15	--	--
23	--	.11	--	.11	.13	--
24	.11	--	--	.18	.03	.09
25	--	--	--	--	.20	.13
26	--	--	--	--	--	--
27	--	--	--	--	.03	T
28	--	--	--	--	.18	.02
29	--	--	--	--	.19	--
30	.01	--	--	--	--	.03
31	--	--	--	--	.24	--

*Trace

Note: First picking of 1957 harvest made on May 29.
First picking of 1958 harvest made on May 26.

Figure 6.

Botrytis petal blight.
Healthy (top row).
Blighted (bottom rows).

Figure 7.

Botrytis sepal blight.
Healthy (top row).
Blighted (bottom rows).

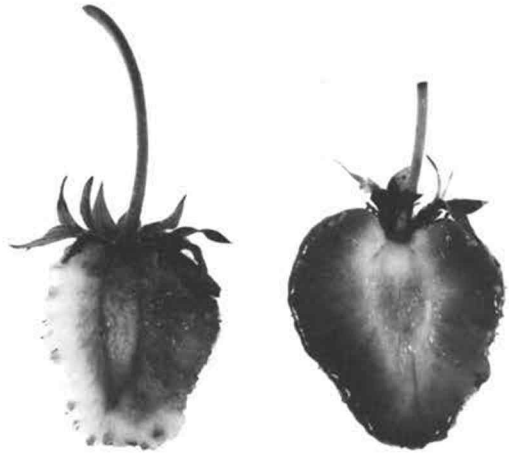


Figure 8. Marshall strawberry sliced to show typical stem-end rot (left). Marketable fruit (right).

stem-end origin (Table 7). This survey showed that 71-87 percent of the rotting strawberries had been infected at the stem-end.

Table 7. The incidence of stem-end infection of rotting strawberry fruits in the Willamette Valley, Oregon, 1958.

Location of field and date surveyed	Rot of stem-end origin per 50 rotted fruits	Percent stem-end rot
Corvallis, June 4	42.8*	85.6
Hillsboro, June 10	42.5	85.0
Silverton, July 1	39.2	78.4
Silverton, July 1	35.5	71.0
Silverton, July 1	43.5	87.0

*Based on four randomized counts per field. Each count consisted of counting the first 50 fruits encountered in a strawberry row where the origin of the rot could be determined.

In many cases, the rot involved only one side of the stem-end and appeared to have originated from infected stamens or sepals. In order to establish whether these floral parts were pathways for fruit infection, isolations were made from symptomless green and ripe fruits, and from attached necrotic and non-necrotic floral organs (Table 8). Necrotic petals, stamens and sepals were found to be frequently infected by B. cinerea. In cases where Botrytis was not isolated from necrotic floral tissues, saprophytic

Table 8. Frequency of isolation of Botrytis cinerea from symptomless green and ripe fruit, and from attached necrotic and non-necrotic floral organs.

	Condition of tissue from which isolations were attempted			
	Fruit		Floral part	
Floral organ	Green	Ripe	Necrotic	Non-necrotic
Petal	--	--	*96/120**	4/90
Stamen	--	--	39/90	3/90
Sepal	--	--	21/90	8/90
Receptacle	6/90	102/190	--	--

*Number of times Botrytis was isolated.
**Isolations attempted.

fungi, e.g. Cladosporium spp. and Penicillium spp., were commonly isolated. Of interest was the frequency with which B. cinerea was isolated from ripe, symptomless strawberry fruits. The method of isolation was to surface-sterilize (see p. 22) the strawberries, remove the cap, and transfer a small piece of exposed fruit flesh to petri plates containing strep-PDA.

Other isolations were made to determine if this latent infection of symptomless fruits was associated with areas other than the stem-end. The fruits were surface-sterilized as previously described and cores taken perpendicular to the fruit axis, from the stem-end, middle and tip-end with a sterile cork borer. The epidermis was removed from each end of the tissue core and the core was plated on

strep-PDA. Isolations made from samples taken at early, middle, and late harvest (Table 9) show that 74 percent of the latent infection was confined to stem-end tissues.

Table 9. Distribution of latent Botrytis cinerea infection in marketable berries.

Date sample was taken	Frequency of isolation from each area			Isolations attempted from each area
	Stem-end	Middle	Tip	
May 26, 1958	16	4	0	30
June 4, 1958	17	4	0	30
June 30, 1958	21	9	2	30
Total isolations	54	17	2	90

Further evidence for the hypothesis that floral parts are pathways by which B. cinerea gains entrance to the fruit was demonstrated in greenhouse experiments. From Marshall strawberry plants growing in number 10 cans, paired berries on the same fruit cluster were selected and the petals, stamens and sepals removed from one berry of each pair. The fruits were selected five to ten days after fertilization, and fruits which later showed signs of incomplete fertilization were discarded. Plants with the above paired fruits were then grown under various combinations of wet and dry environmental conditions (Table 10) where a high density of B. cinerea spores was artificially

maintained (see p. 24) in the air. The total number of fruits that had no symptoms of rot at maturity was 51 where the petals, stamens and sepals had been removed against 22 where these floral organs remained attached. However, if the results obtained under dry conditions are eliminated, 32 fruits reached maturity where the floral organs had been removed and only six where they were left attached. Fruits which had their petals, stamens, and sepals removed were less susceptible to Botrytis rot as shown in Figure 9.

Table 10. The effect of removing petals, stamens, and sepals on the incidence of fruit rot under various combinations of wet and dry environmental conditions in the greenhouse.

Environmental sequence*	Status of floral parts	Initial number of fruits	Symptomless at maturity
Dry-dry	attached	20	16
Dry-dry	removed	20	19
Dry-wet	attached	24	4
Dry-wet	removed	24	12
Wet-dry	attached	23	1
Wet-dry	removed	23	13
Wet-wet	attached	19	1
Wet-wet	removed	19	7

*An attempt was made to simulate various combinations of weather that might occur during the blossom and harvest periods.

Figure 9. Effect of petal, stamen and calyx removal on susceptibility to rot under wet conditions. The fruit on right of each cluster had perianth and stamens removed shortly after fertilization and the calyx remained attached to the others.

The effectiveness of preharvest* applications of fungicides in reducing the incidence of strawberry fruit rot indicates that the preharvest period is the critical period during which primary infections occur. In 1958, tests of fungicides on Marshall strawberries for rot control on the Herb Holman farm, Hillsboro, Oregon, three preharvest applications of captan significantly reduced the incidence of fruit rot, and a fourth application between the first and second picking did not significantly reduce the amount of fruit rot further (Table 11).

Table 11. Effectiveness of preharvest fungicide applications in preventing strawberry fruit rot on the Holman farm, Hillsboro, Oregon in 1958.

Material	Number of applications	Number of fruits harvested per 40 feet of row*		Percent of fruit rotted
		Marketable	Rotted	
Captan**	3 preharvest 1 harvest	2,926	489	14.3
Captan	3 preharvest	2,676	535	16.7
Check-no fungicide		2,170	788	26.6
L.S.D. at 1% level				7.3

*Average of three replications.
**Rate: 3 lbs. captan 80-W per 100 gallons per acre.

*Preharvest is defined as the period between the first appearance of blossoms and the beginning of harvest.

The incidence of fruit rot at each picking, in plots sprayed with three preharvest applications of captan and in unsprayed plots, is represented graphically in Figure 10. The ratio of rotted and marketable berries remained approximately the same throughout the harvest period. This further indicates that most of the primary infection takes place sometime prior to harvest. Otherwise, one would expect this ratio to be proportionately smaller with each subsequent picking during harvest. A cooperative spraying and dusting program was conducted in 1958 with the help of Mr. W. B. Neuberg*, to determine the effectiveness of field applications of fungicides in preventing infection. Data were taken on the incidence of field rot and latent infection of marketable fruits. To determine the amount of latent infection of marketable fruits, the following procedure was used: 1) A random sample of 100 marketable fruits, with the calyx attached, was taken at the time of the second picking from each replication of treated and untreated 30 foot plots; 2) fruits were washed in water containing a detergent; 3) rinsed in tap water; 4) soaked approximately one minute in 20 percent clorox; 5) rinsed in fresh tap water; and 6) incubated in a moisture chamber at room temperature (72°F). The berries did

*Research Horticulturist, Western Branch Laboratory, Birds Eye Division, General Foods Corp., Hillsboro, Oregon.

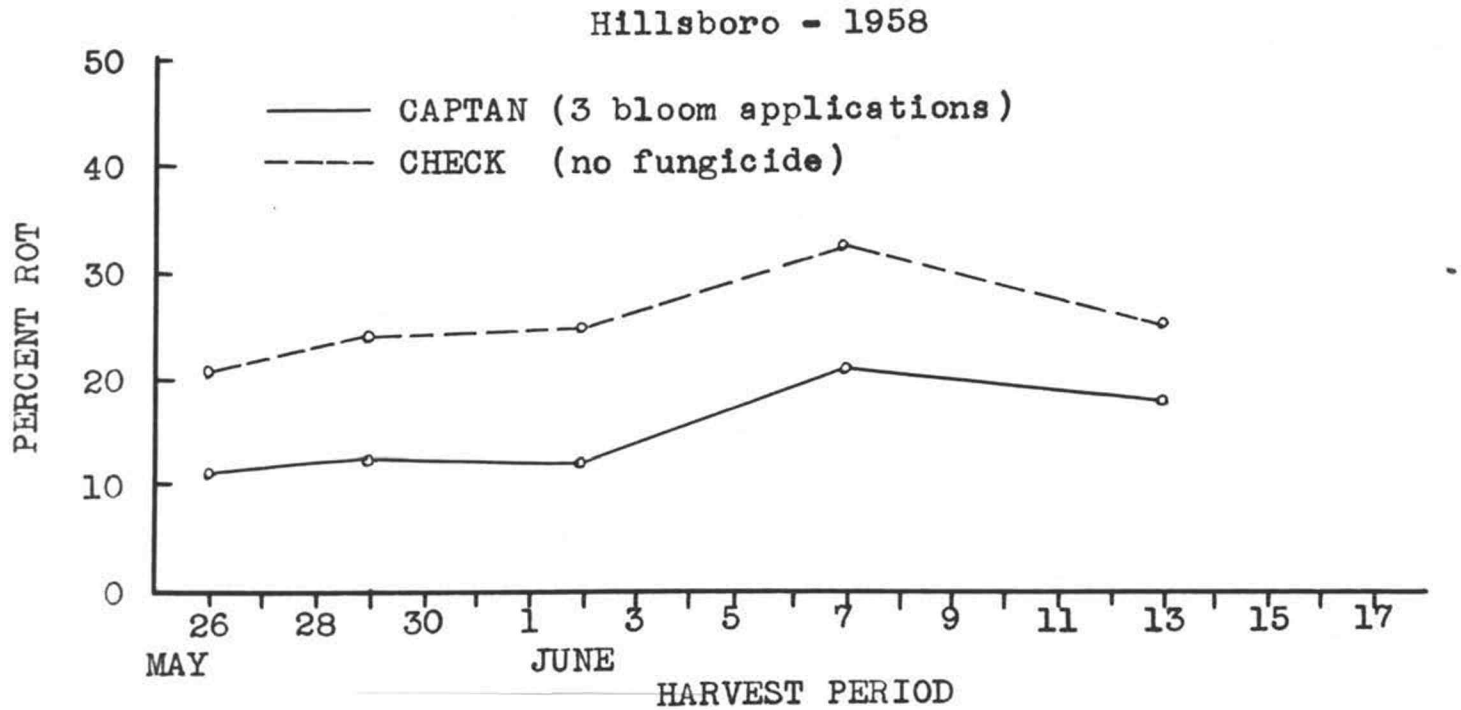


Figure 10. The effect of bloom-applied fungicides on the incidence of strawberry fruit rot at each picking during the harvest period.

not contact each other in the moisture chamber and rested on absorbent paper saturated with a 20 percent clorox solution, which maintained a high humidity in the chamber and minimized the chances of secondary infection. The number of rotted berries was recorded after four days incubation (Table 12). Marketable Marshall strawberries taken from

Table 12. Effect of fungicides on the incidence of field rot and rot of marketable strawberries incubated four days at room temperature (72°F).

Variety	Treatment	Preharvest applications	Percent rot Incubated	Field
Marshall	captan dust	3	27	10.0
Marshall	captan dust	8	--	7.8
Marshall	captan spray	8	28	4.7
Marshall	captan spray	3	--	10.1
Marshall	no fungicide	--	85	29.5
Siletz	captan spray	8	33	3.2
Siletz	no fungicide	--	93	26.5

plots which received three preharvest applications of captan dust and from plots receiving no fungicide, had 27 percent and 85 percent, respectively, of fruit rot when incubated under the conditions outlined above. In the field, these same plots had 10.0 percent and 29.5 percent rot, respectively. Sprays and dusts were equally effective, and three preharvest applications gave control

comparable to eight applications. This evidence further supports the importance of infection during the preharvest period, and indicates that there is a considerable amount of latent infection.

Histological evidence of infection

Limited histological studies were made to determine the site and method of infection. Naturally infected Marshall strawberries with symptoms of petal, stamen, or sepal infection, but no apparent stem-end rot, were examined. Microscopic observations were also made of fruits showing stem-end rot and of fruits where mummification had started. Tissues for sectioning were immersed in Rawlings (59, p. 14) no. 2 formalin-acetic-alcohol killing and fixing solution, and infiltrated and embedded in paraffin according to the tertiary butyl alcohol method of Johansen (34, pp. 130-131). Sections 14 microns thick were cut on the rotary microtome and stained according to the following hematoxylin-safranin schedule:

Xylol five minutes
Xylol rinse
100 percent alcohol
95 percent alcohol
Distilled water
Two percent ferric chloride ten minutes
Distilled water four changes in five minutes
Dilute hematoxylin five minutes
(ten drops of ten percent alcohol-hematoxylin in stain jar)
Tap water five minutes
Dilute safranin ten minutes

Tap water one to five minutes
50 percent alcohol
100 percent alcohol
Xylol

Another shorter and very satisfactory method of staining sections was the use of lactophenol (34, p. 24) containing one percent acid fuchsin. Sections were carried to distilled water (see hematoxylin-safranin schedule), excess water blotted from the slide, sections were flooded with lactophenol-acid fuchsin and then warmed gently for one minute over an alcohol flame. The lactophenol was then removed with running tap water and the sections were dehydrated to 95 percent alcohol. At this point the sections were stained lightly with fast green. Direct mounts of fresh material were made by placing pieces of tissue in heated (fuming) lactophenol containing one percent cotton blue for one or two minutes and then mounting in clear lactophenol.

Pieces of tissue adjacent to those for histological examination, were plated on strep-PDA for positive determination of the presence of B. cinerea.

Hyphae were present in necrotic petal, stamen and sepal tissues (Figures 11, 12 and 13). In cases where necrotic stamens and sepals were attached to the calyx whorl, invasion of the stem-end receptacle tissue was observed (Figure 14). Observations indicated that the invasion of stem-end receptacle tissue originated in infected

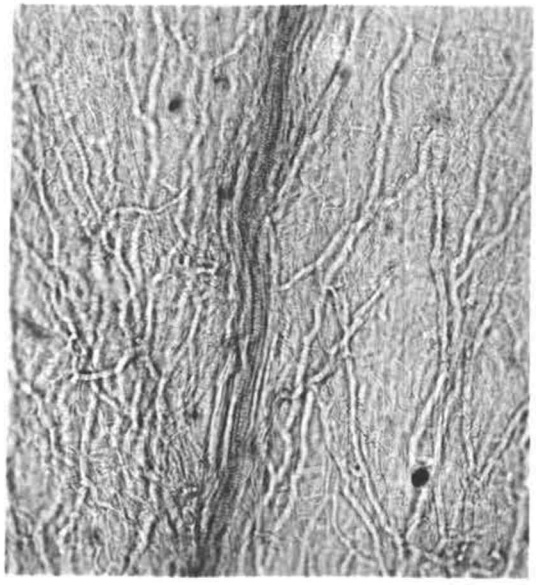


Figure 11.
Botrytis hyphae in tissue of strawberry petal. X100

Figure 12.
Botrytis hyphae in filament tissue of strawberry flower stamen. X100

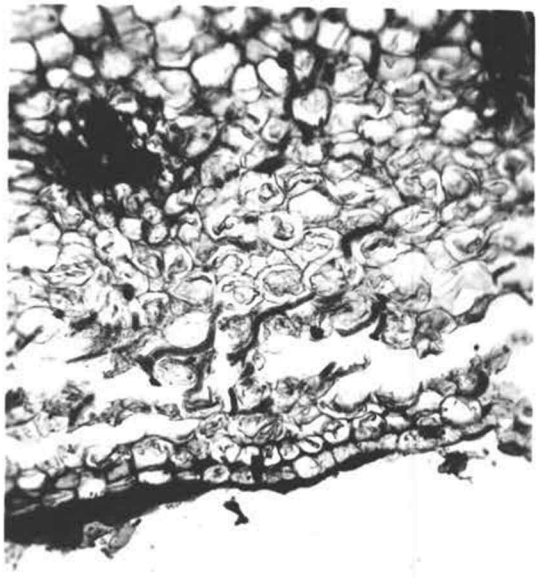


Figure 13.
Cross section of strawberry sepal tissue showing hyphae in subepidermal layers below the lower epidermis. Note separation of cells. X200

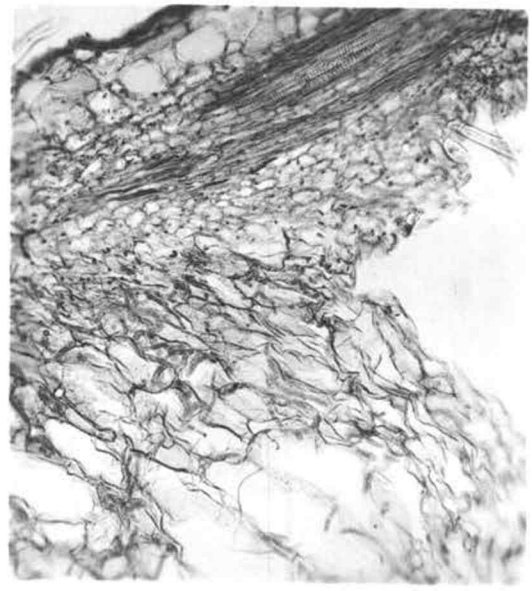


Figure 14.
Botrytis hyphae in strawberry stem-end receptacle tissue in the region of calyx attachment. X100

stamens and sepals and, in most cases, primarily from infected stamens. Invasion of the calyx tissue below the point of stamen attachment (Figure 15) occurs within the two to three cell layers inside the epidermis as evidenced by the presence of hyphae and separation of cells in this area (Figure 13). Latent infection of the stem-end receptacle tissues appeared to be limited to a radial spread of hyphae in tissue between the epidermis and the major vascular network of the fruit, and the density of hyphae was greatest in tissues adjacent to the vascular bundles (Figure 16).

The majority of the hyphae were found to be intercellular in rotted fruit tissue (Figure 17). However, as the rot became more advanced, intracellular hyphae were commonly found (Figure 18). Eventually the entire fruit is ramified with mycelium of the fungus and mummification of the tissues occurs.

In no case was evidence found to indicate that primary infection originates from direct infection of the fruit through the epidermis at the stem-end, or from contact of infected stamens and sepals with the fruit surface. In cases where infected petals are trapped beneath the calyx, infection of the fruit may occur directly from these petals. However, observations indicate that these blighted petals are trapped because of rapid mycelial growth which invades

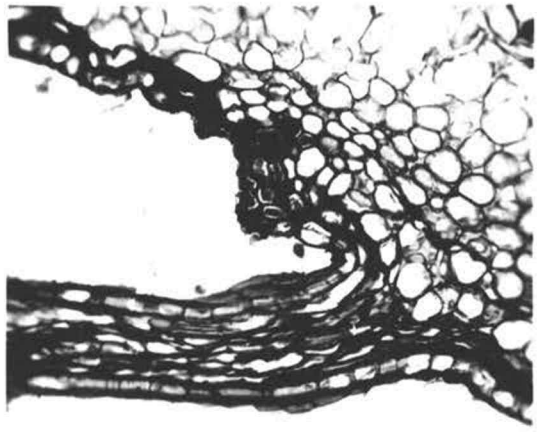


Figure 15.
Cross section of strawberry calyx tissue showing necrotic filament and sepal tissues. X100

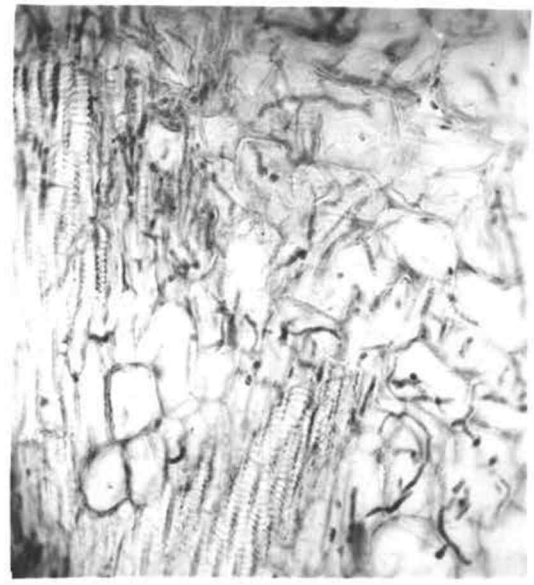


Figure 16.
Portion of a cross section of strawberry stem-end receptacle tissue showing extensive development of mycelia in tissues adjacent to vascular tissues. X200

Figure 17.
Portion of a cross section of rotted strawberry fruit tissue showing intercellular arrangement of hyphae. X200

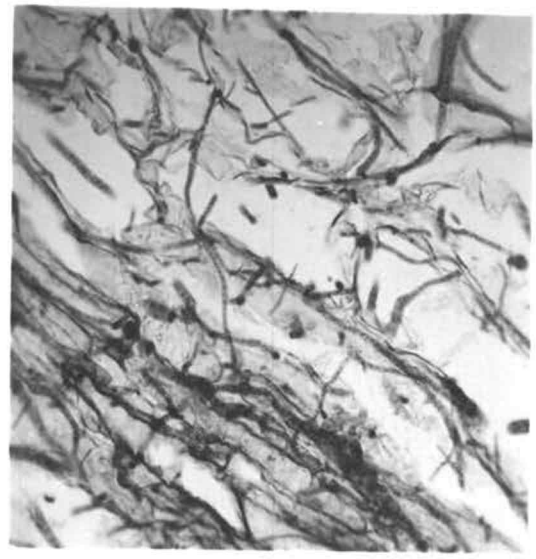


Figure 18.
Portion of cross section of rotted strawberry fruit tissue showing both intercellular and intracellular arrangement of hyphae. X200

the calyx tissue and holds the petal attached to the calyx. Except under conditions conducive to severe petal blight, an abscission layer is formed at the point of petal attachment, and petal fall occurs before invasion of the calyx tissue can occur.

Factors affecting disease development

Some of the factors which are responsible for epiphytotics of strawberry fruit rot caused by B. cinerea have been studied. Investigators have recognized for a long time that severe economic loss from Botrytis rot usually occurs when prolonged periods of rainfall and high humidity occur during harvest. The effect of moisture on the incidence of fruit rot was very evident from the count of rotted and marketable strawberries obtained at the Plant Pathology Farm during the 1957 and 1958 seasons (Figures 19 and 20). The relationship of moisture and disease development was emphasized further in 1957 when the preharvest period was relatively wet and most of the harvest period was dry, whereas in 1958 this was just reversed by having a dry preharvest period and a wet harvest period. These different moisture relationships were reflected in the incidence of blossom blasting and rot of green fruit (Figures 19 and 20).

In the greenhouse, an experiment was designed to determine the effect of four different sets of environmental

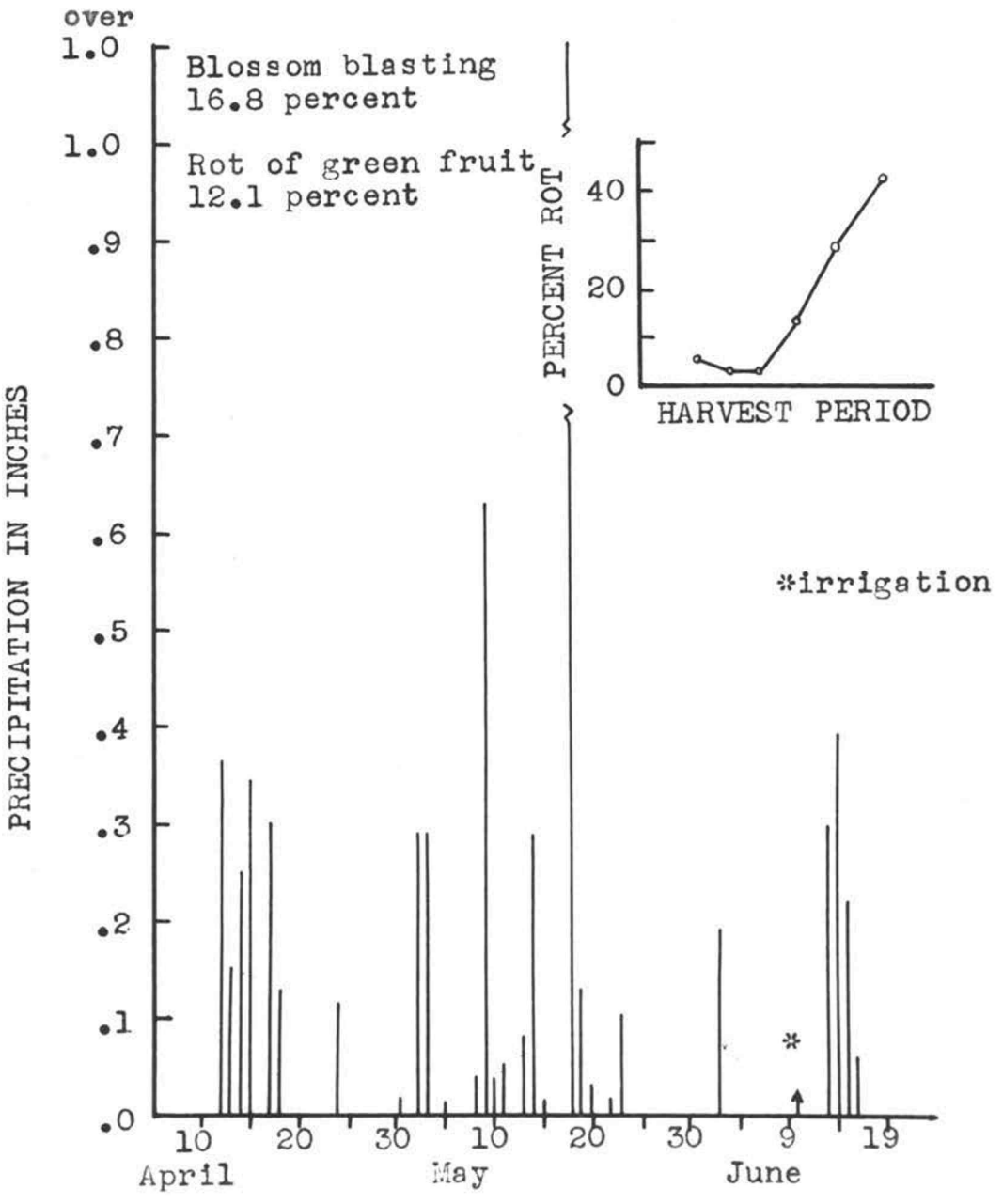


Figure 19. The relationship between daily precipitation (Table 6) and the incidence of blossom blasting, rot of green fruit, and fruit rot during the 1957 season in experimental plots at the Plant Pathology farm, Corvallis, Oregon.

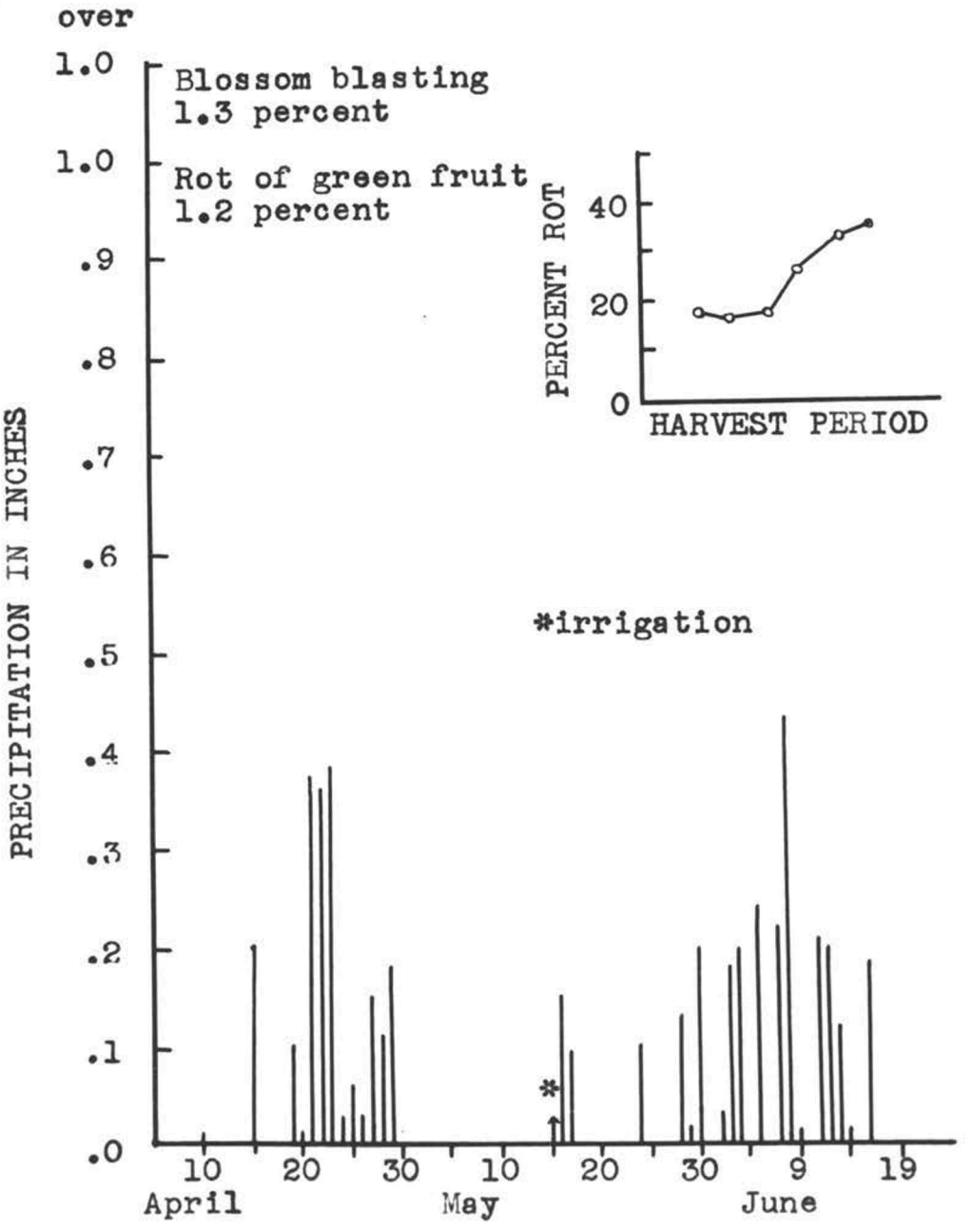


Figure 20. The relationship between daily precipitation (Table 6) and the incidence of blossom blasting, rot of green fruit, and fruit rot during the 1958 season in experimental plots at the Plant Pathology farm, Corvallis, Oregon.

conditions on the incidence of fruit rot. This was done by growing strawberries under the following artificial seasons; "dry bloom-wet harvest", "dry bloom-dry harvest", "wet bloom-dry harvest", and "wet bloom-wet harvest". This experiment was conducted during December and January, since greenhouse temperatures were more easily controlled at this time of year. Active flowering and vegetative growth of Marshall strawberry plants was induced by extending the day length with overhead banks of fluorescent lights. Plants were selected which had one inflorescence with four to five recently fertilized fruits attached, including the primary fruit and three or four secondary fruits. Any additional fruits were removed. Fruits which, during the course of the experiment, showed signs of incomplete fertilization, d.g. "catfacing", were discarded. Previous observations indicated that consistent rot development does not occur in these fruits.

The plants were divided into two lots, one of which was placed inside a moisture chamber where they received intermittent misting from overhead spray jets, and the other remained outside the chamber under dry greenhouse conditions. After two weeks, the plants that were to receive a "wet bloom-dry harvest" treatment were removed to the experimental area outside the moisture chamber, and plants receiving a "dry bloom-wet harvest" treatment were

placed inside the moisture chamber. The relative humidity inside the moisture chamber varied from 85 to 99 percent with a mean daily r.h. of 92 percent, and the temperature varied from 58° to 72°F, with a mean of 62°F. In the experimental area outside the moisture chamber, the r.h. varied from 48 to 60 percent, with a mean daily r.h. of 51 percent and the temperature varied from 58° to 67°F, with a mean of 60°F. A high density of air-borne Botrytis spores was maintained by placing rolled oats, inoculated with B. cinerea, at spaced inoculum centers (see p. 24) in the experimental area. The incidence of fruit rot under the four sets of environmental conditions is shown in Table 13.

Table 13. The incidence of fruit rot under dry and wet conditions in the greenhouse.

	Number of fruits		
Artificial season	Tested	Symptomless after 15 days*	Symptomless at maturity
Dry bloom-dry harvest	32	29	27
Dry bloom-wet harvest	36	27	4
Wet bloom-dry harvest	30	7	5
Wet bloom-wet harvest	32	10	2

*The 15 day period following fertilization is considered the bloom season.

After 15 days in the moisture chamber, only 17 of the 62 experimental fruits showed no symptoms of rot. At the end of this same period outside the moisture chamber, 56 of the 68 experimental fruits were symptomless. When 27 of the symptomless fruits receiving a "dry bloom" period were placed in the moisture chamber, only four reached maturity without rotting.

The effect of supplemental fertilizers on the incidence of fruit rot was investigated. There is much conflicting information on the value of chemical fertilizers in strawberry culture. This is to be expected since conditions vary greatly between different strawberry growing areas.

In the fall of 1956 and 1957, nitrogen (as ammonium sulfate), phosphorus (as superphosphate) and potassium (as muriate of potash) fertilizers were applied as side dressings to replicated plots of Marshall strawberries at the Plant Pathology farm. Experimental applications of N, P and K fertilizers made in 1957 were repeated on the same plots treated in 1956. The last fertilizer application these plants received prior to the above experimental applications was a side dressing of 16-20 ammonium-phosphate (rate unknown) in the fall of 1955.

The influence of N, P and K fertilizers on the incidence of fruit rot in the harvest season following fall

application is shown in Table 14. In 1958, the incidence of fruit rot in the plots received the 200 pound per acre rate of nitrogen was significantly greater than no treatment at the five percent level. The results from all other fertilizer applications, both in 1957 and 1958, were not significantly different from no fertilizer application when the data were subjected to an analysis of variance. However, there is some indication that potassium fertilizers may help in reducing the amount of fruit rot and, while not particularly significant statistically, there was an average of 23 percent less rot in the potassium plots in 1957 and 8 percent less in 1958.

Table 14. Influence of nitrogen, phosphorus and potassium fertilizers on incidence of strawberry fruit rot in 1957 and 1958.

	1957		1958	
Treatment	Percent rotted*	L.S.D. at 5% level	Percent rotted	L.S.D. at 5% level
No fertilizer	12.7		34.8	
N 100 lbs/A	14.1	12.6	37.2	12.1
N 200 lbs/A	20.4	10.2	50.5	11.3
P 150 lbs/A	11.1	7.1	39.2	12.5
K 100 lbs/A	9.8	4.9	31.9	8.8

*Based on the average number of marketable and rotted fruits harvested from four randomized and replicated 40 foot plots of Marshall strawberries.

There appears to be a wide range of varietal resistance to decay among the various strawberry varieties. In an attempt to evaluate the relative susceptibility to fruit rot of the three major strawberry varieties grown in Oregon, the incidence of fruit rot in plots of Marshall, Northwest and Siletz varieties at the Plant Pathology farm, was determined for the 1957 and 1958 harvest (Table 15). In

Table 15. Susceptibility of Marshall, Northwest and Siletz strawberries to fruit rot under field conditions, Corvallis, 1957-1958.

Variety	Percent rot* 1957	1958
Marshall	14	29
Northwest	33	55
Siletz	56	59
L.S.D. at 1% level	5	11

*Based on the number of marketable and rotted berries harvested from randomized 14 foot plots, replicated four times.

1957, Northwest and Siletz varieties had 19 percent and 42 percent, respectively, more rot than did the Marshalls, and in 1958 there was 26 percent and 30 percent more rot in the Northwest and Siletz varieties. However, this difference cannot be explained entirely by host resistance. During the 1957 season, the later-maturing varieties, Northwest and Siletz, were subjected to four days of rainy weather

(Figure 19) during their peak harvest period, while most of the Marshalls were harvested during "dry" weather. In 1958, a prolonged rainy period extended through the peak harvest periods for all three varieties, thus rainfall alone does not account for the increased incidence of rot in the Northwest and Siletz varieties. Another factor which undoubtedly contributed to the high incidence of rot in these varieties, was the increased density of air-borne Botrytis spores from rotting fruits in the adjacent Marshall guard rows during the susceptible "bloom" period for these later-maturing varieties.

In a greenhouse experiment, the susceptibility of these three varieties was determined using fruits of approximately the same maturity, grown under the same conditions of temperature and humidity. Green and ripe fruits were dipped in a heavy suspension of Botrytis spores while still attached to the mother plant and then placed in individual incubation chambers (Figure 1). The entire plant was then placed in a large moisture chamber where a high relative humidity was maintained by allowing water to constantly flow over cloth sheets placed along three sides of the chamber. The number of days between inoculation and symptom expression is given in Table 16. Inoculated ripe Marshall strawberries showed symptoms of rot one to two days later than Northwest and Siletz varieties. When

green fruits were inoculated, the Marshall fruits showed symptoms of rot seven to ten days later than the other two varieties.

Table 16. Rate of fruit rot development in inoculated Marshall, Northwest and Siletz varieties grown under conditions of 99 percent relative humidity in the greenhouse.

Variety	Number of berries inoculated		Days between inoculation and symptom expression	
	Ripe	Green	Ripe	Green
Marshall	52	25	3-4	12-15
Northwest	27	20	2-4	6-8
Siletz	19	21	2-3	4-5

*Inoculations were made at various times during January and February, 1957.

In another experiment, marketable fruits used were all harvested on the same day, with the calyx attached, from variety plots at the Lewis-Brown Horticultural Farm near Corvallis. The fruits were surface-sterilized (see p. 50), dipped into a water suspension of Botrytis spores and then incubated in plastic boxes at room temperature (72°F). The plastic boxes were surface-sterilized with a 20 percent clorox solution and paper toweling saturated with water was placed in the bottom of each container to maintain a high humidity during incubation. Fifteen fruits were placed in each container so as to not touch each other.

Uninoculated checks received the same treatment, except that they were dipped into sterile water. Included in this experiment was the strawberry selection 2414, which has shown promise in field tests conducted by G. F. Waldo*. The susceptibility of fruits to rot was based on a rot index derived by the following method: After 66 hours incubation, the fruits were sliced in half along the axis in a plane perpendicular to the greatest diameter of the rotted portion, and the area of the exposed cut was rated from zero to five.

0 no visible rot
1 slight rot
2 less than half rotted
3 half rotted
4 more than half rotted
5 completely rotted

The results of this experiment are presented in Table 17. At the end of 66 hours incubation, all of the inoculated fruits were rotting and of the uninoculated checks, only two Marshall fruits, one Siletz and two Northwest fruits showed no symptoms of rot. Under conditions of this experiment, the rate of rotting was not significantly different for the commercial varieties; Marshall, Northwest and Siletz. However, the experimental variety 2414 showed considerable resistance to decay. The rot was less extensive in uninoculated fruits, which had a total of 83 percent

*Horticulturist, U.S.D.A., Oregon State College, Corvallis, Oregon.

latent infection.

Table 17. The relative susceptibility to rot of inoculated and uninoculated marketable fruits harvested from selected strawberry varieties and incubated for 66 hours at 72°F.

Variety	Number tested	Mean rot index	Mean percent of fruit rotted*
	Inoculated berries		
Marshall	45	5.00	100
Siletz	45	4.64	93
Northwest	45	4.36	87
2414	45	3.51	70
	Uninoculated berries		
Marshall	15	3.07**	61
Siletz	15	3.20	64
Northwest	15	3.27	65

Rot Index

0 no rot
1 slight
2 less than half rotted
3 half rotted
4 more than half rotted
5 completely rotted

*Based on the rot index.
**Includes only fruits which showed symptoms of rot.

Attempts to determine the length of the incubation period between time of infection and symptom expression under any given set of conditions, proved to be very difficult because of the high incidence of latent infection of apparently healthy fruit. No practical method was found

whereby one could be assured that the fruits being used were not already infected. Even when blossoms were enclosed in individual chambers (Figure 1) shortly after fertilization had occurred, approximately 50 percent were destroyed by Botrytis before they reached maturity. This was not unexpected since Botrytis spores are a common component of greenhouse air spora.

The results of one greenhouse experiment (Table 18) show that 90 percent of the uninoculated fruits were rotted as a result of natural infection. In this experiment, inoculated and uninoculated ripe Marshall strawberries were incubated under conditions of 99 percent r.h. in individual moisture chambers (Figure 1). The mean number of days before any rotting of the uninoculated fruits could be seen was 6.5 days and for inoculated fruits, 3.4 days.

Table 18. The rate of rot development in naturally and artificially infected Marshall strawberries incubated in individual moisture chambers under conditions of 99 percent relative humidity in the greenhouse.

Mode of infection	Berries tested	Percent infection	Number of days before visible symptoms		
			Min.	Max.	Mean
Natural (uninoculated)	97	90	4	8	6.5
Artificial (inoculated*)	52	100	3	4	3.4

*Fruits were dipped in water suspension of Botrytis spores.

DISCUSSION

The most important fruit rot of strawberries in Pacific Coast fields is the rot known as "grey mold" caused by the fungus Botrytis cinerea. Surveys made of strawberry fields in British Columbia, Washington, Oregon and California indicate that over 90 percent of the fruit rot is caused by this fungus. Several other fungi known to cause fruit rot were isolated from rotting fruits but were of minor economic importance in the areas surveyed. One of the common minor rots of strawberries in Oregon was found to be caused by a species of Melanconium. This rot of strawberries is new in the sense that it has never been reported in the literature.

Since B. cinerea was determined to be the only organism causing extensive strawberry fruit rot in the field, investigations were conducted to learn more about the etiology and epiphytology of the rot caused by this fungus.

The principal source of spores for initial infections is overwintering mycelium in mummified fruit and plant debris within the strawberry field. Exposure of strep-PDA plates, over and within a strawberry field, demonstrated that viable B. cinerea spores were present in the air throughout the year. The relative spore density was low during the winter months and did not start to increase rapidly until towards the end of harvest.

The rapid decrease in concentration of spores with increasing distance from the sporulation site is in agreement with the results reported by Jarvis (31, p. 27) and Miller (45, p. 24) who used the first spore trap.

While the efficiency of automatic volumetric spore traps has been shown to be very high when tested against certain air-borne particles (53, p. 240) and 27, pp. 260-262), detection of specific spore types is limited. This is especially true with small nondescript spore types, e.g. B. cinerea, where microscopic identification of spores trapped on glass slides is confounded by the great variety and density of other air-borne particles as well as the similarity to other hyaline single-celled spores. Also in this study, and in studies by Jarvis (32, p. 39) and Miller (45, p. 24) where vaseline-coated slides were used to trap air-borne spores, there was an apparent low efficiency for trapping Botrytis spores. This failure of Botrytis spores to be impacted on glass slides coated with vaseline may be due to such physical properties of the spore as specific gravity and electrical surface charge or the type of impacting medium used.

The method of exposing petri dishes containing strep-PDA used in this investigation gave only limited quantitative data because of the necessity for short exposure times. However, this method was of qualitative value and gave some

measure of relative spore densities.

The cosmopolitan occurrence and sporulation of B. cinerea on almost any damp, decaying vegetation makes it doubtful whether the present sanitation practices used by Oregon strawberry growers have much effect on the incidence of fruit rot. In experimental plots there was no significant reduction in fruit rot from the use of a dinitro, IPC, and diesel oil winter weed spray or from foliage-mowing and debris removal.

Investigations made to determine when and where infection occurs indicate that most of the initial infections are from air-borne spores and occur during the preharvest period. If rainy weather occurs throughout most of the bloom period, considerable blossom blasting and rot of green fruits may occur. However, under dryer conditions, losses from this phase of the disease are usually not significant. The origin of stem-end rot, which is the striking symptom of strawberry fruit rot, was investigated. Isolations made from stem-end tissue of ripe marketable fruits revealed that there was a considerable amount of latent infection. In most cases, necrotic stamens and sepals were associated with these marketable fruits having latent infection. Isolations made from these necrotic floral parts have shown that they were frequently infected by B. cinerea.

Limited histological studies revealed the presence of Botrytis hyphae in infected petal, stamen and calyx tissue and also in stem-end receptacle tissue of marketable fruits. Invasion of the receptacle tissue appeared to have originated as a result of stamen and calyx infection, with invasion being internal through these tissues and not the result of contact of these infected floral parts with the fruit surface.

The infection of senescent floral parts by B. cinerea provides a source of mycelium which is capable of invading the tissues of the healthy strawberry fruit. Brown and Harvey (10, p. 653) have pointed out that "it is a well-known fact that moribund tissues are more readily attacked by Botrytis cinerea than are healthy parts". Shortly after fertilization of the strawberry fruit, petals, stamens, and to a lesser extent the calyx, become senescent and are susceptible to attack by B. cinerea. In a greenhouse experiment, the removal of petals, stamens and calyces shortly after fertilization of the fruit, markedly reduced the incidence of rot in these fruits which were grown under severe epiphytotic conditions. This experiment demonstrated the importance of these floral parts as pathways by which B. cinerea gains entrance to the fruit tissue.

Further evidence which supports the hypothesis that the preharvest period is the time when primary infection

takes place, is provided by the results of preharvest applications of protectant fungicides in reducing the incidence of strawberry fruit rot. During the 1958 harvest season, rainy weather favorable for fruit rot development continued throughout the harvest period and the ratio of rotted and marketable fruits in treated and check plots remained nearly constant throughout the harvest period. The failure of this ratio of rotted to marketable fruits to increase with the increasing number of rotting fruits and air-borne spores towards the end of the harvest period, indicates that direct infection of ripe fruit by air-borne spores during harvest is of minor importance. Preharvest fungicide application not only reduced the incidence of rot in the field but also the amount of latent infection of marketable berries. Incubation of marketable fruits from plots receiving no fungicide has shown that as high as 85 percent of the fruits had latent infection. The reduction of this amount of latent infection by 59 percent with three preharvest applications of a fungicide adds further to the evidence for bloom infection.

That the protective action of fungicides is against petal, stamen and calyx infection seems most probable because between the time of application and maturity of the fruit, the surface area of the small fruit which received the spray would have expanded many times, thus becoming

relatively unprotected. Residue analyses made by Martin and Pickard (42, pp. 85-87) in England show that only 8 ppm. captan was present on marketable fruits harvested 20 days after the last of three preharvest applications of captan applied at the rate of 2½ lbs. per 100 gallons per acre, whereas analysis of the calyx showed 400 ppm. captan. In considering the nature of the protective action of fungicides against strawberry fruit rot, it would be of interest to investigate the suggestion by Newhook (52, pp. 479-80) that as a result of fungicide application, relatively fungicide-resistant saprophytic micro-organisms as Cladosporium spp. and Penicillium spp. have time to colonize senescent floral parts and establish a saprophytic antagonism against Botrytis cinerea.

The most obvious factor responsible for severe epiphytotics of Botrytis rot is the occurrence of prolonged periods of rainfall and high humidities during harvest. The effect of precipitation on the incidence of strawberry fruit rot was clearly shown in yield data for two years taken from plots at the Plant Pathology farm. The amount and distribution of precipitation during the preharvest bloom period and harvest period for one year was almost completely reversed the next year. The amount and rate of rot development was influenced more by prolonged wet periods during harvest than during the bloom period.

During a dry harvest season, latent infected fruits remain symptomless and are harvested as marketable. Even under relatively dry preharvest conditions, there appears to be sufficient moisture associated with susceptible floral parts for infection to take place. This correlation between precipitation and incidence of fruit rot under field conditions was confirmed by growing strawberries under four different sets of environmental conditions in the greenhouse where uniform conditions of temperature, moisture and inoculum were maintained. In this experiment, as mentioned previously, more strawberries reached maturity under wet conditions if the petals, stamens and calyces had been removed than if they remained attached. This occurred even though humidity and moisture were adequate (64, pp. 1-3) for spore germination on the fruit surface. Thus a successful pathogenic relationship is dependent upon moisture in the microenvironment of susceptible plant parts, i.e. petals, stamens and calyces, for spore germination and subsequent infection of these senescent floral organs, with the rate of fruit rot development being correlated with macroenvironment precipitation and humidity.

Prolonged periods of rainfall and high humidities appear to alter the susceptibility of host tissue in a way that allows for active invasion by the fungus and rapid development of rot. A possible explanation lies in studies

by Fernando (22, pp. 108-112) and Mishra (46, p. 339) where active maceration of potato tissue by pectolytic enzymes took place only when the water content of the tissue was raised above normal. Thus factors which would change the susceptibility of the tissues to water-soaking would influence the rate of rot development.

Results from fertilizer trials indicate that high nitrogen and low potassium nutrition of strawberry plants increases the incidence of fruit rot. This agrees with the results obtained by Clayton (12, pp. 260-261), in that high nitrogen and low potassium increase the susceptibility of tobacco leaves to water-soaking, resulting in severe symptoms of wildfire disease caused by Pseudomonas tabaci. A low potassium status of apricot trees was shown by Wade (77, p. 519) to increase the incidence of brown rot (Sclerotinia fructicola). However, any change in the susceptibility of host tissue, resulting from mineral nutrition, may be overwhelmed during prolonged rainy periods. Tukey (73, p. 12) and Culpepper (15, p. 693) have shown that carbohydrate content and chemical composition of strawberry fruits is affected more by rainfall than by fertilizer application. In these investigations, this was reflected in the results from two year fertilizer trials where, during the dry 1957 harvest there was a greater reduction of the amount of fruit rot in the potassium

plots than during the wet 1958 harvest. The significant increase in the amount of rot with the 200 lb/acre rate of nitrogen indicates that the use of nitrogen fertilizers should be limited to the establishment of the planting, and applications thereafter should be minimized.

All strawberry varieties are susceptible to infection and decay caused by B. cinerea. However, the rate of rot development varies among the different varieties. Data obtained for two years from field varietal plots indicated that the Siletz and Northwest were considerably more susceptible to rotting than the Marshall variety. This difference cannot be explained entirely on host resistance since Siletz and Northwest varieties mature approximately two weeks later than the Marshalls and were subjected to a greater amount of air-borne inoculum and more optimum moisture conditions for fruit rot development. In greenhouse experiments, where Marshall, Siletz and Northwest strawberries of approximately the same maturity were inoculated and incubated under similar conditions of temperature and humidity, the rate of rot development was slower in the Marshalls but not as much as might have been expected from field results. In laboratory inoculation-incubation tests, only the experimental variety 2414 showed any appreciable resistance to decay.

Only limited conclusions can be made on the length of

the incubation period for any given set of environmental conditions because of the high incidence of latent infection (over 90 percent in some cases) of strawberries used in these studies. Inoculated ripe strawberries, incubated in individual moisture chambers, rotted twice as fast as uninoculated latent infected fruits. The range of variation between the time the fruits were placed in the individual moisture chambers and the appearance of visible rot symptoms, was four days for uninoculated, latent infected fruits and only one day for inoculated fruits. This indicates that the extent of the latent infection of individual fruits varied considerably, assuming that the rate of rot development would be proportional to the extent of the latent infection.

SUMMARY

1. Botrytis cinerea was found to be the most important fungus causing fruit rot in Pacific Northwest strawberry fields. A minor amount of fruit rot was caused by Rhizopus nigricans, Rhizoctonia spp., Dendrophoma obscurans, Gnomonia fructicola and a species of Melanconium. Melanconium had not been reported to cause strawberry fruit rot.

2. B. cinerea overwinters in mummified fruits and plant debris within the strawberry field. Viable Botrytis spores were present over and within the strawberry field during the winter months. The relative spore density starts to increase rapidly toward the end of harvest. There was also a rapid decrease in concentration of spores with increasing vertical distance from the sporulation site.

3. Minor sanitary practices such as foliage-mowing and debris removal, or the use of a dinitro, IPC and diesel oil winter weed spray, did not reduce the incidence of fruit rot.

4. The disease is characterized by the origin of rot at the stem-end. Isolations and limited histological studies indicate that Botrytis mycelium is present in senescent petal, stamen and calyx tissue associated with marketable fruits. Isolation, incubation and microscopic

examination have shown that a high percentage of the marketable strawberries which are harvested have latent infection which is confined primarily to the stem-end receptacle tissue.

5. Preharvest fungicide applications significantly reduced the incidence of rot in the field, and the amount of latent infection of marketable fruits.

6. Strawberries grown under conditions favorable for severe disease development were less susceptible to attack when their petals, stamens and calyx were removed shortly after fertilization of the fruit.

7. Attempts to correlate the effect of dry and wet environments on disease development, indicate that under relatively dry macroclimatic conditions, sufficient moisture is present in the microenvironment of senescent floral organs for infection to occur. However, active rotting of infected fruits is associated with prolonged periods of precipitation and/or high humidities in the macroenvironment.

8. Mineral nutrition of the strawberry plant affects the innate susceptibility of the fruits to decay. Supplemental nitrogen fertilization greatly increased the incidence of fruit rot. While supplemental potassium fertilization tended to decrease the incidence of fruit rot, the effect on the innate susceptibility of the host tissues to

decay appears to be changed under prolonged rainy periods.

9. The rate of rot development varies between different strawberry varieties. Under field conditions, Siletz and Northwest strawberries appeared to be considerably more susceptible to rotting than the Marshall, but in the greenhouse where berries of the same maturity were inoculated and incubated under similar conditions of temperature and humidity, this difference was not nearly so great. In laboratory inoculation-incubation tests, the three commercial varieties showed no appreciable differences in resistance to rotting, but the experimental variety 2414 showed considerable resistance when compared with the commercial varieties tested.

10. The length of the incubation period for any given set of environmental conditions was not adequately established because of the high incidence of latent infection in the strawberries used. However, the rapid rotting of fruits inoculated with a Botrytis spore suspension and the variation in the length of the incubation period for latent infected fruits indicates that rate of fruit rot development is dependent upon the degree of infection (inoculum potential).

BIBLIOGRAPHY

1. Alexopoulos, Const. J. and Donald Cation. Stem-end rot of strawberries. Phytopathology 38:698-706. 1948.

2. _____ Gnomonia fragariae in Michigan. Mycologia 44:221-223. 1951.

3. Anderson, H. W. Strawberry fruit rots and their control. Transactions of the Illinois Horticultural Society 80:239-243. 1946.

4. _____ Diseases of fruit crops. New York, McGraw-Hill, 1956. 501 p.

5. Angell, H. R. Brown rot of stone fruits. IV. On the peach in wet and dry weather at harvest time in 1956. Journal of the Australian Institute of Agricultural Science 22:293-296. 1956.

6. Beneke, E. S., L. S. White and F. W. Fabian. The incidence and pectolytic activity of fungi isolated from Michigan strawberry fruits. Applied Microbiology 2:253-258. 1955.

7. Blackman, V. H. and E. J. Welsford. Studies in the physiology of parasitism. II. Infection by Botrytis cinerea. Annals of Botany 30:389-398.

8. Bolton, A. T. Gnomonia fructicola on strawberries. Canadian Journal of Botany 32:172-181. 1954.

9. Brown, William. Studies in the physiology of parasitism. I. The action of Botrytis cinerea.

10. Brown, William and C. C. Harvey. Studies in the physiology of parasitism. X. On the entrance of parasitic fungi into the host plant. Annals of Botany 41:643-662. 1927.

11. Chattopadhay, S. B. Effect of age and storage conditions on the susceptibility of certain vegetables to attack by tissue-rotting fungi. Indian Journal of Mycological Research 1:39-74. 1957.

12. Clayton, E. E. Water soaking of leaves in relation to development of the wildfire disease of tobacco. Journal of Agricultural Research 52: 239-269. 1936.

13. Cole, J. S. Studies in the physiology of parasitism: pathogenicity of Botrytis cinerea, Sclerotinia fructigena and Sclerotinia laxa, with special reference to the part played by pectolytic enzymes. Annals of Botany, new ser., 20:15-38. 1956.

14. Cox, R. S. and J. P. Winfree. Observations on the effect of fungicides on grey mold and leafspot and on the chemical composition of strawberry plant tissue. Plant Disease Reporter 41:755-759. 1957.

15. Culpepper, C. W., J. S. Caldwell and H.H. Moon. A physiological study of development and ripening in the strawberry. Journal of Agricultural Research 50:645-696. 1935.

16. Darrow, G. M. Effect of fertilizers on firmness and flavor of strawberries in North Carolina. Proceedings of the American Society of Horticultural Science 28:231-235. 1931.

17. Darrow, G. M. and G. F. Waldo. Effect of fertilizers on plant growth, yield and decay of strawberries. Proceedings of the American Society of Horticultural Science 29:318-324. 1933.

18. Dimarco, G. R. and B. H. Davis. Prevention of decay of strawberries with post-harvest treatment. Plant Disease Reporter 41:460-464. 1957.

19. Dodge, B. O. and Niel E. Stevens. The rhizoctonia brown rot and other fruit rot of strawberries. Journal of Agricultural Research 28:643-648. 1924.

20. Fall, Joan. Studies on fungus parasites of strawberry leaves in Ontario. Canadian Journal of Botany 29:299-315. 1951.

21. Felix, E. L. Phytophthora blight of strawberry. Plant Disease Reporter 42:841-842. 1958.

22. Fernando, M. and Greta Stevenson. Studies in the physiology of parasitism. XVI. Effect of the condition of potato tissue, as modified by temperature and water-content, upon attack by certain organisms and their pectinase enzymes. Annals of Botany, new ser., 16:103-114. 1952.

23. Folsom, Donald. Strawberry fruit rot in Maine caused by Gnomonia fructicola. Plant Disease Reporter 38:796-797. 1954.

24. Gould, C. J. Botrytis diseases of gladiolus. Plant Disease Reporter Supplement 224:1-33. 1954.

25. Gregory, P. H. Spore content of the atmosphere near the ground. Nature (London) 170:475. 1952.

26. Harvey, J. M. A method of forecasting decay in California grapes. Phytopathology 45:229-232. 1955.

27. Hirst, J. M. An automatic volumetric spore trap. Annals of Applied Biology 39:257-265. 1952.

28. _____ Changes in atmospheric spore content: diurnal periodicity and the effects of weather. Transactions of the British Mycological Society 36:375-393. 1953.

29. Horn, N. L. Strawberry fruit rot screening tests. Plant Disease Reporter 36:309-310. 1952.

30. _____ A field method of testing fungicides for control of botrytis rot. (Abstract) Phytopathology 48:343. 1958.

31. Jarvis, W. R. Air spora of soft-fruit plantations. In: Third Annual Report, Scottish Horticultural Research Research Institute, Mylnefield, Scotland, 1956. p. 26-27.

32. _____ Grey mold of soft fruits. Autecology of Botrytis cinerea. In:Fifth Annual Report, Scottish Horticultural Research Institute, Mylnefield, Scotland, 1958. p. 38-39.

33. Jerome, (Mrs.) S. M. R. Brown rot of stone fruits. Latent contamination in relation to spread of the disease. Journal of the Australian Institute of Agricultural Science 24:132-140. 1958.

34. Johansen, D. A. Plant microtechnique. New York, McGraw-Hill, 1940. 523 p.

35. Kluyver, A. J. and J. Visser. The determination of microorganism in air. Antonie von Leeuwenhoek: Journal of Microbiology and Serology 16:299-310. 1950.

36. Kirby, A. H. M., M. H. Moore and Dorothy J. Wilson. Strawberry botrytis rot (grey mold) control: a field trial of captan at East Malling. Journal of Horticultural Science 30:220-224. 1955.

37. Last, F. T. The spore content of air within and above mildew infected cereal crops. Transactions of the British Mycological Society 38:463-464. 1955.

38. Lowings, P. M. The fungal contamination of Kentish strawberry fruits in 1956. Applied Microbiology 4:84-88. 1956.

39. Luttrell, E. S. and M. M. Murphy. Effect of spraying on incidence of diseases and yields of muscadine grapes. Phytopathology 43:629-633. 1953.

40. Maddox, R. L. On the apparatus for collecting atmospheric particles. Monthly Microscopical Journal 3:286-290. 1870.

41. Marsh, R. W. Control of botrytis rot (grey mould) of strawberries, and the effect of fungicide spray residues on the processed fruit. Journal of Horticultural Science 30:225-233. 1955.

42. Martin, J. T. and J. A. Pickard. Spray application problems. XIII. Determination of captan deposits. Progress report. In:1054 Annual Report, Agriculture Research Station, Long Aston, Bristol, 1954. p. 83-89.

43. McCintock, J. A. Spraying for strawberry fruit rots 1946. Hoosier Horticulture 28:147-148. 1946.

44. McDowell, A. M. Crops and markets. Western Fruit Grower 12:45-48. Dec. 1958.

45. Miller, P. M. and P. E. Wagner. Dispersal of spores of Botrytis cinerea among strawberries. (Abstract) Phytopathology 47:24. 1957.

46. Mishra, J. N. Resistance of potato tubers to certain parasitic fungi. Phytopathology 43:338-340. 1953.

47. Moore, M. H. and R. P. Tew. Greenhouse testing of fungicides against botrytis rot (grey mould) of strawberry and other soft fruit. Journal of Horticultural Science 30:213-219. 1955.

48. Nelson, K. D. Effect of humidity on infection of table grapes by Botrytis cinerea. Phytopathology 41:859-864. 1951.

49. _____ The effect of botrytis infection on the tissue of tokay grapes. Phytopathology 46: 223-229. 1956.

50. Newhook, F. J. Microbiological control of Botrytis cinerea Pers.; antagonism by fungi and actinomycetes. Annals of Applied Biology 38:185-202. 1951.

51. Newhook, F. J. and R. M. Davison. Incorporation of fungicides in fruit-setting sprays for control of botrytis fruit rot in glasshouse tomatoes. I. Introduction and screening trials. II. Compatibility of mixtures. III. Tests in commercial houses. New Zealand Journal of Science and Technology 38:166-183. 1956.

52. Newhook, F. J. The relationship of saprophytic antagonism to control of Botrytis cinerea Pers. on tomatoes. New Zealand Journal of Science and Technology 38:473-481. 1957.

53. Ogawa, J. M. and Harley English. The efficiency of a quantitative spore collector using the cyclone method. Phytopathology 45:239-240. 1955.

54. Orillo, F. T. and B. B. Bombay. Melanconium fruit rot of tomato. Phillippine Agriculture 36: 114-130. 1952.

55. Overholster, E. E. and L. L. Claypool. Relation of fertilizers to respiration and certain physical properties of strawberries. Proceedings of the American Society of Horticultural Science 28: 220-224. 1931.

56. Powell, Dwight. The effect of early spring fungicides on Botrytis cinerea. Plant Disease Reporter 36: 97-98. 1952.

57. _____ The effect of captan on grey mold rot incidence and yield of strawberry. Plant Disease Reporter 38:209-211. 1954.

58. Purdy, L. H. Some factors affecting penetration and infection by Sclerotinia sclerotiorum. Phytopathology 48:605-609. 1958.

59. Rawlins, T. E. Phytopathological and botanical research methods. New York, John Wiley, 1933. 156 p.

60. Rose, Dean H. Leather rot of strawberries. Journal of Agricultural Research 28:357-376. 1924.

61. Rose, Dean H., C. O. Brantley and W. T. Pentzer. Market diseases of fruits and vegetables: grapes and other small fruits. Washington, U. S. Government Printing Office, 1939. 27 p. (U. S. Dept. of Agriculture. Miscellaneous Bulletin 340.)

62. Shoemaker, J. S. The strawberry in Ohio. Wooster, 1929, 50 p. (Ohio. Agricultural Experiment Station. Bulletin 444.)

63. Snedecor, George W. Statistical methods. 3d ed. Ames, Iowa State College Press, 1940. 422 p.

64. Snow, D. The germination of mould spores at controlled humidities. Annals of Applied Biology 36:1-13. 1949.

65. Stevens, F. L. and Alvah Peterson. Some new strawberry fungi. Phytopathology 6:258-267. 1916.

66. Stevens, Neil E. Pathological histology of strawberries affected by species of Botrytis and Rhizopus. Journal of Agricultural Research 6:361-366. 1916.

67. Stevens, Neil E. and R. B. Wilcox. Rhizopus rot of strawberries in transit. Washington, U. S. Government Printing Office, 1917. 22 p. (U. S. Dept. of Agriculture. Bulletin 531.)

68. _____ Further studies on the rots of strawberry fruits. Washington, U. S. Government Printing Office, 1918. 14 p. (U. S. Dept. of Agriculture. Bulletin 686.)

69. Stevens, Neil E. Rots of early strawberries in Florida and California. American Journal of Botany 9:204-211. 1922.

70. Stoddard, E. M. and P. M. Miller. Control of grey mold on strawberries under greenhouse conditions. Plant Disease Reporter 40:443-445. 1956.

71. Sturgess, O. W. A strawberry ripe fruit rot. Queensland Agricultural Journal 78:269-270. 1954.

72. Thatcher, F. S. Further studies of osmotic and permeability relations in parasitism. Canadian Journal of Research 20:283-311. 1942.

73. Tukey, H. B., S. H. Whittwer and H. B. Tukey, Jr. Leaching of nutrients from plant foliage as determined by radio-isotopes. East Lansing, n.d. 14 p. (Michigan. Agricultural Experiment Station. Journal Article no. 2092.)

74. U. S. Weather Bureau. Climatological Data. Oregon Section 63:56-113. 1957.

75. U. S. Weather Bureau. Climatological Data. Oregon Section 64:54-111. 1958.

76. Valleau, W. D., E. M. Johnson and Steven Diachum. Angular leafspot and wildfire of tobacco. Lexington, 1934. 60 p. (Kentucky. Agricultural Experiment Station. Bulletin 454.)

77. Wade, G. C. Investigations on brown rot of apricots caused by Sclerotinia fructicola (Wint.) Rehm.. I. The occurrence of latent infection in fruit. II. The relationship of the potassium status of apricot trees to brown rot susceptibility. Australian Journal of Agricultural Research 7:504-526. 1956.

78. Ward. H. M. A lily disease. Annals of Botany 2: 319-382. 1889.

79. Wilkinson, E. H. Observations on grey mould in strawberries. Plant Pathology 3:12. 1954.

80. Zeller, S. M. Some miscellaneous fungi of the Pacific Northwest. Mycologia 27:449-466. 1935.