

STUDIES ON THE WASTAGE OF EXPORT GRAPES

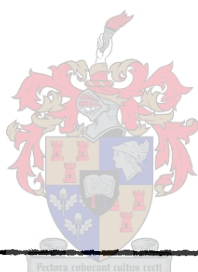
with Special Reference to that

caused by

BOTRYTIS CINEREA, PERS.

by

S.J. DU PLESSIS.



Thesis for the Degree of Doctor of Agri-
culture in the University of Stellenbosch.

Promotor : Dr. P.A. van der Byl.

FOREWORD.

These studies were undertaken at the suggestion of Dr. P.A. van der Byl for whose stimulating encouragement and helpful criticisms the author is gratefully indebted.

Appreciation is expressed to the South African Deciduous Fruit Exchange Ltd., for the financial support of this investigation. To Drs. L. Verwoerd and B.J. Dippenaar the *(to Dr. C. Koen for the identification of the Penicillium and Aspergillus spp.)* writer is indebted for suggestions and advice, and to Dr. J. Reyneke, Messrs. A.M. Skibbe and D.T. Cuthbert for the chemical analyses of grapes in various tests. Sincere thanks are also due to Messrs. J.F.D. Ince, T. Crawford, Em. Spilhaus, S.F. Low and Dr. H.B. Morris for invaluable collaboration and courtesies shown during the course of the field studies.

C O N T E N T S.

Introduction.

Historical review and distribution.

Laboratory studies.

Methods and materials.

Descriptions of the various types of grape wastage.

Descriptions of organisms causing wastage of grapes.

Cultural studies and variations in *Botrytis cinerea*.

Growth studies on different media.

Growth rate.

Colony variations.

Spore production.

Sclerotial production.

Acid Relations.

Effect of hydrogen-ion concentration.

Changes in the acid reaction of the medium.

Effect of various organic acids.

Effect of carbohydrates.

Biometrical differences in spore measurements.

Effect of various disinfectants on spore germination.

Pathogenicity.

Discussion.

Field studies.

Methods and materials.

Relative importance of various organisms in wastage of export grapes.

Influence of environmental conditions on the occurrence of *Botrytis rot* in the vineyard and in storage.

Effect of the size of bunch on *Botrytis rot*.

Effect of mechanical damage on wastage of grapes.

Effect of fertilizers on grape wastage.

Effect of time of picking and packing on the occurrence of *Botrytis rot* in storage.

Effect of "Buller Caps" on the occurrence of *Botrytis rot* in the vineyard and in storage.

Control...

Control studies.

Dusting and spraying experiments in the Vineyards in the seasons 1933-1934 and 1934-1935.

Comparative effectiveness of the fungicides.

Influence of mechanical damage efficiency of fungicides

Effect of time of application of dusts and the time of picking of treated grapes on the efficiency of these dusts.

Effect of the number of dust applications on Botrytis control.

Prestorage treatments.

Addition of chemicals.

Dipping.

Chemically treated wrappers.

Sulphur dioxide fumigation.

Formaldehyde fumigation.

Formalin spray.

Discussion.

Summary

Summary in Afrikaans.

Literature.

I N T R O D U C T I O N .

The export of table grapes from South Africa to Europe, and especially to England, dates back as far as 1886 (52), when grapes, carefully thinned, wrapped in tissue paper and packed in charcoal or cork dust, arrived in England in an excellent condition. A small experimental consignment, shipped in 1888 (46), reached its destination in a very poor condition, it being stated that no sound berries were amongst them. A private consignment of Red and White Hanepoot grapes which was shipped during the same season, however, arrived in England in an excellent condition.

Another consignment of grapes (46) were delayed before shipment, with the result that these grapes arrived overseas in such a condition that they were unfit for sale. Other consignments sent at the same time, but not delayed, were reported to have arrived in a good condition. The packing of grapes in lugs or in sawdust (46) apparently showed very little difference in condition, as some of the grapes in both these consignments arrived wasty, while the condition of others were satisfactory. It is mentioned however that grapes packed in wadding, arrived in excellent condition.

From 1896 to 1905 a few thousand boxes of grapes were annually exported to England, the quantity remaining fairly steady throughout. The condition of grapes of these shipments were, however, on the whole very unsatisfactory and in 1898 (1) complaints were again raised against the arrival of grapes in England in such a wasty condition, that they were unfit for sale. It was very much doubted at the time whether the export of grapes from South Africa was ever to become a practical proposition.

Van Dyk (110), in summarizing the further development of the grape export trade, states that the demand was good during 1901-1902 but the grapes a failure. During 1902 - 1903 the condition of the South African grapes was even worse; but the climax was reached in 1903 - 1904, when it was advised that as a result of the very poor

condition.....

condition of the grapes on their arrival, the export should be stopped until such time when farmers would be able to deliver grapes in a saleable condition on the overseas markets.

Up to 1908, the wastiness of export grapes was generally ascribed to faulty methods of handling and packing, and to unsatisfactory transport conditions. During the 1907 - 1908 season however, methods had reached such a stage of improvement that on arrival of wasty grapes in England, the Trade Commissioner in London reported (3) : "I noticed that though the grapes appeared to have been well-grown, well developed and well packed, yet they were very wet and wasty, and after the fruit had been a few days in London, it deteriorated, much more so than it did last year". He could not ascribe this difference in soundness to differences in packing, because the grapes of the 1907 - 1908 season were generally better packed than were those of the previous season. He expressed the opinion that "...this bad condition of the grapes should be gone into, as it is probably due to their either having been grown under rather wet conditions, in damp or shady places or due to rain previous to packing".

In 1910 (110) however, excellent results were obtained, and the necessity of further investigations was lost sight of. The quantity of grapes exported increased annually, except for a slight set-back during the war period of 1914 - 1918 up. Table grapes always have been the most important of the deciduous fruits exported, and the value of the grape export during the 1931-1932 season amounted to £230,086 (110).

As will be seen from the above, no attempt was made to analyse the different factors contributing to and the causes of the repeated failures in the export of table grapes. The first contribution in this direction, was that of Putterill (89) in 1923, when he described the types of wastages of export grapes caused by Botrytis cinerea; Penicillium spp.,
(particularly P. expansum;

particularly.....

Rhizopus spp.

particularly R. nigricans? and an Aspergillus sp., probably

A. Niger. Botrytis cinerea and the Penicillium spp. were

stated to have been the most common organisms causing wastage of grapes during the 1923 season.

In 1931, Dreyer (41) also mentions Botrytis, Penicillium and Aspergillus as the organisms mainly responsible for wastage of export grapes.

Following the epidemic occurrence of wastage, chiefly ascribed to Botrytis cinerea, in table grapes exported during 1927 - 1928 and succeeding seasons, the Stellenbosch-Wilsonburg College of Agriculture was instructed by the Secretary for Agriculture to investigate the problem and the investigation deputed to the author. The South African Deciduous Fruit Exchange financially supported the investigation. In this investigation a more intensive study was made of the organisms contributing to the heavy losses suffered by grape farmers during recent years, and of possible methods for the control of Botrytis and other rots occurring in export grapes in the vineyard or in storage.

HISTORICAL REVIEW AND DISTRIBUTION.

As demonstrated in a subsequent section, Botrytis cinerea is undoubtedly the most important of the organisms causing or associated with the wastage of export grapes, and will therefore be specially referred to in this review.

Botrytis cinerea was first described by Persoon in 1801 (85) as occurring on decaying leaves and organic matter in soils. In 1822 (86) Persoon again described Botrytis acinorum as being the cause of a gray rot of grape berries. In 1886 Sorauer and in 1888 Muller-Thurgau (79) expressed the opinion that the differences found to exist between these two described species of Botrytis were very likely due to the difference in substrate and that they are probably identical.

The berries infected with Botrytis cinerea in the vineyard during moderately dry weathers, was utilized in the production of special sweet wines in Europe and this rot became known as "noble rot". Studies of Muller-Thurgau (79) indicated that this refinement of the juice of berries was as follows: When properly ripe berries are attacked, the transpiration from them is increased because of the death of the epidermal cells. This increased transpiration results in a more concentrated juice of affected berries. The free acids are also more readily destroyed by the fungus than the sugars and various bouquet compounds undergo desirable changes. The Nitrogen content of berries affected with "noble rot" berries is also lower than that of sound berries, so that a slower and more incomplete fermentation of the juice from the former becomes possible. If the berries are attacked when not fully mature, the sugar content is lowered to such an extent that only wines poor in alcohol, can be produced from them.

The studies of de Bary (34) led him to believe in 1884 that Botrytis cinerea, Pers. was the conidial stage of Sclerotinia Fuckeliana Fkl. This conclusion was drawn mainly from the fact that these two fungi were constantly found to be associated on various hosts or on organic matter and to the

fact that both produce sclerotia. Direct evidence to show that the one stage may develop from the other was, however, not forthcoming (34) and comparatively recent studies (101) show that Botrytis cinerea and Sclerotinia Fuckeliana are definitely two distinct fungi.

Gray rot of grapes occurs in most of the countries where grapes are grown extensively. Muller-Thurgau (79) states that Botrytis attacks may be beneficial in regions of France and Germany under fairly dry conditions; but that it may be detrimental under ~~severe~~^{more} moist conditions. In 1897 Brizi (14) reported the infection of vine leaves and shoots by Botrytis cinerea, resulting in such a severe drop of the leaves that the vines had in midsummer the appearance as if in midwinter. Various methods for the control of Botrytis attacks on loaves, shoots and bunches in France were advised by Lafforgue (68) and Ravaz (91). De Istvanffi (36) in 1907 advised the use of various Copper compounds and Sodium bisulphite for the control of Botrytis on grapes in Hungary, and in 1922 Stummer (102) found that Botrytis infection was very effectively controlled by the use of parchment paper covering in a vineyard in Austria, even under conditions conducive to its epidemic occurrence.

Large quantities of grapes are exported annually from Spain to England and other European countries. Berro Aguilera (7) states that various organisms may be responsible for wastage of export grapes from Spain, and mentions Saccharomyces sp., Penicillium glaucum, Botrytis cinerea, Sterigmatocystis nigra, Cladocporium sp., Monilia sp., Mucor sp. and bacteria as causes of wastage in packed grapes from Almeria in 1925.

In 1897 McAlpine and Robinson (73) discussed various parasitic and saprophytic grape-rot organisms occurring in Australia. The export of table grapes from Australia at that time being only in its infancy the control measures recommended by these authors were mainly directed against the attacks of the organisms in the vineyard. In 1926 Fish (44) and de Castella (35) reported various experiments carried out for the control of wastage of....

scald on the fruit. A brown mark, which is at first small and extends till it covers the grape which then seems rotten and dries up. When the mark covers about half the grape the skin comes off then with a very slight touch, and the grape is very juicy underneath and tastes very sweet...". This farmer deferred from sending samples for identification of the trouble, because the affected grapes made such a mess in the box. To the above letter P.M.O.^x replied as follows:-

"...Following along the lines of your description, it is very probable that the injury complained of is caused by the fungus Sphaceloma ampelinum, De By, and is known as Anthracnose, carbon or black spot..." Judging from the description in the letter quoted above, this trouble would appear to have been Botrytis rot rather than Anthracnose. Another report (2) made in 1903, stated that grapes from Modder River rotted extensively following the summer rains, where the foliage was left intact to keep birds out. No definite identification was made; but very likely this rotting was also caused by Botrytis cinerea.

Botrytis cinerea, Pers., so common on organic matter and a host of hosts (123), may either have been indigenous in South Africa or might have been introduced into this country with the importation of vine cuttings from European countries during the earlier years of settlement at the Cape.

As has been indicated, Botrytis cinerea, may attack the leaves, shoots and berries of the vine, though the first two types of Botrytis attacks have not yet been observed to occur in South Africa. In countries e.g. France, Germany, Austria and Hungary, where grapes are produced mainly for wine-making or where table grapes need not be transported over long distances, the control of Botrytis rot practically ends in the vineyard. In countries, such as Spain, Australia, New Zealand, the United States of America and South Africa, from which grapes are to be transported over relatively long distances, Botrytis rot in the vineyard and in storage becomes

x These initials probably refer to P.M.C. Owan.

an ever increasing menace. In the latter countries more saprophytic organisms, such as Penicillium, Aspergillus, Cladosporium, Rhizopus spp., etc. also become important factors contributing to the amount of wastage and of subsequent loss to grape farmers. In these countries, the control measures for Botrytis and other rots of grapes are to be effective until these grapes reach the consumer, very often six weeks after they have been picked.

That the condition in which fruits, and especially grapes, arrive overseas, is causing grave concern even in South Africa, is evidenced by the extensive studies undertaken during recent years on various phases of the export problem e.g. those of Copeman^{and Foster} (30,31) on the physical and chemical changes in grapes during ripening, those of de Villiers (37, 38) on the physiology of the grape and its relation to keeping quality, and those of Reyneke (95) on the keeping quality of various fruits, including grapes, and the effect of various cultural practices on the keeping quality etc.

LABORATORY STUDIES.

Methods and Materials.

The descriptions of the various types of wastage encountered in export grapes were drafted from naturally and artificially infected berries. The natural infections were those collected from grapes which had been cold stored for three weeks, and kept at room temperatures for seven to fourteen days, being grapes used in the field experiments reported in the second part of this paper. Artificial infections on mature grape berries were made in the laboratory with single spore cultures through small incision wounds. These inoculated berries were suspended in jars under fairly dry conditions and at room temperature for fourteen days.

The.....

The cultures, used in all the laboratory studies, were those originally obtained from single spores of the particular fungus, either directly from the host or from isolations originally made from the host. Difficulty was at first experienced in isolating and transplanting the single spores of the organisms concerned. Ultimately the method, quoted by Dickinson (39) as having been used by him to teach the single-cell isolation technique to students, was modified as follows and used with very successful results:

The glass slide with the L-shaped glass rod, tapering to a fine point was placed on top of the condenser, as described by Dickinson (Fig. 2 d). Instead of the single microscopic slide with a hole in the middle bearing the cover glass with fungous material for isolation, a simple apparatus, illustrated in figure 1, was constructed. This consisted of a microscopic slide on which two narrow and even glass slips were ^{cemented} one on top of the other at both ends and in the middle. The width of the upper strips being so as to form two sliding racks on both sides as illustrated, and wide enough to allow a 15 x 15 mm. cover slide to move freely to and fro in each rack.

This slide rack is sterilized in absolute alcohol and inverted. Two cover slides are then dipped in absolute alcohol and flamed. On the one a drop of a light spore suspension of the particular fungus is placed, inverted and shifted into rack B, as is shown in figure 1. Clear Conn's glucose asparaginate agar is plated in thin layers in sterilized Petri dishes and allowed to cool. When cold, small agar squares, approximately 3 x 3 mm., are cut out with a platinum needle, flattened and bent in a spade shape as illustrated in figure 1 C. The second cover slide is then moved into rack A. The agar squares are then

carefully.



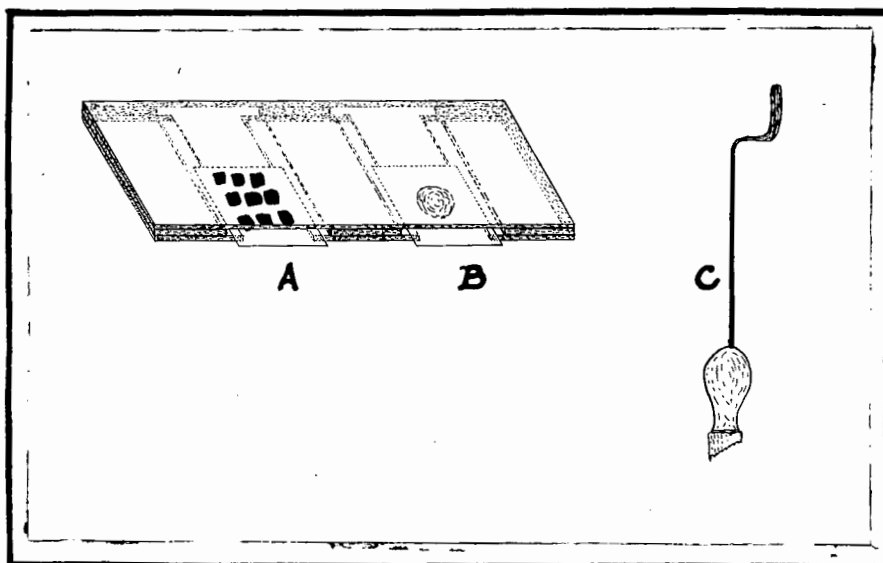


FIG. 1. - Diagram of the cover glass rack and spade-shaped platinum needle used for single spore isolations.

- A - Rack for cover glass ~~for~~ with agar squares
- B - Rack for cover glass with the spore suspension
- C - Spade-shaped platinum needle.

carefully scooped out one by one from the Petri dish and deposited on the lower surface of the cover glass in rack A, the whole slide apparatus being held in an inverted position as is shown. In this way contamination from the atmosphere becomes practically negligible. The number of agar squares placed on the cover glass in rack A, depends on the size of the space between the two strips constituting this rack, and on the number of single spore isolations to be made from any particular fungus culture.

This apparatus is then placed on the mechanical stage of the microscope with the hanging drop of spore suspension directly over the condenser. By manipulating the stage screws, the position of the fungous material may be shifted to the desired position so that an isolated spore is directly over the point of the glass needle. The needle is then raised by means of the condenser screw until its point touches the particular spore, and then lowered. This process is repeated until the spore is seen to adhere to the point of the glass needle, then the needle is lowered until its point is well below the level of the microscope stage. The cover glass rack is then shifted by means of the stage screws, until the point of the needle points to the centre of a particular piece of agar. The needle is raised so that its point just touches the surface of the agar and lowered again. This process is repeated until the spore is seen to be deposited on the agar after the needle has been retracted. At times it becomes necessary to shift the agar with the needle touching its surface, before the spore is left behind on the agar.

When a spore has been deposited on every agar square, these squares are removed with the spade-shaped platinum needle and transferred to a sterile Petri dish containing plated agar medium. Several agar squares can be placed in each dish at short distances from one another. At regular intervals, the dishes are inverted on an ordinary

microscope....

microscope stage and the squares examined under the low power of the microscope for germinating spores and for possible contaminations. The squares of successful single spore germinations are marked with an ink dot and later transferred to test tube slants.

As pointed out by Dickinson (39) the type of glass needle to be used depends on the size and shape of the spore to be isolated. For the isolation of medium-sized spores such as are produced by the fungi dealt with in this contribution, the best results were obtained by using the rod-type needle described in the Dickinson method.

As a matter of precaution against possible contamination the point of the needle may be dipped in absolute alcohol, before starting and between isolations from different cultures. The thickness of the agar squares is of importance because, if too thick, they are less transparent and may come in contact with the surface of the microscope stage during the transfer; if too thin difficulty is experienced, especially in removing these squares from the microscope slide after isolation, as they are then apt to bundle or to tear apart. A thickness of about 1 mm. gave the best results.

This method of single spore isolation has the advantages of being economical, easy, quick to handle, and renders it possible to make up to 12 single spore isolations from a spore suspension without the need of replacement of agar or slides. Owing to the thickness of the glass of the microscope slide and the distance of the spore material and agar surface from the objectives, this apparatus can only be manipulated under the low power of the microscope. Possibilities of contamination are very slight, very few instances having been experienced during the isolations made since the construction of this apparatus.

The....

The following single spore cultures were used in the laboratory studies:-

Culture No.	Organism	Source of Culture	Date of Isolation.
E 3	<u>Penicillium cyclo-</u> <u>podium</u> , West.	Grapes, Constantia	1935.
E 4	<u>Penicillium expan-</u> <u>sum</u> , (Lk.) Thom	Grapes, Constantia	1935.
E 5	<u>Penicillium elon-</u> <u>gatum</u> , Dierckx	Grapes, Constantia	1935.
E 6	<u>Aspergillus car-</u> <u>bonarius</u> , (Bain.) Thom	Grapes, Constantia	1935.
E 11	<u>Aspergillus niger</u> , v. Teigh.	Grapes, Constantia	1934.
E 2	<u>Rhizopus nigri-</u> <u>cans</u> , Ehr.	Grapes, Constantia	1935.
E 8	<u>Fusarium oxy-</u> <u>sporum</u> , Schl.) var. <u>aurantiacum</u>) (Lk.) Wr.)	Grapes, Constantia	1935.
E 9	<u>Cladosporium bac-</u> <u>cae</u> , Verw. & Dipp.	Grapes, Constantia	1935.
E 12	<u>Sphaeropsis Malo-</u> <u>rum</u> , Berk.	Grapes, Constantia	1935.
E 1	<u>Sphaeropsis Malo-</u> <u>rum</u> , Berk.	Grapes, Constantia	1935.
B1b	<u>Botrytis cinerea</u> , Pers.	Grapes, Stellenbosch	1932.
B1c	<u>Botrytis cinerea</u> , Pers.	Grapes, Stellenbosch	1932.
B11a	<u>Botrytis cinerea</u> , Pers.	Grapes, Constantia	1934.
B12c	<u>Botrytis cinerea</u> , Pers.	Grapes, Constantia	1934.
B4c	<u>Botrytis cinerea</u> , Pers.	Pears, Constantia	1934.
B6d	<u>Botrytis cinerea</u> , Pers.	Apples, Constantia	1934.
B8c	<u>Botrytis cinerea</u> , Pers.	Quinces, Constantia	1934.

The single spore cultures of Botrytis cinerea enumerated above, are those which differed most markedly amongst a very large number of isolants, some of which only showed minor differences.

The amount of total acids in the media, used in the culture studies, was determined by titration with a standardised N/10 NaOH solution, using phenolphthalein as indicator. The hydrogenion concentration was determined according to the quinhydrone method.

For determining the colours encountered during these studies, Ridgeway (96) was consulted.

Descriptions of the Various Types of Grape Wastage.

1. The Blue Moulds - Penicillium cyclopodium : Penicillium expansum; and Penicillium clongatum.
Plate 1 c and d.

Young infected spots on the berries steadily spreading, sunken, circular to semi-circular, not sharply demarcated, wrinkled on the surface, soft, pulpy, penetrating into the interior of the berry; colour of the infected areas on berries of coloured varieties very slightly paler than that of healthy surrounding tissues, on berries of white varieties pale, yellowish green; the pigment of coloured varieties mostly unchanged, liberated as a purplish fluid after destruction of the epidermal cells; velvety, pale green masses of conidia (E 3), or slightly darker green conidia (E 4) or conidia and fascicles with conidia (E 5) formed on the surface of infected areas.

2. The black Moulds - Aspergillus carbonarius and Aspergillus niger (Plate 1 d).

Before sporulation the type of berry rot produced by the Aspergillus spp, is on all grape varieties practically indistinguishable from that which is produced by the Penicillium spp. Only when sporulation is about to or has set in, can these two types be distinguished from each other. Berries, infected with Aspergillus, are then covered with conidiophores and with black, and at times closely aggregated conidial heads.

3. Pinhead Mould - Rhizopus nigricans (Plate 1 f).

Infection spreading rapidly; spots not clearly defined, not sunken or wrinkled, remaining fairly firm for a comparatively long period; colouring matter of coloured berries very little affected; epidermis of berries remaining at first normally intact, may crack with slight pressure, but with a lack of the crisp crack so typically encountered.....

encountered with Botrytis infected berries; epidermis of infected areas may at times slip with slight rubbing, but the exposed tissues are wetter than in the case of Botrytis infections; infected and sound berries very soon covered with fairly loose and coarse masses of mycelia with abundant, erect ^{Sporangia} conidiophores and pinhead ^{Sporangia} conidia.

4. Woolly Mould - Fusarium oxysporum Var. aurantiacum
(Plate 11 b).

Very seldom found in cold stored grapes and when present mostly associated with one of former types; the infected area effuse, not sharply demarcated at first very slightly sunken, irregular in outline; ultimately sunken and shrivelled and affected berry covered with a mass of fine fluffy, white mycelium and Olive Buff sporodochia of conidia scattered over the infected area; colouring matter of coloured berries unaffected.

5. ^CCladosporium Mould - Cladosporium baccae, (Plate 11 c)

Spots on berries more or less circular, sunken, sharply demarcated, spreading very slowly and not penetrating very deeply into the berry, firm and comparatively dry, ultimately covered with a compact, velvety, dark olive green mass of conidia.

6. Sphaeropsis Rot - Sphaeropsis Malorum, (Plate 11a and d)

Infected spots soft, even and flabby, sharply defined, sunken, colour of affected berries of the white varieties at first dull, ultimately darkening to black; pigment of coloured varieties remaining unimpaired; ultimately infected berries shrivel and are mummified, after which pycnidia of the causal organism appear beneath the epidermis of such berries as small, black, punctiform dots (Plate 11d).

Another type of Sphaeropsis rot of grapes found on berries differs from the former in that pycnidia are very often lacking and in that the affected berries are ultimately covered
with...

with dirty, blackish gray, velvety aerial mycelium (Plate 11 a).

7. Yeast Rot - Saccharomyces sp. (Plate 11 e).

Of very rare occurrence in grapes which have been cold stored; infected areas mostly sunken, very soft and pulpy, not sharp-defined, shrivelled, young infections very much resembling the type of rot produced by the Penicillium spp. epidermis partly destroyed and yeast cells multiply in ruptures to form a colourless slimy mass, which is very often tinted purplish by the liberated pigments of coloured berries.

8 Gray Mould or Botrytis Rot - Botrytis cinerea, Pers. (Plate 1 a and b).

Infected areas at first circular, at times sharply demarcated, brownish in colour on white varieties, not sunken or shrivelled or soft, slightly pale brownish in colour on the red varieties e.g. Red Hanepoot, On the black varieties such as Henab Turki and Gros Colman the colour of the infected spots is ~~at~~ the same as that of the healthy surrounding portion of the berry, so that macroscopic detection by sight becomes practically impossible. On all varieties however, the skin of portions of berries infected with Botrytis, manifests a peculiar crisp crack at the points of infection with the slightest pressure. The skin of such areas slips off very easily and exposes the inner tissues in an apparent^{ly} perfect condition. The latter phenomenon is in practice usually designated as the "slip-skin stage" of Botrytis rot infection. As can be gathered from this description, the presence of this early stage of Botrytis infection can be more easily felt than seen on the darker coloured varieties, before spore production and growth of aerial mycelium has set in. The above described peculiarities of Botrytis infection makes it possible for the distinction of this rot from the other types, even in the very early stages of decay. Ultimately the whole berry is covered with a fine woolly, almost white mycelial mat, with.

with ^sclerotia of varying sizes imbedded in this mat, or the berries may in some cases be covered with a dark gray, loose fluffy mass of aerial mycelium accompanied with abundant spore production. As will be pointed out in subsequent sections, the types of Botrytis infections mainly depend upon the strain of Botrytis cinerea by which the berries are infected. Under dry conditions, the berries infected with Botrytis rot may be ultimately mummified.

Descriptions of the Organisms causing Grape Wastage.

1. Penicillium cyclopodium, Westling - E 3.

Colonies white, with a homogenous, velvety pale green layer of conidia; surface of the colony smooth and regular in outline. Mycelium fairly thick, wrinkled or bulged. Conidiophores slender, hyaline, smooth, septate, 15.3 - 170 x 2.4 - 3.4 μ ; penicilli usually borne on fairly long, slender branches of submerged hyphae, and consists of sterigmata and metulae, with or without branches of one or two series; two to three bottle-shaped sterigmata branching from the tip of each metula, 6.8 - 11.2 x 2.6 - 3.3 μ ; metulae 6.8 - 11.2 x 2.4 - 5.1 μ , produced at different levels on the main branch or on secondary branches which measure 8.5 - 22.1 x 2.6 - 5.1 μ . Conidia continuous, smooth, globose, light green in colour, 3.4 - 6.9 in diameter. Sclerotia absent.

2. Penicillium expansum (Lk) Thom - E 4.

Growth slightly raised, floccose, with uneven margin, Pea Green in colour, yellow to red shades lacking in culture. Conidiophores closely aggregated or at times in erect club-shaped fascicles, simple conidiophores predominating; penicilli up to 146 μ long, consisting of sterigmata, metulae, with or without branches of one or two series, asymmetrical; sterigmata 6.8 - 11.2 x 2.3 - 3.4 μ , two to three on each metula, acuminate, bearing long, entangled chains of conidia; metulae 11.2 - 17.0 x 3.4 - 5.1 μ , broad and apparently

flat....

flat. Conidia continuous, smooth, globose to slightly elliptical, light greenish in colour, $1.7 - 4.2\mu$ in diameter. Sclerotia absent.

3. Penicillium elongatum, Dierckx - E 5.

Growth raised, floccose, knotted, outline fairly irregular, spore producing area Sage Green in colour.

Conidiophores smooth, slender, $340-510 \times 4.2 - 6.8\mu$; penicilli $109.5 - 146.0\mu$ long, on aerial mycelium, forming a fairly compact asymmetrical brush, consisting of sterigmata, metulae and branches of one or two series; sterigmata $8.5 \bar{9} 11.2 \times 1.7 - 3.4\mu$, slightly fusoid to swollen bottle-shaped, pointed; metulae $11.2 - 20.4 \times 3.4 - 5.1\mu$. Conidia continuous, smooth, ovoid to elliptical, seldom subglobose, $3.4 - 8.5 \times 1.7 - 5.1\mu$.

4. Aspergillus carbonarius, (Bainier) Thom. - E 6.

Medium not coloured, mycelium hyaline, aerial mycelium mainly absent, with the development of abundant conidia which are of a black colour in mass. Stalks $1168 - 1606 \times 17 - 20.4\mu$, smooth, not pitted, fairly thick-walled, stable, vesicle $73 - 87.6\mu$ in diameter, semi-globular; sterigmata $13.6 - 20.4 \times 5.1\mu$, in one series, pointed at the apices, elongated teat-shaped. Conidia borne in globose heads, splitting ultimately at the periphery into columnar masses or at times into isolated conidial chains, $146 - 365\mu$ long; conidia continuous, thick-walled, bearing densely massed echinulations, dark brown to black, globose, $6.8 - 10.2\mu$ in diameter. Sclerotia fairly abundant on potato dextrose agar slants, globular, pale brown in colour $1 - 1.5$ mm. in diameter.

5. Aspergillus niger, Van Tieghem - E 11.

Medium not coloured, aerial mycelium practically absent, outer margin of the culture white, changing from Mineral gray..

Gray to black towards its centre. Stalks 365 - 584 x 11.9 - 17.0 μ smooth, with walls 1.2 - 1.9 μ thick; vesicle 36.5 - 110.5 μ in diameter, globose; sterigmata 13.6 - 17.0 x 3.4 μ , in one series, at first elongated, afterwards constricted at the apices, bearing conidia in chains. Conidia continuous, smooth, globose to slightly elliptical, grayish smoke brown in colour which is diffused in the conidia, 3.1 - 4.3 x 2.7 - 3.4 μ . Sclerotia absent.

6. Rhizopus nigricans, Ehr. - E. 2.

Aerial mycelium at maturity slightly grayish white, rhizoids fairly numerous, fuscous to brown, branched, 182.5 - 730 μ long; stolons ferulate, unbranched, dark brown in colour, smooth, 730 - 1022 μ long. Sporangia 182.5 - 225.5 x 146.0 - 219.0 μ , globose to subglobose; sporangiophores fasciculate, erect, aseptate, smooth, dark brown, 323 - 714 x 17.0 - 32.3 μ ; columellae subglobose to slightly elongate, 15.3 - 37.4 x 13.6 - 30.6 μ . ^{Sporangiospores} Conidia 6.8 - 20.4 x 5.1 - 13.6 μ , continuous, smooth, light fuscous brown in colour, subglobose, ovoid to elliptical, fairly thick-walled.

7. Fusarium oxysporum, Schl. var. aurantiacum, (Lk.) Wr. E 8.

Aerial mycelium abundant, fine, fluffy, white; Conn's Glucose Asparaginate agar coloured Buff Pink, Potato dextrose agar Pleroma Violet to Petunia Violet. Microconidia borne on false heads on simple long, slender, conidiophores (up to 119 x 1.7 - 2.6 μ) or on short, stout, bottle-shaped conidiophores (8.5 - 17.0 x 2.6 - 4.2 μ), on aerial mycelium, abundant, smooth, hyaline, elliptical or at times subfusoidly bent, dominately 0-septate :-
 0-septate : 4.2 - 9.3 x 1.7 - 3.4 μ ; 1-septate : 10.2 - 17.0 x 3.4 μ ; 2-septate : 17.0 - 22.1 x 3.4 - 4.2 μ . Macroconidia borne on irregularly branched, stout conidiophores, scattered or in sporodochia, fuscoid, distinctly pedicellate, attenuated towards the apices, broadest $\frac{1}{2}$ - $\frac{3}{4}$ from the pedicellate end, dominately 3-septate : 17.0 - 40.8 x 3.4 - 5.1 μ ; 4-septate : 18.7 - 51.0 x 4.3 - 5.1 μ ; 5-septate : 45.9 - 47.6 x 5.1 μ ; 6-septate : 42.5 - 50.2 x 5.1 μ .

Chlamyospores abundant, terminal and intercalary, solitary, in pairs short chains or clusters, mycelial and conidial, depressed or irregularly globose, smooth, light brown in colour, 8.5 - 17.0 x 8.5 - 13.6 μ . Sclerotia scant, dark blue in colour, irregular, up to 8 mm. in diameter.

8. Cladosporium baccae, Verw. & Dipp. - E. 9.

The morphology of this fungus which was considered to be ^{of a berry rot at Stellenbosch} the cause was fully described by Verwoerd and Dippenaar (112).

9. Sphaeropsis Malorum, Berk. - E 13 & 14.

Described as the cause of a berry rot and wilt of grapes in several districts of the Western Province (113).

10. Botrytis cinerea, Pers. - B 1 b, B 1c, B 11a, B 12c.

The results of a detailed study of the morphology, physiology and variations of this fungus are fully described in the following sections.

Cultural Studies and Variations in Botrytis cinerea.

Growth Studies on Different Media.

The seven single spore isolates of Botrytis cinerea from grapes, apples, pears and quinces were inoculated in duplicate series on Petri dishes, containing one of the following agar media; meat extract agar, potato dextrose agar, mealie meal agar, and Conn's glucose aspartate agar (70). Two liquid media were used in these culture studies, namely Czapek's sucrose nitrate solution and the salt medium of Letcher and Willaman (69) modified by the author as below.

	Czapek's solution	Modified Letcher and Willaman's salt medium.
Sucrose	30 gm	50 gm.
FeSO ₄	.01 gm.	-
MgSO ₄	. 5 gm.	.25 gm.
KCl	. 5 gm.	-
K ₂ HPO ₄	1.0 gm.	.50 gm.
NaNO ₃	2.0 gm.	-
NH ₄ NO ₃	-	1.0 gm.
Distilled water	1,000 cc.	1,000 cc.

When...

When any of these two media were to be used in the solid condition, 1.5% agar - ~~agar~~^{agar} was dissolved in the medium. Cultures on these liquid media were made in duplicate series and by transferring a platinum loop full of a conidial suspension of the culture concerned to each of a number of Erlenmeyer flasks containing 100 c.c. of the nutrient solution.

The cultures in the Petri dishes or in the flasks were kept under standard laboratory conditions and at a temperature of 25°C. Readings of the growth characters and colony diameters were taken daily.

When the cultures on the liquid media were nine days old, the mycelial mats were filtered separately on the previously weighed filter papers, dried at 100°C until no loss in weight was recorded, cooled and finally weighed.

Growth rates:-

The various isolates of Botrytis cinerea exhibited marked differences in colony diameters after four days' growth on the agar media, as is shown in Table 1. The daily increase in diameter of these isolates are graphically presented in Figure 11.

From Table 1 and Figure 11, it is apparent that, according to the growth of these isolates on potato dextrose agar, they ^{be} may be divided into three groups viz. relatively fast growing: B 8c, B 1c, B 12c and B 6d; moderately fast growing: B 4c and B 1b; relatively slow growing : B 11a.

On meat extract agar, however, as is apparent from Figure 11, it is not possible to group all the various isolates into relatively fast, moderately fast or relatively slow growing groups. The differences in the diameter measurements of the four day old colonies, recorded in Table 1, are very slight. Isolate B 11a, however, distinguishes itself again

and....

TABLE I: Culture characters of various Monopore cultures of Botrytis on different Media.

Culture Co	Medium	Diameter in m.m. (4 days)	Aerial mycelium	Conidia	Microconidia	Sclerotia
B1b	Conn's Glucose Asparaginate Agar.		+	+++	++	-
E1c			+++	+	+	-
B4c			++	++	±	-
B6d			++	++	++	±
B8c			+++	++	+	-
B11a			±	±	++	-
B12c			++	++	+	-
B1b	Meat Extract Agar	21.0	±	++	-	-
E1c		16.8	±	+	-	-
B4c		22.2	++	+++	++	-
B6d		25.0	+	+	+	-
B8c		19.5	+	++	+	-
B11a		18.3	±	±	-	-
B12c		21.0	+	++	-	-
B1b	Potato Dextrose Agar	53.0	+	++++	++	-
E1c		73.3	+++	+	+	-
B4c		35.3	+++	+++	+++	± (2)
E3d		74.5	+++	++	++++	++ (35)
B8c		80.0	+++	+	±	++ (37)
B11a		30.5	++	++	+	+
B12c		74.3	+	++	±	++ (38)
B1b	Malt Meal Agar	30.0	++	+++		-
B1c		53.8	+++	+		-
B4c		53.3	++	+++		-
B6d		54.0	++	+		-
B8c		49.0	++	+++		-
B11a		30.7	±	++		-
B12c		53.8	++	+++		-

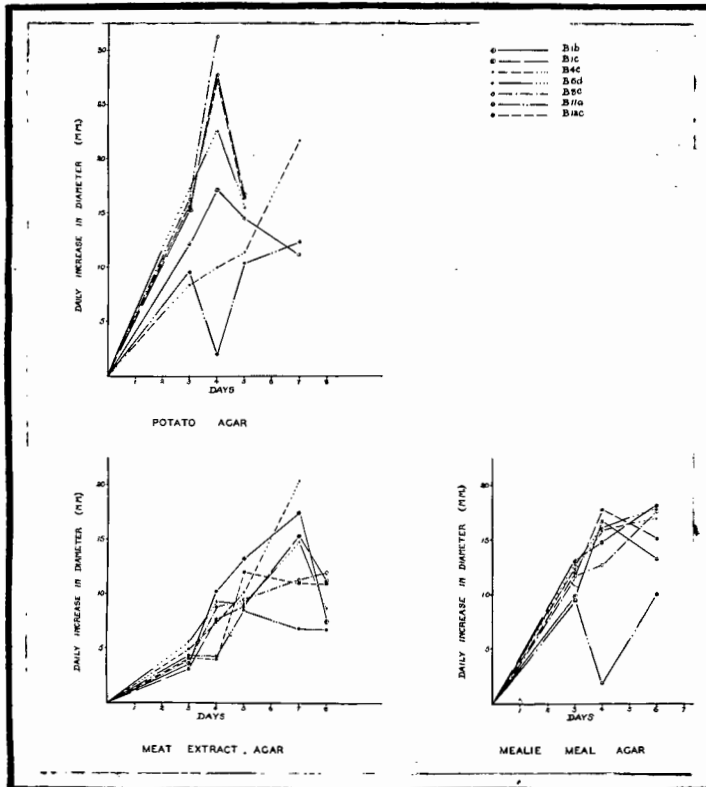


FIG. 11. - Daily increase of the diameter of cultures of various Botrytis isolates on potato dextrose agar, meat extract agar and on mealie meal agar.

on this medium as being a relatively slow grower.

The same slow growing character of isolate B 11 a as compared with the others, is manifested on mealie meal agar as represented in Figure 11. Differences in growth rate between the other six isolates of Botrytis are also very slight on this medium.

From the readings in Table IV, the weight of the mycelial mat of nine day old cultures on Czapek's sucrose nitrate solution, was highest in cultures of B 12 c and B 8 c, second highest in those of B 4 c, B 1 c and B 6 d and lowest in cultures of B 1 b and B 11 a. On modified Letcher and Willaman's salt medium the mycelial weight of equally old cultures of B 8 c, and B 4 c was highest, of B 6 d. and B 12 c the second highest and of B 1 b, B 1 c and B 11 a the lowest.

As regards their growth rates on these media, these isolates of Botrytis may therefore be divided into three groups.

Relatively fast growing: B 1c, B 8c, B 6d and B 12c.

Moderately fast growing: B 4c and B 1b.

Relatively slow growing: B 11a.

Differences in staling characters could however not be observed in these cultures. The petri dishes being only 90 mm. in diameter, they were usually already overgrown or nearly overgrown before any reduction in the daily increase of diameter of the colonies could be noticed.

Colony Variations:

The differences between types of growth produced by the isolates were on some media less distinct than on others. On potato dextrose agar the differences in types of growth were particularly striking, as can be seen in Plates IV and V.

On potato dextrose agar, Conn's glucose asparaginate agar and mealie meal agar B 1b, developed a fairly even layer of aerial mycelium, fairly dark gray in colour. The aerial mycelium of B 1c was more floccose, lighter gray and dense; that of B 4c
fairly...

fairly dense, more so than that of B 1b, dark gray in colour and raised. The aerial mycelium of B 6d showed a peculiarity on these media in that it developed mainly in the central portion of the colony as a fairly dense, raised, light gray, velvety mass. On the remaining portion of the colony aerial mycelium was usually lacking. The aerial growth of B 8c was again less dense and of a lighter gray colour than that of B 4c. B 11a is characterized by a well-developed, fairly loose, raised, woolly mass of light gray aerial mycelium and by the growth in the substrate not being dense. The type of growth produced by B 12c closely resembled that produced by B 8c, from which it differs mainly in a more scant development of aerial mycelium on Conn's glucose asparaginate agar and on potato dextrose agar.

On meat extract agar differences in the appearance of the various isolates were very slight, aerial mycelium being very scant and loose and the substrate covered with only a very thin mat of mycelium.

Spore Production:-

As can be seen in the summary of the results in Table 1, some isolates e.g. B 1c and B 6d are poor producers of conidia on most of the media used; B 4c, B 8c, B 11a and B 12c produce conidia in fair abundance. In this respect, however, B 1b is outstanding in that it produces abundant conidia on most of the media.

The production of microconidia does, however, not appear to be correlated with the production of conidia on any of the media.

Meat extract agar is the least favourable medium for the production of conidia by most of the cultures of Botrytis cinerea. On potato dextrose agar conidia are produced in abundance.

Sclerotia..

Sclerotia productions-

Only the isolate B 6d produced a few sclerotia on Conn's glucose asparaginate agar. No sclerotia were produced by any of the isolates on meat extract agar or on mealie meal agar. On potato dextrose agar sclerotia were produced in fair abundance by B 6d, B 80 and B 12c, a few by B 11a and B 4c (See Plates IV and V - Sclerotia were produced by B 6d and B 11a after these photographs were taken).

The modified Letcher and Willaman's salt medium was solidified by the addition of 1.5% agar-agar, 100 c.c. quantities being standardized at different hydrogen-ion concentrations, and sterilized. Sterile and plugged test tubes were each filled with 10 c.c. of this agar medium under aseptic conditions, a quantity being set aside for hydrogen-ion determinations, which varied for the different lots from pH 6.1 to pH 7.22. The medium was then plated and inoculated in triplicate series with B 1b, B 1c, B 11a and B 12c. The cultures were kept in incubators at 25°C for 30 days.

Of the isolates tested only B 1b did not produce any sclerotia on this medium at any of the reactions. B 1c produced from 8 to 62 sclerotia per Petri dish during the period of study, the greatest number of sclerotia being produced on the medium at pH 6.77. B 11a produced 125 to 179 sclerotia per dish, with a maximum number at pH 6.93. B 12c produced a multitude of confluent sclerotia and accurate counting was practically impossible. The optimum hydrogen-ion concentration for sclerotia production was in this latter case also pH 6.93.

It is furthermore of interest to note that, in cultures of the same isolate at different reactions on this medium, the size of the sclerotia increase with a decrease in sclerotial numbers and as the medium becomes more alkaline, i.e.

above..

above pH 6.93, and more acid than the optimum, i.e. below pH 6.77.

Distinct differences in the measurements of sclerotia produced by the isolates on potato dextrose agar were noticed. Those of B 4c were 1 to 3 mm. in diameter, of B 8c measured 1 - 3 x 1 - 4 mm., of B 11a .5 - 1 mm. and of B 12c 1 - 4 x 1 - 7 mm.

That environmental factors may have a profound retardatory or stimulating effect on the production of conidia and sclerotia of Botrytis cinerea, has been demonstrated by several workers. Ralph Smith (100) found that no conidia are produced by this fungus at 5°C. Erierley (12) states that conidia production by Botrytis cinerea, is at its optimum between temperatures of 16° and 25°C, whereas at temperatures above 25°C and below 16°C the production of conidia is reduced. He further found that conidia production was more profuse in blue and violet rays than in the dark. Beauverie and Guillermond (5) found that only small sclerotia, but no conidia, were produced by Botrytis cinerea at temperatures between 4° and 10°C.

Reidemeister (93) stated that the variation of the temperature and other environmental conditions, under which cultures of Botrytis cinerea were grown might adversely or favourably affect the production of conidia and sclerotia, e.g. light is more favourable for the production of conidia and darkness for the production of sclerotia. Conditions favourable for rapid transpiration were favourable for the production of conidia, but the reverse was the case for the production of sclerotia. Busgon (24) found that the production of conidia in cultures of Botrytis cinerea was more abundant, when they were grown in Erlenmeyer flasks than when the cultures were grown in petri dishes, because of transpiration being more rapid in the Erlenmeyer flasks than in the Petri dishes.

The....

The amount of conidia and sclerotia production are therefore of a very variable nature and dependent upon the medium on which and the environmental conditions under which isolates of Botrytis are grown. These isolates of Botrytis cinerea, studied under identical environmental conditions in the course of these studies do, however, show very striking differences in growth habits, which, though of a variable nature, are clearly distinct.

Acid Relations.

Effect of the Hydrogen-ion Concentration.

Two series of experiments were carried out to determine the hydrogen-ion concentration limits, and the optimum hydrogen-ion concentration, at which conidia of the isolates of Botrytis cinerea would germinate and develop, and at the same time to determine whether any of the four isolates from grapes i.e. B 1b, B 1c, B 11a and B 12c differ in their reactions to different hydrogen-ion concentrations.

In the first series, prepared modified Letcher and Willman's salt medium was measured out in quantities of 1500 c.c. and the medium in each flask adjusted at different hydrogen-ion concentrations by the addition of varying amounts of N/10 NaOH or N/10 HCl solutions. The media were then autoclaved at 12 pounds working pressure for thirty minutes, transferred under aseptic conditions in 100 c.c. quantities to sterilized, plugged Erlomeyer flasks. The medium in one flask of each set was used for the determination of the hydrogen-ion concentration, and also for the determination of the initial amount of titrable acid as is indicated in Table 11.

A fairly heavy spore suspension of each isolate to be tested was prepared and each flask inoculated with two platinum loops full of the spore suspension of the particular isolate. This was carried out in triplicate for each of the four cultures, of Botrytis in every set. The inoculated flasks were then kept in closed incubators at 25°C for six days, when the mycelial

TABLE II : The effect of the H-ion concentration on the growth of monospore cultures of Botrytis from grapes.

Initial H-ion concentration	Dry B1b		Increase in H-ion concentration		Dry wt. B1c		Increase in H-ion concentration		Dry wt. B1d		Increase in H-ion concentration	
	of mycelium	concentration	of mycelium	concentration	of mycelium	concentration	of mycelium	concentration	of mycelium	concentration	of mycelium	concentration
2.32	0.0	0.00	0.0	0.00	0.0	0.00	2.5	-0.08	0.0	0.00	0.0	0.00
2.76	25.7	-0.29	23.2	-0.36	23.2	-0.36	18.4	-0.04	25.2	-0.17	25.2	-0.17
3.33	29.9	0.67	41.3	0.54	41.3	0.54	26.2	0.49	42.1	0.72	42.1	0.72
5.33	31.0	1.51	39.7	1.27	39.7	1.27	25.6	0.96	48.6	1.81	48.6	1.81
6.13	42.5	2.43	45.1	2.32	45.1	2.32	34.2	1.04	54.3	3.02	54.3	3.02
6.47	68.7	2.52	46.4	2.61	46.4	2.61	36.8	1.14	57.7	3.50	57.7	3.50
6.71	53.1	2.28	47.5	2.38	47.5	2.38	41.4	2.64	59.0	3.40	59.0	3.40
6.93	48.6	2.15	45.2	2.90	45.2	2.90	57.9	2.91	67.0	3.49	67.0	3.49
7.35	41.3	1.88	40.7	2.03	40.7	2.03	40.4	1.59	64.5	3.11	64.5	3.11

Mat of each flask was filtered from its medium on to previously weighed filter papers. The paper and mycelium were then dried at 100°C until no more loss in weight was recorded, and the paper and mycelium of each was then weighed separately. The dry weights of mycelium, tabulated in Table 11, are the averages of the triplicates weighed, and this data are also graphically represented in Figure 111. The filtrate of every triplicate set was mixed and the hydrogen-ion concentration and total acidity of the inoculated medium determined.

In the second series some of the medium, prepared for the first series, was adjusted to a few other hydrogen-ion concentrations. The sterilized solution of each in this series was transferred in 10 c.c. quantities to each of a number of sterile, plugged test tubes. The remaining solution in each flask was utilized for hydrogen-ion determinations which were ^{found} to vary from pH 2.32 to pH 10.64.

The test tubes were then inoculated in triplicate series with a platinum loop full of a spore suspension of each of the four isolates of Botrytis used in the first series. The cultures were then kept at 25°C and examined at regular intervals. The comparative amounts of germination of conidia in each test tube were determined by placing all the test tubes in a row, by comparing them with each other and estimating the comparative germination and growth. These were indicated by plus and (or) minus signs. In the above comparisons and estimations, the relative amount of germination, depth of growth and density of mycelial mats were determined and annotated as follows:-

- no visible germination.
- ± germination just discernable.
- ± germination fairly distinct.
- ± germination and growth distinct, but no mycelial mat formed.
- ± ± germination and growth good, thin mycelial mat formed and so on.

The data, taken after 5 days' incubation, are given in Table III and the results plotted in Figure IV. From Tables II and III and Figures III and IV, it is apparent that all four isolates of Botrytis tested, have a wide range of hydrogen-ion concentrations at which germination and growth can occur, i.e. from pH 2.32 to pH 8.85. The optimum growth was manifested in solutions of pH 6.47 to pH 6.93, whereas the estimated germination was the best at pH 6.93. The type of curve obtained from growth figures of all four isolates were practically the same, i.e. a rapid improvement of growth with the increase of the hydrogen-ion concentration of the medium from pH 2.32 to pH 3.33. Between pH 3.33 and pH 6.93 germination and growth improved less rapidly but steadily, but diminished rapidly again on media of hydrogen-ion concentrations beyond pH 6.93.

The four isolates of Botrytis tested showed only minor differences in their germinative and growth capacities at the different hydrogen-ion concentrations. B 1c is apparently more sensitive to the more acid media than the rest. The optimum growth of B 1b, as indicated by the dry weight of mycelium, is at pH 6.47, whereas the optimum germination of all isolates takes place according to the results obtained in the second series, at pH 6.93. Furthermore some conidia of B 1b still germinated at pH 8.85, at which hydrogen-ion concentration the conidia of the other three isolates failed to germinate.

That Dotrytis cinerea is able to grow on media, showing a wide range of hydrogen-ion concentrations, was also found to be the case by Webb (115). By using different media he could still obtain germination at pH 1.6 and at pH 9.8. The type of germination curve, obtained by Webb (115) differs from those obtained in the above discussed tests, in that Webb observed the optimum germination at pH 3.2 with a steady decrease as the media became more alkaline.

TABLE III : Effect of the H-ion Concentration of the Germination and Growth of Spores of various Botrytis Isolates from Grapes.

H-ion Concentration. ^{can}	B1b	B1c	B11a	B12c.
2.32	±	-	+	±
2.35	+	±	+±	+±
2.46	2+±	+±	2+	+±
2.76	3+	2+	4+	3+
3.96	4+±	4+±	4+±	6+
4.92	5+	5+	5+±	7+
5.48	6+	6+	6+	7+±
6.47	6+±	6+±	7+	8+
6.93	7+	7+	8+	9+
7.35	5+±	5+	6+±	7+
8.21	+	±	±	+
8.86	±	-	-	±
10.64	-	-	-	-

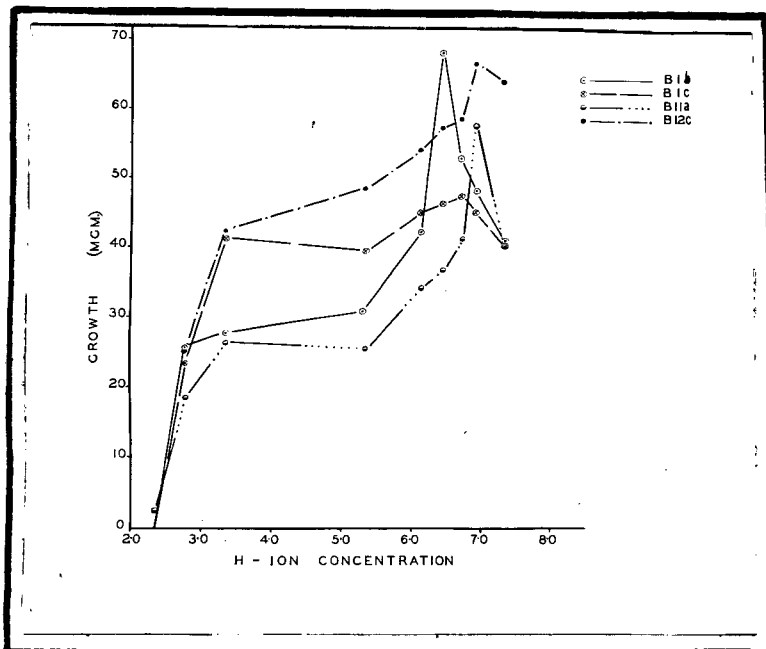


FIG. 111. - The effect of the hydrogen-ion concentration of the modified Letcher and Willamans salt medium on the growth of Botrytis isolates.

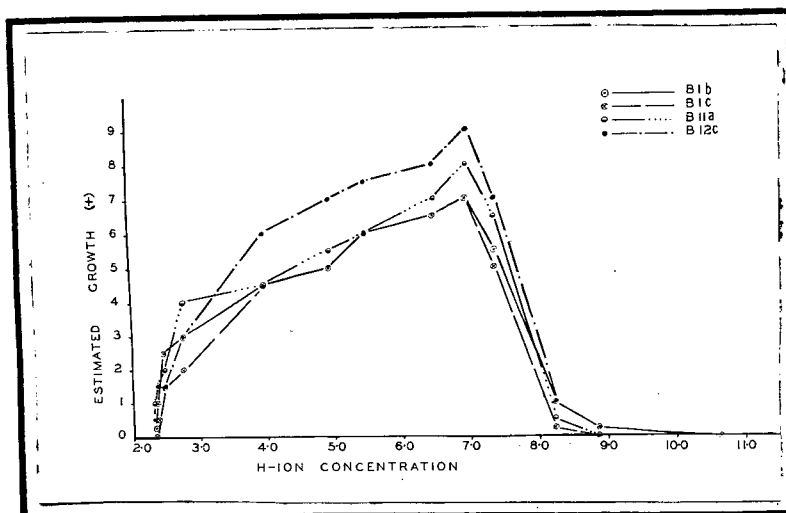


FIG. 1V. - The effect of the hydrogen-ion concentration of modified Letcher and Willaman's solution on the estimated amount of germination and growth of Botrytis conidia.

Boyle (11) in his studies on the effect of the hydrogen-ion concentration on the germination of conidia of Botrytis cinerea on different media, found the lowest pH value at which germination occurred to range from pH 1.7 to pH 2.2 the optimum for germination to range pH 5.4 to pH 7.2, and the maximum from pH 6.8 to pH 10.0, depending on the media used. According to Chona (27), the optimum growth of the culture of Botrytis cinerea which he used occurred at pH 6.0, but no growth was observed at pH 8.2 The type of curve obtained by him somewhat resembles those in figures III and IV. Cultures of Botrytis cinerea accordingly appear to be fairly sensitive to alkaline media.

The differences observed between the four isolates, used in these tests on modified Letcher and Willaman's salt medium, are however of such minor importance that distinction between them on these lines is hardly possible.

Changes in the Reaction of the Medium.

As early as 1888, Müller-Thurgau (79) found that Botrytis cinerea reduces the acidity of the juice of grape berries, being one of the reasons why the juice of berries affected with "noble rot" are specially suited for the production of the sweet wines required.

Weimer and Harter (116) showed that Rhizopus tritici, Diplodia tubericola, Mucor racemosus, Penicillium sp. and Botrytis cinerea increased the acidity of Czapek's nutrient solution when glucose was used as the source of carbon. In a later paper (117) these authors found that Rhizopus nigricans, when grown on Czapek's solution of different hydrogen-ion concentrations from pH 2.62 to pH 8.53, increased the acidity of all sets to between pH 2.0 and pH 3.0. According to Weimer and Harter (55) the acidity of Czapek's.....

Czapek's, Pfeffer and Richard's solutions and of sweet potato decoction is increased by Botrytis/cinerea, whereas that of string bean, prune and Irish potato decoctions are decreased by this fungus. Büsgen (24) found that Botrytis cinerea generates acids on meat extract and peptone and contended that oxalic acid was one of the products of this fungus.

From Table 11 it is apparent that the acidity of the modified Letcher and Willaman's salt medium at all hydrogen-ion concentrations above pH 2.76 is increased, but the acidity of sets of this medium at pH 2.76 show a very slight decrease.

An experiment was carried out to determine on which of the two liquid media, Czapek's solution or the modified Letcher and Willaman's solution, the growth of the isolates of Botrytis cinerea was the most satisfactory, and also to establish the changes in acidity induced by them on these media. These media were prepared, adjusted at the same hydrogen-ion concentration and autoclaved at 12 pounds working pressure for 30 minutes. Quantities of 100 c.c. of each medium were then transferred under aseptic conditions to each of a number of sterile, plugged Erlenmeyer flasks. The hydrogen-ion concentration and the amount of titratable acidity of each medium was determined in triplicate. Sets of three flasks of each medium were then inoculated, as in the previous experiments, with the isolates of Botrytis and the cultures in the liquid media kept at 25°C for nine days. The dry weights of mycelium, final hydrogen-ion concentrations and total acidities of the stale media in each set were then determined as previously described. The results are tabulated in Table 1V.

Table 1V.

Table IV : Amount of growth of the seven isolates of Botrytis cinerea on and the changes in acidities of Czapek's sucrose nitrate solution and the modified Letcher and Willaman's salt medium.

Culture Co.	Initial hydrogen-ion concentration.	Titrable acidity (c.c. N/10 NaOH to neutralize 1000 c.c.)	Dry weight of Mycelium (mgm)	Ultimate.	
				hydrogen-ion concentration	Titrable acidity (c.c. N/10 NaOH to neutralize 1000 c.c.)
(a) Czapek's sucrose nitrate solution				pH	
B 1b	pH 3.00	78.0	35.6	5.93	37.5
B 1c			44.0	4.55	150.8
B 4c			50.0	5.11	91.3
B 6d			43.4	5.46	79.4
B 8c			66.2	5.84	33.3
B 11a			30.6	6.07	55.4
B 12c			73.4	6.93	27.3
(b) Modified Letcher and Willaman's salt medium.					
B 1b	pH 3.74	30.4	56.0	2.38	127.0
B 1c			45.8	2.12	111.1
B 4c			100.3	2.33	103.2
B 6d			80.4	2.91	101.2
B 8c			132.4	3.29	111.1
B 11a			61.0	2.35	121.0
B 12c			83.1	3.25	111.1

The acidity of Czapek's sucrose nitrate solution, as is indicated by the pH values was decreased by all isolates of Botrytis, whereas the opposite is the case with the modified Letcher and Willaman's salt medium. The titration readings, however, of the changes in the total amounts of acid during this period, show that there was an increase in the total amount of acid in Czapek's solution wherein B 1c, B 4c, B 6d and B 8c had grown. On modified Letcher and Willaman's solution all sets showed an increase in total acidity.

The....

The apparently ^{radically} controversial results obtained by comparison of the pH and titration readings on Czapek's solution, may be due to the fact that the acids present in the media at inoculation are probably replaced through the growth of certain cultures by varying quantities of weaker acids, eg. the inorganic acids present in the medium may be replaced by organic acids, which would if the latter is not produced in large enough quantities result in a drop in the dissociation figures, but a rise in titration figures. The total amount of an acid produced in modified Letcher and Willaman's solution are more than in the Czapek's solution, whereas the pH readings were lower.

From the composition of these media on page , it is clear that they are very much the same, the main differences being the difference in concentration of salts and sodium nitrate being included in Czapek's solution and ammonium nitrate in the modified Letcher and Willaman's solution.

According to the summary by Zimmerman (123), several investigations found that nitrogen is essential for the growth of Botrytis cinerea. Laurent (according to Zimmerman (123)) came to the conclusion that Botrytis cinerea may utilise both ammonium salts and nitrates, but that this fungus thrives best on the ammonium salts. Whether it is mainly due to this difference in nitrogen supply or also to the greater percentage of sugar, that growth is greater on the modified Letcher and Willaman's solution is not yet clear.

It is, however, apparent that the changes in hydrogen-ion concentration of the media are dependent on their composition, and inoculate. The determination of hydrogen-ion concentration alone may however lead to misinterpretations of the activities of fungi in a medium, as a reduction in pH values of the medium does not always signify a reduction in amount of total acids.

Effect....

Effect of Various Organic Acids.

The studies of Ralph Smith (100) revealed that Botrytis cinerea developed vigorously on stock solution containing 2% tartaric acid or 2% malic acid. No growth, however, occurred on stock solutions which contained 2% oxalic acid.

De Tótvánffi (36) showed that Botrytis conidia developed fairly satisfactorily on media containing 1 - 10% malic acid, tartaric acid or citric acid.

In order to determine the effect of various concentrations of malic, oxalic and tartaric acids on the four isolates of Botrytis from grapes, Czapek's sucrose nitrate solution was prepared in the ordinary manner and different quantities of these acids added to 200 c.c. quantities of the solution. The different sets of media were then autoclaved in plugged Erlomeyer flasks, after which the media were transferred in 10 cc. quantities to each of a number of sterilized, plugged test tubes. Sufficient medium of each set was set aside for hydrogen-ion determinations. The test tubes were then inoculated in triplicate series with \bar{d} equal quantities of the spore suspensions of the four isolates, B 1b, B 1c, B 11a and B 12c, as previously described.

After 5 days at 25°C, readings were taken of the comparative amount of germination and growth in each test tube, as was done in the former test tube cultures. The results are presented in Table V.

From this it is apparent that the germination and growth of B 1b, B 1c and B 11a was fairly satisfactory at all concentrations of \bar{m} malic acid; but B 12 c is apparently more sensitive to the presence of \bar{m} malic acid than the former three. A reduction in germination and growth did occur with the increase of the percentage of malic acid from 1.5% to 3%, but in the case of B 11a growth in a 2% malic acid medium was apparently, though very slightly, better than in 1.5% malic acid. Where 3% malic acid was added, the germination and growth was in all cases fairly distinct, though the hydrogen-

TABLE \checkmark : Effect of Different concentrations of organic acids on the germination and growth of spores of *Botrytis mono-spore* cultures from grapes.

Acid	Concentration. (%)	H-ion concentra- tion. ^{con} h	B1b	B1c	B11a	B12c
Malic	1.5%	-	9+	5+	8+	4+
	2.0	2.32	7+	4+	9+	3+
	3.0	2.19	4+	\pm	4+	+
Oxalic	1.0	1.97	-	-	-	-
	2.0	1.67	-	-	-	-
	3.0	1.63	-	-	-	-
Tarta- ric.	1.0	2.33	8+	4+	9+	6+
	2.0	2.11	3+	\pm	3+	2+
	3.0	2.18	3+	\pm	2+	+

hydrogen-ion concentration of the medium was only pH 2.19.

No germination or growth could be detected in any of the inoculated test tubes containing 1, 2 or 3% oxalic acid. The hydrogen-ion concentrations were in all three cases however below pH 2.00.

Tartaric acid in these quantities, was less favourable for the germination and growth of these Botrytis conidia than malic acid, but germination was nevertheless fairly distinct, even in the sets containing 3% of this acid.

From Table V it is furthermore clear that isolates B 1b and B 11a are less sensitive to the presence of organic acids than B 1c and B 12c.

Effect of Carbohydrates.

According to Smith (100), Botrytis cinerea grows fairly slowly during the first few days on media containing 3% of sucrose or of lactose, on the media, however, containing 3% of dextrose Maltose, laevulose and of galactose, the growth was comparatively vigorous from the start. Hawkins (57) found that conidia of Botrytis cinerea were still able to germinate in media containing ^omálecular concentrations of 1.6 glucose and of 1.6 sucrose. ^mWeiner and Harter (116) showed that Botrytis cinerea grow in media containing 42 to 50% glucose. According to Peltier (from Zimmerman (123)), the growth of Botrytis cinerea reached an optimum on media containing 20 to 30% glucose.

Czapek's agar medium was prepared with the omission of the sucrose. To 100 c.c. quantities of this medium various amounts of lactose, glucose, sucrose and maltose respectively were added. One set of 100 c.c. medium was left without sugar for checks. The media were then autoclaved at 12 pounds pressure for 30 minutes and measured off, under aseptic conditions, in 10 c.c. quantities into sterile, plugged test tubes. The media were then plated in sterilized Petri dishes and inoculated in triplicate series with conidia of the four Botrytis isolates from grapes.

The...

The average diameter of the cultures, when 4 days old, are tabulated in Table VI. The growth on Czapek's agar without sugar was very scant. Only here and there a few spreading and slightly branched mycelia were visible. On all of the other media, growth was dense and profuse.

The optimum glucose content for the growth of isolate B lb was apparently 20%, but this fungus made better growth on 10% sucrose and 15% maltose than on the higher concentrations of these sugars. Isolate B lc grew best on 30% glucose, 10% sucrose and 15% maltose, B 1la on 20% glucose, 20% sucrose and 15% maltose; B 12c on 20% glucose, 20% sucrose and 45% maltose.

There are, therefore, distinct differences between the four isolates of Botrytis from grapes in regard to their sugar requirements, eg. B 12c. is more favoured by the higher concentrations of sugars, followed in descending order by B 1la, B lc and B lb.

The initial slow growth of Botrytis on media containing sucrose, as described by Ralph Smith (100) was, however, not encountered in the above experiments.

Biometrical Differences in Spore Measurements.

Physiological forms of fungi may differ in several respects from one another, e.g. noticeable differences in the size and shape of spores has been demonstrated in several comparatively recent studies. Levine (71) in a detailed statistical study of the size variation of uredospores of Puccinia graminis tritici, found that the physiologic forms of this variety of Puccinia graminis may differ as much from one another in spore sizes as the tritici variety differs as a whole from the avenae or secalis varieties in this respect.

Broadfoot.....

TABLE VI : Effect of Different concentrations of Sugars on the growth of monospore cultures of Botrytis from grapes.

Sugar	Concentration (%)	Diameter of colonies (m.m.) after 4 days growth.			
		B1b	B1c	B11a	B12c
No Sugar	-	28.0	24.0	30.0	20.5
Lactose	10.0	50.5	30.0	58.5	84.5
Glucose	10.0	53.0	53.0	42.0	51.0
	20.0	63.0	60.0	49.0	53.0
	30.0	55.0	77.0	32.5	28.5
Sucrose	10.0	72.0	75.0	62.5	48.0
	20.0	53.5	65.0	69.0	87.0
	30.0	49.5	26.0	59.5	82.0
Maltose	15.0	57.0	80.0	46.0	41.0
	45.0	44.5	63.0	38.5	48.0

Broadfoot (15) in his studies on the variation of physiologic forms of Fusarium Lini, found that some of the forms of this fungous showed significant differences in length and width of their spores, and others not when grown under the same environmental conditions on slightly acid peptone agar. He also showed that the size of spores of a particular form of Fusarium Lini, when grown under the same environmental conditions but on different media, may also show significant differences. He came to the conclusion that forms of F. Lini could not be separated on the basis of spore size alone.

Palmiter (80) demonstrated that differences in spore sizes exist between particular cultures of Venturia inaequalis (Cke.) ~~Witt.~~^{Witt.}, when grown under identical conditions. The length of the conidia of forms of this fungus was, however, also shown to be influenced by the variety of apple on which the conidia were produced.

Berkeley (6) found that the four cultures of Botrytis cinerea which he studied not only showed cultural differences, but also differences in the range of sizes of their spores. Paul (81) also detected differences in the spore sizes of the different forms of Botrytis cinerea which he had studied.

The seven single spore isolates of Botrytis, used in these studies, were subjected to a more critical study of the variation length and width of their conidia, as exhibited on different media. It was found that a normal distribution curve could be obtained from measurements of 130 conidia. This number of conidia was consequently measured for each isolate on a particular medium.

These isolates were grown under the same environmental conditions on three different media viz. Conn's glucose asparagenate agar, potato dextrose agar and meat extract agar. The required number of conidia of each isolate was measured, when the cultures were 20 days old, the arithmetical mean, and the standard error of the mean calculated

according...

according to the following formulae (quoted from Chaddock (26)).

$$\bar{X} = \frac{\sum X}{N} \quad \text{and} \quad \sigma = \sqrt{\frac{\sum x^2}{N}}$$

where \bar{X} = arithmetical mean

X = individual spore measurements.

N = number of spores measured.

σ_M = standard error of the arithmetical mean

x = deviation from the mean.

These results are tabulated in Table VII.

The differences in spore sizes of the various isolates on the three media, as embodied in Table VII, were then analysed, as regards their degree of significance in terms of the standard deviations of the differences, from the formulae:-

$$\sigma_{(M_1 - M_2)} = \sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}$$

and the degree of significance = $\frac{M_1 - M_2}{\sigma_{(M_1 - M_2)}}$

where $\sigma_{(M_1 - M_2)}$ = the standard error of the difference.

σ_{M_1} = the standard error of the mean of one isolate.

σ_{M_2} = the standard error of the mean of the other isolate

M_1 = arithmetical mean of one isolate.

M_2 = arithmetical mean of isolate to be compared with the former.

The results obtained are tabulated in Table VIII. Where the ratio of $M_1 - M_2 : \sigma_{(M_1 - M_2)}$ is more than 3.0, the differences in the length or width of spores of the two isolates compared are considered to be significant, but where this ratio is less than 3.0, the differences are considered nonsignificant. The conclusions drawn as to which of the Botrytis isolates studied, showed significant differences in the length or width of conidia are presented in Table IX (a) and (b) respectively.

From....

TABLE VII:- Spore Measurements of Monospore Cultures of Botrytis Isolates.

Culture NO.	Medium	Spore Lengths (μ)	Range (μ)	No. Measured	Spore Lengths (μ)	Range (μ)
B1b	Conn's Glucose Asparaginate Agar.	11.50 \pm .18	6.4-19.2	132	8.4 \pm .12	4.8-12.8
B1c		9.15 \pm .16	5.4-17.6	132	7.62 \pm .07	5.4-11.2
B4c		8.96 \pm .18	4.8-16.0	135	7.94 \pm .09	4.8-11.2
B6d		11.57 \pm .19	6.4-20.8	135	8.77 \pm .10	6.1-12.8
B8c		10.25 \pm .18	6.4-17.9	134	8.29 \pm .10	5.9-11.2
B11a		11.47 \pm .25	6.4-20.8	134	8.60 \pm .10	5.4-11.2
B12c		9.85 \pm .16	5.6-16.0	135	7.85 \pm .11	5.6-10.2
B1b	Potato Dextrose Agar	11.96 \pm .27	6.4-17.6	133	7.87 \pm .03	4.8- 9.9
B1c		10.23 \pm .24	7.0-16.0	84	8.07 \pm .11	6.4- 9.6
B4c		9.64 \pm .17	4.8-16.0	135	7.98 \pm .06	6.4- 9.6
B6d		9.49 \pm .18	6.4-19.2	135	9.37 \pm .09	4.8-11.2
B8c		11.29 \pm .19	6.4-17.6	135	8.30 \pm .03	6.4-10.4
B11a		11.72 \pm .20	6.4-19.2	134	8.15 \pm .06	6.4- 9.6
B12c		10.98 \pm .15	6.9-16.0	135	8.15 \pm .05	6.4-10.4
B1b	Meat Extract Agar	12.48 \pm .25	6.4-22.4	135	8.73 \pm .09	6.4-11.2
B1c		12.87 \pm .28	6.4-22.4	135	8.23 \pm .13	6.4-14.4
B4c		10.35 \pm .19	5.9-16.7	135	8.06 \pm .06	5.9- 9.6
B6d		13.03 \pm .21	6.4-19.2	135	8.49 \pm .07	6.4-12.0
B8c		11.58 \pm .22	6.4-24.0	134	8.44 \pm .06	4.8-11.2
B11a		13.28 \pm .20	8.0-19.2	135	8.37 \pm .07	6.7-12.0
B12c		11.46 \pm .19	6.4-16.8	135	8.13 \pm .06	6.4- 9.6

TABLE VIII: Degree of Significance of the difference in spore lengths and widths between monospore cultures of *Botrytis*.

Strain		B1b	B1c	B4c	B6d	B8c	B11a	B12c	Medium
Isolate		L E N G T H S							
B1b		-	9.6	10.0	0.3	4.9	0.1	6.7	Conn's Glucose Asparaginate Agar.
B1c		5.6	-	0.8	9.7	4.5	8.0	3.1	
B4c		3.1	2.8	-	10.0	5.1	7.1	3.6	
B6d		2.4	9.4	6.1	-	5.0	0.3	6.9	
B8c		0.7	5.5	2.6	3.4	-	4.0	1.7	
B11a		1.3	8.0	4.9	1.2	2.2	-	5.6	
B12c		3.4	1.7	0.6	6.2	2.9	5.0	-	
B1b	G	-	4.9	7.2	7.6	2.0	0.7	3.2	Potato Dextrose Agar.
B1c	H	1.7	-	2.0	2.5	3.5	4.8	2.7	
B4c	H	1.6	0.7	-	0.6	7.1	7.9	5.9	
B6d	H	5.2	2.1	3.6	-	6.9	8.3	6.4	
B8c	A	10.2	5.5	4.8	0.7	-	1.2	1.3	
B11a	A	4.2	0.6	2.0	2.0	2.2	-	2.9	
B12c	H	4.9	0.7	0.9	2.1	2.6	0.0	-	
B1b	D	-	1.0	6.8	1.7	2.7	2.5	3.2	Leaf Extract Agar.
B1c		3.2	-	7.5	0.5	3.0	1.2	4.2	
B4c		6.2	1.2	-	9.4	4.3	10.6	4.1	
B6d		2.1	1.8	4.7	-	4.7	0.9	5.6	
B8c		2.7	1.5	4.5	0.5	-	5.7	0.4	
B11a		3.1	0.9	3.4	1.2	0.8	-	6.6	
B12c		5.0	0.7	0.9	3.9	3.7	2.6	-	

TABLE IX: ^{Isolates} ~~Strains~~ of Botrytis showing significant differences in lengths and breadths from one another.
(a) Length.

Strain Isolate	Differs significantly from strains isolates						on
B1b	B1c	B4c		B8c		B12c	Conn's Agar
	B1c	B4c	B6d			B12c	Potato Agar
		B4c				B12c	Meat Agar
B1c	B1b		B6d	B8c	B11a	B12c	Conn's Agar
	B1b			B8c	B11a		Potato Agar
		B4c		B8c		B12c	Meat Agar
B4c	B1b		B6d	B8c	B11a	B12c	Conn's Agar
	B1b			B8c	B11a	B12c	Potato Agar
	B1b	B1c	B5d	B8c	B11a	B12c	Meat Agar
B6d	B1c	B4c		B8c		B12c	Conn's Agar
	B1b			B8c	B11a	B12c	Potato Agar
		B4c		B8c		B12c	Meat Agar
B8c	B1b	B1c	B4c	B6d		B11a	Conn's Agar
		B1c	B4c	B6d			Potato Agar
		B1c	B4c	B6d		B11a	Meat Agar
B11a	B1c	B4c		B8c		B12c	Conn's Agar
	B1c	B4c	B6d				Potato Agar
		B4c		B8c		B12c	Meat Agar
B12c	B1b	B1c	B4c	B6d		B11a	Conn's Agar
	B1b		B4c	B6d			Potato Agar
	B1b	B1c	B4c	B6d		B11a	Meat Agar

Table 1x (b): ~~width~~.

Factor Treatments	Differs significantly from control Treatments						on
B1b	B1c	B4c				B12c	Conn's Agar
			B6d	B8c	B11a	B12c	Potato Agar
	B1c	B4c			B11a	B12c	Meat Agar
B1c	B1b		B6d	B8c	B11a		Conn's Agar
				B8c			Potato Agar
	B1b						Meat Agar
B4c	B1b		B6d		B11a		Conn's Agar
			B6d	B8c			Potato Agar
	B1b		B6d	B8c	B11a		Meat Agar
B6d	B1c	B4c		B8c		B12c	Conn's Agar
	B1b	B4c					Potato Agar
		E4c				B12c	Meat Agar
B8c	B1c		B6d				Conn's Agar
	B1b	B1c	B4c				Potato Agar
		B4c				B12c	Meat Agar
B11a	B1c	B4c				B12c	Conn's Agar
	B1b						Potato Agar
	B1b	B4c					Meat Agar
B12c	B1b		B6d		B11a		Conn's Agar
	B1b						Potato Agar
	B1b		B6d	B8c			Meat Agar

From the results in Tables VII to IX, it is evident that the lengths and (or) width of conidia of all seven isolates of Botrytis differed significantly from one another on one or more media. The poorest distinction in this respect was found in the comparison of B 11a and B 6d where only a difference between the lengths of their conidia was obtained on ^Potato agar.

From Table XI (a) and (b) it is evident that these isolates of Botrytis could be biometrically distinguished in the size of their conidia on not less than the three media used. The use of one medium alone for this purpose, as was done by Broadfoot (15) for Fusarium Aini, appears inadequate.

It is furthermore of interest to note that the isolate, with the longest and (or) broadest conidia on one medium, is not necessarily the one with the longest and (or) broadest conidia on any of the other media. To quote extreme instances, the conidia of B 1c were the shortest of those of all isolates on Conn's glucose asparaginate agar, and on potato dextrose agar, but the average length of its conidia on meat extract agar was one of the longest of the isolates on this medium. The length of conidia of B 6d averaged the smallest of the series on potato dextrose agar, the greatest of that on Conn's glucose asparaginate agar, and second greatest of that on meat extract agar. B 1b again had the narrowest conidia of the series on potato dextrose agar, whereas it had the broadest conidia of that on meat extract agar and one of the broadest of that on ^Potato dextrose agar.

This variance in sequence of the lengths and widths of spores of Botrytis cultures, when compared on different media, makes the comparison of ultimate average spore measurement readings on more than one medium unreliable. A difference between spore measurements on one medium may be largely counterbalanced by that obtained upon another medium, resulting..

resulting in ultimate, apparently nondifferential readings in spore measurements of the cultures to be compared.

The range in spore dimensions of the different cultures differ only very slightly from one another as may be readily seen from Table VII, and differentiation of the size of spores on this basis is apparently not sufficient.

From Table VII it also appears that the size of the conidia of these isolates on meat extract agar are on the whole larger than those on potato dextrose agar, and more so than those on Conn's glucose asparaginate agar, B 6d being the only isolate which produced longer conidia on the latter medium than on the former. The conidia of this isolate were however again very much broader on potato dextrose agar than on Conn's glucose asparaginate agar. The medium upon which conidia of these isolates of Botrytis are produced has therefore very distinct effects on length width and on the shape of these conidia.

Effect of Various Disinfectants on Spore Germination.

The effect of a number of chemicals in different concentrations was tested on the conidia of the B 1c isolate, with the intention of selecting some of them to be used in the field experiments for the control of the Botrytis rot of grapes to be discussed subsequently.

The chemical and its concentration used are listed in Table X, the procedure adopted being as follows:- The chemicals were dissolved in distilled water in such concentrations that, when 9 c.c. of this stock solution was added to 1 c.c. water, the required concentration mentioned in Table X was obtained. A heavy spore suspension from ten day old cultures of isolate B 1c was prepared, well shaken and measured off aseptically in 1 c.c. quantities into sterile, plugged test tubes. Quantities of 9 c.c. of the particular stock solution was then added to the spore suspension in each test tube, shaken well and left for the shortest period required. The mixture of solution
and...

and spore suspension was then poured off into another sterile test tube and plugged. Molten nutrient agar (Conn's glucose asparaginate agar) was then poured into the former test tube, well shaken and plated into sterile Petri dishes. The second test tube was then left for the further period to obtain the second time series with this particular solution, when the procedure of removal and plating, as described above, was repeated. In this manner the same mixture of spore suspension and solution can be used for one concentration for any number of periods of immersion required. The temperature of the solutions were noted and recorded in each instance. In the case of boric acid, the temperature of the mixture in some test tubes, was raised by immersion in water baths which were regulated at the desired temperature.

Comparative checks were obtained by the treatment of the spore suspensions in the same manner, except that 9 c.c. pure water, instead of the stock solution was added to 1 c.c. of the spore suspension, instead of the stock solution. No comparative checks were however kept for temperatures above normal room temperatures.

The Petri dishes were kept at 25°C for two days, when the comparative amounts of conidial germination were estimated as recorded in Table X. Very abundant germination, such as occurred in the check plates, were marked ∞∞∞; abundant germination ∞∞, scant germination ∞, and trace ∞ and no germination -.

From Table X it is apparent that 15% and 20% sodium chloride solutions had very little effect on the germination of conidia of the B 1c isolate. The germicidal effect of 4 and 5% boric acid, ^{and} hydrochloric acid in concentrations of 1 to 10%, a more powerful germination. Copper sulphate, tested for 5 and 15 minutes, had an inhibitory effect on the amount of conidial germination. No germination, however, occurred where the conidia were treated with any of the concentrations of..

TABLE X : Germination of Botrytic spores after immersion in various solutions.

Solution	Concentration (β)	Temperature ($^{\circ}$ C)	Germination of spores after immersion period (minutes)				
			5	15	30	45	60
Sodium Chloride	15	25	+++	+++			
	20	25	+++	+++	+++	+++	++
Boric acid	4	25		++			
	5	25		+++	++	++	++
	5	70		±	-	-	-
	5	90		-	-	-	-
	5	98		-	-	-	-
Hydrochloric acid	1	27		++	++	++	++
	3	26.5		-	-	-	-
	5	27.2		-	-	-	-
	10	27.0		-	-	-	-
Copper sulphate	2	25.2	+	±			
	4	25.0	+	±			
	6	25.3	±	-			
	8	25.2	±	-			
Formalin	1	24.5		-	-	-	-
	2	24.8		-	-	-	-
	3	25.0		-	-	-	-
	4	24.6		-	-	-	-
Nianin	0.25	25.2		+	±	-	-
	0.50	25.0		+	±	-	-
	1.00	24.8		-	-	-	-
	2.00	25.2		-	-	-	-
Potassium permanganate	0.005	25.0		-	-	-	-
	0.050	25.0		-	-	-	-
	2.0	25.2		-	-	-	-
	4.0	25.2		-	-	-	-
	6.0	25.0		-	-	-	-
	8.0	25.2		-	-	-	-
SO ₂ in saturated solution	-	24.5		-	-	-	-
Borax	1.5	25.3		++	±	+	-
	3.5	25.0		++	+	±	-
	5.0	25.0		+	±	±	-
Potassium iodide	1.5	24.5		++	±	+	-
	3.5	24.5		+	+	-	-
	5.0	24.5		+	-	-	-
Check	-	25.0	+++	+++	+++	+++	+++

of formaldehyde, potassium permanganate or with a saturated aqueous solution of sulphur dioxide. Nianin appeared to be one of the strongest disinfectants for conidia of isolate B 1c, whereas borax and potassium iodide solutions were less effective.

Pathogenecity.

Several fungi, causing decay of various fruits, have recently been more closely studied, as regards differences in pathogenetic activities amongst species of the same or different genera and strains of the same species. Harvey (56) infected apples with various strains of Fusarium fructigenum and found significant differences in virulence when inoculated into apples of a number of different varieties. He contended that the virulence of these strains can in a general way be correlated with their morphological characters. Das Gupta (32), using saltants of Cytosporina ludibunda in infection studies on apples, showed that some of the saltants differed very markedly from others and in many cases from their parents in their parasitic relations. He obtained similar results with strains of Diaporthe pernicioso (33)

The parasitic activities of Botrytis cinerea have received considerable attention from a large number of research workers, as this fungous causes disturbances in such a large number of hosts. Paul (81) found that differences existed between the parasitic activities of different strains of Botrytis cinerea, but he could not find any evidence that selective parasitism existed in this fungus. Klebahn (66) compared various species and forms of Botrytis, such as B. douglasii, B. parasitica and B. cinerea/vitis. He observed differences in virulence of the different species and forms on their respective hosts, but he considered the differences obtained to be so slight, that they might be grouped...

grouped as Botrytis cinerea. Morguer (78) also detected differences between the forms of Botrytis cinerea as regards their virulence, parasitic ability and host specificity. Schneider-Orelli (97) inoculated three varieties of apples with a culture of Botrytis cinerea and found that the "Horberenapfel" was the most susceptible to Botrytis attack, Harry's GoldreINETte being more resistant and Grüner Slettiner the most resistant of the three at temperatures of 4.5°C and 14°C.

In the comparative studies of the pathogenicity of the seven isolates of Botrytis, mature and sound apples of the Delicious and Rokewood varieties, which had been cold stored for four weeks at 35°F, were utilised. The apples were first immersed in a .1% solution of mercuric chloride for 15 minutes, rinsed in water and dried. The method of inoculation adopted was the "cork borer method" first used by Van der Byl (103) in his studies on Cephalosporium sacchari and later also described by Granger and Horne (49) for inoculating apples.

The extraction of the cylindrical plug of apple tissue with the borer could under ordinary circumstances not always be accomplished. This difficulty was however overcome by denting a small portion of the lower edge of the borer, which was then twisted as soon as it was inserted to the desired depth before the borer was removed. The cylindrical plug of apple tissue was then very easily extracted from its position with the extraction of the borer. To ensure an even depth of inoculation in the apple, the cork borer was passed through a few corks and only the lower $\frac{3}{4}$ inch left exposed to penetrate into the apple. The inoculum for each apple consisted of two drops of a heavy spore suspension of the particular isolate. This suspension...

suspension dropped into the bottom of the cavity by means of a dropper, ensured a standard method of inoculation with the particular isolate for all apples in this series and facilitated the technique.

Twenty eight apples of each variety were inoculated by the above method with each of the isolates, the plug replaced and sealed with paraffin wax. The inoculated apples were then kept in ordinary paper bags under similar laboratory conditions for 12 days.

These apples were then weighed individually, the affected tissues carefully scooped out and the unaffected remaining portions of the apples again weighed. The percentages of the invaded apple tissues were then calculated for each apple individually.

Gregory and Horne (51) described a method, in which the "radial advance" of fungal invasion in the apple tissue is used for the testing of the parasitic activities of *Gungi* on apples of different varieties and discussed the advantages of using "radial advance" readings for the comparison of different fungi. In a subsequent paper (62) these authors also indicated how this method may be applied to certain specific problems.

For the proper comparison of the pathogenetic activities of the monoconidial cultures of *Botrytis*, used in these tests, the "radial advance" of each individual inoculation was obtained from the percentage tissue invaded as described by Gregory and Horne (51).

The standard error was calculated for each set of inoculations using the formulae previously quoted. The results are tabulated in Table XI.

At the same time Barlinka grapes were inoculated, through small incision wounds, with mycelia and conidia of each of these *Botrytis* isolates. The berries were then suspended in jars, under fairly dry laboratory conditions

when...

TABLE X/ : "Radial Advance" of monospore cultures of *Botrytis* inoculated into Delicious and Rokewood apples.

Culture No.	Radial Advance after 12 days.	
	Delicious	Rokewood
B1b	1.50 ± .02	1.00 ± .04
B1c	1.29 ± .03	1.07 ± .03
B4c	1.78 ± .03	1.58 ± .05
B6d	1.20 ± .03	1.04 ± .04
B8c	1.34 ± .03	0.96 ± .04
B11a	1.74 ± .02	1.28 ± .04
B12c	1.57 ± .03	1.20 ± .04

when the relative amounts of decay resulting from these inoculations were determined by comparison and estimation. The isolates of Botrytis were arranged in order of declining virulence on the Barlinka grapes as follows:-

B 11a (++++++) B 4c (++++); B 12c and B 6d (++++);
B 8c and B 1b (+++); B 1c (++) .

The data in Table X1 indicates that B 4c is the most virulent culture on both Delicious and Rokewood apples. The standard errors of the differences between the radial advances acquired by these Botrytis isolates and the degree of significance of these differences were then calculated according to the formulae previously quoted. Taking the radial advance of B 4c as basis for comparison of the different isolates, they may be grouped in order of declining pathogenetic activities on the two apple varieties as below:-

Degree of significance from basis (B 4c)	On Delicious	On Rokewood
< 3	B 4c	B 4c and B 11a
3 - 10	B 11a and B 12c	B 12c
10 - 15	B 1c, B 6d, B 1b	B 1b and B 8c
> 15	B 8c	B 1c and B 6d

The results obtained in the inoculation experiments on apples and grapes reported above, show very close resemblances. B 4c was in these three experiments practically the most active parasite, followed in order of declining virulence by B 11a, B 12c, B 1b, B 6d, B 8c and B 1c. B 4c, B 11a and B 12c may be grouped as being comparatively virulent; B 1b, B 6d, and B 8c as being fairly virulent and B 1c being a weak parasite in the majority of infections.

Any one of the seven isolates of Botrytis showed significant differences from any of the other six in their virulence, as expressed by "radial advance" figures, on one or both of the apple varieties used.

Discussion.....

Discussion.

Botrytis cinerea has been studied intensively during recent years, as regards the morphological characters of members of this species and the bearing of these characters on the taxonomical position of isolates belonging to this group.

Busgen (24) demonstrated in 1918 that this fungus was able to infect a large number of host plants and that the infected spots spread on some hosts slower than on others. Berkeley (6) made a study of the morphology and physiology of four strains belonging to the Botrytis cinerea group and isolated from geraniums, squash, sunflowers and hemp. He found that he could divide these strains into two distinct groups on the basis of spore size, rate of germination of the spore and cultural characters.

Westerdyk (118) discussed the relations of Botrytis cinerea to other species of Botrytis. Some so-called species such as Botrytis vulgaris and Botrytis cana, were considered to be so closely related to B. cinerea, that they could be hardly separated. Species like B. parasitica could however be distinguished from B. cinerea in morphological as well as in cultural characters.

Paul (81) divided the Botrytis cinerea cultures which he studied into three distinct groups; the one possessing a strongly marked tendency to form sclerotia, the other a general tendency to form aerial mycelium and the third possessing a freely sporing habit. He, however, found that certain growth characters varied somewhat when grown on different media and under different environmental conditions.

Klebahn (66) came to the conclusion that the differences between Botrytis Douglasii, B. parasitica, B. Trifolii and several other species of Botrytis studied, are so slight that these species may be grouped together under the collective species Botrytis cinerea.

Hansen....

Hansen and Smith (54) studied the variation in strains of Botrytis cinerea, which showed distinct cultural differences from one another. They found that anastomosis between neighbouring hyphae, consisting of multinucleate cells, are abundant in cultures of Botrytis cinerea. This anastomosis resulted in a mixture of nuclei of two or more strains in the resultant hyphae and conidia. To this nuclear heterocaryotic condition, forms of this fungus apparently owe their instability. In a later study these authors (53) were able to show that anastomosis occurred between Botrytis Allii and B. Ricini, which resulted in a segregation of three types in later generations for which new varietal or even specific rank appeared to be justified.

Mor^guer (78) also came to the conclusion that Botrytis cinerea might be rather considered as a collective species embracing forms differing in morphological characters and parasitic abilities.

From the literature referred to above, it is apparent that the taxonomy of Botrytis species, and particularly that of B. cinerea is in a fair state of confusion as has so adequately been pointed out by Brierley (13). This state of confusion may be further aggravated by inter-specific anastom^sosis and subsequent segregation into new types, as was found by Hansen and Smith (53). Their researches are, however, bound to lead the way to an elucidation of the conception of variation in the Fungi Imperfecti, and ultimately to that of the taxonomic confusion. These researches all point to the necessity of broadening "species concept" in mycological systematics and of taking into account not only morphological characters, but also physiological and cultural characters in species delimitation.

Brierley.....

Brierley (13) for instance, considers Botrytis cinerea to be comprised of a number of varieties, each of these consisting of a number of races or strains. The races or strains are again subdivided into physiological forms, and the culture isolated from a host Brierley calls an "isolate", a term which was used in this sense in the laboratory studies described in this contribution. According to Brierley, the physiological forms of one strain are a group of those isolates which are indistinguishable on culture criteria, but which differ in their parasitic qualities. Different races or strains of one variety are groups of physiological forms which differ from other groups in their cultural characters and behaviour. Varieties again are made up of somewhat elastic cultural groups, the total of which is the species.

The Botrytis isolates, used in these laboratory studies show, as has been discussed, marked differences in cultural characteristics and may in this respect be grouped according to the classification of Paul (81) as follows:-

Strongly marked tendency to form sclerotia : B12c, B3c, B3d,
B11a.

General tendency to form aerial mycelium: B1c.

Freely sporing habit : B1b, B4c.

Significant morphological differences in the size and shape of the spores have been shown to exist in the different isolates and also significant differences between parasitic activities of these isolates on apples and to a certain extent on grapes.

These differences are therefore distinct, but not of a sufficiently constant character to warrant specific rank. According to the subdivision^{of} Botrytis cinerea by Brierley (13), these isolates may all be considered as belonging to different varieties of this species. The

number...

number of isolates studied are however not enough for a proper indication of all the types of variations possible within this species. The isolates selected, for the laboratory studies, were only those from about 70 single spore isolations, which in the beginning showed the most distinct cultural differences. It is evident that in this selection isolate cultures, belonging to different varieties of Botrytis cinerea would have been chosen. The rest of the single spore isolations showed comparatively minor differences in growth and were therefore not used. The seven cultures of Botrytis cinerea used for these studies, were therefore only a small proportion of forms, strains, and possibly varieties of Botrytis cinerea handled, and a still smaller proportion probably of those occurring in South Africa.

As regards the relation of morphological characters to parasitism, it has already been cited that Harvey (56) found "the degree of virulence shown (by a number of strains of Fusarium fructigenum on apples) is correlated in a general way with the morphological character of the strain".

In comparing the data in Tables I to VII with that in Table XI and with the results of the inoculation experiment on Barlinka grapes, it is evident that no correlation exists between growth rate, amount of aerial mycelium, spore production, sclerotial production, acid toleration, sugar requirements or size of spores and parasitic activity.

Furthermore, if the growth of the different isolates of Botrytis cinerea under different cultural conditions be compared with the analyses made of grapes by Capeman (30) and Frater (31), it appears that the hydrogen-ion concentration, total acidity and sugar content will seldom be factors prohibiting infection and decay of grapes berries by Botrytis cinerea, especially during the ripening stages of the grapes.

Brooks (18), from his studies of the effect of mineral starvation of lettuce plants on infection by Botrytis cinerea, concluded....

concluded that "whatever may be the causes at work in the living cell which confer immunity or ^epr^edisposition on the species of the host plant or which confer virulence or impotence on the spore, they lie deeper than nutrition". The studies of Brown (19-22) and of Blackman and ^eWilsford (8) on the infection of hosts by Botrytis cinerea tend to show that Botrytis cinerea enters its host mainly by mechanical pressure. Brown (19) furthermore concludes that "the ability of certain tissues to resist the action of the extract (of Botrytis cinerea) is dependent upon the special properties of their cell walls. Therefore the nature of the cell wall affords the key to the resistance of the particular tissue to the action of the fungal extract and therefore also of the fungus, because it is demonstrated that if the cell wall is disintegrated, the death of the cell ensues".

This aspect will be further discussed in the subsequent part of this paper.

FIELD STUDIES.

According to Reinecke (94), the Constantia area still ranks the first amongst the areas in South Africa from which table grapes are exported, the amount exported from this area in 1932-1933 totalling 7882 shipping tons. In spite of the lead in the export of table grapes, however, grapes from the Constantia area are also those most severely affected during storage and shipment by wastage, and particularly by Botrytis rot. This latter condition occurs mainly on account of the fact that grapes from this area generally ripen rather late in the season and that growers attempt to place them on the London markets as late as possible. The position in the De Doorns section and..

and to a limited extent in Banhoek, Stellenbosch, is of a similar nature. As pointed out subsequently, the amount of Botrytis and other storage rots increase as the picking season advances, resulting in greater wastage in grapes from these areas.

The field experiments were carried out in vineyards and on picked grapes from the Constantia area. In these experiments the effect of various factors on the occurrence of Botrytis rot in the vineyard and in storage was studied, and various methods for the control, mainly of this rot, were experimented with. The data embodied in this part of the paper constitute the results of such experiments obtained during the 1933-1934 and the 1934-1935 grape seasons.

Methods and Materials.

The experiments in the vineyards were conducted on the farms at "Höhenort", "Under-the-Hatch", "The Vineyards" and "Clunie", all in the Constantia area. Their location are indicated in Figure IV by the letters A, B, C and D respectively. As pointed out by Reinecke (94), these farms are all situated in Zone 11 of the topographical division of this area.

In all cases the vines used in the field tests were planted 6 by 5 feet and grown on three-wired trellises according to the Perold system (vide 84 p. 523). At "Höhenort" and "Clunie" the vines were on Constantia loamy fine sandy type of soil of the Constantia series (according to the soil classification of Reinecke (94)), at "The Vineyards" they were on the Constantia fine sand, and at "Under-the-Hatch" on the fine sand type of the Vlei Series.

For the studies during 1933-1934, grapes of the Henab Turki variety were used from the vineyards of "Under-the-Hatch", "The Vineyards" and "Clunie" and

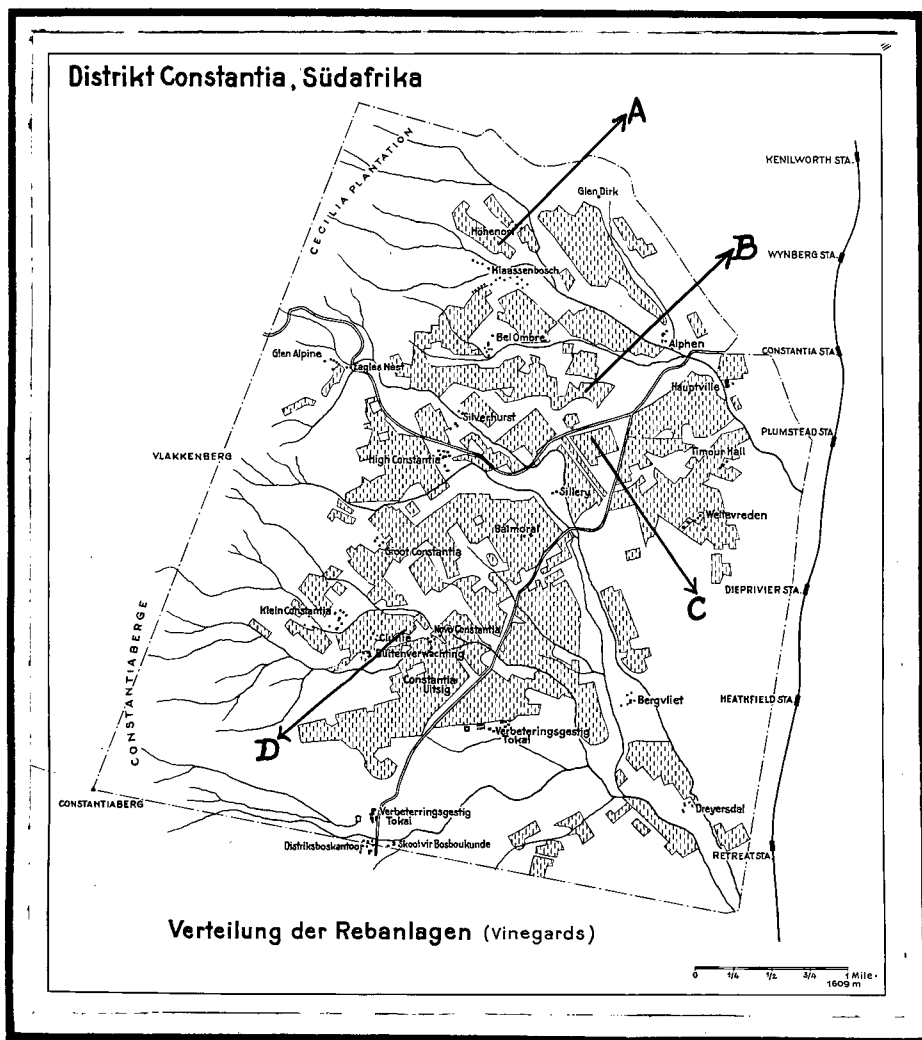


FIG. V. - Map of the Constantia area showing the distribution of the vineyards in this area.

(By permission from Reinecke (94)).

- A - Höhenort .
- B - Under-the-Thatch .
- C - The Vineyards .
- D - Clunie .

Red Hanepoot from "Höhenort". During the 1934-1935 season, experiments were carried out with Honab Turki from "Under-the-Thatch" and with Henab Turki, Raisin Blanc, Red Hanepoot and White Hanepoot grapes from "The Vineyards".

The grapes, intended for experimentation, were thinned moderately to fairly severely during the early part of the growing season and again during midseason. The general cultural conditions of the vines in the experimental plots were those usually applied in this area for the production of sound good quality export grapes.

The experimental grapes were carefully picked and cleaned, but no attempt was made to specially sort the bunches into "Select" and "Choice" grades. They were carefully wrapped in thin sulphite tissue paper and packed in woodwool, approximately 10 lb. of grapes to a box, 18 x 12 x 5½ inches, as was recommended by Bulmer (23)^x. In the experiments at "Under-the-Thatch", the open pack method, as has been referred to and illustrated by de Castella (35) was adopted, but the closed pack was used in the other experiments.

After packing, the grapes were transported by lorry to Plumstead station, 3-5 miles distant, railed to the Imperial Cold Storage, Cape Town, where they were stored 12-24 hours after having been packed and held at 34-35°F for three weeks. The grapes were then again railed to Stellenbosch and kept at ordinary room temperatures for 7 to 14 days, when the boxes were opened and the grapes examined.

In this examination, the bunches were removed one by one in the reverse sequence of packing. Each bunch was

x

Standard method adopted in South Africa.

unwrapped, the general condition and position of wasty berries in the bunch annotated. The bunch was then clipped into smaller portions, so that each individual berry could be carefully examined by sight and by touch. All infected berries were removed, classed into the different types of waste according to the differential characters described on page . The total number of berries in the bunch, and the numbers affected by each type of rot were counted and the figures recorded for each individual bunch in each box. No distinction was however made between rots caused by the different species of Penicillium, Aspergillus etc., all being classed under Penicillium rots etc..

It was, however, found that the different bunches examined from the same box, or from different boxes in the same treatment, showed a very great variation in the amounts of mechanical damage. This variation in the amount of mechanical damage in the bunches considerably influenced the amounts of wastage. For a proper comparison of the data, it was therefore considered essential to estimate the amount of mechanical damage of each bunch and to arrange and compare the infections between different treatments on this basis. The relative amount of mechanical damage was indicated by minus or plus signs as follows:-

Mechanical Damage	Berries		
	With cracked necks	Punctures	or Crushed.
-	Absent	Absent	Absent
o	Very scant	Absent	Absent
oo	Scant	Very scant	Very scant
ooo	Fairly abundant	Scant	Scant
oooo	Abundant	Fairly abundant	Fairly abundant
ooooo	Very abundant	Abundant	Abundant
oooooo	Very abundant	Very abundant	Very abundant.

The....

The percentages of infection by Botrytis, Penicillium, Rhizopus species etc., were then calculated for bunches showing an absence or similar amounts of mechanical damage in each treatment.

Relative Importance of Various Organisms in the
Wastage of Export Grapes.

It is regrettable that from the reports from overseas representatives, it is wellnigh impossible to determine to which extent each type of rot has been responsible for wastage of export grapes. This absence of information of a definite character makes it practically impossible to determine to what extent varying percentages of each of the types of wastages affected the prices obtained for South African grapes.

In 1923 Putterill (89) mentioned Botrytis cinerea, Penicillium spp., Rhizopus spp, and Aspergillus sp. as organisms being most commonly found in wasty grapes. He also stated that Botrytis cinerea and Penicillium spp. were the most important during the 1923 season. Van Niekerk (10()) drew attention to the inadequacy of previous reports in which the reporters satisfied themselves with terms such as "damp, mildewed or showing signs of mouldiness and in some cases even wasty". No attempt was, however, made by him to differentiate between and evaluate the various types of rots occurring in export grapes. De Villiers (37) attempted to describe an Aspergillus species which appeared to him to be the most prevalent type of rot in grapes from Paarl, Stellenbosch (Elsenburg) and de Doorns. Boyce, Beyers and de Villiers (10) stated that "the comprehensive term of wastage of grapes has become to be considered as indicating wastage due to Botrytis and Penicillium glaucum, as superficially these appear to be predominant in the sporulating stage".

Tho...

The total percentage of wastage occurring in check boxes of grapes, used in the various dusting, spraying and other experiments during the two seasons of experimentation, were calculated. The proportion of Botrytis and other rots in relation to the total amount of wastage expressed in percentage are given in Tables XII and XIII. The experiment numbers referred to in Table XII are those of the spraying and/or dusting experiments carried out during the 1933-1934 season, and discussed in a later section of this paper. In Table XIII the percentage wastage of the grapes, used as checks, are given in order of picking, the same method of packing and storage being followed in all cases.

Table XIII: The total percentage of wastage which occurred in boxes of untreated grapes used in the field experiments of the 1934-1935 season, and the proportion of Botrytis, Penicillium, Rhizopus, Cladosporium and Aspergillus rots in relation to the total amount of wastage of stored grapes. Data taken 10 - 12 days after cold storage.

Experimental Series	Date of picking	Total percentage wastage	Percentage of Total Wastage.					Variety.
			Botrytis	Penicillium	Cladosporium	Aspergillus	Rhizopus	
1	28.2.35	2.25	33.3	15.7	50.0	34.0	-	White
2	5.3.35	5.64	0.53	0.18	98.29	-	-	Manepoot
3	28.2.35	3.94	10.40	3.6	75.60	10.4	-	Raisin
4	5.3.35	6.62	0.76	0.30	97.73	-	1.21	Blanc
5	5.3.35	3.80	1.58	3.13	95.25	-	-	Red Hants
6	13.3.35	2.90	37.4	4.8	51.71	14.8	0.5	Donab
7	18.3.35	6.18	70.0	6.14	23.86	-	-	Donab
8	19.3.35	7.71	59.5	6.8	22.99	0.16	0.55	Purki.
9	23.3.35	28.10	34.0	2.4	13.60	-	-	Purki.
10	25.3.25	71.30	91.6	3.6	4.60	-	-	

Experimental series 1-9 picked from adjacent vineyards of "The Vineyards".

Experimental series 10 picked from a vineyard at "Under-the-hatch".

From Table XII it is evident that generally the amount of wastage of grapes was very high during the 1933-1934 season whereas it was again relatively low in the majority of the experimental series of 1934-1935, as is shown in Table XIII.

The...

TABLE XII : The Percentage Botrytis, Penicillium, and Rhizopus wastages of the total wastage, which occurred in the check boxes of the dusting and spraying experiments during 1934.

Experiment No.	Total Percentage waste.	Percentage of the total waste		
		Botrytis	Penicillium	Rhizopus
A	3.6	69.2	7.6	1.5
B	10.0	77.0	13.4	0.4
C	8.3	99.1	0.9	-
D	29.4	95.4	3.0	1.6
E	22.2	99.5	0.5	-
F	69.8	99.7	0.3	-
G	23.2	83.6	15.5	0.9
H	75.5	99.9	0.1	-
I	92.0	99.96	0.04	-
J	41.2	99.9	0.1	-
K	64.4	99.8	0.2	-
L	81.9	99.7	0.3	-
Average	43.5	95.2	3.6	0.4

The grapes of the experiment numbers A, B and G in Table XII were of the Red Hanepoot variety, picked from the same vineyard plots. The grapes of A and B were picked at the same time, but those of A were examined 7 days after cold storage and those of B 14 days after cold storage. The grapes of experiment G were picked three weeks after those of A and B and examined 7 days after cold storage, the results of G are therefore comparable with those of experiment A. According to the particulars of dates of picking, packing and examination, recorded in Table XXII, the results of experiment E is comparable with those of J, those of H with those of K and those of I with those of L.

From these comparisons, where the only difference is practically the difference in the date of picking, it is of interest to note that the percentage of waste was in all the later pickings higher than that in the earlier pickings. The opposite was, however, the case in the comparisons of percentages of H and I with ^{those of} K and L, where the infection in the first picking was abnormally high. This was due to the fact that grapes of experiments H and I were picked only shortly after a rain. This aspect will be further discussed in a later section. From Table XIII it is also clear that the total amount of wastage gradually increases on all varieties as the picking season progresses.

During the 1933-1934 season Botrytis rot constituted from 69.2 to 83.6% of the total amount of waste occurring in Red Hanepoot grapes from "Höhenort" and from 77.0 to 99.96% of the amount of waste in Henab Turki grapes from the other farms. Penicillium rots were of importance in experiments A, B and G in which it averaged from 7.6 to 15.5% of the total amount of waste of these Red Hanepoot grapes. On Henab Turki in the other experiments, however, Penicillium rots were only of very rare occurrence.

Rhizopus rot occurred rather sporadically and in relatively small amounts, it being present only in the Red Manepoot grapes from "Höhenort" and in the second count (experiment D) of Henab Turki from "Clunio". Cladosporium and Aspergillus rots were of such rare occurrence during this season that their percentages were not included in Table Xll. Only occasional grape berries were found to be affected.

The data in Table Xlll indicate that Botrytis rot constituted a smaller proportion of the total amount of wastage, though relatively low, during the 1934-1935 season than during the previous one. Penicillium and Cladosporium rots were, however, present in much higher percentages during the 1934-1935 season. Aspergillus and Rhizopus rots occurred again rather sporadically in only a few of the experimental series. It is of interest to note that the proportional amount of Botrytis rot increased on most of the varieties as the 1934-1935 season advanced and as the amount of the total wastage increased. The proportional amounts of Penicillium and Cladosporium rots showed a decrease with the increase of total wastage. This apparent decrease in the relative percentages of rots due to Penicillium spp. and Cladosporium sp. does not however indicate that a decrease in the actual percentage of these rots in stored grapes occurred with the advance of the season. The actual percentages of Penicillium and Cladosporium infections on the contrary showed an increase as the season progressed. In the four Henab Turki experimental series at "The Vineyards" the percentages of Penicillium infections increased from 0.25% to 6.83% and Cladosporium infections from 1.51 to 3.70%. ~~The proportional increase of Botrytis~~

The equations for the linear trend of the graphs of the logarithms of the percentages infection of Henab Turki berries, picked between the 13th and the 26th of March, 1935, and calculated from Table Xlll are:-

For the total amount of waste :	$\log y = .9355 + .0382 x$
" Botrytis rot	$\log y = .7433 + .0510 x$
" Penicillium rot	$\log y = 1.6561 + .0158 x$
" Cladosporium rot	$\log y = .3173 + .0168 x$

where the origin of x is between the 19th and the 20th March and y the percentage infection by specific rot organism.

In comparing the b constants of the above equations, it is evident that the proportional rate of increase of Botrytis rot on Henab Turki grapes during this period was much greater than that of either Cladosporium or Penicillium rots respectively.

From this it may be concluded that wastage of grapes becomes an increasingly impeditive factor as the Constantia picking season advances. This increase in wastage is also mainly due to an increase in Botrytis rot which becomes of major importance as the season draws to an end.

Fusarium, Sphaeropsis and Yeast (Saccharomyces sp.) rots were encountered in cold stored grapes, but in such small percentages that their presence can hardly be considered of any economical importance in the present consignments of grapes from the Constantia area.

The above conclusions as regards the relative importance of grape rot organisms are to be considered as applying only to the farms and to the varieties used in experimented with. The comparative amounts of these rots will be influenced not only by different varieties, but also ^{by} ~~on~~ seasonal and climatic variations as well as local conditions under which these grapes are grown.

As regards the economic effect of the presence of various amounts of wastage in export grapes, Mr. G.L. Dykes* reports that "... any extensive wastage has a depressing effect on prices generally, so that the financial loss is not entirely confined to the consignments showing wastage. Moreover, buyers naturally discount the possibility of a further development of wastage, so that, say, 10 per cent of wastage in a box would result in much more than 10 per cent fall in price.

....It is reasonable to suppose that buyers pay more attention to a small percentage of wastage on a heavily loaded market when ample alternative supplies are available than they do at times of short supply when the demand is active.

* Correspondence with Mr. G.L. Dykes, Overseas Representative of the South African Deciduous Fruit Exchange Ltd.

Influence of Environmental Conditions on the Occurrence of Botrytis Rot in the Vineyard and in Storage.

It has already been pointed out that, during the development of the grape export trade, the amount of wastage, and particularly that of Botrytis rot greatly varied from season to season. In this respect^{*} the 1931-1932 season was outstanding in that grape farmers suffered severe losses in the export of table grapes, due to a very extensive rotting of the grapes in shipments which arrived in the London market during the latter part of this season. The occurrence of wastage in 1933 was however, sporadic and not of very much importance. The 1934 season was again outstanding in that grapes were fairly severely affected by Botrytis rot and other rots during the later part of the season, though, generally speaking, infections during this season were not as severe as in 1932. The 1934-1935 season is considered to have been relatively favourable for the export of grapes and losses suffered through Botrytis rot in particular were relatively low.

If the rainfall during recent years, as compiled by Reinecke (94) for the Constantia and other grape growing areas, be compared, it will be readily seen that the rainfall during the months of January to May of 1932 was distinctly higher than that during these months of any of the succeeding years. The epidemic occurrence of Botrytis rot during the 1931-1932 season was ascribed mainly to the comparatively heavy rains immediately before and during the late picking season, in which respect the Constantia area was one of those mostly affected.

Attention has been drawn in the discussion of Tables XII and XIII, to the fact that the total amount of wastage, as well as the percentage infection by each type of rot, occurring in packed grapes generally show a distinct upward trend as the packing season of Constantia advances.

This...

* According to Mr. G.M. Dykes

This observation has been confirmed by Mr. G.M. Dykes, who stated that the severity of wastage in each variety of grape increases with the advance of the picking season. In referring again to the rainfall and humidity table of Reincke (94), it is evident that the monthly rainfall and mean monthly humidity increases from February to May.

It would appear that the amount of wastage, and particularly Botrytis rot, of grapes during storage is in relation to the amount of rainfall and relative humidity immediately prior to and during the time of picking and packing.

In order to determine the possible effect of slight differences in relative humidities and other environmental factors on the occurrence of Botrytis rot in the vineyard and during storage, the following experiment was carried out:-

Two spots about 200 yards apart, were chosen in a Red Hanepoot vineyard at "Höhenort". Both were situated in an easterly slope, A being lower down than B and was much later in receiving the morning sun than B. This was due to an avenue of oak trees 50 yards lower down from A. Standardized self-recording hydro- and thermographs were installed at each spot in a somewhat crude, wooden box shelter (Plate V). Care was taken to have the recording apparatus at each spot in the centre of two vineyard rows and at about the same height as the majority of the bunches on the vines.

The percentage infection by Botrytis occurring in the vineyard was determined at different periods by counting the total number of berries and the number of berries infected by Botrytis on three vines on each side of each stand.

The environmental conditions at the two spots differed mainly in the following respects:-

1. The...

1. The period of high humidities (i.e. relative humidity above 70%) was generally slightly longer at A than at B (Fig. VI).

11. The daily period of sunlight at A was of shorter duration than at B. This difference increased as the season drew to a close. The rays of the morning sun struck A $1\frac{1}{2}$ hours later than B during February and A was generally in shadow an hour before B in the afternoon. In April again B received the morning sun 2 hours before A. This resulted in dew which was deposited fairly abundantly on the leaves and bunches nightly during April and May, drying so much slower at A than at B.

111. The temperature recorded at A was at any time always 1°C lower than that at B.

IV. The grapes at A were on the whole a week later in colouring than those at B and the colouring of grapes at B was very much superior to that at A. This latter difference was also associated with the production of a much firmer, sweeter and more attractive grape at B than at A.

The percentages of Botrytis infection in the vineyard increased at certain periods at A, as is indicated in Figure VI. These increased infection were always preceded by periods of long duration of high humidity (24 hours per day) and by varying amounts of rain e.g. ^a large increase in percentage of Botrytis infection was recorded on the 12th of March, following 2.35 inch precipitation between 7-9 March and a long period of high humidity. After this rain, several fairly severe outbreaks of Botrytis rot in vineyards were reported in the Constantia area. Several farmers had the experience to have their grapes, which had been packed just after the rain, rejected for export on inspection at the Docks. As a result packing operations

at....

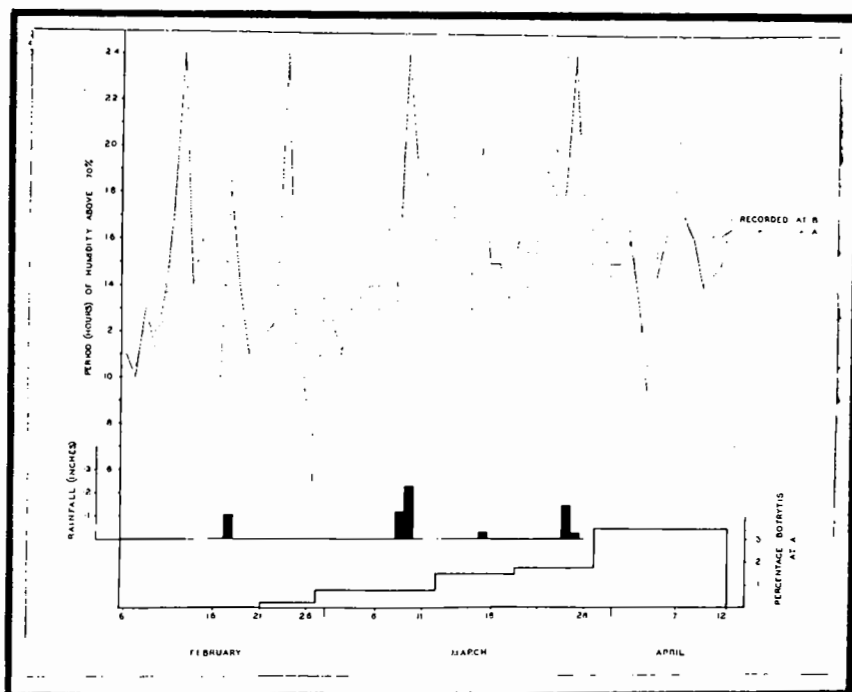


FIG. VI. - The daily periods with relative humidity above 70% at two selected spots, A and B, in a vineyard at "Höhenort", Constantia, from February to April, 1934, and the daily rainfall and percentage Botrytis infection at A.

at several farms were terminated for the season. Another big increase in the percentage of Botrytis infection was observed at A on the 29th of March, 1934. This was again preceded by a precipitation of .18 inch and a long duration of high humidity on the 25th and 26th of March. On the 28th of March, the grapes at A were considered to be totally unfit for export as 100% of the bunches was affected by Botrytis rot.

At the time of concluding this experiment, i.e. 15th April, no sign of infection of grapes at B by Botrytis rot could be observed.

As previously mentioned, the general appearance of the grapes at A was very much inferior to that of the grapes at B. It was found that the epidermis and pulp of the grapes at A was less tough and firm than those grapes at B. These differences in the general condition of the grapes at the two spots were most probably due to the slightly lower temperature, slightly higher humidity, and poorer soil conditions at A than at B. It would appear that the difference in physical and chemical properties of the grapes might have resulted in a difference of their resistance to Botrytis infection, and which could have been responsible for the vast difference in infection observed in this vineyard.

In this connection Reyncke (95) found that the chemical constitution of grapes and other fruit may be affected by ^asoils and methods of their cultivation and that the chemical constitution of the fruit had a very important bearing upon the keeping quality of these fruit and on their liability to be infected by storage rot organisms.

In order to determine whether the difference in the resistance of grapes to Botrytis rot, as observed in the vineyard, will also be manifested by these grapes after they have been picked and cold stored, the following experiment was carried out:-

On the 24th of February, 1934, one box of grapes was picked..

picked from the six vines in the immediate vicinity of A, and one from those at B. They were packed, and kept in cold storage as previously described and examined seven days after cold storage when the data in Table XI V were obtained.

Table XIV: Inspection of Red Hanepoot grapes picked from vines in two selected spots (A and B) in a vineyard "Höhenort", Constantia. Data taken 7 days after cold storage.

Grapes picked at	Date of picking	Number of			Percentage infection	
		boxes	bunches	berries	Botrytis	Penicillium
A	24.2.34	1	13	788	21.1	-
B	24.2.34	1	13	805	0.2	0.5
A	14.3.34	1	12	533	15.6	2.4
B	14.3.34	1	13	815	2.9	-

The grapes from A and B, although picked, packed and stored in the same way, still differed even more markedly in their amounts of Botrytis rot. ^{the} here amount of mechanical damage was approximately the same in all the boxes, the grapes from A showed a much higher percentage of Botrytis infection than those from B. From these results it is evident that the grapes from these two spots differed definitely in their susceptibility to infection.

The foregoing observations and results tend to show that climatic conditions such as precipitation and prolonged periods of high humidity, are of very great importance as factors influencing the occurrence of Botrytis rot in the vineyard and that during shipment. They are considered to be the main factors responsible for the differences of amounts of Botrytis infections in export grapes during different seasons and also for an increase of these infections as the season advances. Further it is evident that apparent minor differences of temperatures, humidities, period of sunlight, soil conditions etc., may have a very marked effect on the resistance of grapes to Botrytis infection either in the vineyard or during storage.

Effect....

Effect of the Size of Bunch on Botrytis Rot.

Severe thinning of export grapes ~~which~~ has become a regular practice in all vineyards, the object being mainly to obtain a large sized and an attractive berry. To determine whether the size of a bunch had any effect on the relative amount of Botrytis infection, the number of berries per bunch was taken as a unit for comparison. The percentages infection of sixty boxes of Henab Turki grapes, used as checks in some of the field experiments at "The Vineyards" during the 1933-1934 season, were utilised for the subsequent determinations.

To compare the relative percentages of Botrytis infections of different sized bunches in the boxes picked and packed on different occasions, it was considered necessary to obtain a comparative value for every bunch in a box. This unit value is to indicate the relative amount of Botrytis infection of a particular bunch as compared with the average percentage of Botrytis infection of a particular box of grapes. This comparative value was termed "Tendency of Infection" was calculated as follows:-

Percentage Botrytis infection of the bunch = x

Average percentage Botrytis infection of the box = y

Tendency of infection = $\frac{x - y}{y}$ or $\frac{x}{y} - 1$,

The "Tendency of Infection" of every bunch contained in the sixty boxes of grapes was calculated separately. In this calculation the bunches were grouped in accordance with their number of berries, and an average "Tendency of Infection" of the bunches in every class obtained. Only in this manner was it possible to compare readings of bunches in different boxes of different experiments.

The data are graphically presented in Figure VII. From this it may be seen that the inclusion of small-sized bunches

in...

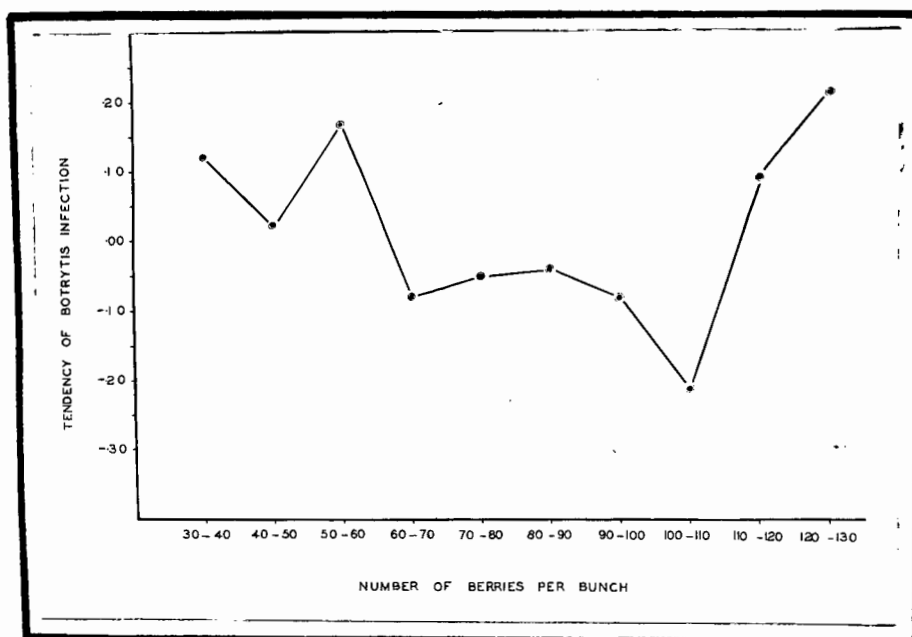


FIG. VII. - The effect of the size of Henab Turki on the
"Tendency of Botrytis Infection."

in packs tends to raise the percentage of Botrytis infection in the box. Small-sized bunches are mostly those which had received a severe thinning in the vineyard. The inclusion of very large bunches (i.e. bunches which had not been thinned at all or thinned very moderately) also tends to raise the amount of Botrytis rot considerably in the box. It is apparent that the least amount of Botrytis infection can be obtained by aiming at the production of only medium-sized bunches for export.

Reyneke (95) carried out more detailed experiments to determine whether thinning had any effect on the keeping quality of Waltham Cross, Barlinka and Henab Turki grapes. He found that thinning of grapes had the effect of lowering the dissociation figure and specific electrical resistance of the juice of these berries when ripe, and of increasing the percentage of waste occurring in grapes. He ascribed the poorer keeping quality of grapes, which had been thinned, to an increase in the rate of respiration, due to an increase in the concentration of respirable compounds in such berries. Reyneke also found that, though the dissociation figure and specific electrical resistance of juice of berries from unthinned bunches were higher, than those of thinned bunches, the amount of wastage in these bunches were higher than in the bunches which were moderately thinned. This apparent inconsistency in his results he ascribed to the occurrence of mechanical damage in such tightly packed bunches.

In general there is a very close similarity between the results obtained by the author and those of Reyneke (95), though the methods of investigation differ considerably. The results lead one to conclude that too severe thinning as well as no thinning at all may both be detrimental to
the....

the keeping quality of grapes. The latter is a well known fact as far as Botrytis infection in the vineyard is concerned, unthinned bunches being always the first to be ^{come} infected, as has been mentioned in the case of Fleming Tokai in a previous report (43).

Effect of Mechanical Damage on Damage of Grapes.

The occurrence of mechanical damage in fruit packs has been an impeditive factor in the marketing of most fruits, not that its occurrence affects their salcability to any appreciable extent, but mainly due to the fact that wounds afford points of entrance for most of the storage rot organisms. In 1908 Powell (88) carried out a very thorough investigation into the occurrence of mechanical damage in oranges from various packing sheds and on the effects of mechanical injury on the occurrence of decay in such packs. He concluded that this type of damage was one of the most important factors contributing to the severe losses suffered in the Californian citrus industry.

In 1923 Putterill (89) drew attention to the fact that mechanical injury to South African grapes, however carefully handled, was fairly common and considered the main type of injury to be the punctures caused by contact of the berries with protrusions of the stalk.

Molz (75) discussed the relation of the thickness of the epidermal layer of grape berries of different varieties to their liability to be mechanically injured. He stated that berries with a thin epidermal layer were very apt to crack during rainy weather and are then readily infected by Botrytis cinerea.

De Villiers (37) came to the conclusion that the tendency of berries to rupture on vines after rain is also correlated with the elasticity of the skin of such berries. He further demonstrated the relation of Aspergillus infection to mechanical injury at the pedicel end and found that infection was high in injured berries when.

kept under relative humidity of 90%, but that no Aspergillus infection of injured berries was obtained when they were kept at a relative humidity of 40%.

Of the organisms responsible for wastage of South African export grapes, Botrytis cinerea is the only fungus which is definitely known to be able to infect grape berries through the uninjured skin. The occurrence of mechanical damage in the form of cracked necks, punctures or crushed berries, have a direct bearing on the occurrence of these rots including Botrytis in the vineyard and in storage.

At the outset of these field studies, mechanical damage was found to have such an important bearing on the occurrence of the various types of wastage in packed grapes, that the classification of data, according to varying amounts of mechanical damage in the bunches, was considered essential for the proper interpretation of most of the results of the field studies. The amounts of mechanical damage referred to in this discussion was unless otherwise stated, determined by estimation as previously explained on page .

The occurrence of mechanical injury in the vineyard is of importance to varieties like Gros Colman and Raisin Blanc, which are very apt to crack after rains. If the period following a rain is fairly dry, these cracks very seldom lead to any serious consequences. Under these conditions the cracks may at times be infected with Cladosporium baccatae, but it hardly ever results in wasty masses. Should wet weather, however follow on rains, these cracks may at times lead to extensive infections mainly by Botrytis cinerea and Penicillium spp.

Mechanical damage is also of common occurrence in bunches which have not been thinned. This is due to the fact that the crowdedness of the berries in such bunches does not leave very much space for expansion of the berries.

Then....

When such expansion occurs during ripening, some of the berries may be crushed or even forced from their pedicels.

The type of injury which was found to be of a much more serious character than the above-named in some vineyards than the former, was that which was caused by various insects, such as the fruit fly (Ceratitia capitata, Wied.). Where fruit fly was abundant, vinegar flies (Drosophilidae) were usually also present in large numbers. The presence of these two insects in a vineyard may have very serious consequences. Punctures or cracks which would otherwise have dried up or become only slightly infected with saprophytic fungi, very soon attract vinegar flies with at times as serious consequences as following fruit fly attack on uninjured berries.

The presence of these insects in large numbers in a vineyard lead to insanitary conditions and contribute to an abundance of wastage inoculum in the air and on fruit. This ultimately leads to relatively high infections during the storage of grapes originating from these vineyards.

The percentages of bunches showing various estimated amounts of mechanical injury were determined for Henab Turki and Red Hanepoot grapes, used in the field experiments during the 1933-1934 and 1934-1935 seasons. The results are graphically presented in Figure VIII.

It may be seen from this graph that the majority of the bunches show 0 to 40 mechanical damage, i.e. crushed berries, berries with cracked necks or punctures were scant or absent in most of the bunches of these two varieties. A small percentage of the bunches were, however, badly damaged to an extent of 40 to 60. The percentage of badly damaged Red Hanepoot bunches were higher than that of Henab Turki of the same season; but Henab Turki bunches of the 1934-1935 season were apparently more injured than those...

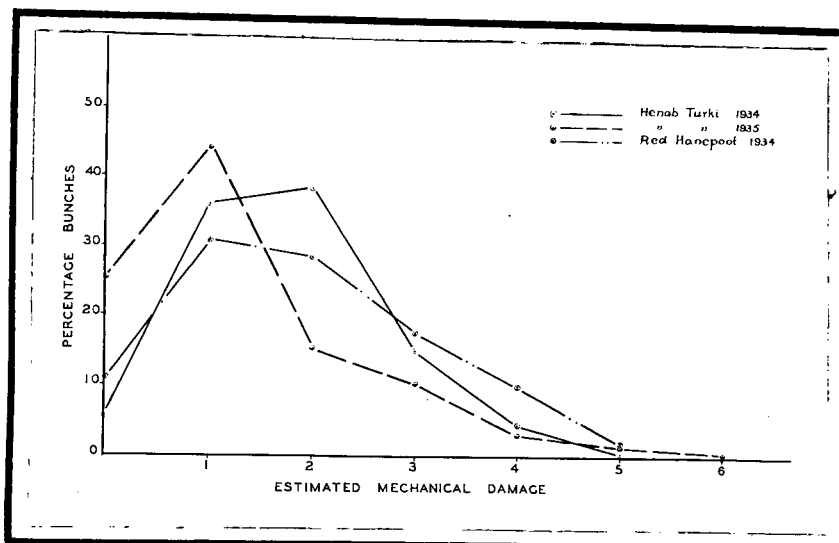


FIG. VIII. - Percentage of Henab Turki bunches showing various degrees of mechanical injury during 1934 and 1935.

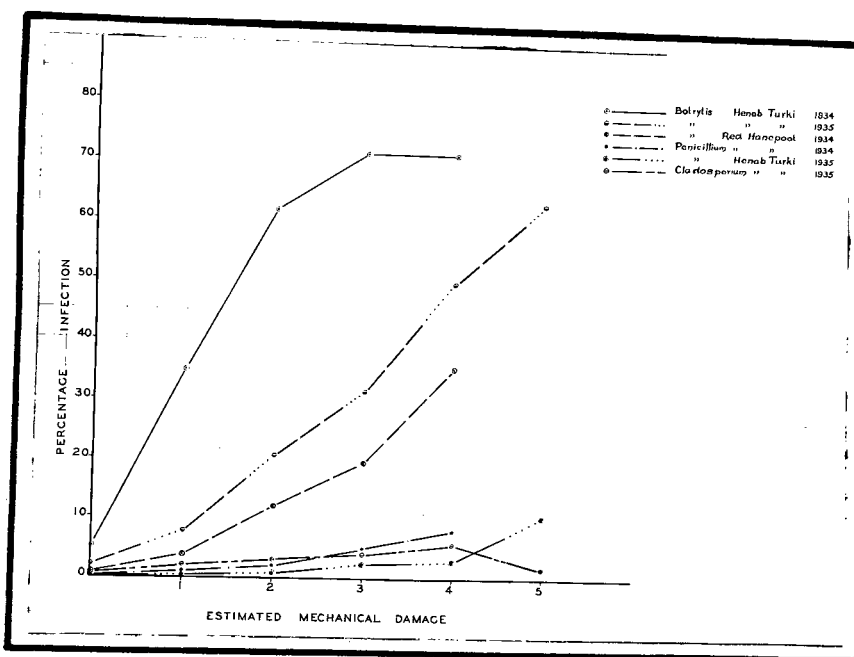


FIG. IX. - ~~Index~~ Percentage Botrytis, Penicillium and Cladosporium infection of Henab Turki and Red Hanepoot grapes showing various amounts of mechanical damage.

those of the previous season. The experience of the author has been that Henab Turko berries are generally not so susceptible to injury as Red Hanepoot berries.

Varieties differ greatly in their liability to mechanical damage. Of the export varieties handled during the course of these experiments, they may be arranged in the following sequence of increasing resistance to mechanical injury during packing and storage:- Raisin Blanc, White Hanepoot, Red Hanepoot, Gros Colman, Prune de Cazane^{oul}, Henab Turki.

The amount of mechanical injury occurring in bunches of any particular variety will however vary from one season to another, and will further depend on the time of picking, and packing and on the method of packing and handling. De Villiers (37) has, for instance, demonstrated that a turgid berry was more likely to be cracked at the necks, punctured or crushed, than a berry which was less inflated.

The amounts of infection by the various organisms occurring on bunches which showed different estimated amounts of mechanical damage during the 1933-1934 and 1934-1935 seasons are shown in Figure 1X. The percentage of Botrytis rot showed a steady increase with an increase in the amount of mechanical damage. During the 1933-1934 season the rate of increase in the percentage of Botrytis infection on Henab Turki was somewhat less in the badly damaged bunches. These infections were on the whole very high and proper estimation was found to be difficult in the very wasty bunches. The amount of Botrytis infection in Henab Turki of the 1934-1935 season and in Red Hanepoot of the 1933-1934 season show a steady proportional increase with the increase of the amount of mechanical damage. The same was found to be the case with the occurrence of Penicillium and Cladosporium rots. When the bunches were apparently free from injury, the percentages of Botrytis infection were low in

both...

both seasons, and Penicillium and Cladosporium rots practically absent.

The amounts of infection by Rhizopus and Aspergillus are not included in Figure IX. On account of their sporadic occurrence in the Constantia grapes no continuous curve could be obtained. Both of these rots were, however, favoured by severe mechanical injury to the berries.

In comparing the 1934 data of Botrytis infection on Henab Turki and Red Hanepoot respectively the former variety appears to have been much more susceptible to Botrytis infection than the latter variety when having the same estimated amount of mechanical damage.

To determine whether the position of bunches in a packed box of grapes had any effect on the amount of Botrytis rot occurring in them, the Henab Turki grapes, used as checks in the different experiments at "The Vineyards", were again made use of. The "Tendency of Infection" of each bunch of grapes was determined as previously explained. The bunches were classed according to their position. They were then grouped into the four quarters in which they had been packed and an average "Tendency of infection" by Botrytis rot determined for each quarter.

	Tendency of infection.
First quarter (nearest to packer)	-0.61
second quarter	-0.13
third quarter	+2.49
fourth quarter	+0.095

The third quarter showed the highest "Tendency of Infection". In this quarter mechanical damage was also found to be the most severe. In packing it is usually this quarter which is pressed backwards by most packers so as to obtain sufficient space for the insertion of the last few bunches. The danger of an overcrowded packed box of grapes is evident and results generally in badly damaged and rotted grapes.

During...

During the 1934-1935 season the occurrence of mechanical damage and of wastage in White Hanepoot and in Raisin Blanc grapes was more closely studied during transport and storage. A number of boxes of grapes were taken at random from ordinary commercial packs in the packshed at "The Vineyards". Immediately after packing on the 28th of February, 1935, two boxes of each variety were examined in the packshed and the data recorded as given in Table XV. The grapes were forwarded the same day to the Imperial Cold Storage, Cape Town. On the 1st March two further boxes of each variety were examined, and the remainder placed in cold storage. On the 7th and 15th of March, two boxes of each variety were taken out of cold storage and examined. On the 21st of March the remaining boxes were railed to Stellenbosch, kept at ordinary temperatures and examined. The data are recorded in Table XV.

The percentages of cracked necks, punctures or crushed berries were relatively low in the grapes examined immediately after packing, though Raisin Blanc packs, as is commonly known, exhibited a fair percentage of berries which were crushed. The amount of mechanical damage was however almost doubled in many cases following the motor and train transport from the packshed to the cold storage chambers, Cape Town. During the period of cold storage the percentage injured berries showed little increase. Then followed another rapid increase after railage to Stellenbosch. During the period of storage at ordinary room temperatures at Stellenbosch, only a slight rise in the percentage punctures was observed, most probably due to contact of berries with the drying protrusions of the stalks of the bunches. The results of this experiment

tend...

TABLE XV: The occurrence of various types of mechanical damage and of storage rots in ordinary export grapes.

Date of inspection	Number of		Percentage berries cracked at the necks	Percentage berries crushed	punctured	F e r c e n t a g e					Rhizopus.	
	Boxes	Bunches				Berries	Botrytis	Penicillium	Cladosporium	Aspergillus		
<u>White Hanopoot</u>												
28 Febr.	2	23	6.80	-	-	-	-	-	-	-	-	-
1 March	2	1589	11.60	0.13	0.19	-	-	-	-	-	-	-
7 March	2	1509	16.40	0.86	0.13	-	-	-	-	-	-	-
15 March	2	1512	12.80	0.79	0.26	-	-	0.07	-	-	-	-
22 March	2	1579	22.00	2.83	0.57	0.46	-	-	-	0.06	-	-
2 April	1	798	23.40	2.63	0.88	0.19	0.25	1.25	-	-	-	0.38
2 April	1	801	34.40	15.00	-	0.75	5.49	-	-	13.21	-	0.38
15 April	4	2907	28.10	0.53	1.82	4.61	4.85	3.55	-	0.62	-	1.13
<u>Raisin Blanc</u>												
28 Febr.	2	20	13.80	0.08	0.53	-	-	-	-	-	-	-
1 March	2	1452	15.20	2.30	-	-	-	-	-	-	-	-
7 March	2	1428	16.30	4.62	0.49	-	0.28	-	-	-	-	-
15 March	2	1460	17.90	5.21	0.75	1.09	-	0.27	-	-	-	-
22 March	2	1527	28.80	6.55	0.39	0.07	-	-	-	0.07	-	-
2 April	1	739	24.00	9.75	0.14	0.41	0.14	2.98	-	0.41	-	-
2 April	1	843	27.40	2.85	0.24	0.59	5.82	-	-	1.42	-	-
15 April	5	3279	34.00	6.87	0.70	1.04	3.69	8.48	-	1.95	-	14.06

(a) Grapes not kept in cold storage.

tend to show that the ultimate amount of mechanical damage occurring in grapes which are exported, occurs not only during the actual ^{packing} operations of the grapes. Certain methods of packing may possibly decrease the probability of injury to grapes during transport and shipment. This aspect, however, was not further investigated, but the matter is of sufficient importance to warrant intensive investigation to develop a less cumbersome, safer and more economical pack for export grapes than is at present used.

The percentages of infection by the various organisms also increased during this period of investigation, but no conclusive evidence was obtained to show the relation between various amounts of the different types of injury and the wastage caused by the different organisms.

Effect of Fertilizers on Grape Wastage.

The general observations of Reinecke (94) on the occurrence of wastage in grapes in different fertilizer experiments in the Constantia area tend to show that heavy applications of nitrogenous fertilizers always had the effect of increasing the amount of wastage in the vineyard. This was especially the case where these fertilizers were applied late in the season, i.e. in November. The plots, which had been dressed with both potassic and phosphatic fertilizers during the late dormant season, i.e. August, yielded the soundest grapes. Grapes of lower resistance were obtained from plots fertilized with a mixture consisting of potassic, phosphatic and nitrogenous fertilizer. The resistance of grapes was still lower in plots which had received applications of phosphatic and nitrogenous fertilizers together with a late application of a nitrogenous fertilizer in November. Grapes showed
the.....

the least resistance to wastage infection in plots where a mixture of potassic, phosphatic and nitrogenous fertilizers had been applied during August and an extra nitrogenous dressing during November.

The Red Hanepoot grapes used by Reinecke, in his fertilizer experiments at "Silverhurst" during the 1933-1934 season, were examined by the author two weeks after being cold stored. The results obtained however failed to show any reduction of Botrytis storage rot through the application of extra potassic fertilizers or through the application of lime or iron sulphate.

A full fertilizer experiment, in coöperation with the Department of Agricultural Chemistry, was carried out at "The Vineyards", during the 1934-1935 season, to investigate the influence of specific fertilizers on the incidence of Botrytis and other storage rots of grapes. A block of Honab Turki vines, growing on a loamy sandy soil, was used in this experiment. The treatments were given in duplicate series to plots consisting of three rows of 18 vines each. The two interrow spaces in each plot were fertilized. The following were the treatments given:-

1. Superphosphate and sulphate of potash at the rate of 500 and 200 lb. per acre respectively (P & K)
2. Sulphate of ammonia and superphosphate at the rate of 200 and 500 lb. per acre respectively (N & P).
3. Sulphate of ammonia at the rate of 200 lb. per acre (N)
4. Sulphate of ammonia, superphosphate and sulphate of potash, at the rate of 200, 500 and 200 lb. per acre respectively (N & P & K).
5. Sulphate of ammonia, superphosphate and sulphate of potash at the rate of 200, 500 and 200 lb. per acre and nitrate of soda at the rate of 200 lb. per acre (2N & P & K).

The sulphate of ammonia, superphosphate and sulphate of potash were applied by broadcasting on the 23rd of August, 1934, and the nitrate of soda in series 5 on the 20th of November, 1934.

The grapes in the experimental block were regularly culphured for Oidium and fairly heavily topped and thinned during January. No differences in growth could be observed between the different plots in this experiment. The grapes were picked, when they were fully mature, only the centre row of each plot being used. The crop of one vine at each end of every centre row was not included in these tests, on account of the border effect. The picked grapes were thinned, trimmed and packed in the customary way and held in cold storage for three weeks.

At the time of picking, representative samples of berries were taken from every bunch and analysed for percentage of nitrogen and percentage of total solids, according to the methods described by Reyneke (95). These analyses were made in duplicate series, the average readings obtained are tabulated in Table XVI.

After cold storage, the grapes of this experiment were divided into two series. The one series was examined 7 days after cold storage to determine the amounts of Botrytis and Penicillium storage rots. The grapes of the other series were unpacked immediately after cold storage and kept separate for three weeks at ordinary room temperatures, when the total amount of wastage was determined. The results are presented in the table below.

Table XVI : The effect of fertilizers on the amount of wastage, in grapes, and on the nitrogen and total solid content of the berries.

Fertilizer	Number of berries	Percentage.			Percentage in berries.	
		Botrytis(a)	Penicillium(a)	Total Waste (b)	Nitrogen	Total Solids.
PeK	3581	1.42	0.17	10.5	.383	14.07
MeP	2904	1.93	0.17	11.3	.400	14.17
M	5911	1.49	0.08	13.2	.407	
MePeK	5757	2.72	0.47	14.9	.4115	14.395
2MePeK	5209	2.21	0.21	19.5	.4312	14.91

(a) Data taken 7 days after cold storage.

(b) Data taken 21 days after cold storage.

The percentages of Botrytis and Penicillium infections in the grapes, which were examined 7 days after cold storage, were exceedingly low as is evident from the above table. The infection differences between the treatments are small, yet the least amount of Botrytis infection was encountered in grapes from the E plots, which received phosphatic and potassic fertilizers. The results of the total percentage waste in the grapes, examined 21 days after cold storage, were, however, striking. It appears from this result that the amount of wastage in the grapes increased with the increase in the amount of nitrogen in the berries and with an increase in the amount of total solids in the fruit.

These results are in full accord with those obtained by Horne (59, 60). He inoculated apples with various cultures of fungi and established that the concentration of nitrogen in the fruit was in direct relation to the "radial advance" of the resultant rot. In other words, the amount of nitrogen and the resistance to invasion, were inversely related. Reyneke (95), in his experiments on the effect of fertilizers on the keeping quality of pears and of Kelsey plums, also found that the amount of wastage in these fruits varied in direct proportion to the amount of nitrogen in the fruit.

It is evident that the amount of nitrogen in the fruit is one of the factors of major importance influencing the amount and severity of fungous attack. The above effects of increasing amounts of nitrogen in the fruit on its susceptibility to wastage may be ascribed to:-

(a) The direct influence of nitrogen on the nutrition of fungi. Vasudeva (111) for instance, demonstrated that even Botrytis Allii could be made to attack apples by adding a certain amount of nitrogenous nutrient to the inoculum.

(b) The...

(b) The fact that nitrogen has a direct influence on the respiratory activities of the fruit. Reyneke (95) has reviewed the literature bearing on this aspect, and from his own results, came to the conclusion that a higher nitrogen content in fruit results in higher respiratory activities. This fact is also demonstrated in the results, reported above, where the amount of total solids in the berries is proportional to the amount of nitrogen in these berries. As was stated, no appreciable difference in growth could be observed between the different fertilizer plots. It is therefore unlikely that the increased amount of total solids in the berries could mainly be the result of an increased photosynthetic capacity of the foliage. More likely this greater percentage of total solids was a result of increased respiration of the berries themselves, whereby large amounts of respiratory products were liberated in the juice of the berries.

It was furthermore shown by Reyneke (95) that the longevity of fruit is dependent upon their rate of respiration. The higher this rate, the sooner will the fruit reach the stage of senility and of internal breakdown. When this latter stage is reached, the fruit will also be more easily attacked by various storage rot organisms. This was well illustrated in the experiment reported above. In this the infection differences between the different treatments were only distinctly noticeable after the fruit has been kept for three weeks at ordinary room temperatures after its removal from cold storage. It was apparent that a period of seven days storage at ordinary temperatures was too short for the respiratory effect discussed above to manifest itself.

(c) The fact that nitrogen has an effect on the nature and thickness of the cell walls. Lyon, Fippin

and....

and Duckman (72), for instance, stated that nitrogen has the effect of thinning and of softening the cell wall. The results of Brown and Harvey (22) furthermore indicate that the penetration ability of the germ tubes of Botrytis cinerea through paraffin-wax membranes was dependent on the hardness of these membranes. They also found that this fungus could penetrate into harder membranes than could species of Penicillium or of Rhizopus. It is therefore evident that the effect of nitrogen on the nature of the cell wall and the cuticle will also influence the susceptibility of grapes to wastage infection.

It is also of interest to note that the amount of nitrogen in the berries was greatly influenced, not only by the amount and time of application of nitrogenous fertilizers, but also by the applications of potash and phosphate fertilizers.

A single application of a nitrogenous fertilizer (see Table XVI) had the effect of increasing the amount of nitrogen in the berries, which was still more evident in the case of late applications of this fertilizer.

Potash dressings (^{see Table XVI} ~~e.g. PN and KPN~~) also raised the amount of nitrogen in the fruit considerably. This effect of potash fertilizers was also observed by Reyneke (95) and by Horne (60). This effect is fairly easily explained in the light of the results of Collison and Harlan (28), who indicate that potassium nitrate was the best carrier of nitrate nitrogen for plants. The addition of potash fertilizers to soils will, therefore, result in an increased availability of the nitrogenous nutrients in the soil and therefore in an increased amount of nitrogen in the fruit.

The application of phosphate fertilizer ~~(e.g. P and P₂O₅)~~, on the other hand, diminished the inflow of nitrogen into the fruit. This effect may be due to a probable combination between these two elements in the plants to form insoluble compounds, by which their translocation to

the fruit is prevented. This was demonstrated by Heyneke in his results on pears, in which the P_2O_5 concentration in fruit from the plot fertilized with nitrogenous and potassic fertilizers was slightly less than that of fruit from the plot which received an application of nitrogenous and phosphatic fertilizers, whereas the nitrogen content of the first was higher than of the latter plot.

From the results of this fertilizer experiment, it is clear that the applications of heavy or late dressings of nitrogenous fertilizers to Constantia soils is detrimental to the resistance of export grapes to wastage. If nitrogenous fertilizers is to be used, only light dressings should be applied. Potassic fertilizers should also only be applied in relative small quantities where necessary, whereas heavy dressings of phosphatic fertilizers may be applied with advantage. In this respect, Reinecke (94) has recommended different quantities of each to be applied to the different soil types in the Constantia area.

Effect of the Time of Picking and Packing on the Occurrence of Botrytis Rot in Storage.

No regular practice is followed by grape growers as to a time of picking and a period of ^Wgtilting grapes before packed for export. On most farms in the Constantia area, picking is commenced in the morning, as soon as the vines and bunches are dry, and continued until 4 p.m. on some farms the grapes are cleaned and packed as soon as they reach the packshed. The majority of growers, however, leave the grapes in the lug boxes which are stacked till the following day before these grapes ^{are} cleaned and packed.

The conclusions of de Villiers in this respect are somewhat contradictory. From his results, mainly laboratory tests (37), he concluded that "the ideal time to pick grapes is at thermal noon, i.e. between 1.30 and 2.30 p.m., except during hot days, when the cooler parts of the days...

days should be selected". Grapes, picked at this time, was found to be less turgid and therefore less liable to injury and subsequent fungous infection. For this reason he also recommended periods of ^wilting varying for the different varieties according to the thickness of their skin (epidermal layer). In a later paper, de Villiers (38), in discussing storage experiments which were ^{conducted} concluded with grapes, states that "From the results it became evident that most grape varieties keep better if picked during the morning, whereas those picked between 2 and 3 p.m., i.e. the hottest part of the day, do not keep so well".

Van Niekerk (109) quotes the results of de Villiers, and states that, in his field experiments, apparently no difference in the condition of packed grapes observed when they were picked at 10 a.m. or 4 p.m. and wilted for 0, 12 or 24 hours. Fish (44) also recommended the picking of Australian grapes to be done during the afternoon of a dry day.

The conclusions of these authors are indefinite particularly as regards the best time of the day to pick. No definite percentages of infection and environmental data are furnished in their discussions and it is therefore not possible to compare the exact environmental conditions, during their picking and packing operations, with the results obtained.

An experiment was carried out with Honab Turki grapes of "The Vineyards" to find whether any difference could be obtained by picking grapes during the different hours of the day, and by allowing them to wilt for different periods before being packed. This experiment was carried out in three series simultaneously. A number of boxes of grapes were picked at 8 a.m. on the 21st March 1934, some packed within an hour of being picked, and equal quantities packed at 12 noon and 4 p.m. of the same day and again at 8 a.m., 12 noon and 4 p.m. on the following day...

day. The grapes of the second series were picked at 12 noon on the 21st March, 1934, equal quantities being packed immediately and at 4 p.m. and at 8 a.m., 12 noon, 4 p.m. on the 22nd of March, and at 8 a.m. on the 23rd March. Grapes for the third series were picked at 4 p.m. on the 21st March, 1934, and packed in equal quantities immediately, and at 8 a.m., 12 noon, 4 p.m. on the 22nd, and at 8 a.m. and 12 noon on the 23rd of March. The packed grapes were railed to cold storage in the afternoon of each day.

The humidity and temperature readings in the vineyard were obtained from a standardised, self-recording hydro-thermograph, which was placed in a wooden box shelter in between the vineyard rows as is shown in Plate VI. The relative humidity and temperature at the different stages in this experiment are given in Table XVII.

The percentage of Botrytis infection of grapes in this experiment, as found seven and fourteen days after three weeks' cold storage, are tabulated in Tables XVIII and XIX respectively.

Table XVII. Relative humidities (%) and temperatures (°C) at different stages during the period of experimentation from 21 to 23 March, 1934.

Date	Time	Relative Humidity (%)	Temperature (°C)	Remarks.
21.3.34	3 a.m.	95	11	Max. Hum., Min. Temp.
	8 a.m.	55	28	1st picking, 1st packing stage.
	11 a.m.	35	35.5	Min. Hum., Max. Temp.
	12 noon	36	35	2nd picking, 2nd packing stage.
	4 p.m.	65	25	3rd picking, 3rd packing stage.
22.3.34	4 a.m.	-	13	Min. Temp.
	5 a.m.	98	-	Max. Hum.
	8 a.m.	55	28	4th Packing stage.
	10 a.m.	49	-	Min. Hum.
	11 a.m.	-	31	Max. Temp.
	12 noon	52	30	5th packing stage.
	4 p.m.	76	25	6th packing stage.
	23.3.34	5 a.m.	98	15
8 a.m.	72	24	(7th packing stage.	
	12 noon	51	32	(8th packing stage. (Min. Hum., Max. Temp.

Min. = Minimum; Max. = Maximum; Temp = Temperature;
Hum. = Relative Humidity.

TABLE XVIII. The effect of the time of picking and packing on the occurrence of Botrytis rot during storage :
Data taken 7 days after cold storage.

Time of picking	Time of packing	Number of			Percentage Botrytic infection	Mechanical damage.
		boxes	bunches	berries		
8 a.m.	8 a.m.	1	1	109	93.5	+++
			6	441	91.1	++
			1	65	92.5	+
8 a.m.	12 noon	1	3	302	37.4	++
			5	554	11.9	+
8 a.m.	4 p.m.	1	1	75	89.4	++++
			2	153	93.0	+++
			4	302	82.9	++
8 a.m.	8 a.m.	1	1	70	91.5	++++
			3	276	59.1	+++
			2	160	66.2	++
8 a.m.	12 noon	1	4	340	90.6	+++
			4	251	84.1	++
			2	171	32.2	+
8 a.m.	4 p.m.	1	4	365	50.4	+++
			3	169	32.5	++
			1	64	-	+
12 noon	12 noon	1	4	247	80.5	++
			5	294	24.2	+
12 noon	4 p.m.	1	1	117	8.6	++++
			1	190	79.0	+++
			2	242	62.0	++
			4	324	50.7	+
12 noon	8 a.m.	1	1	135	68.1	++++
			1	75	82.6	+++
			5	524	55.2	++
12 noon	12 noon	1	1	151	63.6	+++
			5	403	36.0	++
			1	39	43.6	+
12 noon	4 p.m.	1	3	155	66.2	+++
			3	286	45.8	++
			2	69	7.2	+
12 noon	8 a.m.	1	1	128	75.8	++++
			4	400	66.0	+++
			3	231	40.3	++
4 p.m.	4 p.m.	1	2	169	87.6	+++
			5	302	61.3	++
			1	65	18.5	+
4 p.m.	8 a.m.	1	2	112	53.5	+++
			1	64	6.3	++
			5	481	10.4	+
4 p.m.	12 noon	1	5	523	29.7	++
			3	200	6.5	+
4 p.m.	4 p.m.	1	1	114	73.6	+++
			4	297	28.3	++
			3	178	19.1	+
4 p.m.	8 a.m.	1	2	231	69.3	++++
			1	103	36.8	+++
			2	214	11.7	++
			1	35	-	+
4 p.m.	12 noon	1	2	166	46.3	+++
			5	482	30.7	++

TABIE XIX : The effect of the time of picking and packing on the occurrence of Botrytis rot during storage : Data taken 14 days after cold storage.

Time of picking	Time of packing	Number of			Percentage Botrytis rot	Mechanical Damage
		boxes	bunches	berries		
8 a.m.	8 a.m.	1	8	625	99.6	++
	12 noon	1	7	493	87.0	++
	4 p.m.	1	8	454	82.2	++
	8 a.m.	1	1 7	63 536	100.0 89.5	+++ ++
	12 noon	1	1 7	105 584	100.0 94.4	+++ ++
	4 p.m.	1	2 5	176 348	96.0 87.7	+++ ++
	12 noon	12 noon	1	9	623	73.0
4 p.m.		1	8	537	81.9	++
8 a.m.		1	2 6	131 420	93.1 90.5	+++ ++
12 noon		1	2 5	134 338	94.0 91.4	+++ ++
4 p.m.		1	6	464	90.4	++
8 a.m.		1	7	585	57.5	++
4 p.m.		4 p.m.	1	5 2	392 188	41.8 4.3
	8 a.m.	1	8	614	77.6	++
	12 noon	1	1 6 1	82 376 46	100.0 57.5 2.2	+++ ++ +
	4 p.m.	-	-	-	-	
	8 a.m.	1	8	665	69.8	++
	12 noon	1	9	645	60.0	++

From the data in Tables XVlll and Xlll, it is apparent that the highest percentages of Botrytis infection occurred in grapes which were picked at 8 a.m., and the lowest in grapes which were picked at 4 p.m. This is also evident in comparing the grapes of the three picking series, packed at 4 p.m. on the 21st March. At any time of packing, the percentage of infection was least in the grapes picked at 4 p.m. and the highest in grapes picked at 8 a.m.

The average infection of the individual boxes (i.e. disregarding the classification of data according to the amounts of mechanical damage), as was obtained 7 days after the grapes have been removed from cold storage, are graphically presented for each separate series in Figure X. The striking effect of the time of picking on the amount of Botrytis rot during storage can be readily seen. In every case the lowest percentage of Botrytis infection was observed in grapes picked at 4 p.m.

No regular effect of the period of wilting on the amount of Botrytis rot developing in storage was evident, although there appears to be a general tendency for infection to decrease as the period of wilting is prolonged. If the percentages Botrytis infection of bunches showing ~~no~~ mechanical damage ^{are} plotted against the length of the wilting period, such a reduction of Botrytis infection readily demonstrated. The actual relative humidities and temperatures at the time of picking of these grapes differed slightly from one another, but more so during the periods immediately prior to picking. (Table XVll). A period of very high humidity, preceded 8 a.m. (21.3.34) (of more than 12 hours, an optimum of 95% being recorded at 6 a.m. At 12 noon the relative humidity was very close to the minimum for the day and the temperature again very close to the maximum. The period immediately prior to 12 noon was characterized by relatively low humidity during the forenoon. Increases in

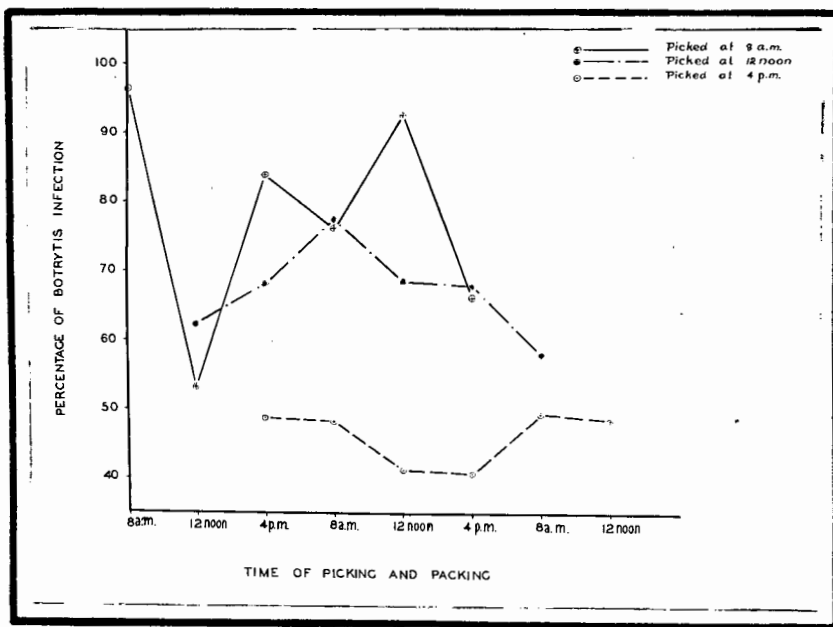


FIG. X. - Effect of the time of picking and packing on the percentage of Botrytis infection seven days after cold storage.

in relative humidity were on the whole accompanied by decreases in temperatures. It would appear that the amount of Botrytis infection in storage is also affected by the evaporating power (37) of the air during the period prior to the time at which these grapes had been picked.

De Villiers (37) made a detailed study of the effect of the Evaporating Power on the transpiration of grapes and on the turgidity of grape berries. He found that the root pressure of vines was at its lowest at the thermal noon when transpiration was at its highest, the berries were therefore the least inflated at this time and, as a result the least liable to mechanical injury during packing operations. It is however, more likely that the turgidity of the berries would reach its minimum ^{not} just at the thermal noon, but some time later.

The time of picking of grapes influences the amount of Botrytis rot in storage mainly because the turgidity of the berries are affected.

The effect of wilting on the liability of the berry to Botrytis infection would be the same as previously discussed. There is, however, a danger of a too long wilting period through which additional difficulties may be encountered in the packshed.

From the data collected, it would appear that the practice to be recommended, to ensure the least amount of Botrytis infection during storage, would be to pick the grapes during the hotter part of the day and to pack the day following picking.

The above experiments were repeated during the 1934-1935 season, but with rather inconclusive results. The failure of a differential effect of the time of picking and packing was probably mainly due to the exceedingly low percentages of Botrytis infection in all sets (varying from 0.08 to 2.22%). It may also have been due to such low

percentages...

percentages of infection, that van Niekerk (109) failed to observe any difference between grapes which had been picked and packed at different times of the day.

The differences in this respect are apparently more prominent under conditions conducive to high percentages of Botrytis infections. The necessity for regulating the times of picking and of packing appears to be of particular importance during seasons, or under conditions, when Botrytis rot in storage is apt to occur in epidemic severity.

Effect of "Buller Caps" on the Occurrence of
Botrytis Rot in the Vineyard and in Storage.

In order to protect grapes from attacks by birds, Mr. A.C. Buller at Banhoek (Stellenbosch) devised an umbrella-shaped paper cap for covering the bunches. These caps consist of ordinary brown paper, cut in squares and with a slit from one side to the centre. Each paper square is then folded round the stalk of a bunch and the one side of the slit drawn over the other and pinned on to it in such a way that a cone is formed over the shoulder of the bunch, but the lower part of the bunch left fairly exposed. Observations during previous seasons appeared to show that these caps were effective in not only preventing bird injury but also Botrytis infections. St^Wemmer (104) had also found that Botrytis infection was reduced in the vineyard by covering the bunches with parchment paper bags. This effect he ascribed to the absence of air currents which ~~could~~ should supply sources of spore contamination and to the absence of dew deposits on the bunch by which Botrytis infection is reduced.

Several experiments were carried out on Red Hanepoot grapes at "Höhenort" and on Honab Turki at "The Vineyards" during the 1933-1934 season to determine the effectiveness of these "Buller Caps" for the control of Botrytis rot in

the...

the vineyard and in storage. The bunches on the vines were covered as soon as the berries were about 50% coloured and the caps were left on them until the time of picking. The covers were then removed and the grapes packed as previously described. An equal number of bunches of the same maturity from the same vines were left exposed and ultimately packed, at the same time to serve as checks.

Observations in the vineyard indicated that these caps were generally effective in preventing rain and dew drops from collecting on the berries, an account of which the Botrytis infection may be actually less on covered than on uncovered bunches. In some cases, however, dew drops were noticed to have collected on berries under these caps, and if such berries were once moist they remained so for a much longer period than those exposed. Where rains and dews frequently occur, the caps may hence tend to aggravate the dangers of Botrytis infection in the vineyard through lack of proper aeration of the covered bunches.

A much more serious effect resulting from the use of these caps, was that the colouring of the berries was very much retarded. This effect was particularly noticeable in certain spots where colouring was generally poor. The use of "Buller Caps" where sunlight and heat radiation is already insufficient is therefore undesirable.

The data, obtained seven days and fourteen days after cold storage from grapes used in these experiments are recorded in Table XX. In experiments 1 and 2 at "Höhenort" Red Hancock grapes were used and at "The Vineyards" Monab Turki. From Table XX it appears that the results which were obtained, are not absolutely conclusive. In general no improvement of any importance is evident in any of these experiments, that would warrant the expense of covering especially.....

TABLE XX : The effect of "Butler paper cap" coverings of the bunches in the vineyard on the occurrence of Botrytis and Penicillium rots in storage : Data taken 7 and 14 days after cold storage.

Bunches covered or not covered	Number of			Percentage		Mechanical damage	Days after storage.	
	boxes	bunches	berries	Botrytis	Penicillium			
+	1	20	947	13.0	1.3	+	7	
	1	20	929	0.4	0.3			
+	1	1	67	9.0	-	++	14	
		6	309	4.5	-			
		10	392	0.5	-			
	-	1	6	413	18.9	4.4	*+++	14
			1	76	10.5	-		
			3	163	14.7	-		
		2	83	1.2	-	++		
+	1	4	211	30.8	10.4	++++	7	
		4	177	11.3	13.5			
		4	181	6.6	1.1			
		3	168	1.8	-			
	-	1	2	101	31.0	3.0	++++	7
9			443	8.1	1.4			
		5	215	1.9	0.5	++		
+	2	1	37	65.0	2.7	+++++	14	
		17	719	21.8	13.2			
		12	559	18.3	3.6			
		1	82	7.3	-			
	-	1	3	142	16.2	4.9	+++++	14
6			335	38.2	0.3			
1			37	48.7	16.2			
+	4	6	400	84.5	0.5	+++	7	
		28	2281	73.4	0.2			
		1	61	60.6	-			
	-	4	1	68	100.0	-	+++++	7
			1	60	71.7	1.7		
			10	685	95.0	0.9		
		16	995	81.0	0.2	++		
		5	409	75.4	-	+		
+	3	2	212	98.0	-	++++	14	
		11	690	96.8	0.1			
		11	628	96.0	-			
		2	63	87.4	-			
	-	3	1	48	98.0	2.0	++++	14
15			882	88.4	1.1			
9			542	92.5	2.8			
1			62	23.2	-			
+	1	2	125	48.0	10.4	+++	7	
		4	215	17.7	5.1			
		2	152	16.5	1.3			
	-	1	1	70	42.9	-	++++	7
			2	135	38.2	0.7		
			1	70	20.0	-		
		4	291	10.3	-	+		
+	1	8	601	21.4	0.3	+	14	
		3	217	61.8	1.8			
		4	230	26.5	0.9			

- bunches covered in Butler caps.

- bunches not covered.

especially where there exists a possible danger of grapes being under coloured. These covers also provide an excellent shelter for spiders and mealy bug, resulting in further difficulties in thoroughly cleaning the bunches before being packed.

Control Studies.

Dusting and Spraying Experiments in the Vineyards in the seasons 1933 - 1934 and 1934 - 1935.

A number of fungicidal mixtures has been tested by various investigators for the control of Botrytis rot of grapes in the vineyard, and with varying degrees of success. Kramer (67) and Schmidt (93) found that soft soap solutions were effective in controlling the stalk rot stage of Botrytis infection.

De ^Istvanffi (36) recommended the dusting of vines with a mixture of $\frac{1}{2}$ lb. sodium sulphite and 3 lb. charcoal to control the infection of grape berries by Botrytis.

Putterill (59) stated that sanitation, a dormant wash of the vines with a solution of 5% calcium bisulphite and a late dust application of 10 parts calcium bisulphite to 90 parts powdered clay, were measures found to be effective for the control of Botrytis rot of grapes.

Kirchner (65) reported in 1903 that he failed to control the Botrytis disease of the vine by spraying with copper containing mixtures Ravaz (91), however, found that treatment with cuprous powders was effective as a preventative against Botrytis infection of the bunches in the vineyard. Moreau and Vinet (77) reported satisfactory control of this rot by applying a cupric sulphur dust. They found that copper percentages in the mixture varying from .6% to 2%

did....

did not materially affect the effectiveness of the fungicide. Zaccarevitz (122), again, stated that he obtained the best control of Botrytis rot by dusting with a copper containing dust, such as the following mixture:- 55 kg. calcined plaster of Paris, 40 kg. sulphate containing 80% copper sulphate and 5 kg. caponaphtha powder. He further recommended that the dust should be applied after the vines were sprayed with Bordeaux mixture and as soon as they were dry.

Lafforgue (68) reported that Truchot in 1912 was able to control Botrytis rot in the vineyards of France very satisfactorily by dusting with a mixture consisting of 85 parts sifted lime or plaster of Paris and 15 parts pulverised potassium permanganate. His second application contained 45 parts of sifted lime or Plaster of Paris, 10 parts pulverised potassium permanganate and 5 parts pulverised alum. Woodfin (120) also recommended dusting of grapes with a mixture consisting of 85 parts hydrated lime and 15 parts finely pulverised potassium permanganate for the control of Botrytis rot in New Zealand.

From this review of the literature on the control of Botrytis infection of grapes, it is apparent that attention was mainly directed to the use of dusts rather than sprays for grapes. Soft soap solutions were used in some of the European countries, but mostly for the control of Botrytis attacks on stalks and leaves of vines. Apparently the copper containing dusts gave a fair amount of control for Botrytis berry rot in most cases, whereas some workers found the inclusion of pulverised potassium permanganate in dusts beneficial for the control of this rot.

The above results mainly deal with the effect of the fungicides on Botrytis infections in the vineyard. In none of these contributions is it mentioned whether the

effects....

effects of the fungicidal applications were noticeable even during the storage of grapes treated in this manner. As has been indicated previously, the grapes in most of these countries are consumed shortly after picking and storage rots, by Botrytis and other organisms, are practically of no importance.

That fungicides, applied to fruit in orchards, may reduce the amount of wastage occurring in storage was first demonstrated by Brooks and Fischer (17) and by Brooks and Cooley (16). They found that the amount of Anilia infection of cherries and prunes in storage was about four times higher on unsprayed than on sprayed fruit, but that spraying had very little effect on the amounts of Penicillium and Rhizopus storage rots. The results obtained by Fulton and Bowman (47) show that the amount of Phomopsis stem-end rot on citrus fruits, stored over a prolonged period, may be reduced by half or more by the application of a Bordeaux spray to the trees, before harvesting. Diplodia stem-end rot was reduced by about one fifth, but blue mould and other minor storage rots were apparently not much affected by this spray.

In this investigation extensive dusting and spraying experiments were carried out to establish the relative effectiveness of various commercial fungicides for the control of Botrytis and other rots of grapes in the vineyard and during storage. The following fungicides were included in these tests:- (1) Zinfandelite; (2) Sodium sulphite (15 parts) and Knolin (85 parts); (3) Copper Sulphur dust (Capex) containing 9% metallic copper; (4) Copper Lime dust (Springbok) containing 20% anhydrous copper sulphate and 80% chemical lime; (5) Cupric Sulphur dust; (6) Cuprite; (7) Verderame Sulphur dust consisting of 20% Verderame (Copper-oxide-chloride, having a copper content....

content of 13% and a chlorine content of 5%) and 80% "Lighting" sulphur; (6) Bouisol (15% copper content by weight) and Sulsol (55% Sulphur content by weight) mixed in proportions of 1 pint of the former and $\frac{1}{2}$ pint of the latter in 100 gallons of water.

The dusting and spraying experiments during the 1933-1934 season were conducted at "Höhenort", "Under-the-Thatch", "The Vineyards" and at "Clunie" (Figure V; A, B, C and D respectively) on Red Manspoot grapes on the first farm and on Honab Durki grapes on the other three farms. The blocks used for these experiments consisted of 4 to 6 rows of 20 to 35 vines each. These blocks were each divided into a number of small plots varying in size from 3 to 3 vines in a row. Each one of the different fungicides used were applied to separate plots in each row. Each treatment was replicated 4 to 6 times and in such a manner that its plots were randomised over the whole experimental block.

The dusts and sprays were applied on sunny days, when *W'd* there was only a slight breeze, the dustings and sprayings being begun at about 10 a.m. or as soon as the vines were quite dry. In order to prevent drifting of the dusts from a plot which is being dusted on to adjoining rows, a hessian cloth was used to screen the side of the row opposite to that which was being dusted. No attempt was made to dust the foliage of these vines; but the dust or spray was directed exclusively on to the bunches. The flow of dust was regulated to ensure uniformity and moderate distribution, and in all cases a maximum pressure of the dusts was aimed at. Every individual bunch was dusted or sprayed from all sides and the plots dusted or sprayed from both sides of the vineyard row. Care was taken to prevent the overloading of the bunches with a particular fungicide, but

the.....

the bunches were dusted until a fine, even, bloom of the fungicide was visible, as far as possible, on all the berries in a bunch.

Unless otherwise stated, only one dusting or spray was applied to every crop of grapes which were picked from the vines in the experimental blocks. The fungicides were applied, when the grapes had attained 50% of their colour and when they had already started to sweeten.

The grapes from each of the experimental plots were picked and packed separately, but the dusts were left on the bunches during packing, in order to test the effect of these fungicides during storage. The grapes were cold stored for three weeks and examined at different periods after cold storage as has been previously described. The results are tabulated in Tables XXII to XXXIII and the percentages of Botrytis and other storage rots are the averages of those of the plots dusted with a given fungicide on a particular farm. As will be noticed from these tables, not all of the tested fungicides were included in the experiments on each of the farms, on account of the fact that the blocks available for experimental purposes were not large enough to allow of a proper test and comparison of all the various fungicides on each of the farms.

The dates on which the grapes in these experiments during the 1933-1934 season were dusted, picked, packed and examined are listed in Table XXI.

The percentages of Botrytis and Penicillium infections in the vineyard, were determined, when present in sufficient quantities, just before the grapes from these plots were picked. In these determinations random samples were taken from each of the plots and the number of infected and sound berries established and from this data the average percentages
of.....

TABLE XXI: Particulars concerning the various dusting and spraying experiments carried out in the Constantia area during 1934 for the control of Botrytis and other rots of export grapes.

Experiment	Locality <i>Farms</i>	Date of application of fungicides	Date of picking	Date of packing	Dates of cold storage	Dates of inspection.
A	Höhenort	7 Febr.	21 Febr.	22 Febr.	23 Febr.	23 March.
B	Höhenort	7 Febr.	21 Febr.	22 Febr.	to 16 March	30 March.
C	Clunie	24 Jan.	27 Febr.	27 Febr.	28 Febr.	28 March
D	Clunie	24 Jan.	27 Febr.	27 Febr.	to 21 March	4 April
E	The Vine-	6 Febr.	12 March	13 March	14 March	11 April
F	yards	6 Febr.	12 March	13 March	to 4 April	18 April
G	Höhenort	7 Febr.	14 March	14 March	15 March to 5 April	12 April
H	Under-	9 Febr.	28 March	29 March	30 March	27 April
I	the- Thatch	9 Febr.	28 March	29 March	to 20 April	4 May
J	The Vine-	5 April	30 April	1 May	2 May to 23 May	31 May
K	yards					
L	Under-	6 April	3 May	4 May	5 May	2 June
	the- Thatch	6 April	3 May	4 May	to 26 May	9 June

TABLE XXII : Experiment A - Dusting and spraying experiments at "Hohenort", Constantia, 1934 : Data taken 7 days after cold storage.

Fungi- cide	Number of			Percentage infection by			Mechani- cal Damage.
	boxes	bunches	berries	Botry- tis	Penicil- lium	Clado- sporium	
Check	6	2	93	6.5	6.5	7.5	+++
		20	1015	3.6	1.9	0.1	++
		49	2353	2.8	0.5	0.5	+
		34	1538	1.0	0.1	-	-
Zinfandelite	6	2	108	21.8	15.7	-	+++++
		1	45	8.9	-	-	++++
		10	576	1.4	3.1	0.2	+++
		14	846	2.6	0.7	1.1	++
		62	3140	2.8	0.5	-	+
		9	398	0.3	-	0.3	-
Copper Sul- phur Dust	7	2	139	13.0	-	-	+++++
		3	216	6.5	0.5	-	++++
		11	573	3.5	1.2	0.4	+++
		30	1519	1.4	2.3	0.5	++
		39	1602	0.6	0.7	1.5	+
		11	414	0.2	0.7	2.1	-
Copper Lime Dust	6	1	83	9.6	2.0	-	+++++
		4	269	23.8	12.6	2.1	++++
		14	812	15.0	4.4	0.9	+++
		20	950	5.6	6.4	0.7	++
		29	1462	2.5	1.5	-	+
		31	1358	0.7	1.8	0.8	-
Cupric Sulphur Dust	7	5	329	4.9	0.9	1.5	++++
		13	909	4.6	1.3	0.2	+++
		31	1771	4.1	2.9	-	++
		39	2255	1.1	0.4	-	+
		5	260	-	-	-	-
Bouisol and Sulsol	6	5	243	8.2	1.2	1.6	++++
		16	822	4.0	1.3	-	+++
		23	1234	9.9	5.3	0.5	++
		38	1994	3.4	0.9	0.3	+
		24	1250	0.8	0.1	-	-

Table XXIII: Experiment 3 - dusting and spraying experiments at "Löhendorf", Langkloof, 1934: data taken 14 days after cold storage.

Fungicide	Number of			Percentage infection by			Mechanical Damage.
	boxes	bunches	berries	Botrytis	Penicillium	Rhizomas	
Check	7	3	238	20.4	5.4	0.7	++++
		15	733	14.6	3.4	-	+++
		21	1134	11.7	0.8	-	++
		44	2153	4.4	1.0	-	+
		23	1077	1.6	-	-	-
Zinfundo- lito	6	30	1662	12.9	2.3	-	++++
		24	1353	7.8	1.9	0.1	+++
		19	858	6.6	1.3	-	++
		19	1113	1.3	-	-	+
		4	175	-	-	-	-
Copper Sulphur Dust	6	4	292	18.1	3.4	10.0	+++++
		22	1117	17.9	2.5	0.4	++++
		21	1165	13.5	4.1	0.2	+++
		21	1056	9.0	2.4	0.9	++
		22	1238	4.3	0.9	0.1	+
Copper lime Dust	7	7	381	26.8	1.0	-	+++++
		14	861	14.3	1.3	-	++++
		13	689	19.0	11.5	4.5	+++
		46	2321	1.6	1.6	-	++
		15	549	0.4	-	-	+
4	204	-	-	-	-		
Cupric Sulphur Dust	7	12	645	27.4	1.2	-	+++++
		23	1484	22.5	2.8	0.9	++++
		44	2350	10.9	1.5	0.5	+++
		31	1809	3.2	1.2	0.2	++
		9	478	2.5	0.4	-	+
9	455	-	-	-	-		
Sulcol and Sulcol	5	4	249	22.0	2.4	-	+++++
		14	749	24.7	3.7	-	++++
		10	523	20.1	4.2	0.1	+++
		25	1389	10.7	1.7	0.1	++
		20	1111	4.9	0.8	0.1	+
6	335	2.4	-	-	-		

TABLE XXIV : Experiment C - Dusting experiments at "Clunie", Constantia, 1934 : Data taken 7 days after cold storage.

Fungicide	Number of			Percentage infection by		Mechanical damage.
	boxes	bunches	berries	Botrytis	Penicillium	
Check	5	2	206	39.8	-	+++
		5	615	7.6	-	++
		27	1571	6.1	0.1	+
		13	714	4.2	-	-
Zinfandelite	6	1	77	22.1	-	+++++
		2	155	25.8	-	++++
		6	460	22.6	-	+++
		12	853	9.1	0.4	++
		20	1351	4.7	0.1	+
		16	965	2.5	0.1	-
Sodium Sulphite and Kaolin	4	1	91	38.5	-	++++
		3	232	15.1	1.6	+++
		6	388	8.0	0.3	++
		13	799	2.1	-	+
		15	851	0.8	-	-
Copper Sulphur Dust.	4	5	305	6.6	-	++++
		5	292	3.8	0.3	+++
		8	605	5.8	0.2	++
		11	783	2.4	0.5	+
		7	427	2.1	0.5	-
Copper Lime Dust	6	8	508	16.1	-	+++
		11	710	11.1	-	++
		14	915	4.7	-	+
		19	1127	2.6	-	-

TABLE XX : Experiment 9 - Dusting experiments at "Clunie", Constantia, 1934 : Data taken 14 days after cold storage.

Fungi- cide	Number of			Percentage infection by		Mechani- cal damage
	boxes	bunches	berries	Botrytis	Penicil- lium	
Check	4	1	75	50.7	9.3	++++
		5	297	62.0	2.4	+++
		16	1080	27.8	0.6	++
		14	842	15.9	-	+
		1	47	4.3	-	-
Zinfan- delite	5	3	232	38.5	-	++++
		15	1078	41.8	0.8	+++
		19	1196	7.7	0.2	++
		10	453	3.1	-	+
Sodium Sulphite and Kaolin	4	8	479	51.0	0.8	+++
		20	1242	28.8	0.2	++
		9	558	14.1	0.4	+
Copper Sulphur Dust	3	1	69	56.5	-	+++++
		3	210	24.2	0.5	++++
		9	516	17.8	-	+++
		10	556	3.6	-	++
		9	457	0.4	0.4	+
Copper Lime Dust	4	2	116	77.5	2.6	+++++
		8	443	62.4	1.4	++++
		9	584	50.0	0.5	+++
		13	819	24.4	-	++
		9	484	3.5	0.2	+

TABLE XXVI : Experiment E - Dusting and spraying experiments at "The Vineyards", Constantia, 1934 : Data taken 7 days after cold storage.

Fungi- cide	Number of			Percentage infection by		Mechani- cal Damage
	boxes	bunches	berries	Botrytis	Penicil- lium	
Check	6	2	210	54.4	2.4	++++
		9	706	63.3	-	+++
		18	1392	57.1	-	++
		20	1369	36.2	-	+
		2	199	3.2	-	-
Zinfandelite	8	3	319	79.4	-	+++++
		15	1276	37.4	0.2	++++
		14	1247	26.7	-	+++
		18	1377	12.9	0.1	++
		7	454	1.1	-	+
1	59	-	-	-		
Sodium Sulphite and Kaolin	7	7	678	49.0	0.3	++++
		14	1181	58.4	0.3	+++
		22	1837	38.7	0.3	++
		9	600	18.9	0.2	+
		3	177	4.5	-	-
Copper Sulphur Dust	7	8	608	48.6	0.3	++++
		18	1674	19.1	-	+++
		14	1295	6.8	0.1	++
		7	544	0.7	0.1	+
		1	54	-	-	-
Copper Lime Dust	4	2	184	58.2	-	++++
		6	508	54.4	-	+++
		12	973	36.0	-	++
		14	1057	15.4	-	+
		1	83	-	-	-
Verderams Sulphur Dust	5	1	79	78.5	-	+++++
		2	177	33.9	-	++++
		12	1152	19.1	0.2	+++
		17	1472	7.9	0.2	++
		9	821	1.7	-	+
Bouisol and Sulisol	10	1	94	76.6	1.1	++++
		11	1048	43.3	0.1	+++
		30	2247	49.2	0.1	++
		17	1367	15.7	-	+
		9	731	2.5	-	-
Cuprite	8	3	284	72.5	0.4	++++
		8	733	49.0	0.4	+++
		23	1983	41.8	0.1	++
		21	1801	29.8	0.1	+
		2	139 139	0.7	-	-

TABLE XXVII : Experiment F - Dusting and spraying experiments at 'the Vineyards', Constantia, 1934, : Data taken 14 days after cold storage.

Fungicide	Number of			Percentage infection by		Mechanical Damage
	boxes	bunches	berries	Botrytis	Penicillium	
Check	8	1	72	100.0	-	++++
		17	1518	75.6	0.3	+++
		30	2441	71.4	0.04	++
		14	980	57.4	0.1	+
		1	46	-	-	-
Zinfandelite	9	5	426	87.5	-	++++
		21	1785	61.9	-	+++
		31	2516	43.4	-	++
		7	541	28.6	-	+
Sodium Sulphite and Kaolin	8	4	345	84.9	-	++++
		22	1947	73.2	-	+++
		31	2575	61.6	-	++
		3	164	33.5	-	+
Copper Sulphur Dust	6	10	933	65.2	0.5	++++
		12	862	64.1	0.6	+++
		20	1381	33.7	-	++
		3	158	0.6	-	+
Copper Lime Dust	1	5	435	45.0	0.9	++
		5	341	13.5	-	+
Verderame Sulphur Dust	5	3	270	85.0	-	++++
		11	909	45.1	0.1	+++
		19	1714	36.8	0.06	++
		8	668	7.5	-	+
Boulsol and Sulsol	8	3	271	90.4	1.8	++++
		14	1210	80.6	0.5	+++
		25	2240	48.4	0.1	++
		13	928	48.2	-	+
Cuprite	7	4	367	85.0	-	++++
		8	637	65.8	0.5	+++
		30	2355	45.3	0.1	++
		9	718	21.5	-	+

TABLE XXVIII: Experiment G - Dusting and spraying experiments at "Eshenort" Constantia, 1934 : Data taken at picking and 7 days after cold storage.

Fungi- cide	Number of			Percentage infection by				Mechanical damage	
	boxes	bunches	berries	Botrytis		Penicillium			Rhizopus (b)
				(a)	(b)	(a)	(b)		
Check	6	9	435	1.29	50.6	0.38	10.8	1.1	++++
		9	361		37.4		5.3		+++
		34	1385		20.9		3.4		++
		28	1153		5.5		1.6		+
		10	316		-		-		-
Zinfandelite	6	6	244	0.48	39.4	0.45	15.9	2.9	++++
		17	774		22.9		5.4		+++
		23	1170		7.7		5.1		++
		25	1040		2.9		1.5		+
		3	106		-		-		-
Copper Sulphur Dust	7	14	779	0.59	14.5	0.10	1.5	0.3	++++
		23	931		11.9		4.0		+++
		40	1639		6.4		1.2		++
		16	511		2.3		0.4		+
Copper Lime Dust	6	7	344	0.59	36.0	0.03	3.8	1.5	++++
		18	861		23.1		1.5		+++
		28	1230		12.1		1.2		++
		29	1171		4.8		0.6		+
		4	136		0.7		-		-
Cupric Sulphur Dust	6	25	1076	0.78	11.8	0.09	1.8	-	++++
		30	1287		10.7		1.1		+++
		18	685		1.8		0.7		++
		1	36		-		-		+
Bouisol and Sulsol	5	2	82	1.10	63.4	0.95	3.7	-	++++
		16	1204		10.1		2.6		+++
		25	1090		13.6		2.1		++
		20	730		4.8		1.6		+
		3	77		-		-		-

(a) Data taken immediately before picking.
 (b) Data taken 7 days after cold storage.

TABLE XXIX : Experiment H - Dusting experiments at "Under-the-Thatch",
Constantia, 1934 : Data taken immediately before picking
and 7 days after cold storage.

Fungicide	Number of			Botrytis infection %		Mechanical damage
	boxes	bunches	berries	(a)	(b)	
Cheek	9	6	298	4.92	97.5	+++
		65	3165		81.2	++
		12	538		29.2	+
Zinfandelite.	6	11	655	2.69	96.5	+++
		34	1578		73.2	++
		4	149		14.1	+
Sodium Sulphite and Kaolin	5	7	396	2.91	96.2	+++
		41	1873		82.2	++
		1	42		78.6	+
Copper Sulphur Dust	6	15	828	2.54	72.2	+++
		38	2028		61.3	++
		1	58		-	+
Copper Lime Dust	5	3	150	2.95	88.6	+++
		40	2019		67.8	++
		4	180		37.2	+
Cuprite	6	3	120	4.81	74.1	+++
		41	2052		74.5	++
		4	146		61.6	+

(a) Data taken immediately before picking.
(b) " " 7 days after cold storage.

TABLE XXX : Experiment I - Dusting experiments at "Under-the-Thatch", Constantia, 1934 : Data taken immediately before picking and 14 days after cold storage.

Fungicide	Number of			Botrytis infection(%)		Mechanical damage
	boxes	bunches	berries	(a)	(b)	
Check	6	45	2314	4.92	87.6	-
Zinfandelite	6	46	2452	2.69	94.7	-
Sodium sulphite and Kaolin	3	22	1150	2.91	29.1	-
Copper Sulphur Dust	6	42	2194	2.54	98.2	-
Copper Lime Dust	7	57	3012	2.95	96.2	-
Cuprite	4	31	1295	4.81	97.6	-

(a) Data taken immediately before picking.
 (b) " " 14 days after cold storage.

TABLE XXXI : Experiment J - Dusting and spraying experiments at 'The Vineyards', Constantia, 1934 : Data taken 7 days after cold storage.

Fungicide	Number of			Percentage Botrytis infection	Mechanical Damage.
	boxes	bunches	berries		
Check	5	2	154	55.8	++++
		9	639	61.3	+++
		13	910	47.6	++
		11	788	21.6	+
		3	155	6.5	-
Zinfandelite	5	1	54	68.5	++++
		7	373	60.6	+++
		15	990	32.1	++
		15	943	17.3	+
Sodium Sulphite and Kaolin	4	4	212	74.1	+++
		10	835	37.8	++
		11	768	22.0	+
		4	276	5.4	-
Copper Sulphur Dust	5	6	385	58.7	++++
		15	840	38.0	+++
		14	830	20.5	++
		4	189	6.9	+
		1	47	-	-
Copper Lime Dust	4	4	329	59.0	+++
		15	1002	50.8	++
		11	833	22.0	+
		2	114	3.5	-
Verderame Sulphur Dust	6	4	289	66.1	++++
		11	791	50.4	+++
		17	1214	22.7	++
		12	690	9.7	+
		2	94	1.1	-
Bouisol and Sulsol	5	3	228	70.6	++++
		7	556	55.4	+++
		15	1070	40.5	++
		7	474	19.4	+
		6	492	8.3	-
Cuprite	4	3	172	92.5	++++
		11	757	68.4	+++
		11	751	39.1	++
		5	306	19.9	+

TABLE XXXII : Experiment K - Dusting experiments at "Under-the-Thatch", Constantia, 1934 : Data taken immediately before picking and 7 days after cold storage.

Fungicide	Number of			Botrytis infection(%)		Mechanical damage
	boxes	bunches	berries	(a)	(b)	
Check	6	1	47	7.00	100.0	+++
		10	393		97.0	++
		48	1980		65.9	+
		9	439		24.2	-
Zinfandelite	7	2	75	3.04	94.7	+++
		24	974		68.2	++
		57	2330		47.5	+
		2	97		4.1	-
Sodium sulphite and Kaolin	5	12	391	4.22	73.5	++
		29	1265		48.5	+
		7	362		1.4	-
Copper sulphur Dust	7	5	171	1.59	81.3	+++
		26	1070		50.6	++
		56	2364		18.0	-
Copper Lime Dust	5	3	120	3.86	93.5	+++
		6	212		72.2	++
		37	1338		38.0	+
		8	307		4.9	-
Cuprite	4	8	316	3.71	66.8	++
		33	1216		34.6	+
		8	332		8.4	-

(a) Data taken immediately before picking.

(b) " " 7 days after cold storage.

TABLE XXXIII : Experiment L - Dusting experiments at "Under-the-Thatch", Constantia, 1934 : Data taken immediately before picking and 14 days after cold storage.

Fungicide	Number of			Botrytis infection (%)		Mechanical damage
	boxes	bunches	berries	(a)	(b)	
Check	5	14 49	554 1949	7.00	93.6 78.5	++ +
Zinfandelite	6	17 50	630 2008	3.04	87.9 65.0	++ +
Sodium sulphite and Kaolin	5	16 45	603 1742	4.22	97.4 74.3	++ +
Copper sulphur Dust	2	9 18	501 724	1.59	79.5 27.8	++ +
Copper Lime Dust	6	18 54	753 2294	3.86	92.8 62.5	++ +
Cuprite	6	16 51	511 2179	3.71	92.0 56.8	++ +

(a) Data taken immediately before picking.
 (b) " " 14 days after cold storage.

of vineyard infections were calculated. Although Botrytis and Penicillium infections were practically absent in the vineyard in the majority of these experiments, these rots occurred in fair amounts during the storage, especially during the 1933-1934 season.

In order to compare the efficiency of the various fungicides for the control of Botrytis and other grape rots an "Index of Control" was originated. This "Index of Control", calculated for a particular fungicide, is a numerical fraction indicating the extent to which this fungicide controls the rot concerned.

This figure is obtained as follows:-

If a, b, c, d = average percentages infection of bunches from the check boxes showing +, ++, +++, +++++ amounts of mechanical damage resp.

and p, q, r, s = average percentages infection of treated bunches showing +, ++, +++, +++++ amounts of mechanical damage respectively.

Then the average percentage infection in

the check boxes $= \frac{1}{4} (a + b + c + d) = A$

and the average difference between infection of treated

and untreated grapes $= \frac{1}{4} ((a-p) + (b-q) + (c-r) + (d-s))$

$= D$

Then "Index of Control" of the fungicide $= \frac{D}{A}$ or

$$\frac{(a-p) + (b-q) + (c-r) + (d-s)}{a + b + c + d}$$

The closer the control of the rot through the application of a fungicide approaches 100%, the less will the difference be between D and A, and therefore the closer will the "Index of Control" approach + 1.00. An "Index of Control" of 0.00 would likewise indicate that no control of the rot was obtained by a particular fungicide and a minus "Index of Control" would indicate that the treated grapes were more severely affected by the rot than the untreated grapes of the corresponding checks.

After the results of the 1933-1934 season had been analysed using the method just described, attention was drawn to a paper by Moore (76) who, in spraying experiments against pear scab, had also evolved a somewhat similar method for interpreting his results. The numerical figure, which he used to express the respective merits of the particular sprays, was called the "Relative Efficiency". This he calculated from the "Scab Equivalent" figures or from the percentages of scab infection. For the determination of the "Scab Equivalent", Moore classified the fruit according to the size of the scab lesions, whereas in these Botrytis studies, the data was classified for the sake of comparison according to the amount of mechanical damage in the grapes. Further Moore multiplied his results with a constant, in order to avoid decimals in the ultimate readings, whereas in these studies decimals were used. An "Index of Control" of 0.53, for instance, would correspond to a ~~his~~ "Relative Efficiency" of 53.

The Comparative Effectiveness of the Fungicides.

Only in experiments G, H, I, K and L was the percentage of Botrytis infection in the vineyard high enough to yield comparative results, as regards the control of this rot in the vineyard. From the results of these experiments, it would appear that the different fungicides which were included in them may be arranged in order of merit as follows:- Copper sulphur dust, infandelite, Copper lime dust, Cupric sulphur dust, sodium sulphite and Kaolin, Cuprito, Bouicool and Sulsol.

In reviewing Tables XXII to XXXIII, it is evident that the main type of wastage which occurred in these grapes during the 1933-1934 season was Botrytis rot. In a few experiments Penicillium rots occurred in large enough percentages and sufficiently frequent to warrant reliable comparisons to be made between the effects of the different treatments on this rot. In the above mentioned tables the

extent to which the occurrence of varying amounts of mechanical damage complicates proper comparisons of the merits of the various fungicides, is also clearly demonstrated. All the other types of wastage of grapes occurred in such small quantities, or so sporadically, in some of the packs, that no definite conclusions could be drawn from these tables as to the possibilities of their control by the various fungicides.

If the data on Botrytis control are summarized as in Table XXXIV, the comparison of the results is very much simplified. The ultimate result of the comparative effectiveness of the different fungicides could be presented graphically as in Figure XI.

From Table XXXIV and Figure XI, it is clear that copper sulphur dust and Verdorame sulphur dust in the 1933-1934 dusting and spraying experiments proved to be the best fungicides for the control of Botrytis storage rot of grapes from Constantia. Their "Indices of Control" were however practically the same and it was hence decided to continue the dusting experiments in the 1934-1935 season to determine which of these two actually gave the best control of Botrytis rot.

In these experiments during the 1934-1935 season, the plots dusted with each fungicide were randomized over an experimental block and the treatment replicated three times. Two vines were left between every two adjoining plots in a row. These vines were left untreated and served as buffer plots to minimize, as far as possible, the drifting of dusts from one plot to another in the same row. Drifting of dusts from one row to another was prevented by using a hessian screen as previously described. One series of plots was also left untreated to serve as checks. These comparative experiments were carried out on Kenab Purki grapes at "Under-the-hatch" and "The Vineyards" (Figure V:B and C resp).

Botrytis...

TABLE XXXIV: The "Index of Control" of the various fungicides for the control of Botrytis storage rot of grapes as calculated from the dusting and spraying experiments carried out at Constantia during 1934.

Fungicide	Average infection of checks	Average difference between treated and untreated grapes.	Index of Control	Calculated from
Copper Sulphur Dust	3.5	2.1	0.60	A
	10.6	1.6	0.15	B
	14.4	10.9	0.76	C
	39.1	27.6	0.71	D
	42.8	27.8	0.65	E
	76.1	35.8	0.46	F
	28.6	19.8	0.69	G
	52.0	24.6	0.47	H
	87.6	-10.6	-0.12	I
	38.6	13.7	0.35	J
	87.6	37.7	0.43	K
	86.1	32.4	0.38	L
Average Index of Control			0.46	
Zinfandelite	3.5	1.7	0.47	A
	10.6	4.9	0.47	B
	14.4	4.7	0.32	C
	39.1	18.8	0.48	D
	42.8	27.2	0.63	E
	76.1	20.8	0.27	F
	28.6	10.4	0.36	G
	52.0	8.0	0.15	H
	87.6	-6.9	-0.08	I
	46.6	2.0	0.05	J
	71.8	18.8	0.26	K
	86.1	9.2	0.11	L
Average Index of Control			0.29	
Sodium sulphite and Kaolin	14.4	5.4	0.38	C
	35.2	3.9	0.15	D
	42.8	8.5	0.20	E
	76.1	12.8	0.17	F
	52.0	-16.6	-0.32	H
	87.6	-8.7	-0.10	I
	34.3	-0.6	-0.02	J
	62.4	21.2	0.34	K
86.1	0.3	0.03	L	
Average Index of Control			0.09	
Copper Lime Dust	3.5	-2.5	-0.71	A
	12.8	4.3	0.24	B
	14.4	5.8	0.40	C
	39.1	4.1	0.10	D
	42.8	10.0	0.23	E
	64.2	35.2	0.55	F
	28.2	7.7	0.27	G
	52.0	4.4	0.08	H
	87.6	-8.6	-0.10	I
	34.3	0.4	0.01	J
	71.8	19.6	0.27	K
	86.1	8.4	0.10	L
Average Index of Control			0.14	
Sulphur Dust	3.5	0.8	0.23	A
	10.6	2.7	0.25	B
	16.0	9.9	0.62	C
Average Index of Control			0.37	
Sulphur Dust	52.5	37.1	0.71	E
	76.1	32.5	0.43	F
	38.6	8.6	0.22	J
Average Index of Control			0.45	
Sulsol and Sulsol	3.5	-0.8	-0.23	A
	10.6	-2.0	-0.19	B
	42.8	5.4	0.13	E
	76.1	9.2	0.12	F
	28.6	4.5	0.16	G
	38.6	-0.3	-0.08	J
Average Index of Control			-0.02	
Sulphite	42.8	4.1	0.10	E
	76.1	22.0	0.29	F
	52.0	-0.4	-0.01	H
	87.6	-10.0	-0.11	I
	46.6	-8.4	-0.18	J
	62.4	25.4	0.42	K
	86.1	11.7	0.14	L
Average Index of Control			0.09	

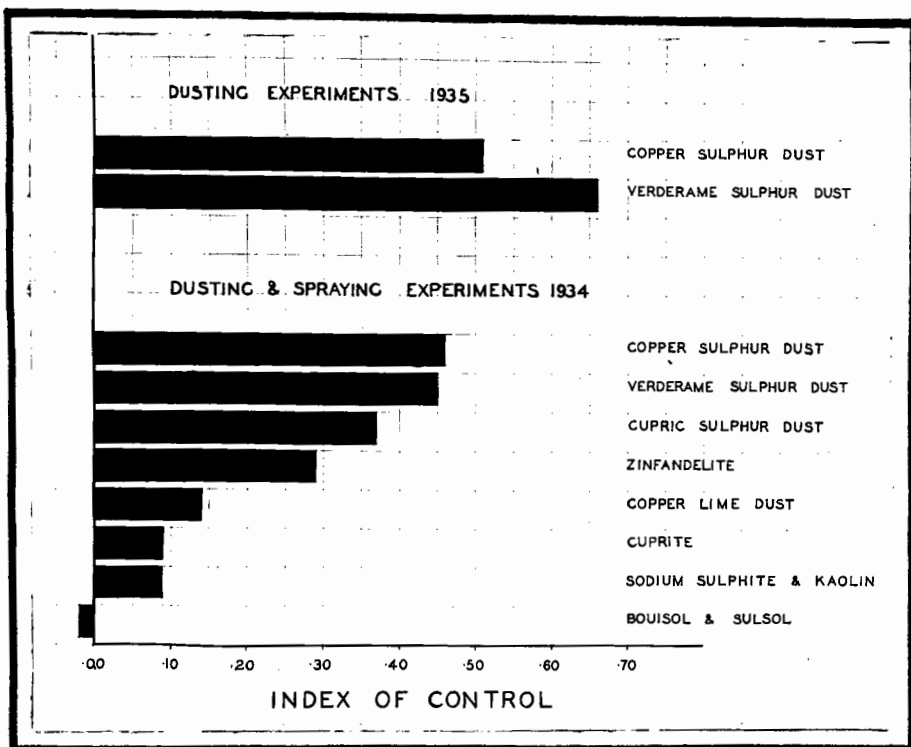


FIG. XI. - The "Index of Control" of different fungicides in the dusting and spraying experiments of 1934 and 1935.

Botrytis rot in these vineyards was totally absent at the time when the grapes of these experiments were picked. These grapes were picked, packed, stored and examined as in the experiments of the previous season. The results are given in Tables XXXV and XXXVI.

The "Index of Control" of Botrytis storage rot of Copper sulphur dust is 0.47 as derived from Table XXXV and 0.55 as from Table XXXVI, while that of Verderame sulphur dust is 0.63 and 0.69 respectively. The averages of these figures are presented graphically in Figure XI. From these results it is concluded that both Copper sulphur dust and Verderame sulphur dust control Botrytis rot during storage to an appreciable extent, but that Verderame sulphur dust gives better results.

The "Indices of Control" of Penicillium storage rots were also determined from the results of these dusting and spraying experiments during these two seasons, where infection was sufficiently consistent. (From tables XXII, XXIII, XXV, XXVIII, XXXV and XXXVI) These calculations are given in the following table.

Table XXXVII : "Index of Control" of Penicillium storage rots for the different fungicides used in dusting and spraying experiments carried out during the 1933-1934, and the 1934-1935 seasons.

Zinfandelite	Index of Control of						Calculated from Table
	Sodium Sulphite and Koalin	Copper sulphur dust	Copper Lime Dust	Cupric Sulphur Dust.	Boöisel and Gulsol	Verderame Sulphur Dust.	
.52	-	.46	-.59	.49	.16	-	XXII
.48	-	.07	-.35	.44	.02	-	XXIII
.92	.53	.93	.72	-	-	-	XXV
-.32	-	.67	.67	.75	.53	-	XXVIII
.40	.53	.53	.11	.56	.24	-	Average 1934
-	-	.58	-	-	-	.31	XXXV
-	-	.75	-	-	-	.59	XXXVI
-	-	.67	-	-	-	.45	Average 1935

From this table it would appear that Cupric sulphur dust gave the best results for the control of Penicillium storage...

TABLE XXXV: Dusting Experiments at "Under-the-Thatch", Constantia during 1935 : Data taken 14 days after cold storage.

Fungi- cide	Number of			Percentage infection by			Mecha- nical Damage
	boxes	bunches	berries	Botry- tis	Penicil- lium	Clado- sporium	
Check	8	4	307	98.80	10.09	-	+++++
		10	647	78.30	4.18	7.32	++++
		20	1112	75.70	2.61	3.86	+++
		18	1027	52.70	1.56	2.73	++
		15	614	34.25	0.34	1.79	+
		6	237	29.50	-	-	-
		-	-	-	-	-	-
Copper Sulphur Dust	12	8	746	57.60	3.89	11.39	+++++
		11	957	64.90	0.73	4.88	++++
		9	706	40.40	2.12	8.50	+++
		25	1684	25.52	0.71	4.27	++
		27	1786	4.87	0.11	1.72	+
		10	449	-	-	-	-
		-	-	-	-	-	-
Verderame Sulphur Dust	9	1	75	57.40	6.66	25.38	+++++
		7	577	40.01	6.58	13.51	+++++
		9	632	36.10	3.96	9.98	++++
		19	1380	24.58	1.52	4.72	+++
		18	1328	7.62	0.83	5.19	++
		16	835	5.27	0.25	1.08	+
		5	242	0.41	-	-	-

TABLE XXXVI: Dusting experiments at 'The Vineyards', Constantia, 1935 : Data taken 10 days after cold storage.

Fungicide -	Number of			Percentage infection with				Mechanical damage
	boxes	bunches	berries	Botrytis	Penicillium	Rhizopus	Cladosporium	
Check	16	2	223	17.48	11.21	-	4.49	+++++
		7	567	9.70	2.65	-	2.82	++++
		27	2479	9.87	1.90	-	2.54	+++
		30	2695	5.24	0.37	-	1.71	++
		33	2537	0.83	0.08	-	0.51	+
		10	676	0.15	-	-	-	-
		-	-	-	-	-	-	-
Copper Sulphur Dust	19	7	720	23.20	16.95	-	7.23	+++++
		13	940	10.75	1.81	0.21	5.65	++++
		20	1611	4.84	1.43	-	4.10	++++
		46	3603	2.84	0.53	-	1.36	+++
		41	2923	1.13	0.14	0.10	0.34	++
		13	927	0.22	-	-	-	+
		7	420	-	-	-	-	-
Verderame Sulphur Dust	16	1	66	3.03	1.51	-	16.70	+++++
		5	372	7.00	4.57	1.61	30.40	++++
		6	511	3.33	1.57	-	5.09	++++
		42	3929	1.04	0.20	-	1.63	+++
		40	2946	0.88	0.13	-	0.14	++
		10	691	0.14	0.14	-	0.43	+
		7	601	0.83	-	-	0.14	-

storage rots during the experiments of the 1933-1934 season. Copper sulphur dust, sodium sulphite and Kaolin were slightly less effective against these rots. In the experiments of 1934-1935 copper sulphur dust proved to be better for Penicillium control than Verderame sulphur dust.

No evidence could however be obtained to indicate any control of Cladosporium, Rhizopus and Aspergillus rots by any of the tested fungicides. The data obtained concerning these rots are however considered insufficient to warrant any definite conclusions in this matter.

In summarising the results, obtained in these different tests, it may be stated that Verderame sulphur dust proved to be the best dust for the control of Botrytis rot of grapes in storage. Copper sulphur dust, on the other hand, was again one of the best dusts for the control of Penicillium storage rots of grapes and at the same time very effective against Botrytis rot in the vineyard and in storage, although less effective in this latter respect than the former fungicide. Taking into account that Botrytis is evidently the main cause of wastage in export grapes, preference would hence be given to the Verderame sulphur dust.

Influence of Mechanical Damage on Efficiency of Fungicides.

It has already been demonstrated that the amounts of the various types of grape storage rots increase directly with the increase in the amount of mechanical damage (see Figure 1). In reviewing the individual results of the dusting and spraying experiments in Tables XXXI - XXXVI, it is at once apparent that the relative difference between percentages of Botrytis infection of treated and untreated grapes generally decrease as the amounts of mechanical damage increase.

FIG....

The "Indices of Control" were there^{fore} determined for each fungicidal application on grapes showing different amounts of mechanical damage in each of the dusting and spraying experiments of the 1933-1934 season. The average "Indices of Control" for each fungicide, on grapes showing different amounts of mechanical damage, were then obtained and the results plotted as in Figure XII.

From these graphs it is evident that the efficiency of all these fungicides were very distinctly affected by injury to grapes during their packing or storage. The various fungicides, however, react differently in this respect. The changes in the "Index of Control" of Copper sulphur dust, Verderame sulphur dust, Cupric sulphur, Zinfandelite and Cuprite show more or less the same tendency of variation with varying amounts of mechanical injury. Variation in already badly injured grapes, does apparently not affect the effectiveness of Copper lime dust to the same extent as in the case of the former fungicides. The effectiveness of sodium sulphite and Kaolin, and that of Bouisol and Sulcol were apparently very little affected by any variation in the amount of mechanical injury. The "Index of Control" for Botrytis rot of these two fungicides were however low, even on grapes showing no visible signs of injury.

These results further stress the emphasis already laid on the deleterious effects of mechanical damage in grape packs. Good results for the control of Botrytis storage rot by some of the more promising fungicides may be expected only if the dusted grapes are packed and transported with such care that very little injury is inflicted to them.

The above effects on the occurrence of varying amounts of mechanical damage on the "Index of Control" figures of

the.....

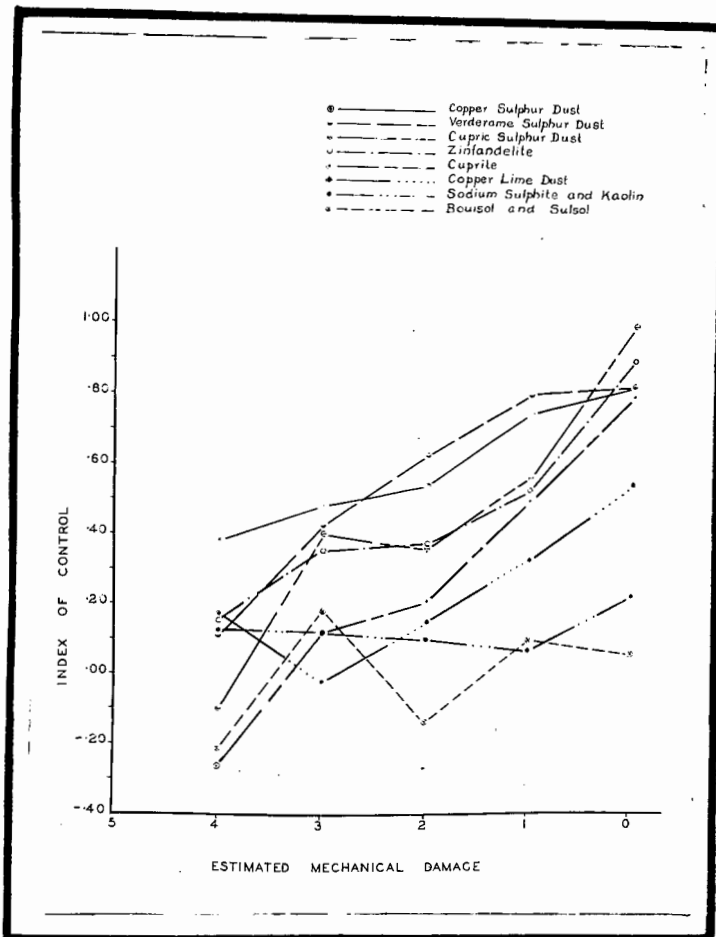


FIG. XI1. - The effect of mechanical damage on the "Index of Control" of the different fungicides in the dusting and spraying experiments during the 1934 season.

the different fungicides are readily understood. Dusts applied in an uniform layer to the surface of grape berries may protect these surfaces against possible Botrytis infection. As soon as rupture of any type is caused to the berry, the inner tissues of the berry are exposed and unprotected by the fungicidal film. These parts are then liable to and more easily infected by Botrytis and other storage rot organisms. It is also evident that the effectiveness of fungicides, which normally are poor controllers of Botrytis rot even on uninjured grapes, will hardly be much decreased by an increase in the amount of mechanical damage.

These conclusions are also confirmed by data on the effect of the fungicidal dusts or sprays on the initiation of wastage in a bunch, especially that caused by Botrytis. As previously stated, information was also recorded, during the examination of the experimental grapes, as regards the position of wasty berries in every bunch. These observations, made during the 1933-1934 season on grapes used in the various dusting and spraying experiments on the different Constantia farms, are tabulated in Table XXXVIII.

A more critical study of the ratios of the positions in a bunch of the berries affected by Botrytis rot presented in this table, reveals that the ratios of bunches treated with Zinfolite, sodium sulphite and kaolin, copper lime dust, cuprite, Bouisol and Sulsol are very close to the ratio in the untreated bunches of the checks, i.e. top (shoulder of bunch): middle: ⁶Bottom (tail of the bunch): 1.00 : 1.21 : 1.12. In the copper sulphur, cupric sulphur and Verderame sulphur treated bunches, however, the ratio of the incidence of Botrytis rot at the bottom of the bunch to that at the top is increased and that of the middle to the top is still more increased. As was indicated and shown in Figure XI, these latter three fungicides were found to be the best for the control of botrytis storage rot during the experiments of the

TABLE XXXVIII Percentage bunches in the dusting experiments at Constantia during 1934 infected with Botrytis at their top, middles and bottoms and the respective ratios of these positions with the various fungicides.

Proof Experiment	Zinfandelite		Sodium sulphite and Kaolin.		Copper Sulphur Dust.		Copper Lime Dust.		Cuprite		Cupric Sulphur.		Verderame Sulphur.		Bouisol and Sulsol.		Check.
	T	M B	T	M B	T	M B	T	M B	T	M B	T	M B	T	M B	T	M B	
A	8	25 - 17	4	12 - 13	9	38 - 17	2	30 - 15	7	33 - 24	4	33 - 12	7	33 - 24	4	33 - 12	
B	23	54 - 39	40	64 - 61	33	41 - 41	41	62 - 57	49	72 - 64	24	47 - 44	49	72 - 64	24	47 - 44	
C	35	63 - 46	25	53 - 39	27	69 - 33									45	68 - 43	
D	43	77 - 64	27	50 - 54	54	90 - 73									70	95 - 87	
E	58	83 - 66	40	83 - 54	72	89 - 80			81	86 - 84			37	76 - 63	90	92 - 94	
F	89	97 - 80	64	93 - 76	30	80 - 60			74	92 - 74			54	85 - 63	97	99 - 92	
G	26	49 - 35	18	33 - 41	29	57 - 49					19	51 - 48			28	58 - 56	
H	96	96 - 92	89	93 - 94	98	100 - 90			94	94 - 92					90	97 - 93	
I	100	100 - 100	100	100 - 100	100	100 - 100			100	100 - 100					100	100 - 100	
J	84	92 - 90	62	82 - 82	91	97 - 94			97	100 - 100			74	96 - 85	84	95 - 93	
K	89	97 - 93	56	84 - 69	67	89 - 80			61	90 - 76					91	100 - 94	
L	91	99 - 96	74	94 - 74	93	100 - 95			90	96 - 90					97	100 - 100	
Average	62	78 - 68	50	70 - 63	59	79 - 68			85	94 - 88			55	86 - 70	68	82 - 76	
Ratio	1 : 1.26	1.1	1 : 1.40	1.26	1 : 1.34	1.15	1 : 2.35	2.00	1 : 1.13	1.02	1 : 1.56	1.28	1 : 1.35	1.24	1 : 1.21	1.12	

T - top shoulder portions of the bunch.
M - middle portions of the bunch.
B - bottom or tail portions of the bunch.

the 1933-1934 season.

The lower half of the bunches were found to be those which generally showed signs of most severe mechanical injury. In applying fungicides, which are effective against Botrytis rot to grapes, the greatest proportional control of this rot would be obtained in those sections of the dusted bunches, which were the least mechanically injured. The least proportional control would also be obtained in those sections of the bunch, which were badly injured. This would result in a change of the said ratios as was found in the bunches treated with the three best fungicides. Where fungicides applied are less effective against this rot, the effect of dusting with them on the ratios of Botrytis rot initiation in bunches would be less distinct.

The apparent big change in ratios produced by cupric sulphur is due to the fact that this dust was used only in Red Hanepoot and not on Henab Turki grapes as was the case with the other fungicides. The ratios of Botrytis rot initiation on Red Hanepoot bunches are different from those on Henab Turki. Actually there is no difference in the ratios of Cupric sulphur when compared with those of copper sulphur dust on Hanepoot alone.

The above observations therefore also tend to show that the best results from fungicides, effective for the control of Botrytis rot, is to be expected in bunches or in sections of bunches which are the least mechanically injured.

Effect of the Time of Application of dusts and the
Time of Picking of Treated Grapes on the Efficiency
of these Dusts.

From Table XXXIV it is apparent that the "Index of Control" figures of any of these fungicides, which had been used in the dusting and spraying experiments of the 1933-1934 season, vary considerably. Various factors might have been responsible for this great variation in results. A factor, suggesting itself as possible contributing to this variation

variation, is the period between dusting and the next rain.

Attention was first drawn to the possible relation of this period to the efficiency of fungicides by the results obtained in experiments *On* the control of almond anthracnose (42). The season, during which these experiments were carried out, was characterized by abundant precipitation at frequent intervals, conditions considered extremely conducive to the development of the disease. In spite of this fact excellent control was obtained by Bordeaux applications, some of which were applied only a short time before rain. These results are to some extent in accordance with those of Young and Day (121), who found that the most effective sprays against apple scab were those applied just before the so-called infection periods, which were mostly periods of rain. Keitt and James (34) concluded from the results of field tests for the control of apple scab in Wisconsin that "in emergencies considerable benefit may be derived from dust applications during infection periods before the fungus becomes well established in the host".

In order to determine whether the period between dust application and the next rain had any effect on the efficiency of the dust for the control of Potrytis rot of grapes, the variation in the "Index of Control" figures of the copper sulphur dust were more closely studied. This fungicide was selected for further study because it had proved to be the best fungicide for the control of Potrytis rot in the experiments of the 1933-1934 season and also because it was one of the fungicides used in all these tests.

The rainfall records of the Tokai Forest Station for the 1933-1934 season was used in analysing the results of experiments C and D conducted at "Clunie" in the neighbourhood of this station (see figure V). For the analysis of

the....

the results of the experiments on the other three farms, the rainfall data recorded at "Silverhurst" were used. These data for January to May 1934 are presented graphically in Figure XIII. By comparing the dates of application in Table XXI with Figure XIII, the periods between the different applications of copper sulphur dust and the next rain were obtained for the 1933-1934 experiments. These periods were plotted in Figure XIV against the "Index of Control", figures of copper sulphur dust from experiments A, C, D, G, H, I and K (i.e. "Index of Control" from results obtained 7 days after cold storage).

In the case of experiment H, however, it was found that the "Index of Control" of Copper Sulphur dust was very low when compared with those figures from the other experiments of the first part of the season (i.e. experiments A - H). If the dates of the picking of grapes in the experiments listed in Table XXI be compared with the rainfall data in Figure XIII, it will be found that the grapes of experiment H were the only ones in these series picked as short as $1\frac{1}{2}$ day after a rain. The "Index of Control" figures of copper sulphur dust were again plotted against the period between picking of treated grapes and the rain immediately preceding it. These figures were also plotted against the ratio of $\frac{\text{number of days dusted before a rain.}}{\text{(number of days picked after a rain)}}$ in order to establish the possible combined effect of these two factors on the efficiency of copper sulphur dust. The latter two curves obtained for the early experiments (1933-1934, A - H) were regular as is shown in Figure XIV.

From this figure it may be concluded that the best control of Botrytis rot by copper sulphur dust is to be obtained by dusting as shortly before a rain as possible - as in experiment C - and to pick as far as possible not less than three days after a rain.

In....

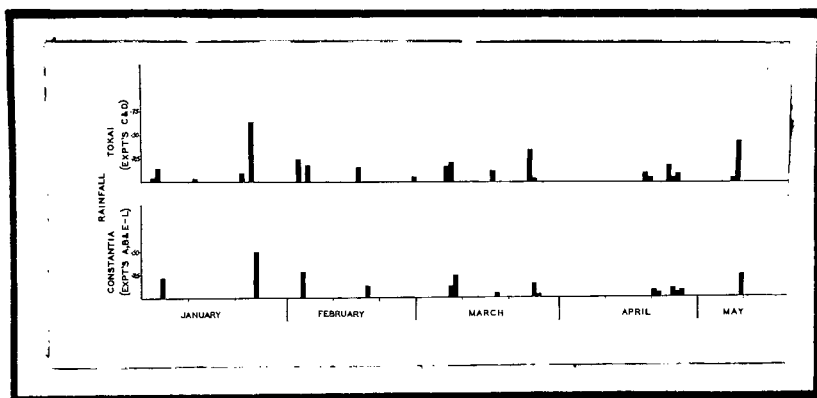


FIG. XIII. - Daily rainfall from January to May, 1934, at Tokai and at "The Vineyards", Constantia.

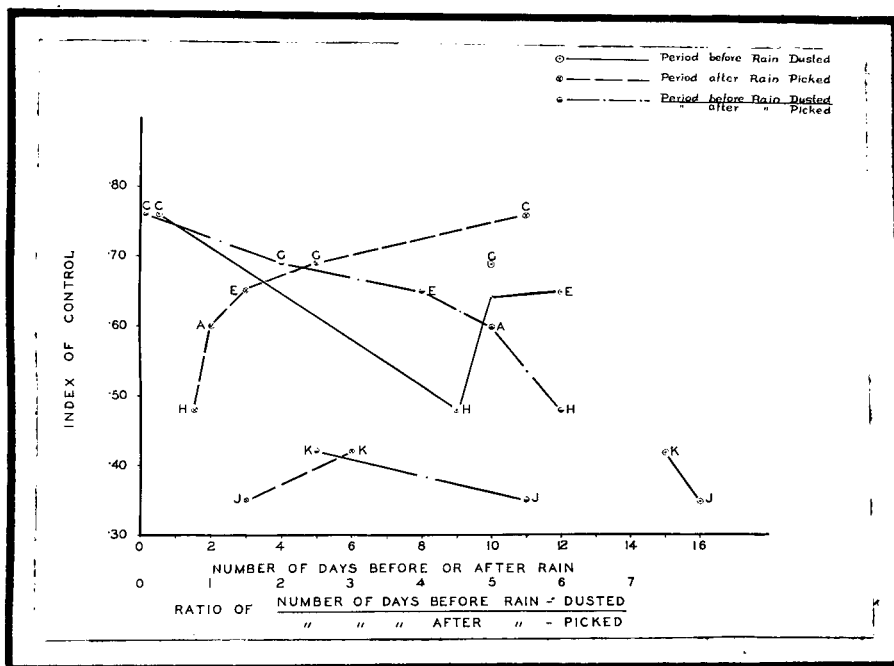


FIG. XIV. - Effect of the period before rain of application of Copper Sulphur dust, the period of picking of dusted grapes after rain, and the ratio of period of dusting before rain: period of picking after rain on the "Index of Control" of Copper Sulphur dust.

A, B, C, etc - dusting and or spraying exp. no.

In Figure XIV, the lines joining the "Index of Control" figures of copper sulphur dust, obtained from experiments J and K, were not concurrent with the curves obtained from the previous experiments. The grapes used of the latter two experiments were, however, those of the last crops of Henab Turki, dusted two months after the former grapes had been dusted, and picked more than a month after the last pickings of the first series of the experiments. It is therefore obvious that the environmental conditions governing experiments J to K could not be comparable with those governing the earlier experiments. The above results, though striking, were obtained from dusting and spraying experiments carried out on different farms in the Constantia area and with two grape varieties viz. Red Hanepoot and Henab Turki.

In order to study more accurately the effect of the time of application of copper sulphur dust before a rain on the efficiency of this dust, the following experiment was initiated during the 1934-1935 season:-

A block of Henab Turki, consisting of six rows of 35 vines each, was divided into thirty six experimental plots, six plots in each row. In between every two plots in a row one vine was left untreated to minimise dust drifting from one plot to an adjoining one in the same row. During the particular applications, the same general procedure of dusting was adopted as in the previous experiments, using anessian screen on one side of the plot to prevent drifting of dust on to adjacent rows. The different plots were treated only once with copper sulphur dust, but ^{on} different dates, six plots being treated at any particular time. These plots were situated in such a manner so that a randomised Latin square of the plots treated at different times, was formed. One series of plots similarly distributed over the experimental block

were left untreated to serve as checks.

The dates on which the different series of plots were dusted with copper sulphur dust were 21:2:1935, 27:2:1935, 4:3:1935, 6:3:1935 and 11:3:1935. About .54 inch of rain fell on the 5th of March, i.e. one day after the third series of copper sulphur dust applications and $1\frac{1}{2}$ day before the fourth of this series. No further rain intervened between these applications and the time when these grapes were picked i.e. the 21st of March, 1935.

The grapes were picked, packed, cold stored and examined 10 days after cold storage as described^c for the previous experiments. The data of this experiment are given in Table XXXIX, from which it is evident that the results of this experiment confirms those of experiments carried out during the previous season. The "Index of Control" of copper sulphur dust increased with the shortening of the period between its application and the following rain. A fair amount of control was also obtained by applications soon after the rain, but much less than in the former case.

To establish the effect of the time of picking of treated grapes after a rain on the efficiency of copper sulphur dust, three rows of Henab Turki, of 35 vines each, were dusted thoroughly with copper sulphur dust on the 27th of February, 1935. Three adjoining rows were left untreated as checks. Excepting the rain on the 5th of March reported in the previous experiment, another light drizzle of about .25 inch fell during the afternoon of the 25th March. Grapes were picked at random from the treated and untreated rows on the 26th, 27th and 28th of March, 1935. The treated and untreated grapes were packed separately immediately after picking and forwarded for cold storage. The periods at which the grapes of the different series were picked after the rain on the 25th March, the data on percentages infection and "Index of Control" of Botrytic rot of copper sulphur dust are given in Table XL.

The...

TABLE XXXIX: Dusting experiments at "The Vineyards", Constantia, 1935, to establish the effect of the time before or after rain of application of Copper Sulphur Dust on its efficiency: Data taken 10 days after cold storage.

Time of application	Number of		Percentage infection by				Clado- sporium	Mechani- cal damage.
	boxes	bunches berries	Botrytis Perce- tage	Index of Control	Penicil- lium			
Check	19	964	15.98		1.04	7.68	++	
		5855	7.98		0.43	2.90	+	
		4789	2.09		0.29	1.13	-	
11 (b)	19	341	39.00		7.04	29.61	+++	
		227	15.41		10.12	17.59	+++	
		1883	8.55		1.27	7.83	++	
		5860	4.54	0.40	0.09	1.96	+	
		3437	2.42		0.23	1.49	-	
6 (b)	22	69	7.26		-	10.13	+++	
		854	4.46		1.17	11.96	++	
		7436	1.10		0.55	2.92	+	
		4757	1.09	0.70	0.34	1.55	-	
1 (b)	19	427	12.88		3.28	9.84	+++	
		1162	1.98		1.29	11.18	++	
		8255	1.77		0.22	2.52	+	
		2101	1.43	0.80	0.14	1.43	-	
1.5 (a)	21	1097	18.88		3.38	10.53	+++	
		2049	12.02		1.12	5.42	++	
		9198	2.48		0.35	1.50	+	
		910	0.22	0.43	0.11	1.10	-	
6 (a)	19	224	4.92		11.11	12.50	+++	
		453	17.89		0.88	13.00	+++	
		1663	13.89		1.14	6.56	++	
		6835	2.28		0.48	3.00	+	
		2890	1.00	0.34	0.31	1.12	-	

(a) Number of days applied after rain.
(b) Number of days applied before rain.

TABLE X: Dusting experiments at "The Vineyards", Constantia, 1935, to establish the effect of the time of picking of grapes dusted with Copper Sulphur dust on the efficiency against Botrytic rot in storage. Data taken 10 days after cold storage.

Period after rain picked (days)	Grapes dusted or not dusted	Number of		Percentage infection by			Mechanical damage.	
		boxes	bunches	berries	Botrytis			Cladosporium
					Dotyrtis	Index of control.		
0.5	not dusted	3	3	295	64.20	0.34	7.12	++++
				593	52.80	0.34	6.58	+++
				336	41.90	0.32	4.96	++
				520	7.69	0.58	3.85	+
				38	2.64	-	-	-
0.5	dusted	3	4	288	65.70	4.86	6.94	++++
			5	487	40.30	2.26	7.18	++++
			3	241	54.80	1.66	3.32	+++
			9	789	2.28	-	1.78	++
			1	67	-	0.23	-	+
2	not dusted	3	1	70	50.00	2.71	10.00	+++
			6	634	35.86	-	3.32	++
			6	497	29.00	-	1.61	+
			10	881	2.95	-	1.02	-
2	dusted	2	1	51	17.62	7.84	43.20	+++
			3	280	37.80	1.79	9.67	+++
			3	225	7.57	-	12.43	++
			5	511	7.83	0.39	2.74	+
			2	132	-	0.44	3.78	-
3	not dusted	3	2	186	19.88	-	10.74	+++
			10	771	22.72	0.78	4.02	++
			8	750	4.67	0.93	2.67	+
			2	172	4.07	-	-	-
3	dusted	3	2	187	15.50	0.53	10.12	+++
			4	388	22.18	1.29	5.93	+++
			7	626	7.18	0.16	4.79	++
			5	319	2.82	-	-	+
			1	69	-	0.37	-	-

The lowest "Index of Control" figures for copper sulphur dust were obtained, where the grapes were picked half a day after this rain and the highest when picked 2 days after the rain. A slightly lower "Index of Control" was recorded on grapes picked 3 days after the rain. The rain was, however, only very light and its effects on the efficiency of the dust would therefore not have been quite the same as the observed effects of the comparatively heavy rains during the previous season.

From the results of these two seasons, it would appear that the best control of Botrytis rot in storage can be obtained by dusting as shortly as possible before a rain, when the grapes are in an advanced ^{stage of} colouring and sweetening, and when the dusted grapes are picked not too soon after a rain.

From Table XL it is also clear that Botrytis infection was the highest in untreated grapes, picked shortly after the rain. The infection of grapes picked three days after a rain was much less than that of grapes picked two days after as also less than in grapes picked half a day after a rain. Apparently the length of the danger period of picking grapes with regard to rain is also correlated with the amount and duration of the precipitation prior to picking.

The possible cause of the effects of rain on the amount of Botrytis rot during storage, and on the effectiveness of fungicides, have not been further investigated. Young and May (121) and Keitt and Senne (34), however, found that the amount of ascospore discharge of the apple scab fungus is closely correlated with the duration and amounts of rainfall. In many cases therefore, sprays against apple scab are timed just before these periods of maximum spore discharge, i.e. before predicted rains. Horne (59) furthermore reported, from investigations carried out on apple storage rots and on the distribution of fungi in the orchards, that

"The..

"the fungi present in the tissues of apples contracting disease are also present on the fruit before it is detached from the tree".

An interesting phase of further research would be the determination of the effect of rain and other climatic conditions on the distribution of Botrytis spore^s in relation to Botrytis storage rot, as also the efficiency of fungicides applied to grapes in the vineyard under different climatic conditions.

Effect of the Number of Dust Applications on Botrytis Control.

In the spraying and dusting experiments reported in the foregoing paragraphs, only one application of dust or spray was given to the bunches, and this when the berries were about 50% coloured and starting to sweeten. This stage was chosen for the applications, because observations in the vineyard failed to reveal any Botrytis infection on the more immature berries. From the results obtained in the dusting and spraying experiments of the 1933-1934 season, the question naturally presented itself as to what extent the efficiency of these fungicides may still be increased by increasing the number of fungicidal applications.

Copper sulphur dust was also selected for further studies in this direction during the 1934-1935 season. The experimental plots were arranged according to the Latin square system in the same way as in the experiment to establish the effect of the time of application of this dust on its efficiency.

One series of plots received only one application of copper sulphur dust, and each of the other series received two, three, four or five applications of this dust respectively and at weekly intervals. One series of plots similarly randomised as the others, was left untreated and used as corresponding checks.

From the results of this experiment, recorded in Table XL1, it is evident that the efficiency of copper sulphur dust was increased by increasing the number of applications. It is further evident that the increase of the "Index of Control" figures is hardly such as to warrant repetition of dust

TABLE 24 : Dusting experiments at the Vineyards, Constantia, 1955, to establish the effect of the number of applications on the efficiency of Copper Sulphur Duct. Data taken 10 days after cold storage.

Number of Applications	Number of boxes		Number of bunches per box	Percentage infected by Botrytis Index of control	Percentage infected by Cladosporium		Mechanical damage.
	Boxes	bunches			Cladosporium	Cladosporium	
0	19	6	412	0.07	5.09	+++	+
			1296				
			5238				
			5360				
1	18	2	217	0.46	4.61	+++	+
			496				
			1566				
			4663				
			4840				
			0.19				
2	18	2	177	4.72	30.06	+++	+
			297				
			477				
			778				
			4716				
			0.26				
3	18	2	164	6.25	27.32	+++	+
			365				
			1024				
			6174				
			3916				
			0.13				
4	17	1	70	0.86	8.22	+++	+
			670				
			1414				
			4979				
			3055				
			0.12				
5	18	3	296	1.35	19.41	+++	+
			270				
			1555				
			5362				
			3579				
			0.02				

dust applications under these climatic conditions. It should be borne in mind, though, that the 1934-1935 season, during which this experiment was conducted, was characterized by a relatively low rainfall during the period intervening the dusting and picking of these experimental grapes. This latter fact is also reflected in the very low percentages of Botrytis storage rot infection.

During seasons with heavier rainfall the increase of the efficiency of ^copper sulphur dust may be proportionally larger where the number of dust applications are increased. This will very likely be the case if these applications are to be properly timed in relation to rainfall.

Prestorage Treatments.

Addition of Chemicals.

Barker and Grove (4) found that fruit intended for jams or stews could be effectively preserved by storing such fruit in weak concentrations of sulphur dioxide in water. Sodium metabisulphite is known to be one of the compounds which evolves a strong odour of sulphur dioxide gas, so that the addition of this chemical to the bunches, before being wrapped, would possibly serve as a preservative during storage.

During the 1933-1934 grape season, weighed quantities of this compound were added to each of a number of Henab Turki bunches, immediately before being wrapped and packed. Some of the bunches were packed without being treated and served as checks. These grapes were stored and examined at two different periods after cold storage and the results tabulated in Table XLII (a).

From the data in this table, it is evident that Botrytis storage rot of grapes may be effectively controlled by the addition of various quantities of sodium metabisulphite to the bunches just before they are wrapped. It was, however, found that....

that the addition of even 2 gms. of this compound to a bunch caused a discolouration of Honab Turki berries to an extent of 20% (see page). This compound, moreover, leaves a residue in the bunch after having been stored for the given period. It was also found that the flavour of the grapes were very distinctly affected by the addition of this chemical.

In 1931 Jenkins and Frout (107) found that they were able to control green rot of oranges (Penicillium digitatum) by, either passing air, first blown over ammonium bicarbonate over oranges or by adding crystals of the salt to vessels containing the fruit. The addition of crystals of ammonium bicarbonate to wraps, before the fruit was packed, did not yield as distinct beneficial results as the former treatments, because ammonium bicarbonate failed to dissociate in the absence of moisture. These workers also mentioned that injured grapes remained free from decay, when stored in a jar containing small amounts of this salt. In 1933 Jenkins (105) further reported satisfactory results in the control of Botrytic rot of stored tomatoes by passing air currents, containing 1.5 to 12.5 c.c. ammonia per 10000 c.c. of air, over these tomatoes. Graboskiy and Skiff (50) obtained good results for the control of blue moulds and Diplodia rot of Palestinian oranges by inserting ammonium bicarbonate crystals in the wrappers before wrapping the oranges. A certain amount of injury was reported as being caused by the latter treatment, which was, however, more than counterbalanced by the control of the moulds.

Weighted quantities of ammonium carbonate were added to each of a number of wrappers before covering Honab Turki bunches with them. These grapes were packed, stored and examined as usual and the results obtained are presented in Table XLII(b).

The control of Botrytic storage rot of Honab Turki grapes was satisfactory in bunches to which 1 gm. or more of ammonium carbonate was added before wrapping. It was, however, found that the additions of quantities larger than .5 gm. per bunch caused a deadly blackening of the stalks and of the berries

of this variety. The intensity of the blackening increased with an increase of the amount of salt added. This type of injury was very similar to that described by Ramsey and Butler (80) as being caused to onions and fruit by exposure to ammonia gas. The grapes which received 5 to 10 gms. of ammonium carbonate had a distinct ammonia taste. The only bunches which were absolutely normal in appearance and taste were those to which .2 gm of this salt had been added. Where these smaller quantities were inserted, a total volatilization of the salt occurred, leaving practically no trace of its addition.

The danger of overdosing with ammonium carbonate is however so great, especially in the midst of hard-packed packing activities, that is it doubtful whether it can be applied to grapes in practice. Quantities up to 1 gm. per medium-sized bunch may still be used with a fair degree of safety, but the addition of 2 gms. per bunch may be detrimental to the appearance and flavour of Konab Purki grapes.

Dipping.

From the results recorded in table X on the immersion of Botrytis spores in various solutions, it is evident that formalin and potassium permanganate possess the highest degree of toxicity of the chemicals used in tests with conidia of isolate B 1c. These two compounds were then included in tests during the 1933-1934 season to establish whether the dipping of bunches before packing would control Botrytis storage rot of grapes. In these tests ammonia solution was also tried in different concentrations, as well as boiling water, a method said to be in vogue for the home-storage of grapes in Arabia.

The....

The concentrations used are given in table XLIII. Freshly picked and sound Ronab Turki bunches from "The Vineyards" were dipped in these solutions for thirty seconds. Some of the bunches were then dipped in water, and half of every treatment was packed immediately, i.e. while the bunches were still wet, whereas the other half was first allowed to dry before packing.

The results recorded 7 days after cold storage, are given in table XLIII. A fair amount of control of Botrytis storage rot was obtained by dipping the bunches into a 1% ammonia solution. The best results with this solution were obtained when the grapes were first allowed to dry after packing. The same phenomenon was observed in grapes which were immersed in formalin solution i.e. that Botrytis rot was the least in grapes which were dried immediately after immersion in this solution and before packing. Grapes dipped in water after having been in formalin, then dried and packed, were also more severely affected by this rot, than in the previous series, but still less severely than any of the checks. Where the bunches were packed immediately after immersion in formalin, Botrytis infection was particularly high; relatively high also in packs which were dipped in water after the formalin treatment and packed while they were still wet.

The fact that packing of grapes, immediately after dipping in formalin solution and while they were still wet, aggravated rather than alleviated the Botrytis damage trouble, was also clearly demonstrated in Barlinka grapes during the 1934-1935 season. Some of the late crop of Barlinka, grown in Banhoek, near Stellenbosch, were dipped in a 1.6% solution of formalin and packed while still wet. Corresponding checks were packed without having been dipped. The grapes which were dipped in formalin were found to be about twice as heavily infected as the checks, when they were examined 7 days after a three weeks' cold storage.

TABLE XLIII: The effect of dipping bunches of the Henab Turki variety in various solutions on the occurrence of Botrytis rot in storage : Data taken 7 days after cold storage.

Solution	Concentration (%)	Number of			Percentage Botrytis infection	Treatment
		Boxes	bunches	berries		
Ammonia	1	1	8	662	17.4	(a)
		1	7	448	23.7	(b)
		1	7	475	33.9	(c)
		1	8	657	19.9	(d)
	0.5	1	8	662	25.17	(a)
		1	7	602	16.8	(b)
		1	8	671	23.7	(c)
		1	8	670	20.2	(d)
Formalin	0.1	1	8	593	20.5 5.5	(a)
		1	8	592	20.5 20.5	(b)
		1	8	531	20.5 10.7	(c)
		1	8	609	20.5 37.8	(d)
Potassium permanganate	0.1	1	8	741	21.3	(a)
		1	9	659	42.4	(b)
		1	8	704	19.2	(c)
		1	8	611	46.8	(d)
	0.01	1	8	592	33.6	(a)
		1	8	551	29.0	(b)
		1	8	572	38.8	(c)
		1	8	682	45.3	(d)
	0.005	1	9	589	28.0	(a)
		1	8	646	21.8	(b)
		1	9	638	35.2	(c)
		1	8	593	45.0	(d)
Boiling water	-	1	8	673	64.5	(a)
		1	8	533	66.0	(b)
Check	-	1	8	611	34.0	(e)
		1	8	575	74.0	(f)
		1	8	694	28.9	(g)

- (a) - Dried immediately after immersion and before packing.
 (b) - Dipped in cold water and then dried.
 (c) - Packed immediately after immersion and while still wet.
 (d) - Dipped in cold water after the first dipping and then packed while still wet.
 (e) - Dried after dipping in cold water and then packed.
 (f) - Packed immediately after having been dipped in cold water.
 (g) - Packed ordinarily without dipping.

De Villiers (37) reported satisfactory results by dipping grapes in a 5% solution of formalin, but his method is not described. Similarly Boyce, Beyers and de Villiers (10) used a number of dips for different varieties of grapes, but their report lacks descriptions of the method of experimentation adopted, neither is any mention made of the concentrations of dips used. Their data are, therefore, practically of no value for comparative purposes, especially in view of the fact that the procedure of experimentation has such an important bearing on the ultimate results obtained, as is evident from the above experiment.

Dipping grapes in a .1% solution of potassium permanganate, gave a fair amount of control when they were packed without redipping in water. The dipping of grapes in weaker solutions of this chemical apparently resulted in only a very slight control of Botrytis storage rot.

The dipping of bunches in boiling water rather served to increase the amount of Botrytis storage rot of packed Henab Turki grapes. Most probably the immersion caused a decrease of resistance of the grapes to Botrytis infection and this resulted in a higher percentage of wastage. The packing of grapes, which were dipped in cold water, when they were still wet, was found to be definitely detrimental.

The dipping of grapes, intended for export, is however not considered to be applicable in practice, as this method is not only cumbersome, but is also one which will most likely result in a considerable loss in the packed.

Chemically Treated Wrappers.

The investigations of Cooley and Crenshaw (29) revealed that the Botrytis storage rot of pears may be very effectively controlled by using wrappers which were soaked in 2% and 10% solutions of copper sulphate, nickel sulphate, ferrous sulphate, sodium chromate and sodium carbonate, respectively..

respectively. The most satisfactory results were, however, obtained, when the wrappers were soaked in a 2.5% solution of copper sulphate.

It was considered desirable to test the effect of chemically treated wrappers on the occurrence of Botrytis rot in stored grapes. A number of wrappers were thoroughly soaked in each of the following solutions:- 5% copper sulphate, 5% potassium iodide, 1% potassium permanganate and 5% ferric sulphate solutions. The papers were dried after soaking and pressed.

Menab Turki bunches from "The Vineyards" were then wrapped in these papers, packed and examined after cold storage in the usual way. One series was also included in which the grapes were wrapped in ordinary blotting paper instead of any of the said treated wrappers. The results are given in Table XLIV.

When the grapes were examined 7 days after cold storage, it appeared that the amount of Botrytis infection was the least in grapes which were wrapped in ordinary blotting paper. This reduction of Botrytis storage rot, by the use of ordinary blotting paper, might have been due to the fact that blotting paper served to absorb water, which had condensed on the surface of those berries with which it came in contact. This will result in a general drier condition of the grapes and therefore in a reduction of the amount of Botrytis infection. In this connection, it is also of interest to note that Brown (21) reported reduced percentages germination of conidia of Botrytis cinerea in the presence of wet blotting paper or filter paper. This reduction he ascribed to probable volatilization of compounds in the blotting or filter paper which were repressive to the germination of Botrytis conidia.

Bunches, which were wrapped in paper treated with ferric sulphate and potassium iodide solutions were also in

TABLE XLIV : The effect of paper wrappers, soaked in various solutions on the occurrence of Botrytis rot in storage.

Solution in which soaked	Concentration (%)	Number of			Percentage Botrytis infection	Days after cold store.
		boxes	bunches	berries		
Potassium permanganate	1	3	27	2168	77.1	7
		2	18	1032	89.6	14
Potassium iodide	5	2	22	1495	67.6	7
		2	21	1256	96.0	14
Copper sulphate	5	2	23	1595	79.9	7
		2	24	1207	97.0	14
Ferric sulphate	5	2	19	1442	56.9	7
		2	17	1052	93.3	14
Blotting paper	-	2	17	1068	57.7	7
		2	18	870	92.8	14
Check	-	4	33	2417	77.1	7
		3	26	1634	93.7	14

a better condition than the checks, when they were examined 7 days after cold storage. The differences were, however, not perceptible in any of the packs when they were examined 14 days after cold storage. The percentage of Botrytis infections in the latter case were exceedingly high, as is evident from Table XLIV.

This general failure to control Botrytis storage rot through the use of chemically treated wrappers, may be ascribed mainly to the fact that such wrappers come in contact with only a relatively small proportion of the total surface of the berries in a bunch, and then only with the surfaces of the outer berries. The lower middle parts of the bunch, which are usually the centres of Botrytis rot initiation, hardly ever come into direct contact with the wrappers used. Chemically treated wrappers will therefore only be effective against Botrytis storage rot of grapes, if the treatment of the wrappers is such that a continuous flow of gas or vapour of a particular compound, inhibitive to the germination of the conidia of Botrytis and to infection, originates from the paper.

This has been achieved by Tomkins (106) who devised a method of treating wrappers with iodine and potassium iodide in such a manner that iodine volatilizes steadily from the paper, and acts as a disinfectant and a retardant against various types of rot organisms. He also tested these wrappers on grapes and found that mould was greatly reduced in them and that the storage life of grapes was prolonged to about two or three times through the use of these iodized wraps.

A number of ordinary grape wrappers were accordingly treated in the way prescribed by Tomkins (103 p. 314) Barlinka grapes, grown in Banhoek (Stellenbosch), were picked during May 1935, wrapped in these wrappers and packed in the usual way. Sufficient check boxes were kept in which the grapes were wrapped in the ordinary sulphite wrapping....

wrapping paper. The grapes were cold stored for three weeks and examined 10 days after cold storage as in the previous experiments. The results obtained are given in the following table:-

Table XLV: The effect of Iodized wrappers on the percentage Botrytis storage rot in Barlinka grapes, 1934-1935. Data taken 10 days after cold storage.

Wrappers used	Number of			Percentage Botrytis infection.
	boxes	bunches	berries	
Iodized	4	30	2352	19.0
Ordinary sulphite	3	29	2079	42.0

These data show that wrappers treated with iodine and potassium iodide, controlled Botrytis rot in Barlinka grapes from Stellenbosch very satisfactorily. No analysis was however made of the amount of iodine absorbed by the grapes. Grapes wrapped in these iodized wrappers, appeared perfectly normal and no trace of iodine could be detected by taste. It should, however, be noted that on opening a box of grapes, the bunches ^{of} which were wrapped in iodized paper, the general superficial appearance of the packing is not attractive, as most of the woodwool is stained a yellowish iodine tint. Considering the beneficial effects realised by the use of wrappers, treated in this way, and the comparative ease with which they may be used, this method may however be preferred to most of the prestorage treatments found to be effective against Botrytis storage rot.

Sulphur Dioxide Fumigation.

Sulphur dioxide was first used as a preservative of fruit by Barker and Grove in 1934 (4). They found that small percentages of this gas in water were sufficient to preserve fruit, which was intended for jam making or steaming. In 1925 Linkler and Jacob (119) reported that in comparison with boric acid, formic acid, formaldehyde, benzoate of soda and salicylic acid, sulphur dioxide gas yielded the best results for the preservation of Californian grapes during

during storage. Various apparatus which may be used for fumigation of bunches of grapes with this gas were described by Jacob (33) in a later contribution.

In 1927 de Jantelle (26) tested the effect of sulphur dioxide gas on different varieties of Australian grapes, but found that, though this treatment was effective in preventing mould development, the effect of this gas on the flavour and texture of the grapes was marked, and of undesirable nature.

In 1932 Pentzer, Ashbury and Renner (82) described the effects of sulphur dioxide fumigation on different varieties of grapes. They found that the amount of this gas absorbed by and the amount injurious to grapes differed greatly for the different varieties. Immature, or small and less firm berries were found to absorb the gas more readily than mature, or large and firm ones. More gas was also absorbed by grapes at higher temperatures and higher gas concentrations than at lower temperatures and lower gas concentrations. They concluded that 80 parts per million of sulphur dioxide in the tissues was a safer amount for grapes than in the San Joaquin Valley, than 80 to 150 p.p.m. as recommended by Hinkley and Jacob (33, 110) for Californian grapes.

Boyes, Boyers and de Villiers (16) reported sulphur dioxide fumigation experiments, carried out on different varieties of South African grapes during 1933 and 1934. They stated that the amount of sulphur dioxide was only in a few cases sufficient to control rot of grapes, without being injurious to the fruit.

Fumigation experiments were carried out during the 1933-1934 season, to establish the possibility of the application of the sulphur dioxide fumigation practice for the control of Botrytis storage rot.

The chamber in which these and other fumigation experiments (reported in the following section) were carried out, measured 5 ft 6 in 3 ft. and was constructed

of asbestos boards fitted in such a manner that the walls, floor and roof were practically airtight. The sulphur dioxide gas was obtained in a pure state from a sulphur dioxide gas bomb. The outlet of this bomb was connected to an iron cylinder fitted with a valved outlet and a pressure gauge. This latter cylinder measured 12 inches in inner diameter and 4 feet 5 inches in height. The cylinder outlet was connected to the gas chamber through a small circular hole in the wall.

The gas was emitted from the bomb into the cylinder, while the valve of the cylinder outlet was closed, until the desired pressure was recorded on the gauge. The valve of the bomb was then closed, the cylinder valve opened and the gas in the cylinder allowed to flow into the chamber until the pressure on the gauge fell to zero.

To obtain a 1% concentration of sulphur dioxide gas (volumetric percentage) in the fumigation chamber, the required pressure reading on the gauge of the cylinder was determined as follows:-

Volume of fumigation chamber = 216 cubic feet.

Volume of SO_2 to be introduced = 2.16 cubic feet.

(at normal atmospheric pressure).

Volume of iron measuring cylinder = 3.48 cubic feet.

∴ Total Volume of gas (at normal atmospheric pressure) to be introduced into the cylinder, so that 2.16 cubic feet may still be emitted to the fumigation chamber, must be = 5.64 c. ft.

According to Boyle's Law

$$\frac{V_1}{V_2} = \frac{P_1}{P_2}$$

i.e. $\frac{3.48}{5.64} = \frac{14.7}{\pi}$

∴ $\pi = 23.0$ lb. to the square inch,

there.....

where x = the pressure which would be registered if 5.64 cubic feet of gas at 14.7 lb. pressure (normal atmospheric pressure) is forced into 3.48 cubic foot space.

The pressure gauge, being calibrated in pounds per square inch above normal atmospheric pressure (14.7), must therefore register $23.6 - 14.7 = 9.1$ lb. to the square inch in order to emit sufficient gas into the gas chamber to supply a 1% concentration of the gas. The required pressures for obtaining the other percentages of sulphur dioxide gas concentrations in the chamber were calculated in the same way.

Before the introduction of the gas into this chamber, the pipe leading from the cylinder to the fumigation chamber was disconnected, the valve from this cylinder opened wide and sulphur dioxide gas let in slowly into the cylinder from the bomb to displace the air in the former by pure gas. The gas was allowed to run slowly into the ~~chamber~~ ^{chamber} until strong sulphur dioxide gas was emitted from the cylinder for about ten minutes. All the valves were then closed, the tube from the cylinder connected to the gas chamber through the circular hole, which was situated in one corner of the chamber just below the roof. A fan was placed inside the fumigation chamber at a distance of about 12 inches below the gas inlet.

The grapes, used in these experiments, were stacked against the inner side of the chamber opposite to the gas inlet. The boxes were stacked in such a way that the position of each was known. The same arrangement of boxes in the chamber was adopted in all the fumigation experiments during this season. Some of the grapes, included in each fumigation series, were already packed in woodwool in the usual manner and ready for transport. An equal quantity...

quantity of unpacked grapes was also included in each fumigation series. The latter were placed in lugboxes and had previously been thinned and trimmed for packing. After the boxes had been placed in their respective positions the door of the chamber was securely closed, the valve from the cylinder to the chamber closed and the gas allowed to run into the cylinder, until the required pressure was recorded on the pressure gauge. In some cases it became necessary to heat the sulphur dioxide gas bomb with a blow lamp in order to attain the required pressure in the cylinder. This was due to the fact that the temperature of the bomb dropped to such an extent on account of gas expansion that the pressure was not sufficient to raise the pressure in the cylinder sufficiently. As soon as enough gas was let into the cylinder, the valve of the bomb was closed, the fan set in motion and the gas allowed to run from the cylinder into the fumigation chamber, until the gauge indicator reached zero, when the valve governing this inlet was closed. During the introduction of the gas into the chamber, a plug serving as an air escape which had been inserted in a small circular hole on the opposite side of the chamber, was removed. This plug was securely replaced as soon as sulphur dioxide was noticed to escape from this hole.

The fan was allowed to run during the whole period of fumigation of the particular set of grapes. At the end of this period, the chamber was thoroughly ventilated and the grapes removed as soon as possible. The ⁴³packed grapes were packed immediately after fumigation. Separate series of experiments with a particular concentration of sulphur dioxide gas, were conducted for each period of fumigation.

In each series of experiments one box of grapes, fumigated after being packed and which had been placed about midway between the floor and roof of the fumigation chamber.

chamber, was submitted for quantitative sulphur dioxide analysis. The determination of sulphur dioxide was made on the day following fumigation by Messrs. A.H. Sibbe and D.F. Cuthbert using the following method:-

Random samples consisting of 300 gms. weight of berries were selected from the bunches in each box. After crushing these in a mortar, the pulp etc. was transferred to a round-bottomed distilling flask and 10 c.c. phosphoric acid and approximately 1 gm. of sodium bicarbonate added. The pulp and juice was then distilled in the absence of air and the distillate collected in a flask containing freshly distilled water to which 5 c.c. of a $\frac{N}{10}$ iodine solution had been added. The distillation was continued until about 50 c.c. of the distillate has collected, when the latter was titrated with a $\frac{N}{10}$ sodium thiosulphate solution. Every c.c. of $\frac{N}{10}$ iodine solution found to be neutralised by the sulphur dioxide in the distillate was taken to represent .0032 gm. sulphur dioxide. The percentages sulphur dioxide absorbed by the grapes were thence calculated.

The rest of the treated grapes were examined 7 days after cold storage, as in the case of the previous experiments. In this examination, it was however soon apparent that the flavour and colour of some of the treated Henab Turki grapes were very much affected. The sulphur dioxide taste of the different bunches were determined simply by estimation after tasting a few berries from each bunch. Numerical values, indicating the extent to which the normal taste of the grapes were unfavourably affected by this treatment, were allotted to each bunch, and the average for each series obtained. A discolouration standard was first established to determine the percentage discolouration of the berries. This colour standard consisted of eleven berries showing regular differences in degrees of sulphur dioxide discolouration deviating from the natural Henab Turki berry colour to as near to white as could be found. These berries were arranged

in order of increasing intensity of discolouration, their colours then being determined as given below by consulting the colour charts of Ridgway (93). Each bunch was compared with this discolouration standard and the percentage discolouration of the majority of the berries in this bunch determined.

Stage of Discolouration	Estimated percentage discolouration.	Ridgway's colour standard.
Natural mature colour	0	Taupe Brown.
I	10	Dusk Bull Violet (2)
II	20	Anthracene Purple.
III	30	Dull Indian Purple.
IV	40	Dark State Purple
V	50	Deep Purplish Vinaceous
VI	60	Deep Purplish Vinaceous (slightly paler)
VII	70	Deep Hellobore Red.
VIII	80	Vermora Purple
IX	90	Light Corinthian Red.
X	100	Salmon Buff.

These data, together with the percentages Botrytis infection in each series are given in Table XVI. From this the following conclusions may be drawn:-

(a) The percentage of Botrytis storage rot of Hanab Turki grapes was only reduced by fumigating with a 2% and a 3% concentration of sulphur dioxide gas. The amount of Botrytis rot in grapes fumigated with a 1% sulphur dioxide for $\frac{1}{2}$ to 2 hours, and with a 1% concentration of sulphur dioxide for 14 hours (i.e. overnight), respectively, were notably higher than the amount of rot in the check boxes. This higher percentage of infection would indicate that the concentration for, and period of, fumigation was not sufficient to kill the Botrytis spores, but nevertheless sufficient to diminish the resistance of grapes to infection. Grapes, fumigated with these concentrations of sulphur dioxide gas, were flabby, soft and less crisp than untreated grapes. This detrimental effect on the texture of grapes were also mentioned by de Castella (35) in reporting sulphur dioxide fumigation experiments on Australian grapes. It was further...

further found that fumigated grapes were apt to be wet after storage, whereas the checks were comparatively dry.

Both these effects of sulphur dioxide fumigation will result in a higher percentage of Botrytis infection in cases where the concentrations and periods were not sufficient for a proper disinfection of the grapes.

(b) The percentage Botrytis rot in grapes was reduced by lengthening the period of fumigation by a particular concentration from $\frac{1}{2}$ to 2 hours. In this respect, the treatment with a 1% for 2 hours, for reasons unknown, was an exception.

(c) In the majority of cases the amount of Botrytis rot was less in grapes which were fumigated after having been packed, than in those packed after fumigation. This difference may probably be ascribed to the fact that, where packed boxes were fumigated, the woodwool and paper might have absorbed a certain amount of the gas, and were at the same time disinfected. This adsorbed gas might then have been steadily emitted during storage and have had an inhibitory effect on the germination of the conidia of Botrytis and on infection. This may also be the reason why the percentage discolouration of grapes fumigated after having been packed, was generally higher than that of grapes fumigated before packing them. The latter grapes were further again exposed to recontamination by air-borne Botrytis conidia or conidia present in the woodwool and paper. Fish (44) for instance, indicated that granulated cork, used for packing grapes in Australia, may serve as a source of contamination of grapes by various storage rot organisms, including Botrytis cinerea. The same may also be true for woodwool, used for packing grapes in South Africa.

(d) The differences in the amounts of Botrytis rot of grapes in boxes, placed at different levels in the fumigation chamber, were not consistent. Apparently one box for each level was not sufficient to warrant any definite conclusion in this respect.

(c) The....

(c) The amount of sulphur dioxide absorbed by the grapes was exceedingly low, when compared with the absorption figures of Winkler and Jacob (119), Jacob (63), and Fentzer, Ashberry and Hamner (82). This difference is probably mainly due to the fact that the grapes, used in these experiments were analysed for sulphur dioxide absorption 24 - 48 hours after fumigation. Even in these analyses the amount of sulphur dioxide absorbed diminished with the shortening of the period of fumigation. From Table XLVI it is also evident that the percentage of Botrytis infection was less than that of the checks only where the percentages sulphur dioxide absorption was .0009% or more. The amount of Botrytis rot was further in proportion to the amount of sulphur dioxide in the grapes, as is evident from Figure XV.

(f) The amount of Botrytis storage rot was also proportional to the estimated sulphur taste and the percentage discolouration of fumigated grapes, as is shown in this figure.

From the above conclusions, it would appear that sulphur dioxide fumigation is not a safe practice to be applied for the preservation of Henab Turki grapes from the Constantia area. Where too low concentrations were used, or where the periods of fumigation were too short, the wastage was aggravated more than alleviated. Fumigated grapes, showing the same percentage infection as the corresponding untreated checks, were already discoloured to an extent of between 20 and 30% and had a fairly distinct and peculiar sulphurish taste (estimated at 2).

The results obtained are largely in accordance with those of de Castella (35) for Australian grapes. In the light of results of Fentzer, Ashbury and Hamner (82), it may, however, be that some of the other grape varieties, grown for export in South Africa, may be more resistant

to....

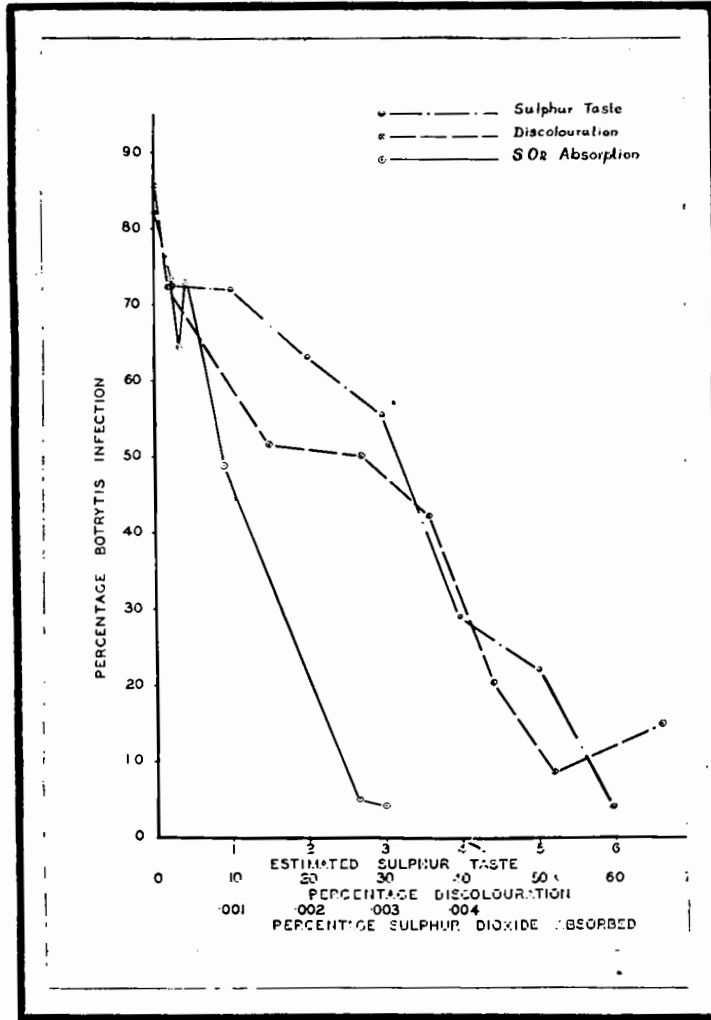


FIG. IV. - The relation of Botrytis infection of ~~Hensab~~ fumigated Hensab Turki grapes to the estimated sulphur taste of these grapes, their percentage discoloration and sulphur dioxide absorption.

to sulphur dioxide injury than the Henab Turki used in these experiments. The authors just mentioned, reported for instance, fairly severe damage of Sultanina grapes, when fumigated with a .63 to 2.0% sulphur dioxide for thirty minutes, whereas Alicante Bouschet and Zinfandel absorbed less sulphur dioxide than the former in similar treatments, and showed practically no injury. Henab Turki is, however, one of the varieties most severely affected by Botrytis rot in South Africa and will need, therefore, a more suitable method of preservation during transport and storage.

Formaldehyde Fumigation.

Fumigation with formaldehyde gas has long since been known to be effective for the disinfection of potato tubers against Rhizoctonia, as discussed by Gloyer (48). The results obtained by him in his disinfection experiments, however, indicated that formaldehyde gas or solution could not be depended upon for a thorough disinfection of potato tubers. Failures in this respect he ascribed to the inability of the gas to penetrate into larger and more compact sclerotia of Rhizoctonia. He also found that, the smaller the amount of potatoes per cubic foot capacity of the fumigation chamber, the greater the efficiency of this treatment.

Formaldehyde gas was however, as far as the author is aware, never applied for the preservation of any of the fruit crops intended for storage. Winkler and Jacob (119) only referred to this gas as having been tried on grapes, but with results less satisfactory than those obtained by sulphur dioxide gas. Experiments are reported below in which the effect of this gas on the occurrence of Botrytis storage rot of grapes and on the appearance of grapes was studied during the seasons 1933-1934 and 1934 - 1935.

The chamber, used for fumigation with this gas, was the same one as was used for the sulphur dioxide fumigation experiments. The formaldehyde gas was obtained by mixing

a 40% solution of formaldehyde (commercial formalin) with potassium permanganate crystals. The particular concentrations of the gas were obtained by using such quantities of formalin and potassium permanganate, which would yield the required concentration (volumetric percentage) of the gas in the chamber at 20°C, when the reaction was completed. This temperature mentioned above was that of the chamber during the major part of these experiments.

During the 1933-1934 season, only Henab Turki grapes were used. Three packed boxes and three unpacked boxes (grapes in lug boxes) were placed in the chamber for each treatment. The boxes were stacked in the same way as in the sulphur dioxide experiments and the position of each box noted. The container used for the generation of the gas was elevated on a stand to about midway between the roof and the ceiling of the chamber and placed against the wall opposite to the grape stacks. An electric fan was placed on a stand next to the container and at such a level that the maximum air current was produced round about the site of gas generation. The fan was set in motion, the desired quantity of formalin poured into the container and a weighed amount of potassium permanganate added. The door of the chamber was immediately closed as securely as possible. After the required period of fumigation, the chamber was well ventilated before the grapes were removed. The unpacked boxes were packed immediately and forwarded with the other boxes of these experimental series to cold storage.

The grapes were examined 7 days after cold storage, when the data recorded in Table XVII were obtained. These data indicate that an excellent control of Botrytis storage rot was obtained by fumigating Henab Turki grapes with from 2% to 6% concentrations of formaldehyde gas. It was also observed that, where these grapes were fumigated for a period of 2 hours with a 4% concentration, and for 1 and 2 hours respectively with a 6% formaldehyde gas concentration,

Table XLV : The effect of Formaldehyde fumigation of Henab Turki grapes on the occurrence of Botrytis rot during storage, experiments 1934 : Data taken 7 days after cold storage.

Concentration (%)	Period of fumigation (hours)	Before or after packing	Position in chamber	Number of boxes			% Botrytis infection
				Boxes	Bunches	Berries.	
2	14	B *	a *	1	8	769	8.7
			b	1	8	570	11.6
			c	1	8	705	3.5
	Average						7.9
	Average	A	a	1	8	664	24.1
			b	1	8	570	31.6
c			1	8	498	10.0	
Average						21.9	
Check	-	-	-	2	16	1085	58.8
4	2	B	a	1	8	579	9.3
			b	1	4	248	5.6
			c	1	8	631	10.8
	Average						8.6
	Average	A	a	1	8	612	5.7
			b	1	4	311	9.3
			c	1	8	556	18.5
	Average						11.2
	1	B	a	1	8	659	13.2
			b	1	4	296	16.9
			c	1	8	653	9.8
	Average						13.3
Average	A	a	1	8	605	9.3	
		b	1	4	274	4.4	
		c	1	8	489	4.9	
Average						6.2	
1/2	B	a	1	8	605	7.9	
		b	1	4	273	13.9	
		c	1	8	413	20.3	
Average						14.0	
Average	A	a	1	8	549	29.7	
		b	1	4	286	11.9	
		c	1	8	573	12.7	
Average						18.1	
6	1	B	a	1	8	694	14.1
			b	1	3	249	6.4
			c	-	-	-	-
	Average						10.3
	Average	A	a	1	8	547	18.2
			b	1	4	244	9.4
c			1	8	635	3.6	
Average						10.4	
1/2	B	a	1	8	705	6.5	
		b	1	4	278	8.6	
		c	1	8	575	22.4	
Average						12.5	
Average	A	a	1	8	536	4.5	
		b	1	4	274	4.0	
		c	1	8	546	4.8	
Average						4.4	
Check	-	-	-	2	15	1061	40.3

* See Table XLVI.

a certain amount of injury was caused to the grapes.

The type of injury observed, very closely resembled that which was described by Stewart and Gloyer (193) on potato tubers and also especially round the lenticels of some apple varieties. Grapes damaged by formaldehyde gas showed small, brownish, sunken and more or less circular spots (Illustrated on Raisin Blanc in Plate VII). These spots were very apt to develop round punctures or cracks, but were seldom very noticeable on a black variety such as Henab Turki. When the injured spots were however situated round about the pedicel end of the berry, their result was of a more serious nature, causing a considerable drop of these affected berries.

At the time of this examination, representative samples from each treatment were analysed for the presence of formaldehyde in the berries. The following qualitative test was carried out by Messrs. A.H. Skibbe and D.F. Cuthbert:-

400 gms. of berries were selected at random from each given sample, crushed in a mortar, the pulp and juice transferred to a 700 c.c. round-bottomed distillation flask and acidified by the addition of phosphoric acid. This acidified mixture of pulp and juice was then distilled and the distillate received in a 100 c.c. glass-stoppered measuring cylinder. When 50 c.c. distillate was received, the cylinder was shaken several times to ensure thorough mixing, 5 c.c. pipetted into a test tube, approximately .03 gm. phenylhydrazin hydrochloride and 3 to 4 drops of a 1% ferric chloride solution added and the tube shaken; 1 to 2 cc. of concentrated sulphuric acid was then slowly added to the mixture, while it was held in a cold water bath and agitated. If formaldehyde was present a red colour would develop in the mixture. It was found that the minimum delicacy of this test for formaldehyde presence was .002%.

Co....

No trace of formaldehyde could however be detected in any of the samples of Henab Turki grapes used in the above experiment, when subjected to this test.

These fumigation experiments with formaldehyde gas were continued during the 1934-1935 season, in order to establish the effect of this gas on Botrytic and other storage rots on various grape varieties and to study the occurrence of formaldehyde injury more closely. In the first series of experiments of this season Red Hanapoot, White Hanapoot and Raisin Blanc grapes were fumigated simultaneously. About 200 lb. of grapes were placed ^(in the chamber) for each treatment. The container in which the formaldehyde gas was generated was again raised on a stand to about midway between the roof and the floor of the chamber. In these experiments, however, this container was placed in the centre of the chamber and the grapes stacked around it, the fan being situated in the same position relative to the container as in the experiments of the previous season. The position of each box in the chamber was noted, the chemicals mixed in the container after the fan had been set in motion, and the door tightly closed for the required period. The grapes, fumigated in lugboxes, were packed immediately after fumigation and one box of each of the packed and unpacked series of every treatment was submitted for qualitative chemical analysis as previously described. These analyses were done within 24 to 48 hours after fumigation.

Henab Turki grapes, also from "The Vineyards", were fumigated a fortnight after the fumigation of the previous three varieties, the procedure of experimentation being adopted as in the former experiments.

These treated grapes were carefully examined 10 to 12 days after cold storage when the results, recorded in Tables XLVIII to LI were obtained. At this examination one box from each treatment was again submitted for a qualitative...

TABLE XLV: The effect of Formaldehyde fumigation on the occurrence of Botrytis and other storage rots of Red Hanepoot grapes : Data 10 to 12 days after cold storage.

Concentration (%)	Period of fumigation (hours)	Before or after packing	Position in Chamber	Number of		Percentage			Cladosporium	
				boxes	bunches	Botrytis	Fenicillium	Rhizopus		
2	14	A*	a*	4	40	3051	-	0.03	-	1.92
			b	4	40	3164	-	-	0.57	1.64
			c	4	41	2917	-	0.21	1.17	1.78
			Average	-	0.08	0.57	1.79	
	B	a	4	42	3478	-	0.06	0.06	0.09	0.32
		b	3	32	2787	-	0.07	0.07	0.54	1.04
		c	6	58	4802	-	0.06	0.06	0.40	0.42
		Average	-	0.06	0.06	0.21	0.54	
	C	a	8	83	5992	0.13	-	-	0.12	2.54
		b	1	11	778	-	-	-	1.29	2.58
		c	3	33	2296	-	0.09	0.09	1.77	1.52
		Average	0.10	0.02	0.02	0.55	2.55	
B	a	6	59	4847	-	0.02	0.02	0.06	0.48	
	b	2	20	1747	-	0.23	0.23	-	1.95	
	c	4	40	3037	-	0.03	0.03	0.03	0.55	
	Average	-	0.06	0.06	0.04	0.77		
A	a	6	59	4578	0.02	0.02	0.02	0.11	3.87	
	b	1	11	763	-	-	-	0.13	1.71	
	c	4	41	3058	-	0.03	0.03	0.07	2.03	
	Average	0.01	0.02	0.02	0.09	3.10		
B	a	2	21	1533	-	0.46	0.46	3.78	1.04	
	b	5	50	3909	-	0.26	0.26	0.38	0.61	
	c	4	38	2924	-	0.82	0.82	5.03	0.51	
	Average	-	0.36	0.36	2.59	0.66		
Check				6	66	4905	0.06	0.12	-	3.62

* See Table XLVI

TABLE 11 : The effect of Formaldehyde fumigation on the occurrence of Botrytis and other storage rots on White Hanepoot grapes : Data taken 10 to 12 days after cold storage.

Concentration (5)	Period of fumigation hours	Before or after packing	Position in chamber.	Number of		Percentage				Cladosporium
				Boxes	bunches berries.	Botrytis	Penicillium	Rhizopus		
2	14	A *	a *	7	65	5918	-	-	0.10	2.01
			b	2	18	1668	-	-	-	0.60
			c	3	29	2495	-	-	0.08	0.92
			Average.....				-	-	0.08	1.51
		B	a	9	94	7761	0.01	0.12	0.21	0.70
			b	2	21	1628	-	0.25	6.58	8.12
			c	3	32	2562	-	0.08	0.73	0.28
			Average.....				0.01	0.13	1.19	1.63
4	2	A	a	7	67	5933	0.27	0.05	0.50	1.66
			b	2	19	1754	-	-	0.06	2.15
			c	3	28	2672	-	-	1.46	1.12
			Average.....				0.16	0.03	0.68	1.61
		B	a	3	33	2537	-	-	0.08,	0.63
			b	5	55	4547	0.20	0.02	0.02	0.15
			c	3	32	2521	-	-	0.16	0.28
			Average.....				0.09	0.01	0.67	0.31
4	2	A	a	4	42	3384	-	-	0.06	0.47
			b	5	54	4362	-	-	-	1.72
			c	4	40	3396	0.06	-	0.06	0.91
			Average.....				0.02	-	0.04	1.09
		B	a	7	77	5782	-	0.69	1.26	0.21
			b	1	11	868	-	-	-	-
			c	1	10	778	-	-	-	-
			Average.....				-	0.54	0.98	0.13
6	1	A	a	4	41	3394	0.24	0.03	0.03	0.29
			b	1	10	877	-	-	-	-
			c	3	30	2446	-	-	-	0.33
			Average.....				0.12	0.01	0.01	0.27
		B	a	4	41	3604	-	-	0.03	-
			b	1	9	868	-	-	2.77	-
			c	3	34	2726	-	-	0.11	0.37
			Average.....				-	-	0.39	0.13
6	1	A	a	3	31	2487	0.28	-	0.16	0.32
			b	1	12	792	-	-	-	0.13
			c	2	22	1690	-	-	0.18	0.41
			Average.....				0.14	-	0.14	0.32
		B	a	5	48	3972	0.03	-	0.10	-
			b	2	17	1433	-	-	-	-
			c	3	31	2539	-	-	-	-
			Average.....				0.01	-	0.05	-
Check				8	80	6822	0.03	0.01	-	5.60

* See Table XVI.

TABEL 2. : The effect of Formaldehyde fumigation on the occurrence of Botrytis and other storage rots on Raisin Blanc grapes : Data taken 10 to 12 days after cold storage.

Concentration (%)	Period of fumigation (hours)	Before or after packing	Position in chamber.	Number of boxes bunches berries	P e r c e n t a g e			
					Botrytis	Penicillium	Rhizopus	Cladosporium
2	14	A *	a *	63	0.08	-	0.45	5.97
			b	33	-	-	-	0.88
			c	30	0.04	-	0.04	1.59
			Average.....	2570	0.05	-	0.24	3.62
2	14	B	a	45	-	0.03	1.63	0.86
			b	36	-	0.27	0.46	0.49
			c	76	0.03	0.06	1.34	0.64
			Average.....	6254	0.01	0.11	1.24	0.67
2	2	A	a	39	0.18	-	2.45	4.66
			b	48	0.07	0.02	0.83	4.99
			c	28	-	0.13	0.09	2.18
			Average.....	2294	0.09	0.04	1.21	4.11
2	2	B	a	41	0.06	0.09	0.89	0.73
			b	32	-	-	0.96	2.22
			c	61	0.02	0.04	0.10	0.69
			Average.....	4794	0.03	0.03	0.50	1.04
4	2	A	a	75	-	-	0.27	3.05
			b	20	-	-	0.06	2.14
			c	31	-	-	0.04	0.54
			Average.....	2383	-	-	0.17	2.27
4	2	B	a	53	-	0.02	1.45	0.48
			b	10	-	-	0.12	0.30
			c	73	-	0.08	0.87	0.69
			Average.....	6108	-	0.05	1.07	0.58
4	1	A	a	44	0.03	-	0.06	1.15
			b	10	-	-	-	0.11
			c	33	-	-	-	0.73
			Average.....	2583	0.01	-	0.03	1.00
4	1	B	a	42	-	-	-	0.05
			b	10	-	-	9.41	0.56
			c	43	-	0.06	4.18	0.58
			Average.....	3229	-	0.03	2.84	0.34
6	1	A	a	33	-	0.04	0.08	0.04
			b	12	0.11	-	-	1.78
			c	22	-	-	0.43	0.55
			Average.....	1634	0.02	0.02	0.17	0.52
6	1	B	a	40	0.03	-	4.74	-
			b	11	-	-	-	-
			c	30	-	0.04	9.50	0.16
			Average.....	2505	0.02	0.02	6.08	0.06
Check				7	0.05	0.03	0.08	6.47

* See Table XLVI.

TABLE 4 : The effect of Formaldehyde fumigation on the occurrence of Botrytis and other storage rots on Henab Turki grapes : Data taken 10 to 12 days after cold storage.

Concentration (%)	Period of fumigation (hours)	Before or after packing	Position in chamber	Number of			Percentage.				
				boxes	bunches	berries	Botrytis	Penicillium	Rhizopus	Cladosporium.	
2	14	A *	a *	3	31	2091	1.00	0.24	0.05	0.19	
				2	19	1425	0.84	-	0.07	0.42	
				2	20	1388	0.87	-	0.07	-	
				Average			0.92	0.10	0.06	0.20	
		B	a	3	21	1887	4.08	0.27	0.11	1.48	
				1	8	575	0.70	0.35	-	2.08	
				2	17	1321	0.61	-	0.30	0.38	
				Average			2.35	0.18	0.16	1.19	
	2	A	a	3	27	2075	0.14	0.05	-	0.68	
				2	18	1412	0.35	-	-	0.14	
				3	32	2191	2.60	0.18	0.18	0.46	
				Average			1.14	0.09	0.07	0.46	
		B	a	2	18	1289	0.78	-	-	0.78	
				1	10	610	0.16	0.16	-	1.31	
				2	18	1355	1.70	0.07	-	0.52	
				Average			1.04	0.06	-	0.77	
4	2	A	a	3	25	2221	0.77	-	-	0.27	
				2	16	1504	1.66	-	0.13	0.53	
				3	25	2179	1.38	-	-	0.27	
				Average			1.22	-	0.03	0.34	
		B	a	2	19	1251	0.96	-	-	0.24	
				2	16	1217	-	0.08	-	-	
				3	24	1748	1.66	0.34	-	0.97	
				Average			0.97	0.17	-	0.47	
6	1	A	a	3	26	1922	1.14	-	-	0.52	
				2	16	1359	5.24	0.88	0.15	0.88	
				3	25	2184	1.28	-	-	0.27	
				Average			2.22	0.22	0.04	0.51	
		B	a	3	25	2016	0.20	-	0.10	0.25	
				1	9	805	0.25	-	-	-	
				2	18	1406	1.56	0.36	-	0.28	
				Average			0.61	0.12	0.04	0.21	
	1	A	a	3	27	2238	0.13	-	0.05	0.05	
				2	19	1502	0.40	-	-	-	
				3	27	2197	0.32	-	-	-	
				Average			0.27	-	0.02	0.02	
		B	a	3	25	1817	0.06	0.11	0.11	0.28	
				1	10	617	0.32	0.32	-	0.32	
				3	24	1881	0.11	-	-	-	
				Average			0.12	0.09	0.05	0.16	
Check				4	35	3388	4.32	0.38	-	-	1.48

* See Table XVI.

qualitative chemical analysis for the presence of formaldehyde.

During the inspection of these grapes a method was evolved by which it was possible to record the varying degrees and intensities of damage to grapes by the formaldehyde gas. One bunch in the middle of every box was selected for these counts, all the berries showing signs of formaldehyde damage removed, and grouped according to intensity of formaldehyde injury into the following categories:-

* Damaged areas less than 1 mm. in diameter (under this category berries showing only a very slight browning of the tissues around the pedicel attachment end of the berry were classed. The latter discolouration was also found to be practically indistinguishable from the ordinary browning as it occurred in those parts of berries of untreated boxes. For the sake of detail this category was however maintained).

++ Damaged areas from 1 - 3 m.m. in diameter.

+++ Damaged areas from 3- 5 m.m. in diameter.

++++ Damaged areas more than 5 m.m. in diameter.

The numbers of berries in each category and the total number of berries in this bunch were determined. These results are graphically presented in Figure XVI.

The percentages of infection by Botrytis, Penicillium, Rhizopus and Cladosporium were however exceedingly low in all the grapes used for these experiments. In reviewing these data, Botrytis, Penicillium and Cladosporium rots appear to have been diminished by fumigation with formaldehyde gas. Rhizopus infection, on the other hand, was present in higher percentages on fumigated than on untreated grapes. Instances were often observed where infection by one of these organisms occurred through damaged areas as is illustrated in Plate VII (b)

The percentages of infection were, however, not high enough to show any distinct differences between the effects

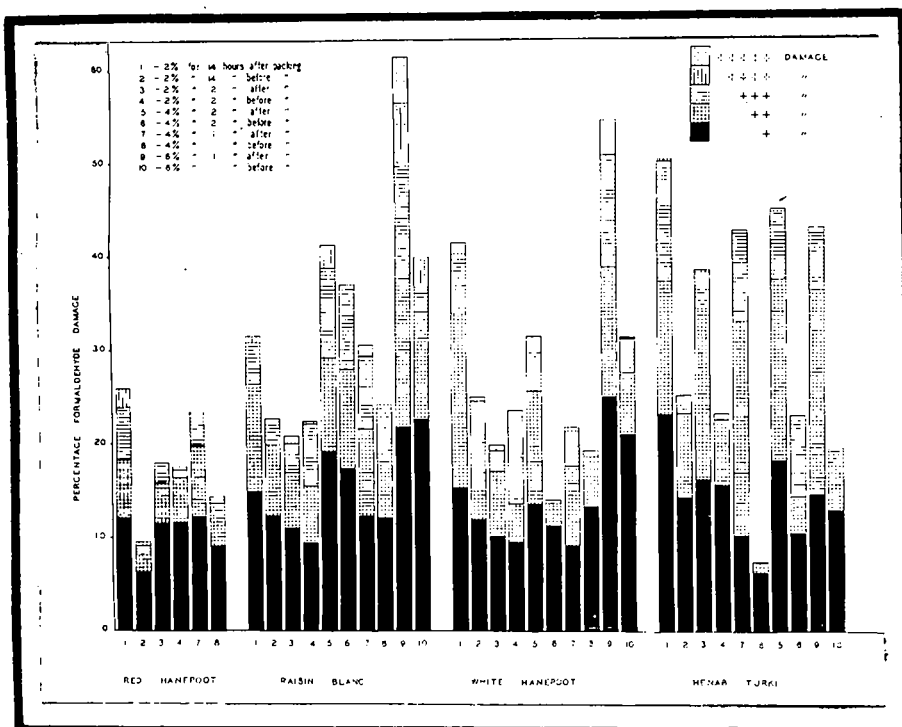


FIG. XVI. - Damage caused by formaldehyde fumigation to Red Hanepoot, Raisin Blanc, White Hanepoot and Henab Turki grapes in the 1935 experiments.

effects of the various concentrations of formaldehyde gas used and the various periods of fumigation. Botrytis control was distinct where a 2% gas concentration was used for 14 hours (overnight) and where 4% and 6% concentrations were used for one hour or more.

From the experiments carried out during these two seasons, it would appear that the control of Botrytic storage rot was generally more effective in grapes fumigated before they were packed. This may be due to the inability of the gas to penetrate into packed boxes in sufficient concentrations to affect control.

From the graphical presentation of the damage inflicted by formaldehyde gas to the different varieties of grapes, it is apparent that Raisin Blanc grapes proved on the whole to be much more susceptible to this type of injury than any of the other varieties tested, and Red Hanepoot the least susceptible. Formaldehyde injury was always the least in grapes fumigated before they were packed.

A 2% concentration of the gas caused a fair amount of injury when used overnight on Herab Turki and White Hanepoot. A fair amount of injury was also recorded on all varieties when they were fumigated with a 4% for 2 hours and with a 6% concentration for one hour.

It transpired during the examination of these grapes, that the occurrence of mechanical damage in grapes ^{was} ~~was~~ very conducive to formaldehyde injury. Many of the injured areas were noticed to have started in a small puncture or crack, the most common of which was the crack round the pedicel attachment of the berry (see Plate VII). It is mainly due to this fact that Raisin Blanc was found to be so severely damaged by the formaldehyde gas treatments.

The.....

The qualitative analyses, made of the treated grapes 24 to 48 hours after treatment and again 12 days after cold storage, all excepting one series showed negative reactions for the presence of formaldehyde in the grapes. A very faint trace of formaldehyde was detected 48 hours after fumigation in a box of Raisin Blanc grapes, which were treated with a 4% concentration of the gas for one hour after the grapes had been packed. The grapes in this box were, however, noticed to have been severely mechanically injured, which may probably account for the absorption of gas by the exposed inner tissues of the berries and for the resulting injured spots. Analyses, made of similarly treated grapes of this variety 12 days after cold storage, yielded negative test reactions.

In all cases the flavour of grapes treated with formaldehyde gas, had not been affected in any way.

From the results of the fumigation experiments with formaldehyde gas on various varieties of grapes from the Constantia area, the following conclusions are drawn:-

(a) The most effective and the safest treatment with this gas is to expose grapes for one hour to a 4% concentration (i.e. 5.8 pints of 40% formaldehyde solution mixed with 46 gms. potassium permanganate for every 1000 cubic feet of space of the chamber).

(b) The fumigation of grapes with this concentration of formaldehyde gas does not appear to be justified in seasons during which Botrytis rot is not apt to occur in fair amounts.

(c) The most satisfactory control of Botrytis and other storage rots and the least amount of formaldehyde damage may be obtained by fumigating the grapes in lugs, after having been cleaned but prior to packing.

(d) Limitation.....

(d) Limitation to a minimum amount of mechanical damage during handling and subsequent packing should be aimed ~~at~~ at, as its occurrence directly influences formaldehyde damage and Rhizopus infection of grapes. Rhizopus rot was apparently less effectively controlled by formaldehyde gas than any of the other storage rots.

(e) Caution should be observed in gassing varieties, such as Raisin Blanc, which are very susceptible to formaldehyde injury. Considering the relative earliness of this variety in the Constantia area, its comparative resistance to Botrytis infection, its liability to mechanically injury and its susceptibility to formaldehyde injury, it is doubtful whether it should be fumigated at all, except perhaps, under conditions extremely conducive to development of Botrytis storage rot.

Formalin Spray.

At "The Vineyards" it had become a regular routine practice to spray the woodwool and sides of the lug boxes, used for picking, with a 4% solution of formaldehyde as soon as they were emptied and before they were again used for picking.

A series of experiments were started during the 1933-1934 season and continued during the 1934-1935 season, to establish the effect of this concentration of formaldehyde solution, applied in different ways, on the amount of wastage, especially Botrytis rot, during storage. Henab Turki grapes from "The Vineyards" were used for these experiments and the formalin sprayed on to the bunches which were either wrapped before packed or packed immediately etc. by means of an ordinary "flit" sprayer. Some of the bunches were not wrapped, but the boxes lined with a fairly even layer of woodwool, which was then sprayed with the solution. The sprayed woodwool was then covered with wrapping paper and the bunches packed flat in the boxes in two layers, almost in the same way adopted for packing x 4% solution of formaldehyde in water. (The 1934-1935 season) pears.....

pears (23). Corresponding checks, in which the grapes were packed similarly but without any treatment with formalin, were kept in each experiment.

Difficulty was experienced in regulating the amount of formalin solution sprayed on to the bunches. No method of measuring the application of small quantities in this form being available, the spray was applied until a fine dewy deposit on the bunches was visible. The wood-wool was sprayed until fairly moist, but not wet.

The results, taken 7 to 8 days after cold storage, of the experiments of these two seasons are recorded in Tables LII and LIII. When the grapes of the 1934-1935 season's experiment were examined, one box of each treatment was again submitted for qualitative chemical tests for the presence of formaldehyde as described for the formaldehyde fumigation experiments.

Owing to the lack of standardization the method of experimentation was not considered entirely satisfactory, but nevertheless the data collected in Table LII reveal some striking results.

The percentage of Botrytis rot on the grapes, packed without being wrapped and in boxes of which the wood-wool linings was sprayed before packing, was the lowest of all the treatments. The control of Botrytis rot obtained by spraying the wrappers, bunches or woodwool in which the bunches were wrapped, were also very satisfactory. The results of the experiments, carried out during the 1934-1935 season also show that the best results for the control of storage rot of grapes were obtained by spraying the woodwool and packing the bunches unwrapped or wrapped. It is at this stage however not possible to express any definite opinion as to whether the former method of packing would be as desirable and attractive as that at present in use for South African grapes.

11 : The Effect of a 4% Formalin spray on the amount of Botrytis rot during storage : Fats taken 7 days after cold storage - 1957 Experiments.

	Bunches wrapped or not wrapped.	Number		of berries	Percentage Botrytis infection
		boxes	bunches		
	wrapped	1	8	673	19.9
	wrapped	1	8	690	22.9
	wrapped	1	3	565	18.0
	not wrapped	1	8	664	7.9
	wrapped	2	16	1085	58.8
	not wrapped	1	9	415	73.2

The effect of a 4% Formalin spray on the amount of Botrytis rot during storage : Data taken 6 days after cold storage - 1935 experiments.

	Bunches wrapped or not wrapped	Number of			Percentage		
		boxes	bunches	berries	Botrytis	Penicillium	Clad sporium
	wrapped	3	24	2304	0.52	-	2.16
	wrapped	4	31	2703	2.62	0.11	5.91
	wrapped(a)	3	24	2039	1.47	0.34	2.31
	Wrapped	3	22	2083	0.53	0.05	0.62
	wrapped(a)	3	22	2020	0.15	0.05	1.88
	not wrapped	3	27	2062	0.19	-	1.79
	wrapped	4	30	2589	1.04	0.19	2.78
	wrapped(b)	4	31	2841	7.77	1.09	6.13
	not wrapped	4	33	2687	13.45	0.15	1.56

(a) - bunches or woodwool allowed to dry before packing.

(b) - bunches sprayed with pure water before packing.

In the experiments of the last season it appeared that there may be a danger in spraying the formalin directly on to the bunches and packing the latter while they are still wet. In such bunches the percentage Gliosporium infection was even higher than in any of the checks. By comparing the different sets of checks, it is apparent that the wetting of bunches with pure water definitely increased the percentage waste, and Botrytis rot especially. This is probably mainly due to the raised humidity and the presence of moisture on the bunches sprayed with water. The use of formalin instead of water, diminished the amount that would have occurred under these conditions, but not sufficiently to compare favourably with the untreated, unsprayed, wrapped grapes in the original checks.

These latter observations are again in accordance with the results obtained in the dipping of grapes in a 0.1% solution of formalin (c.f. Table XLIII).

The danger of wetting grapes by dipping or by spraying with formalin and packing them immediately afterwards, appears to become particularly acute when the weaker solutions of formalin are used. In dipping experiments, carried out on Barlinka grapes from Banhoek (Stellenbosch) during May 1935, the grapes were dipped in a 1.6% formaldehyde solution and packed immediately and while still wet. The grapes of some of the boxes were sprayed with this solution and packed immediately. On examining 7 days after cold storage, it was however found that the treated grapes were about twice as heavily infected with Botrytis as the untreated grapes.

A further disadvantage of spraying the formalin solution directly on to the bunches and packing these while still wet, was revealed in the chemical tests made of the treated Menab Turki grapes from Constantia and referred to above. In these analyses, the grapes treated with formalin solution in this manner were the only ones which showed a distinctly positive reaction for the presence of..

of formaldehyde. It was observed during the latter tests that a number of the berries in this box were cracked or showed considerable skin rupture, particularly in the areas surrounding the pedicel attachments. The qualitative test was repeated on a sample of berries injured in this manner and a separate test made with berries which were sound, unruptured and from the same bunches as the former. The first sample gave a decided positive reaction for the presence of formaldehyde and the second a negative one. Once more these results stress the importance of the occurrence of mechanical injury in grapes.

The flavour and appearance of all the grapes, treated with formalin in the above experiments were absolutely normal and only slight formaldehyde injury was observed on an occasional berry.

In conclusion, it may be stated that Botrytis and other storage rots of grapes may be best controlled by spraying the woodwool lining of grape boxes with a 4% solution of formaldehyde immediately before packing. The formalin may be applied with a "flit" sprayer in such quantities that the woodwool is just moist but not wet. This method was found to be not only one of the most effective for the control of grape wastage, but also safe to use. The possibility of overdoing the spraying of woodwool should, however, be guarded against.

Possibly the use of stronger concentrations of formaldehyde solution would increase the effectiveness of this germicide. Further experiments will however have to be conducted before any definite opinion can be expressed in this respect.

Discussion....

Discussion.

The results of the field tests carried out during the 1933-1934 and 1934-1935 seasons and reported in the second part of this paper, elucidate several interesting features in the occurrence and control of wastage of grapes.

A comparison of the results and observations of experiments of the two seasons reveals that the average percentages of Botrytis infection during the former season was very much higher than those during the latter one. Unfortunately plate exposures were made only during the 1934-1935 season in the vineyard and in the packed at "The Vineyards". From these exposures, it was observed that Cladosporium occurred the most frequently in the air during the early part of the season, Penicillium spp. fairly frequently whereas Botrytis, Rhizopus and Aspergillus were found only in occasional exposures. During the latter part of this season Botrytis occurred more frequently in the plate exposures. The occurrence of these fungi in rotting grapes in storage corresponded more or less with their occurrence in the atmosphere.

The low percentages of Botrytic storage rot during the 1934-1935 season was very likely due to a scant inculum of air-borne Botrytic conidia. As already stated, Horne (53) reported that the fungi causing storage rot of apples were found to be already present on their surfaces when in the orchard. Probably the relatively low rainfall immediately prior to and during the period of experimentation during the 1934-1935 season was mainly responsible for the paucity of Botrytic conidia in the air.

The rate of spore distribution of Botrytis may to a certain extent also account for (a) the steady increase of Botrytic storage rot as the picking seasons advanced, as rains and dews were more frequent during the latter part of the season. (b) The fact that applications of fungicides immediately prior to rain were the most effective for

Botrytis....

Botrytis rot control. (Horne (59) reported that "the fungal numbers recorded after rain were in excess of those recorded for the same plot, 20 minutes earlier by 29.7 per cent"). Fungicides having been applied shortly before the rain are therefore in loco to protect berries against an increase of inoculum. (c) Grapes picked in the afternoon were less heavily infected by Botrytis during the 1933-1934 season, than grapes picked earlier in the day. According to the data supplied by Horne (58), it is evident that fungal numbers were higher at noon than late in the afternoon.

From the results recorded in the foregoing pages, it is also clear that the application of a single dust application and the picking of dusted grapes at the correct time contribute at times more to the control of Botrytis storage rot than the increase of the number of applications of a particular fungicide. Further reductions in the amount of wastage in grapes may be realised by auxiliary measures of practice viz. fertilizing, thinning, time of picking during the day, wilting, handling and packing.

In the discussion of the various field experiments the importance of the occurrence of mechanical damage in regard to the amount of wastage and to wastage control in grapes has occasionally been stressed. Injury to grapes was shown to have a direct influence on the amounts of Botrytis, Penicillium and Cladosporium rots in storage. It has furthermore a diminishing effect on the relative effectiveness of the different fungicides. The occurrence of mechanical injuries not only favoured damage inflicted by formaldehyde gas to grapes, but also promoted the absorption of formaldehyde by the berries during fumigation. Berries, severely injured mechanically, absorbed this gas, whereas sound berries did not absorb any formaldehyde.

It.....

It has also been demonstrated on Raisin Blanc and White Hanepoot grapes, that not all of the mechanical damage observed to occur in grapes after cold storage could be considered as injuries sustained during the packing operations. An appreciable proportion of this damage was also shown to have occurred during handling and transport of the grapes after they were packed.

In the studies of the effect of environmental factors on the amount of wastage and especially on that caused by Botrytis rot of Red Hanepoot grapes in the vineyard and during storage, the results indicated that the physiological condition of the grape berry might have been one of the deciding factors responsible for the reported differences in amounts of Botrytis rots in these grapes. In discussing the susceptibility or resistance of grape berries to Botrytis infection, it should be borne in mind that the problem of infection is mainly one of a two-fold nature, as was pointed out by Zimmerman (123), viz. penetration and invasion.

The researches of Brown (19, 20) and Harvey (22) and of Blackman and Welsford (5) show that Botrytis cinerea may affect its entrance into the host plant through the uninjured cuticular layer mainly by mechanical pressure, exerted by its germ tube on the cuticle of the host. Various factors affecting the resistance of the cuticula and underlying tissues to penetration, have been reported by Brown and Harvey (22). They, for instance, found that exposure of Eucharis leaves to sulphur dioxide or formaldehyde gas, or dipping into boiling water, reduced the resistance of the cuticula of these leaves to such an extent, that they were readily penetrated by Botrytis germ tubes. Under these conditions normal penetration of the cuticula of healthy, intact Eucharis leaves could however not be obtained. By referring to the sulphur dioxide, formaldehyde and dipping experiments reported previously, it is evident that the same diminishing effect in resistance was obtained by similar treatments

treatments to grapes.

It is, however, obvious that, contrary to the results of Brown and Harvey (22), grapes, picked when more or less flaccid and allowed to wilt before packing, were less severely affected by Botrytis storage rot than those picked in the early morning and packed immediately, i.e. when the berries were most turgid. This apparent difference is however most likely due to the greater tendency of turgid berries to be mechanically injured than the flaccid berries, as was indicated by de Villiers (37). Further this difference in results may also be due to the greater abundance of Botrytis conidia during the earlier part of the day than during the latter part.

No reference to direct experimental evidence could be found to indicate that the increase of nitrogen definitely softens and thins the cuticular layer of plants. Moynake (95) concluded that increases in nitrogen in plants result in increased rates of Anabolic and metabolic activities in plants. This results in an increased influx of sap into the berries and in a distension of the cell walls and cuticular covering. This will result not only in a larger grape berry, but in one less resistant to breakdown and decay. The effect of thinning of the cuticular layer, may also favourably affect penetration by germ tubes of Botrytis cinerea.

Zimmerman (123) however considered that the evidence in literature of the effect of the thickness of epidermal and cuticular layers on the susceptibility of Botrytis infection were insufficient to decide whether such a relation actually exists.

If berries are mechanically injured, the penetration difficulty of Botrytis germ tubes through the cuticula are however eliminated and direct invasion of the underlying tissues becomes possible.

That....

That the chemical composition of fruit may directly influence the rate of invasion of particular fruit by fungi, has been shown by various investigators. The results, reported by Horne (59, 60), tend to show that the nitrogen content of apples directly influenced the rate of invasion of these apples by pathogenic fungi. Chona (27) demonstrated that Botrytis cinerea and other fungi exhibited increased virulence when nitrogenous nutrient is added to the inoculum. This addition even led Fusarium coeruleum, normally non-parasitic on apple, to show a certain amount of invasion of inoculated apples. Vasudeva (111) also obtained invasion of apples by Botrytis Allii by similarly adding a trace of nitrogenous nutrient to the inoculum.

From the results obtained in the fertilizer experiments a direct relation was also shown to exist between the nitrogen content of grape berries and the amount of wastage occurring in them during storage.

Brown (19) concluded, however, that the cell wall affords the key of resistance to attack by Botrytis cinerea. This fungus was demonstrated by Brown (19) and by Blackman and Telford (8), Weiner^w and Harter (55), Chona (27) Kenon (74) and others, to secrete a pectinase enzyme which acts on the pectin compounds of the cell walls of host tissues and cause their disintegration. Paul (81) was unable to correlate the enzymatic activities of various strains of Botrytis cinerea with their respective virulence.

From the results, obtained by Vasudeva (111) and by Chona (27), it would appear that the addition of a nitrogenous nutrient to inocula of Botrytis Allii and Fusarium coeruleum might possibly have affected favourably their capabilities of secreting pectinase resulting in increased pathogenetic activities on apple tissue. Both these authors found that the resistance of apples to an attack by these, fungi, normally non parasitic on apples, decreased as the host ripens.

Reynske (95) gave a brief summary of literature dealing with the hydrolysis of protopectin and a resultant weakening of cell walls of fruits during ripening. The cell walls afford therefore less resistance to fungous attack with a resultant increased susceptibility to invasion.

The increase in susceptibility during ripening has also been found to occur in grapes. Hanab Turki grapes from "The Vineyards" were picked in two different stages of maturity during the 1933-1934 and 1934-1935 seasons. The "green" grapes were still slightly undercoloured round the pedicel ends and had a slightly acid flavour. The "ripe" grapes were fully coloured and tasted decidedly sweet. They were packed, cold stored and examined as in the foregoing experiments. The chemical analyses were made according to methods described by Reynske (95). The results of experiments of these two seasons are presented in Table LIV.

From this Table it is evident that the riper grapes were more severely attacked by Botrytis, Penicillium and Cladosporium storage rot than the greener grapes. The comparison of the "total acid" and "hydrogen-ion concentration" readings of the grapes at these two stages with results of laboratory tests with Botrytis isolates, makes it doubtful whether any of these two factors could account for the difference in infection of grapes picked at these two stages. Botrytis isolates grow fairly well on media between pH 5 and pH 7 and their conidia germinated appreciably even in media containing 3% malic or tartaric acid. They were, however, more sensitive to the presence of oxalic acid.

The glucose, soluble nitrogen, acidity and pH readings of the juice of berries of different grape varieties at various stages of ripeness, according to the analyses of Copeman (30) and Copeman and Frater (31) were at all stages such as to allow growth of Botrytis in this juice.

The....

Table LIV : Effect of stage of ripeness on the occurrence of rotage in grapes and chemical analysis of Juico of the grapes at the time of picking.

Stage of ripeness	Number of bunches		Percentage infection by			Percentage total solids.	Total acid	Specific gravity	Hydrogen-ion concentration (pH)	Season	Days after cold storage
	Berries		Botrytis	Penicillium	Glabrosporium						
Ripo	8	499	84.2	-	-	16.0	15.6	-	-	1934	7
Green	8	741	48.1	-	-	15.1	16.4	-	-	1934	7
Ripo	7	575	92.0	-	-	16.0	15.6	-	-	1934	14
Green	6	617	71.8	-	-	15.1	16.4	-	-	1934	14
Ripo	8	701	9.54	1.21	2.42	17.62	19.13	232	3.67	1935	10
Green	8	661	2.00	0.56	0.29	15.42	18.6	254	3.55	1935	10

The results of Hartor and Feimer (55) on pectinase produced by Asizopus tritici and of Menon (74) on that produced by Botrytis cinerea and other fungi indicate that the substrate influences the production of this enzyme considerably. The results of the latter however also show that the activity of the pectinase enzyme of B. cinerea decreases with the increase in the pH value of the substrate.

The total solids and specific resistance readings of these grapes, when compared with those of Royncke (95) show that the "ripe" grapes used in these experiments were already slightly overripe. The percentages total solids of the grapes in these experiments were higher in the "ripe" than in the "green" grapes, i.e. respiration had very likely proceeded to such an extent in the berries at the time of picking, that the amount of respiratory products liberated in the juice showed an increase. In the 1934-1935 experiments, the specific resistance was found to be lower in the "ripe" than in the "green" berries, i.e. more electrolytes were liberated into the juice of the ripe berries at this time as a result of respiration than in that of the "green" berries, with a resultant increased electrolytic conductivity of the juice of the "ripe" berries. It has already been shown that it is during this process of katabolic activities in fruit that the pectin of cell walls undergo hydrolyses, and is therefore weakened.

The difference in susceptibility of different grapes appear also to be closely related to the resistance of the cell wall to the pectinase action of pathogenic fungi, this resistance being less in grapes in which this destruction has advanced somewhat.

In connection with the resistance of uninjured grape berries to attacks, by Botrytis and other fungi, it would appear that the nature of the cell wall is of more importance than the actual chemical constitution of the berry.

SUMMARY.

The wastage, and especially Botrytis rot, of export grapes in South Africa has been studied in detail in the laboratory and in the field.

One of the methods of Dickinson (33) for single spore isolation was modified and a small apparatus constructed which proved very efficient for making single spore isolations of all the cultures studied. The types of wastage caused by the following fungi are described, viz. Penicillium cyclopoium, P. olomatum, P. expansum, Aspergillus carbonarius, A. niger, Rhizopus nigricans, Fusarium oxysporum var. aurantiacum, Cladosporium baccae, Sphaeropsis alorum and Botrytis cinerea.

Comparative culture studies were undertaken of seven monospore isolates of Botrytis cinerea: B 1b, B 1c, B 11a and B 12c from grapes, B 1e from pear; B 6d from apples and B 8c from quince. It was found that they exhibited distinct differences in growth rate, colony characters, conidial and sclerotial production. No appreciable difference in the effect of hydrogen-ion concentration of the medium on the germination and growth of conidia of the four monospore cultures from grapes could be detected. The changes in acid reaction of the medium, brought about by these four cultures, appeared to be dependent mainly on the constituency of the medium. Their conidia were able to germinate in media containing 3% malic or tartaric acid, but did not germinate even in 1% oxalic acid. Apparently these isolates of Botrytis cinerea differed somewhat in their sugar requirements. All cultures showed a fair amount of growth in media containing 45% mallose.

The seven isolates of Botrytis showed significant differences in length and (or) width of conidia, but these measurements did not vary in the same sequence on each of the media used. It would appear that, for the purpose of distinguishing.....

distinguishing these isolates by the size of their spores, they must be cultivated on at least three different media.

The fungicidal effect of various solutions ^{was} tested on conidia of the isolate, B 1c, from which it appeared that the conidia of this isolate were particularly sensitive to potassium permanganate and to formalin solutions, and fairly sensitive to hydrochloric acid.

From pathogenetic studies of the seven isolates of Botrytis on Delicious and Hekwood apples, these cultures could be classified into four groups of pathogenetic activity.

In the discussion of the results of the laboratory studies, it was concluded that the differences observed between the seven isolates of Botrytis were sufficient to consider them as being different varieties of Botrytis cinerea.

Field studies on the wastage of export grapes were conducted on four farms in the Constantia area during the 1933-1934 and 1934-1935 seasons. Botrytis rot of grapes appeared to ^{be} the most common type of wastage encountered during the first season of the investigation. During the 1934-1935 season, however, the percentage of Botrytis rot was relatively low during the early part of the season, but increased with the advance of the season and proportionately more than any of the other rots encountered.

Botrytis infection of grapes in the vineyard was found to be favoured by long durations of high humidities. In these studies the physiological condition of the grapes, suggested itself as one of the main factors influencing the occurrence of Botrytis rot in the vineyard and in storage.

Very small bunches with few and large berries and very large bunches with many and small berries were found to be comparatively more severely affected by Botrytis storage rot (as indicated by the "Tendency of Infection")

than...

than medium sized bunches. Too severe as well as no thinning is hence not desirable and medium thinning is recommended.

The amounts of Botrytis, Penicillium and Cladosporium rots were shown to increase directly with the increase in amount of mechanical injury to the grapes. Tests on Raisin Blanc and White Hanepoot, however, indicated that mechanical damage found on grapes after storage, were not wholly caused during packing; but that a fair amount was also inflicted during handling and transport subsequent to packing.

Heavy or late applications of nitrogenous fertilizers and to a certain extent the application of potassic^s fertilizers, increased the susceptibility of Menab Turki grapes to wastage infection. Phosphatic^{fertilizers} on the other hand increased the resistance of grapes to wastage infection. The amount of wastage was also found to vary indirect proportion with the amount of nitrogen in the berry.

The least amount of Botrytic rot occurred in grapes picked during the afternoon and packed the following day.

The use of the so-called "Buller caps" was found to be ineffective for the control of Botrytic storage rot.

In the interpretation of the results of the dusting and spraying experiments, the data were simplified by calculating an "Index of Control" for each fungicide for the particular type of storage rot. Verderamo sulphur dust yielded the best results for the control of Botrytic storage rot of grapes in extensive experiments, whereas Copper Sulphur dust showed the best control for the Penicillium rots. No evidence to suggest control of any of the other more frequent types of wastage of grapes could be obtained.

The relative efficiency of fungicides was found to decrease with the increase of the amount of mechanical damage

in....

in packed grapes. The effect of mechanical damage on the efficiency of fungicides for the control of Botrytis rot was also demonstrated by comparing the effects of these applications on the position of rot initiation in the grape bunches.

The period between the application of Copper sulphur dust and subsequent rain was found to affect the efficiency of this fungicide very distinctly; the shorter this period the greater the efficiency. The effectiveness of this fungicide was also influenced by the period elapsing between rain and the picking of the treated grapes; the longer this period, the greater being the effectiveness of the fungicide applied. Dusted grapes should be picked not less than two or three days after a rain, dependent on the amount of rain. The beneficial effect of dusting and picking of treated grapes at the correct time appears to be especially marked during seasons conducive to Botrytis rot development.

The efficiency of copper sulphur dust increased with the increase in the number of applications, but, during moderately dry seasons, it would hardly be worth while to apply more than one or two well-timed dust applications.

The effect of adding various quantities of sodium metabisulphite and ammonium carbonate crystals to Kenab Turki bunches before wrapping them as tested. The danger of overdosing, especially with the latter was, however, found to be such that it is doubtful whether this method of combating wastage of grapes can be applied in practice.

The dipping of bunches into various solutions was not considered to be of very much practical value, though fairly effective control of Botrytis rot was obtained by dipping into formalin and ammonia solutions and allowing them to dry before packing.

Wrappers,...

Wrappers, soaked in solutions of potassium permanganate, potassium iodide, copper sulphate and ferric sulphate, respectively hardly had any effect in reducing the amount of Botrytis rot of grapes during storage. Iodized wrappers, prepared according to the formula of Tenkins (103), however reduced the amount of Botrytis rot considerably and can be recommended as one of the methods easily applicable and fairly effective.

The effect of sulphur dioxide gas on the appearance and flavour of denab Turki grapes from the Constantia area was found to be so undesirable that it cannot be recommended for the control of wastage of grapes from this area.

Fumigation with formaldehyde gas yielded promising results, though a certain amount of injury was caused by this gas to grapes. Raisin Blanc grapes were found to be particularly susceptible to formaldehyde damage. The most satisfactory fumigation treatment was found to be with a 4% (volumetric) concentration of formaldehyde for one hour. Mechanical damage promoted not only formaldehyde injury to the grapes, but also facilitated the absorption of this gas by the grapes.

Satisfactory results were obtained by spraying the bunches, wrappers or woodwool with a 4% solution of formaldehyde in water. The wetting of bunches with this solution was to an extent dangerous as the packing of wet grapes proved to be conducive to wastage at all times. The safest method appeared to be the spraying of the woodwool linings of boxes moderately with this solution just before packing. This method proved to be particularly effective if the grapes are packed without being wrapped.

Ripe, and especially overripe, grapes were found to be very much more susceptible to Botrytis and other storage rots than greener grapes. The practice of delaying the packing of late grapes as much as possible, in order to tender for the late overseas market, is therefore to be discouraged.

LESKULING IN AFRICAANS;

Ondersoekings oor die Vrotting van Uitvoer-tafeldruive, veral met betrekking tot die veroorsaak deur Botrytis cinerea, Pers.

Die vrotting van uitvoer-tafeldruive in Suid-Afrika, en veral die veroorsaak deur Botrytis, is noukeurig bestudeer in die laboratorium en in die wingerd.

Een van die methodes, soos beskrywe deur Dickinson (33) vir die isolasie van enkel spore, is gewysig en 'n klein apparaat (sien Figuur 1) is saargestel, waarmee dit moontlik was om enkelspoor isolasies vry gemaklik te maak van al die swamkulture wat bestudeer is. Die tipes van vrotting, veroorsaak deur die volgende swamme is beskrywe: Penicillium cyclopoium, P. elongatum, P. expansum, Aspergillus carbonarius, A. niger, Rhizopus nigricans, Fusarium oxysporum var aurantiacum, Cladosporium baccac, Sphaeropsis Malorum en Botrytis cinerea (sien Plate I en II).

Vergelykende kultuurstudies is gemaak van sewe verskillende enkelspoor kulture van Botrytis cinerea : B 1b, B 1c, B 11a, en B 12c uit druive geïsoleer, B 4c, uit pere, B 6d uit appels en B 8c uit kersers. Dit is gevind dat hierdie sewe kulture duidelik van mekaar verskil in groeisnelheid, in kultuurseienskappe, in konidia en in sclerotia produksie (Plate IV en V). Geen noemenswaardige verskil kon opgemerk word in die invloed van die H⁺-konentrasie van die voedingsbodem op die ontwikkeling en groei van spore van die vier enkelspoor kulture uit druive nie. Die spore van al vier was in staat om te ontwikkel in voedingsbodem bevattende 3% appelsuur of wynsteensuur, maar geen ontwikkeling het plaasgevind in solke 'n 1% oksaaluur nie. Hierdie kulture van Botrytis cinerea het blykbaar iets van mekaar verskil in hulle suikerverdragsvermoë. Al die kulture het egter nog matig goed gegroei in voedingsbodem wat 45% maltose bevat het.

Dio..

Die verskille tussen die lengtes en (of) breedtes van spore van die sewe kulture van Botrytis was statisties meetbaar, maar hierdie sporemate het nie in dieselfde volgorde van mekaar verskil op elkeen van die voedingsbodems wat/op die spore geëra is nie. Dit blyk uit die bevindings dat die sporemate van hierdie kulture op tenminste drie verskillende voedingsbodems verkry moet word, ten einde hulle van mekaar hiervolgens te kan onderskei.

Die swamdodende werking van verskillende oplossings is uitgetoets op spore van kultuur B 1c, waaruit geblyk het dat hierdie spore veral gevoelig is vir kalium permanganaat en vir formaline oplossings en taamlik gevoelig vir soutsuur.

Deur spore van die sewe kulture van Botrytis in te ont in Delicious en Hokewood appels en in Berlinke druiwe, was dit moontlik om genoemde kulture in vier groepe van parasitiese aktiwiteit in te deel, nl.

Betreklik sterk parasities	B 4c
" matig sterk parasities	B 11a en B 12 c
" matig swak parasities	B 1b en B 6d
" swak parasities	B 1c en B 8c

By die bespreking van die resultate wat in die laboratorium verkry is, is dit becluit dat die verskille wat hier opgemerk is tussen die sewe kulture van Botrytis voldoende is om hulle te beskou as behorende tot verskillende variëteite van Botrytis cinerea.

Veldproewe in verband met die verrotting van uitvoerdruiwe is uitgevoer op vier plase in die Constantia strek gedurende die 1932-1934 en die 1934-1935 seisoene (Fig. V: A, B, C, D). Gedurende ooreenstemmende seisoen was Botrytis verrotting van druiwe die mees algemene tipe van bedorf wat voorgekom het. Gedurende die 1934-1935 seisoen, egter, was die persentasie Botrytis verrotting in opgebergde druiwe betreklik laag gedurende die vroeë seisoen, maar hierdie verrotting het geleidelik toegeneem, en in 'n meerdere mate as enige van die ander tipes van bedorf wat aangesitref is.

Dit is gevind dat aanhoudende weerstoestande van hoë humiditeit besmetting van druiwe met Botrytis in die wingerd begunstig het. Uit hierdie ondersoekings het dit ook geblyk dat die fisiologiese toestand van die druiwe een van die faktore is wat die grootste invloed uitoe^fen op die voorkoms van Botrytis verrotting in die wingerd en gedurende opberging (Fig. VI).

Dit is gevind dat baie klein druiwetrosse met min en groot korrels, asook baie groot trosse met baie maar klein korrels, die ^gerste deur Botrytis aangetas word (soos aangedui deur die "Tondens van Besmetting") (Fig. VII). Te strawe uitdunning, sowel as geen uitdunning is dus nie wenslik nie en 'n matige uitdunning van druiwe word aanbeveel.

Die persentasies besmetting met Botrytis, Penicillium en Cladosporium vermeerder in direkte verhouding tot die vermeerdering in die mate van meganiese beskadigings op druiwe (Fig. IX). Proewe met "Aisin Blanc en Wit Kanepoot het egter aangetoon dat die meganiese beserings wat op druiwe aangetref word na opberging nie net veroorsaak word tydens hulle verpakking nie, maar dat 'n aansienlike mate van beserings ook opgedoen word gedurende hantering en vervoer, nadat die druiwe gepak is.

Swaar of laat toedienings van Stikstofbevattende kunsmiste, en tot 'n sekere mate ook toedienings van potasbevatende kunsmiste, verhoog die vatbaarheid van Honab Turki druiwe vir bederf. Fosfaatbevattende kunsmiste, daarenteen, verhoog die weerstand van druiwe teen verrotting. Dit is ook gevind dat die persentasie bederf varieër in direkte verhouding met die hoeveelheid stikstof in die korrel.

Die minste Botrytis verrotting is aangetref in druiwe wat gedurende die agtermiddag gepluk en die volgende dag gepak is (Fig. X).

Geen beheer van Botrytis verrotting kon verkry word deur die gebruik van die sogenaamde "Buller Keppies" nie.

Tensinde die resultate wat verkry is in die stuif- en spuitproewe behoortlik na te gaan, is dié gegewens vereenvoudig

deur 'n "Tabel van Beheer" te bereken vir elke swamdoder vir die bepaalde tipe van verrotting gedurende opberging. Verderome swavel stof het Botrytis verrotting gedurende opberging die beste beheer (Fig. XI), terwyl Koperswavel stof Penicillium verrotting die beste beheer het. Geen aanduiding is gevind om aan te toon dat enige van die ander meer algemene tipes van bederf deur hierdie toedienings vermindert is nie.

Dit is gevind dat die relatiewe doeltreffendheid van swamdoders vermindert namate die hoeveelheid meganiese beskadiging van gepakte druiwe toeneem (Fig. XII). Die invloed van meganiese beskadiging op die doeltreffendheid van swamdoder vir die beheer van Botrytis verrotting is ook aangedui deur 'n vergelyking van die invloed van sulke toedienings op die posities van beginpunte van verrotting in die druiwetrosse.

Die periode wat verloop tussen die toediening van Koperswavelstof en daaropvolgende reën het 'n baie duidelike invloed op die doeltreffendheid van hierdie swamdoder. Hoe korter hierdie periode is, des te meer doeltreffend is die swamdoder. Die doeltreffendheid van hierdie swamdoder word ook beïnvloed deur die periode wat verloop tussen 'n reën en die tyd wanneer die bestuifde druiwe gepluk word, hoe langer hierdie periode, des te groter die doeltreffendheid van die toegediende swamdoder (Fig. XIV). Bestuifde druiwe behoort nie voor twee of drie dae na 'n reën gepluk te word nie, afhanklik van die hoeveelheid reën. Die voordeel van die bestuiving en die pluk van bestuifde druiwe op die regte tyd, blyk veral groot te wees gedurende seisoene met toestande wat baie gunstig is vir die voorkoms van Botrytis verrotting.

Die doeltreffendheid van koperswavelstof neem toe met die vermeerdering van die aantal toedienings, maar dit sal skaars die moeite werd wees om meer as een of twee

maal op die regte tye te bestuur gedurende matige droë seisoene.

Die invloed van die byvoeging van kristalle van natruim metabisulfaat en ammonium karbonaat in verskillende hoeveelhede by Henab Turki trosse, voordat hulle geogedraai word, is uitgetoets. Die gevaar van byvoeging van te veel, van veral laasgenoemde, sout is egter so groot dat dit twyfelagtig is of hierdie metode gebruik kan word in die praktyk vir die bestryding van verrotting van druiwe gedurende opberging.

Die indoping van trosse in verskillende oplossings is beskou as 'n metode van min praktiese waarde, hoewel taamlike bevredigende beheer van Botrytis verrotting verkry is deur die indoping in formaline en in ammonia oplossings en die trosse eers te laat droog word voor verpakking.

Die gebruik van toedraai papier, geweeke in kalium jodied, kopersulfaat en ystersulfaat oplossing onderskeidelik, het skaars enige verminderende invloed op Botrytis verrotting gehad. Papier, wat behandel is met jodium en kalium jodied, soos aangegee deur Tomins (193), het die hoeveelheid Botrytis verrotting egter aansienlik verminder en kan dit aanbeveel word as een van die metodes wat maklik toegepas kan word en ook taamlik doeltreffend is.

Dit is gevind dat beroking met swavel dioksied gas so'n ongunstige uitwerking het op die voorkoms en ssaak van Henab Turki druiwe van die Constantia streek (Fig. XV), dat dit nie aanbeveel kan word vir die beheer van verrotting van druiwe uit hierdie streek nie.

Die beroking van druiwe met formaldehiede gas het belowende resultate gelewer, alhoewel hierdie gas die druiwe tot 'n mate beskadig het (Fig. XVI). Dit is gevind dat Raicin Blanc druiwe veral gevoelig is vir formaldehiede beskadiging (Plaat VII). Die mees bevredigende resultate is verkry deur beroking met 'n 4% (volumeries) konsentrasie...

konentrasie formaldehid vir een uur. Meganiese beserings op druiwe het nie alleen formaldehiede beskadiging op druiwe bevorder nie, maar het ook die absorpsie van hierdie gas deur druiwe vergemaklik.

Bevredigende resultate is verkry deur die druiwe-trosse, die toedraai papier of die houtwol met 'n 4% oplossing van formaldehiede in water te bespuit. Deurdát die verpakking van nat druiwe ten alle tye gevind is om verrotting gedurende opberging te bevorder, kan dit soms gevaarlik wees om hierdie oplossing direk op die druiwe-trosse te spuit. Die veiligste metode is blykbaar om die houtwol uitvoerdel van kassies matig met dié oplossing te bespuit voor verpakking. Hierdie metode is veral effektief as die druiwe hierin gepak word sonder dat die trosse toegedraai word.

Ryp, en veral oorryp, druiwe is baie meer vatbaar vir besmetting met Botrytis en ander verrottingsorganismes as groener druiwe. Dit is dus nie raadsaam om die verpakking van laat variëteite tot so lank as moontlik uit te stel, tensinde die laat markte oorsae te vang nie.

L I T E R A T U R E.

1. Anonymous: Cape Grapes. Agric. Jour., Dept. of Agric., Cape of Good Hope, XIII, p.332, 1898.
2. Anonymous: Summer rains. Agric. Jour., Dept. of Agric., Cape of Good Hope, XVIII, p. 590, 1903.
3. Anonymous: Cape fruit in London. Agric. Jour., Cape of Good Hope, XXIII, pp. 404-403, 1908.
4. Barker, B.T.P. and Grove, O.: Sulphur dioxide as a preservative for fruit. Annual Rep., Agric. and Hort. Soc. Stat., Long Ashton, 1924, pp. 97-103.
5. Beauverie, J. and Guillermond, A.: Etude sur la structure du Botrytis cinerea. Centralbl. f. Bakt., X, pp. 275-281 & 311-320, 1903.
6. Berkeley, G.H.: Studies on Botrytis. Trans. Roy. Canadian Inst., XV, pp. 83-127, 1924.
7. Borzo Aguilera, J.M.: Memoria de las experiencias o investigaciones realizadas en la estacion de patologia vegetal de Almeria durante el ano 1925, sobre la Ceratitis capitata y otras enfermedades de las uvas de Chancoc. 54 p., Almeria, Spain. (From Rev. of Appl. Myc., VI, pp. 73-77, 1927).
8. Blackman, V.H. and Teleford, E.J.: Studies on the physiology of parasitism II. Infection of Botrytis cinerea. Ann. Bot., XXX, pp. 389-392, 1916.
9. Bonnet, L.C.: Enemies of the flower and fruit. Calif. Grape Grower, March 1923, pp. 6-7.
10. Boyes, J.S.; Beyer, E. and de Villiers, D.J.R.: Preliminary experiments on the control of wastage of table grapes. Rep. Low Temp. Res. Lab., Cape Town, Dept of Agric., Union of S.A., pp. 94-95, 1935.
11. Boyle C.: Studies in the physiology of parasitism X. The growth reactions of certain fungi to their staling products. Ann. Bot., XXXVIII, pp. 113-135, 1924.
12. Erieryley, E.B.: The microconidia of Botrytis cinerea. New Bull. Misc. Inf., pp. ~~129~~ 129-146, 1919.
13. _____: Biological races in fungi and their significance in evolution. Ann. Appl. Biol., XVIII, pp. 420-434, 1931.
14. Brixi, H.: Über die Fäulnis der Rebenriebe, durch Botrytis cinerea verursacht. Centralbl. f. Bakt., III, pp. 141-146, 1897.
15. Broadfoot, F.C.: Studies on the parasitism of Fusarium lini, Bolley. Phyt., XVI, pp. 951-978, 1923.
16. Brooke C. and Cooley, J.S.: Time-temperature relations in different types of peach-rot infection. Jour. Agric. Res., XXXVII, pp. 507-544, 1908.
17. Brooke, C. and Fischer, D.F.: Transportation rots of stone fruits as influenced by orchard spraying. Jour. Agric. Res. XII, pp. 467-477, 1921.
18. Brooke, F.T.: Observations on the biology of Botrytis cinerea. Ann. Bot., XXII, pp. 473-487, 1908.

19. Brown, J.: Studies in the physiology of parasitism I. The action of *Botrytis cinerea*. Ann. Bot., XLII, pp. 313-348, 1915.
20. _____ : do. III. On the relation between the "Infection Drop" and the underlying host tissue. Ibid. XXX pp. 329-403, 1913.
21. _____ : do. IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ibid., XLVI, pp. 285-300, 1922.
22. Brown and Harvey, E.C.: do. X. On the entrance of parasitic fungi into the host plant. Ibid, XLI, pp. 643-652, 1927.
23. Eubner, R.J.: Packing succiduous fruit for export. Jour. Dept. of Agric., Union of S.A., XII, pp. 507-513, 1923.
24. Buason, H.: Biologische studien mit *Botrytis cinerea*. Flora, CXL/CXII, pp. 645-620, 1918.
25. Eutler, E.J.: The delimitation of species of fungi on physiological grounds. Proc. Int. Congr. of Plant Sc., II, pp. 1590-1597, 1929.
26. Shaddock, R.E.: Principles and methods of Statistics. Houghton Mifflin Co., New York, 1926.
27. Chonn, B.L.: Studies in the physiology of parasitism. XIII. An analysis of the factors underlying specialization of parasitism, with special reference to certain fungi parasitic on apple and potato. Ann Bot., XLVI, pp. 1033-1050, 1932.
28. Collison, R.C. and Marlan, J.D.: Fertilizer responses of Baldwin apple trees on an acid soil. New York Agric. Exp. Stat. Bull. No. 646, pp. 1-24, 1934.
29. Cooley, J.S. and Crenshaw, J.H.: Control of *Botrytis* rot of pears with chemically treated wrappers. U.S. Dept of Agric. Circ. No. 177, pp. 1-9, 1931.
30. Coneman, F.R.v.d.R.: An investigation into some physiological and chemical changes occurring in grapes during ripening. Dept. of Agric., Union of S.A. Sc. Bull. No. 20, pp. 1-33, 1924.
31. Coneman, F.R.v.d.R. and Arator, G.: Some physical and chemical changes occurring during the ripening of grapes. Dept. of Agric., Union of S.A., Sc. Bull. No. 50, pp. 1-54, 1926.
32. Das Gupta, S.T.: Studies in the genera *Cytosporina*, *Phenopsis* and *Diaporthe*. III On the pathogenicity of *Cytosporina* and its saltants. Ann. Bot., XLVII, pp. 197-223, 1933.
33. _____ : do. IV. On the pathogenicity of certain strains of *Phenopsis* and *Diaporthe*. Ibid XLVII, pp. 385-400, 1933.
34. de Bary, A.: Comparative morphology and biology of the fungi, Mycetozoa and bacteria. Clarendon Press. Oxford, 1887.
35. de Castella, F.: Fresh grape export investigations. Jour. of Dept. of Agric., Victoria, Austr., XLIV, pp. ~~325-330~~, 1927.

Reynolds (95) gave a brief survey of literature dealing with the hydrolysis of protopectin and a resultant weakening of cell walls of fruits during ripening. The cell walls afford therefore less resistance to fungus attack with a resultant increased susceptibility to invasion.

The increase in susceptibility during ripening has also been found to occur in grapes. Harsh Turkî grapes from "the Vineyards" were picked in two different stages of maturity during the 1933-1934 and 1934-1935 seasons. The "green" grapes were still slightly undercoloured round the pedicel ends and had a slightly acid flavour. The "ripe" grapes were fully coloured and tasted decidedly sweet. They were packed, cold stored and examined as in the foregoing experiments. The chemical analyses were made according to methods described by Reynolds (95). The results of experiments of these two seasons are presented in Table LIV.

From this table it is evident that the riper grapes were more severely attacked by Botrytis, Penicillium and Glehnaria storage rot than the greener grapes. The comparison of the "total acid" and "hydrogen-ion concentration" readings of the grapes at these two stages with results of laboratory tests with Botrytis isolates, makes it doubtful whether any of these two factors could account for the difference in infection of grapes picked at these two stages. Botrytis isolates grew fairly well on media between pH 3 and pH 7 and their conidia germinated appreciably even in media containing oxalic or tartaric acid. They were, however, more sensitive to the presence of oxalic acid.

The glucose, soluble nitrogen, acidity and pH readings of the juice of berries of different grape varieties at various stages of ripeness, according to the analyses of Hopson (30) and Hopson and Lester (31) were at all stages such as to allow growth of Botrytis in this juice.

The....

33. de Istvanffi, Gy.: Etudes microbiologiques et mycologiques sur le rot gris de la vigne (*Botrytis cinerea* - *Sclerotinia fuckeliana*). Annuaire de l'Inst. Centr. Agricol. Royal, Hongrie, CXI, p. 163, 1905. (Fren Centralbl. f. Lakt., XVII, pp. 260-269, 1907).
37. de Williera, F.J.: Physiological studies of the grape. Dept. of Agric. Union of S.A., Sc. Bull. No. 45, pp. 1-97, 1923.
38. _____: Studies of grapes in cold storage. S.A. Jour. Nat. Hist., VI, pp. 315-329, 1929.
39. Dickinson, S.: The technique of isolation in microbiology. Phyt., XXIII, pp. 367-367, 1933.
40. Doidge, H.H.: A preliminary check list of plant diseases occurring in South Africa. Bot. Survey of S.A. Mem. 6, pp. 1 - 56, 1924.
41. Dreyer, D.J.: Guide to the grading of grapes for export. Dept. of Agric., Union of S.A., Bull. 67, 4 p., 1931.
42. du Plessis, S.J.: Spraying experiments for the control of the anthracnose disease of almonds. S.E. College of Agric., Union of S.A., Bull. No. 60, pp. 1-9, 1932.
43. _____: Botrytic rot of grapes, and its control during 1933-1934. S.E. College of Agric., Union of S.A., Farmer's Bull. No. 65, pp. 1-6, 1934.
44. Fish, S.: A grape export problem. Microfungi on granulated cork. Jour. Dept. of Agric., Victoria, Austr., XXIV, pp. 316-318, 1925.
45. _____: Grape export problem. Jour. Dept. of Agric., Victoria, Austr., XXV, pp. 31-39, 1927.
46. Fischer, A.: Fruit export. Agric. Jour., Dept of Agric., Cape Colony, 1, pp. 33-34, 51, 67, 75, 76, 1920.
47. Kulten H.R. and Norman, J.J.: Effect of spraying with fungicides on the keeping quality of Florida citrus fruits. U.S. Dept of Agric., Circ. No. 409, pp. 1-13, 1927.
48. Glover, H.O.: The efficiency of formaldehyde in the treatment of seed potatoes for *Rhizoctonia*. New York Agric. Exp. Stat. Bull. No. 370, pp. 419-431, 1913.
49. Grantor, K. and Horna, A.S.: A method of inoculating the apple Ann. Bot., XXXVIII, pp. 212-215, 1924.
50. Granovsky, A. and Schiff, H.: The effect of ammonium bicarbonate on the storage of oranges. Madar, VII, pp. 152-172, 1934 (from Rev. Appl. Myc., XIV, pp. 33-31, 1935).
51. Gregory F.C. and Horna, A.S.: A quantitative study of the course of fungal invasion of the apple fruit and its bearing on the nature of disease resistance. Part 1. A statistical method of studying fungal invasion. Proc. Roy. Soc. E., CII, pp. 427-443, 1928.
52. Hahn, P.D.: Viticulture of the colony. Cape of Good Hope, Official Yearbook, pp. 233-278, 1883.

53. Hansen, H.H. and Smith, R.H.: Interspecific anastomosis and the origin of new types in imperfect fungi. *Abs. Phyt.*, XXIV, pp. 1144-1145, 1934.
54. _____: The mechanism of variation in imperfect fungi: *Botrytis cinerea*. *Phyt.* XXII, pp. 953-964, 1932.
55. Harter, L.L. and Reimer, J.L.: Influence of the substrate and its hydrogen-ion concentration on pectinase production. *Jour. Agric. Res.*, XXIV, pp. 831-878, 1923.
56. Harvey, C.C.: Studies in the genus *Fusicarium* VII. On the different degrees of parasitic activity shown by various strains of *Fusicarium fructigenum*. *Ann Bot.*, XLIII, pp. 245-259, 1929.
57. Hawkins, L.A.: Growth of parasitic fungi in concentrated solutions. *Jour. Agric. Res.*, VII, pp. 255-260, 1916.
58. Horne, A.S.: Biological work on fruit. *Rep. Food Invest. Board*, 1930, pp. 162-172.
59. _____: Biological work on fruit. *Rep. Food Invest. Board*, 1931, pp. 272-280.
60. _____: Biological work on fruit. *Rep. Food Invest. Board*, 1932, pp. 279-300.
61. _____: Biological work on fruit. *Rep. Food Invest. Board*, 1933, pp. 223-245.
62. Horne, A.S. and Gregory, F.G.: A quantitative study of the course of fungal invasion of the apple fruit and its bearing on the nature of disease resistance. Part II. The application of the statistical method to certain specific problems. *Proc. Roy. Soc. B*, CII, pp. 447-466, 1928.
63. Jacob, H.E.: The use of sulphur dioxide in shipping grapes. *Univ. of Calif.*, *Bull. No. 471*, pp. 1-24, 1923.
64. Keitt, G.W. and Jones, Leon E.: Studies of the epidemiology and control of apple scab. *Wis. Agric. Exp. Stat.*, *Res. Bull. No. 73.*, pp. 1-104, 1923.
65. Kirchner, O.: Bericht über die Tätigkeit der Kgl. Anstalt für Pflanzenschutz in Hohenheim im Jahre 1923, pp. 1-19.
66. Klebahn, H.: Zur Kenntnis einiger *Botrytis*-formen vom Typus der *Botrytis cinerea*. *Zeitschr. für Bot.*, XXIII, pp. 251-272, 1930.
67. Kramer O.: Die Stiel- und Blattpilzkrankheiten der Reben und ihre Bekämpfung. *Der Weinbau*, p. 159, 1923.
68. Lafforgue, G.: Le *Botrytis cinerea*. *Rev. de Vitic.*, XXXIX, pp. 245-254, 1913.
69. Letcher, H. and Illman, J.S.: Biochemistry of plant diseases VIII. Alcoholic fermentation of *Fusicarium lini*. *Phyt.*, XVI, pp. 841-849, 1923.
70. Levine, H. and Schoenlein, H.S.: A compilation of culture media Bailliere, Tindall & Co., London, 1930.

71. Levine, M.H.: Biometrical studies on the variation of physiological forms of *Fuccinia graminis tritici* and the effects of ecological factors on the susceptibility of wheat varieties. *Phyt.*, XVIII, pp. 7-124, 1928.
72. Lyon, T.L., Fieppin, E.O. and Buckman, H.O.: Soils, their properties and management. The Macmillan Co., New York, 1924.
73. McAlpine, D and Robinson, G.H.: Additions to the fungi on the vine in Australia. Dept. of Agric. Victoria, Melbourne, 1897.
74. Menon, K.P.V.: Studies in the physiology of parasitism. XIV. Comparison of enzymic extracts obtained from various parasitic fungi. *Ann Bot.* XLVIII, pp. 187-210, 1934.
75. Holz, E.: Über die züchtung widerstandsfähiger sorten unserer kulturpflanzen. *Zeitschr. für Pflanzenzüchtung*, V. pp. 121-224, 1927.
76. Moore H.H.: Spraying trials against pear scab (*Venturia pirina*). Some practical and theoretical aspects of the interpretation of the results. East Malling Res. Stat. Annual Rep. for 1932, pp. 99-108, 1933.
77. Moreau, L. and Vinet, E.: Le mildiou. Evolution et traitements en 1927. Conclusions pratiques. *Rev. de Vitic.*, LXVIII, pp. 255-258; 269-274, 285-287, 1928.
78. Morquer, R.: ^SConsiderations biologiques sur les variations du *Botrytis cinerea* et spécialement sur une nouvelle forme pathogène pour les cucucules. *Bull. Soc. Hist. Nat. Toulouse*, LXV, pp. 603-617, 1933.
79. Müller-Thurgau, H.: Die edelfaule der trauben. *Landw. Jahrb.*, XVII, pp. 83-160, 1888.
80. Palmiter, D.H.: Variability in monoconidial cultures of *Venturia inaequalis*. *Phyt.*, XXIV, pp. 22-47, 1934.
81. Paul, W.R.C.: A comparative morphological and physiological study of a number of strains of *Botrytis cinerea*, Pers. with special reference to their virulence. *Trans. Brit. Myc. Soc.*, XIV, pp. 118-135, 1929.
82. Pentzer, V.T., Ashbury, C.E. and Hamner, K.C.: Effects of different varieties of *Vinifera* grapes with sulphur dioxide gas. *Proc. Amer. Soc. Hort. Sc.*, XXIX, pp. 339-344, 1932.
83. Perold A.I.: The principal diseases of our vineyards. Cape of Good Hope, S.A., *Agric. Jour.* XXXVII pp. 370-377, 1910.
84. _____: Handboek oor Wynbou. Pro Ex^{CS}lesia Drukkery, Stellenbosch, 1926.
85. Pendron, C.H.: *Synopsis methodica fungorum*. Göttingae, 1801
86. _____: *Mycologia Europaea*. Erlangae, 1822.
87. P.M.O.: Diseases in grape. *Agr. Jour. Dept. of Agric.*, Cape of Good Hope, VII, p. 507, 1894.
88. Powell, G.H.: The decay of oranges while in transit from California U.S. Dept of Agr., *Bull. No. 123*, pp. 1-79, 1908.
- 89.

89. Putterill, V.A.: Plant diseases in the Western Cape Province. Jour. Dept of Agr., Union of S.A., VII, pp. 332-333, 1923.
90. Rambooy, G.B. and Bufler, L.F.: Injury to onions and fruits caused by exposure to ammonia. Jour. of Agric. Res., *xxxviii*, pp. 339-348, 1928.
91. Ravaz, L.: Chronique. Prog. Agric. et Vitic., XL, pp. 533-538, 1923.
92. Ravaz, L. and Gouirand, G.: Recherches sur le traitement de quelques maladies de la vigne. 1. Pourriture grise (*Botrytis cinerea*). Rev. de Vitic., VI, pp. 101-103, 1896.
93. Reidemeister, J.: Die bedingungen der sklerotien- und sklerotienbildung von *Botrytis cinerea* auf kunstlichen nährböden. Ann. Mycol., VII, pp. 19-44, 1909.
94. Reinecke, V.: A soil utilisation study of the Constantia area. Thesis, Univ. of Stellenbosch, S.A., 1935.
95. Reyncke, J.: Studies in verband met die goedhou-vermoë van vrugte. Thesis, Univ. Stellenbosch, S.A., 1934.
96. Ridgeway, R.: Color standards and color nomenclature. Washington, (C.D.), 1892.
97. Ritter, G.E.: Ammoniak und nitrate als stickstoffquelle für schimmelpilze. Ber. Dtsch. Bot. Ges., XXVII, pp. 582, 1909.
98. Schmidt, E.W.: Die fungizide wirkung von seifenlösungen. Ber. Dtsch. Bot. Ges., XLIII, pp. 131-135, 1924.
99. Schneider-Orelli, O.: Versuche über die wachstumsbedingungen und verbreitung der fäulnispilz des lagerobstes. Centralblatt f. Bakt., XXXII, pp. 161-169, 1912.
100. Smith, R.E.: The parasitism of *Botrytis cinerea*. Bot. Gaz., XXXIII, pp. 421-433, 1902.
101. _____: *Botrytis* and *Sclerotinia*: their relation to certain plant diseases and to each other. Bot. Gaz., XXIX, p. 369, 1900.
102. Snyder, H.C.: Variability in the pea-wilt organism, *Fusarium orthoceras* var. *pisii*. Jour. Agric. Res., XLVII, pp. 65-88, 1933.
103. Stewart, F.C. and Gloyer, C.O.: The injurious effect of formaldehyde gas on potato tubers. N.Y. Agric. Exp. Stat., Bull. No. 339, pp. 385-416, 1913.
104. Stummor, A.: In gaze eingebeurelte trauben sind *Peronospora* fest. Zeitschr. f. Pflanzenzuchtung, X, pp. 468-469, 1925.
105. Tomkins, R.G.: The prevention of mould on stored fruit by use of gases and volatile substances. Dept. Sc. and Indus. Res., Rept. Food Invest. Board for the year 1932, pp. 65-66, 1933.
106. _____: Iodized wraps for the prevention of rotting of fruit. Jour. Pom. and Hort. Sc., XII, pp. 311-320, 1934.
107. Tomkins, R.G. and Trout, S.A.: The use of ammonia and ammonium salts for the prevention of green mould in Citrus. *Ibid*, IX, pp. 257-234, 1931.

108. van der Dyl, P.A.: Observations on a fungus (*Cephalosporium Sacchari* Butler) which causes a red-rot of sugar-cane stems. Dept. of Agric., Union of S.A., Sc. Bull. No. 11, pp. 1-8, 1919.
109. Van Niekerk : The wastage in export grapes. Jour. Dept. of Agric., Union of S.A., VII, pp. 19-22, 1926.
110. Van Wyk, S.P.: Die uitvoer van Suid-Afrikaanse somervrugte. Thesis, Univ. van Stellenbosch, S.A., 1933.
111. Vasudeva, R.S.: Studies in the physiology of parasitism XI. An analysis of the factors underlying specialization of parasitism, with special reference to the fungi *Botrytis Allii*, *Munn*, and *Monilia fructigena*, Pers. Ann. Bot., XLIV, pp. 469-493, 1930.
112. Verwoerd, L. and Dippenaar, B.J. : Descriptions of some new species of South African fungi and of species not previously recorded from South Africa. S.A. Jour. of Sc., XXVII, pp. 326-330, 1930.
113. _____ : On the occurrence of a berry wilt and rot of grapes (*Vitis vinifera*) caused by *Sphaeropsis malorum*, Berk.; Dept. of Agric., Union of S.A., Sc. Bull. No. 86, pp. 1-16, 1930.
114. von Tubeuf, K.F. and Smith, W.G.: Diseases of Plants. Longmans, Green and Co., London, 1897.
115. Webb, R.W. : Studies in the physiology of the fungi X. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Ann. Miss. Bot. Gard., VIII, pp. ~~191-200~~ 201-222, 1919.
116. Weiner, J.L. and Harter, L.L.: Glucose as a source of Carbon for certain sweet potato storage-rot fungi. Jour. Agric. Res., XXI, pp. 189-210, 1921.
117. _____ : Hydrogen-ion changes induced by species of *Rhizopus* and by *Botrytis cinerea*. Jour. Agric. Res., XXV, pp. 155-164, 1923.
118. Westerdyk, J.: Die Grage der *Botrytis cinerea* und ihrer verwandten. Mededeeling van het Phyt. Lab. "Willie Commelin Scholten", Baarn, X, pp. 35-36, 1927. (from Contr. f. Bakt., LXXIII, p. 435, 1928).
119. Winkler, A.J. and Jacob, H.E.: The utilization of sulphur dioxide in the marketing of grapes. Hilgardia, 1, pp. 107-131, 1925.
120. Woodring, J.C.: Control of vine diseases and pests occurring in New Zealand. N.Z. Jour. of Agric., XXXV, pp. 298-309, 1927.
121. Young, H.C. and May, C.: The timing of apple scab sprays. Ohio Agric. Exp. Stat. Bull. No. 403, pp. 1-28, 1927.
122. Zacharowits, E.: Pourriture grise. Comment la combattre. Prog. Agric. et Vitic., CII, pp. 229-231, 1934.
123. Zimmerman, A.: Sammelreferate über die Beziehungen zwischen parasit und wirtspflanze. Nr. 3. *Sclerotinia*, *Monilia* und *Botrytis*. Centralbl. f. Bakt., LXX, pp. 51-86, 261-313, 411-436, 1927.

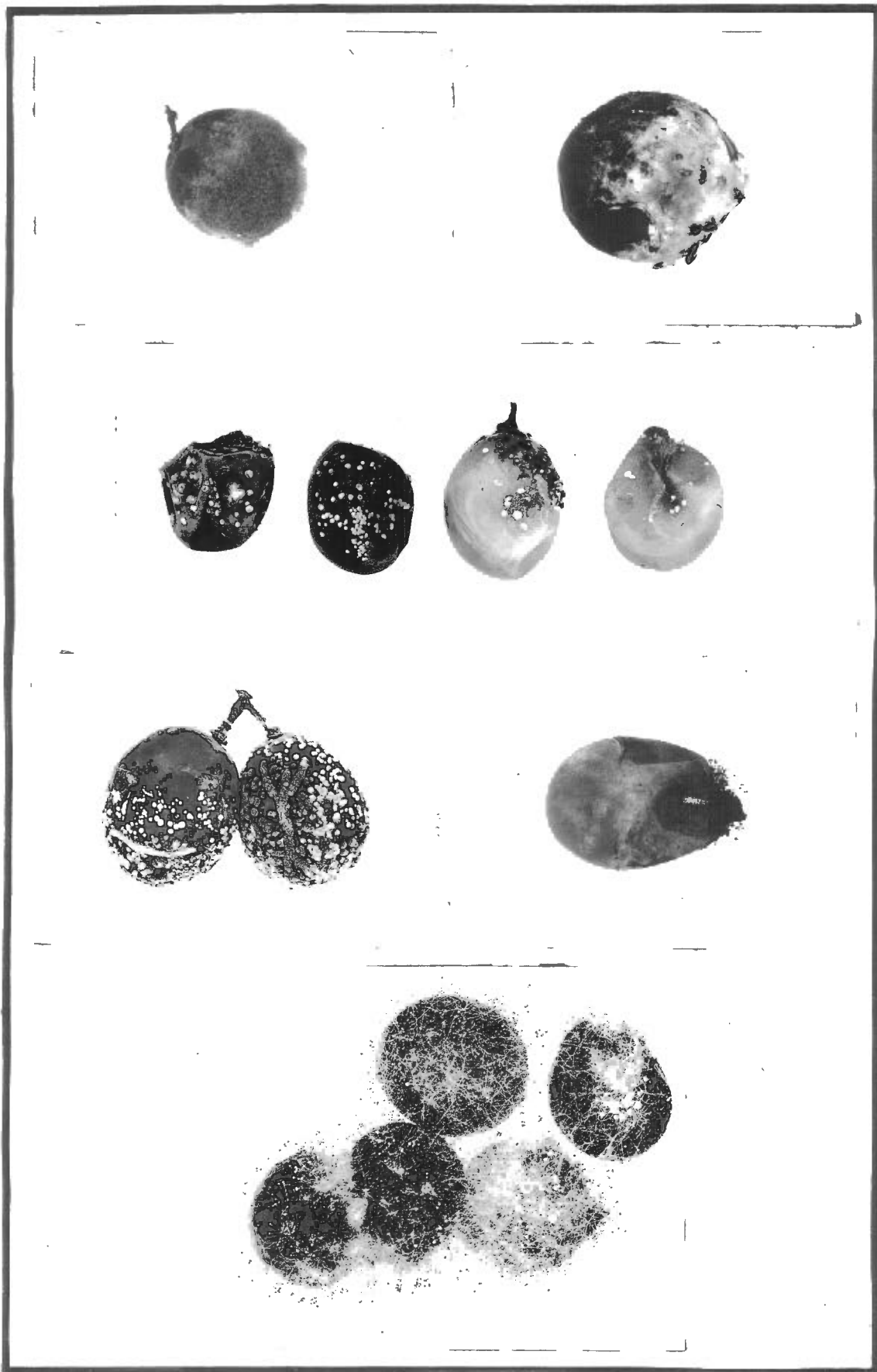


PLATE 1. - Grape wastage organisms. A and B - berries infected by Botrytis cinerea; C - Benicillium ^{Paro.} eructosum, Thom; elongatum, Dierckx; D - Penicillium ^{LLK} expansum, Thom; E - Aspergillus niger, v. Tiegh. schiemani (Schieman) Thom and F - Rhizopus nigricans, Ehr. infection. of grape berries during cold storage.

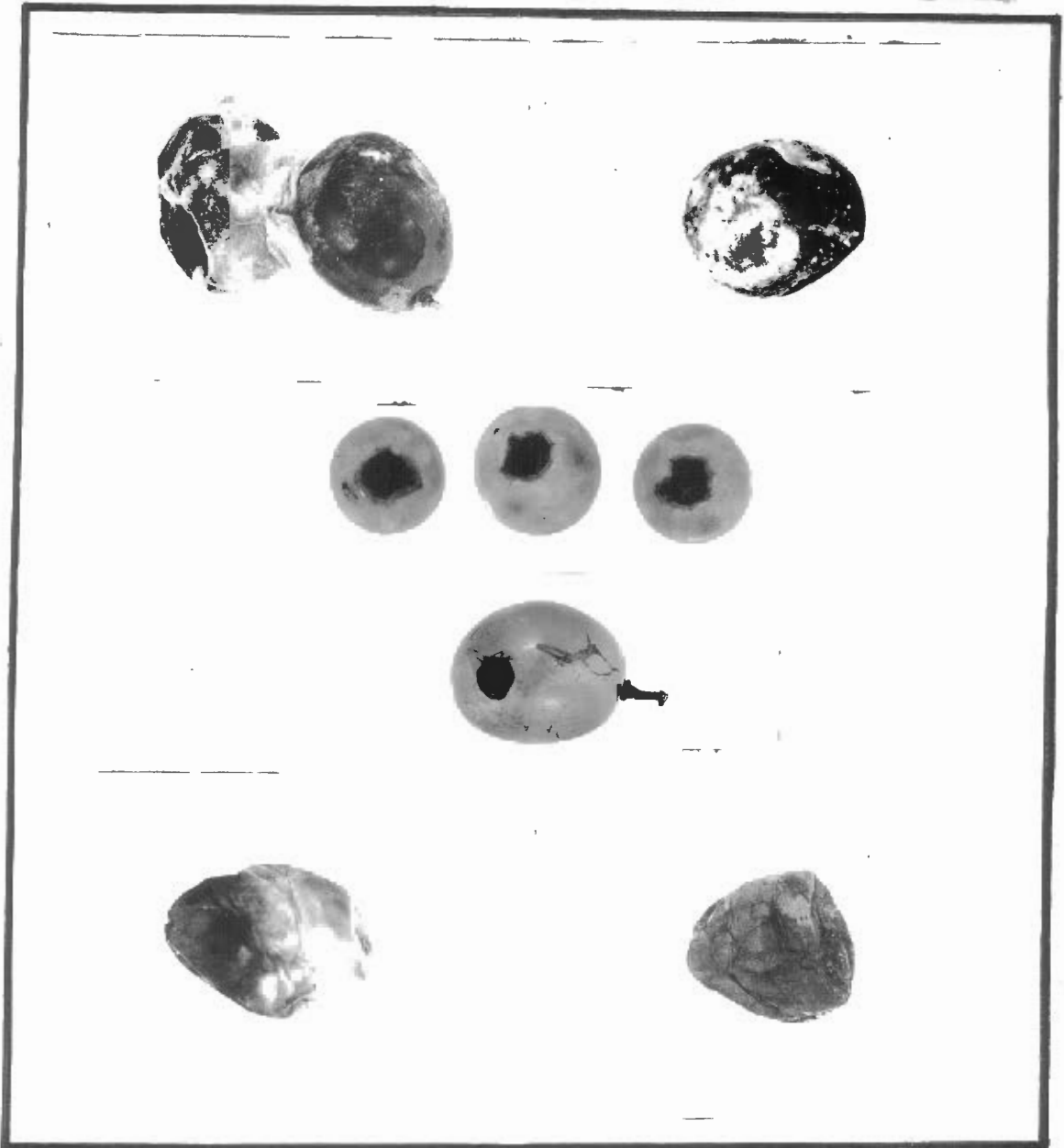


PLATE 11. - Grape wastage organisms. A and D - infections by Sphaeropsis Malorum, Berk.; B - Fusarium oxysporum, Schl. var. aurantiacum (Lk.) Wr.; C - Cladosporium baccae, Verw & Dipp.; E - Saccharomyces on grape berries.

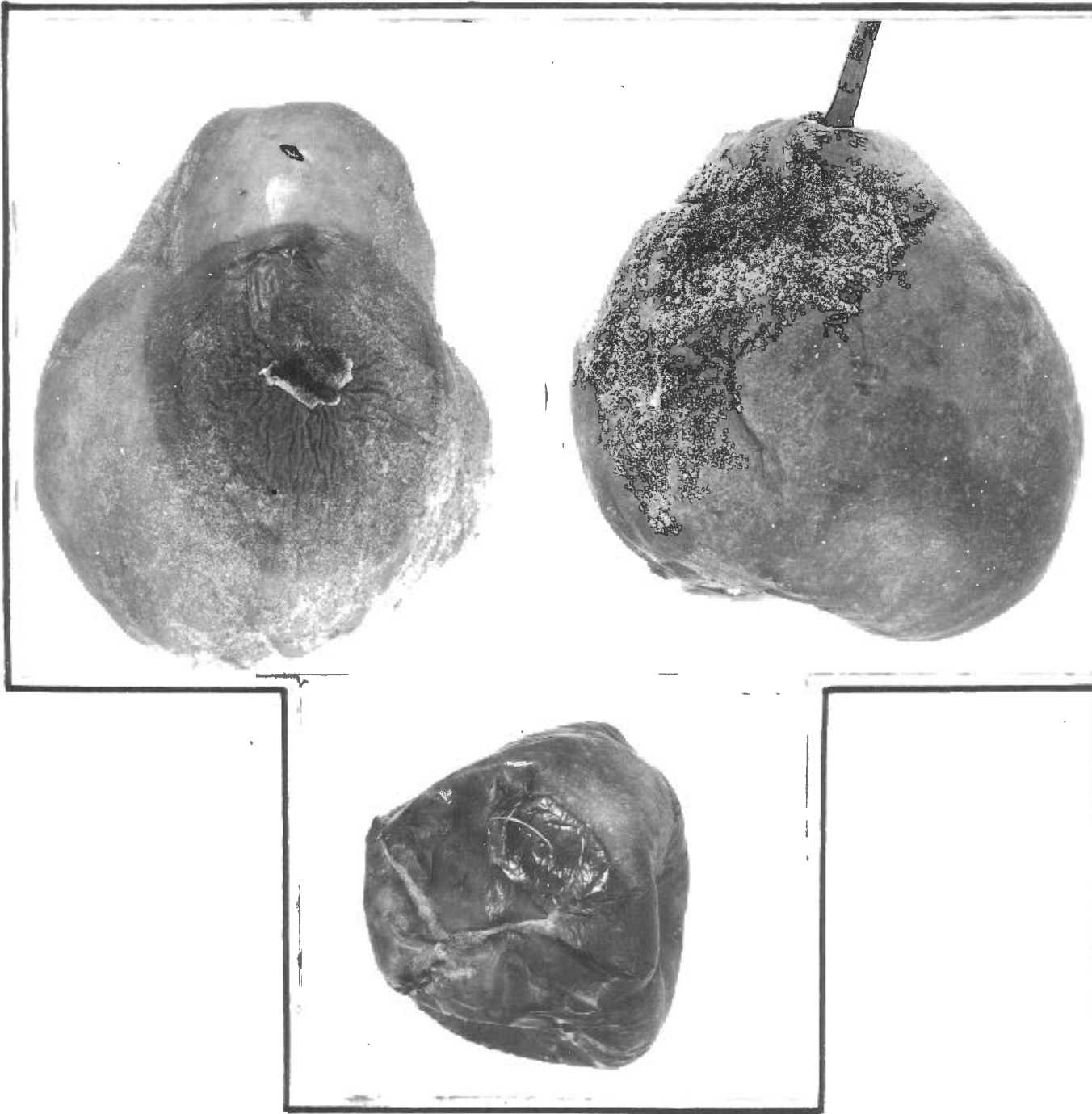


PLATE 111. - Botrytis cinerea, Pers. on A - quince, B - pear,
and C - apple.

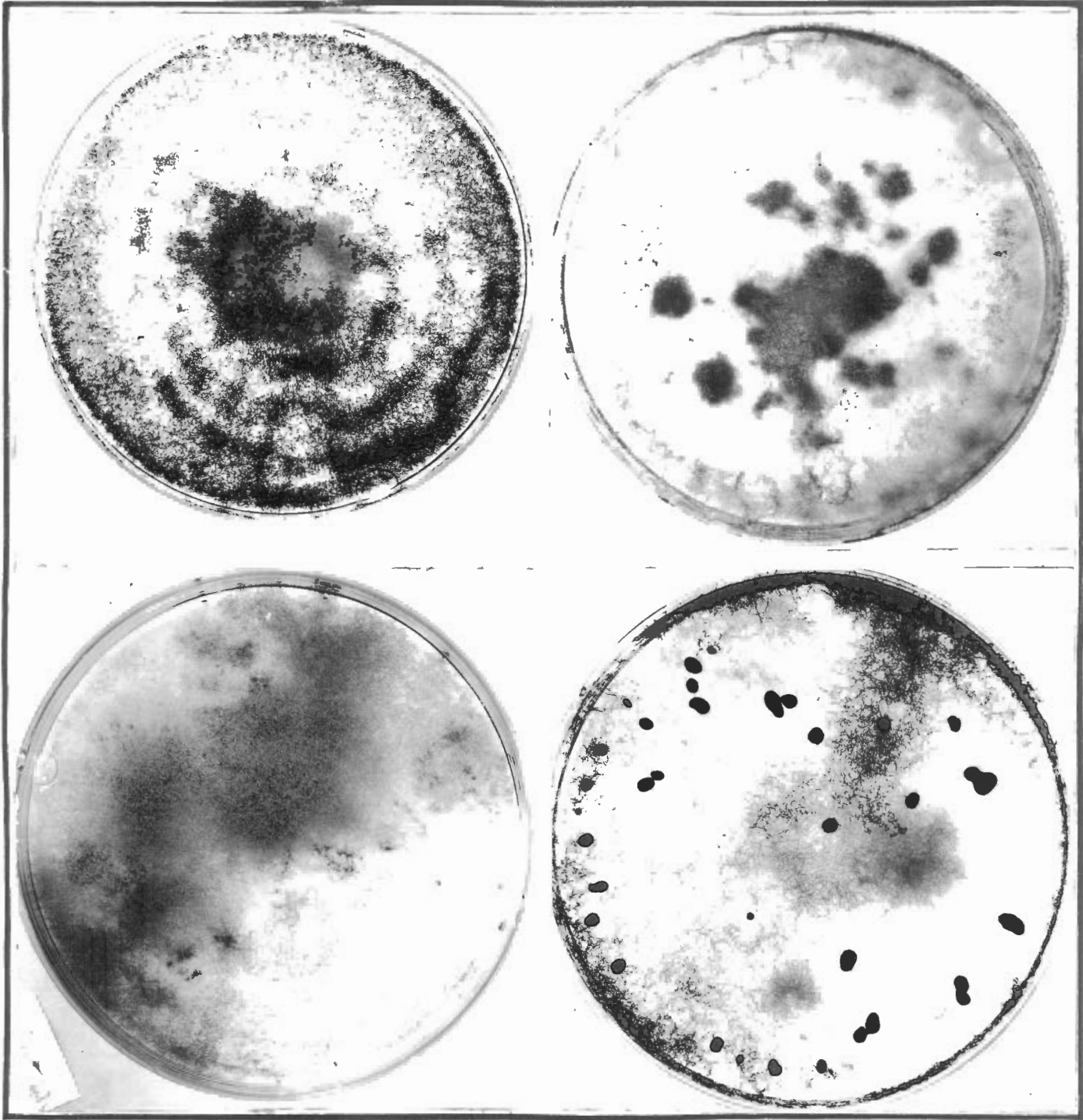


PLATE IV. - The appearance after 20 days on potato dextrose agar of four monospore isolates of Botrytis cinerea from grapes.

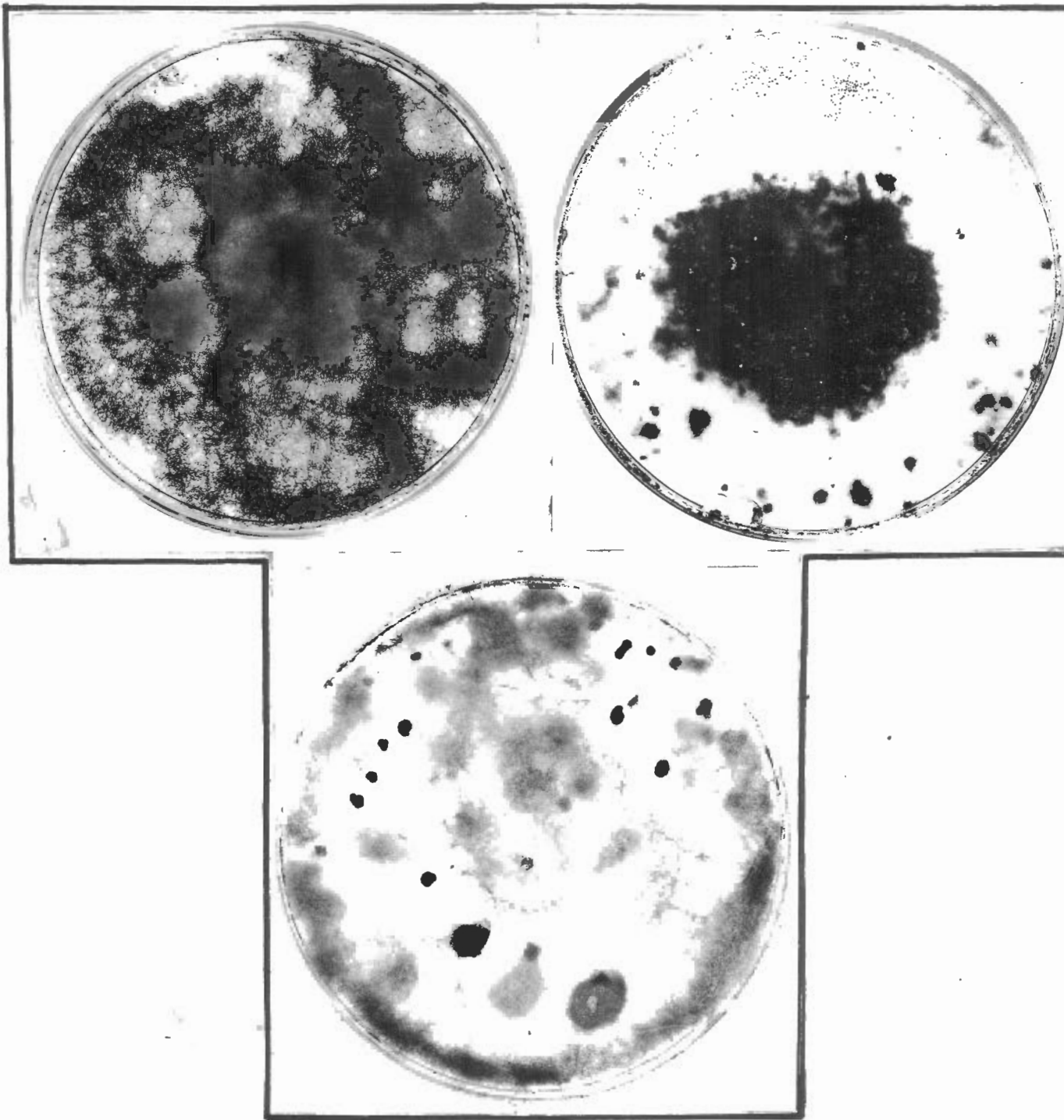


PLATE V. - The appearance after 30 days on potato dextrose agar of monospore cultures of Botrytis cinerea from pears, apples and quinces respectively.



PLATE VI. - The position of the stand for hydro- and thermo-
graphs in between the rows of vines.

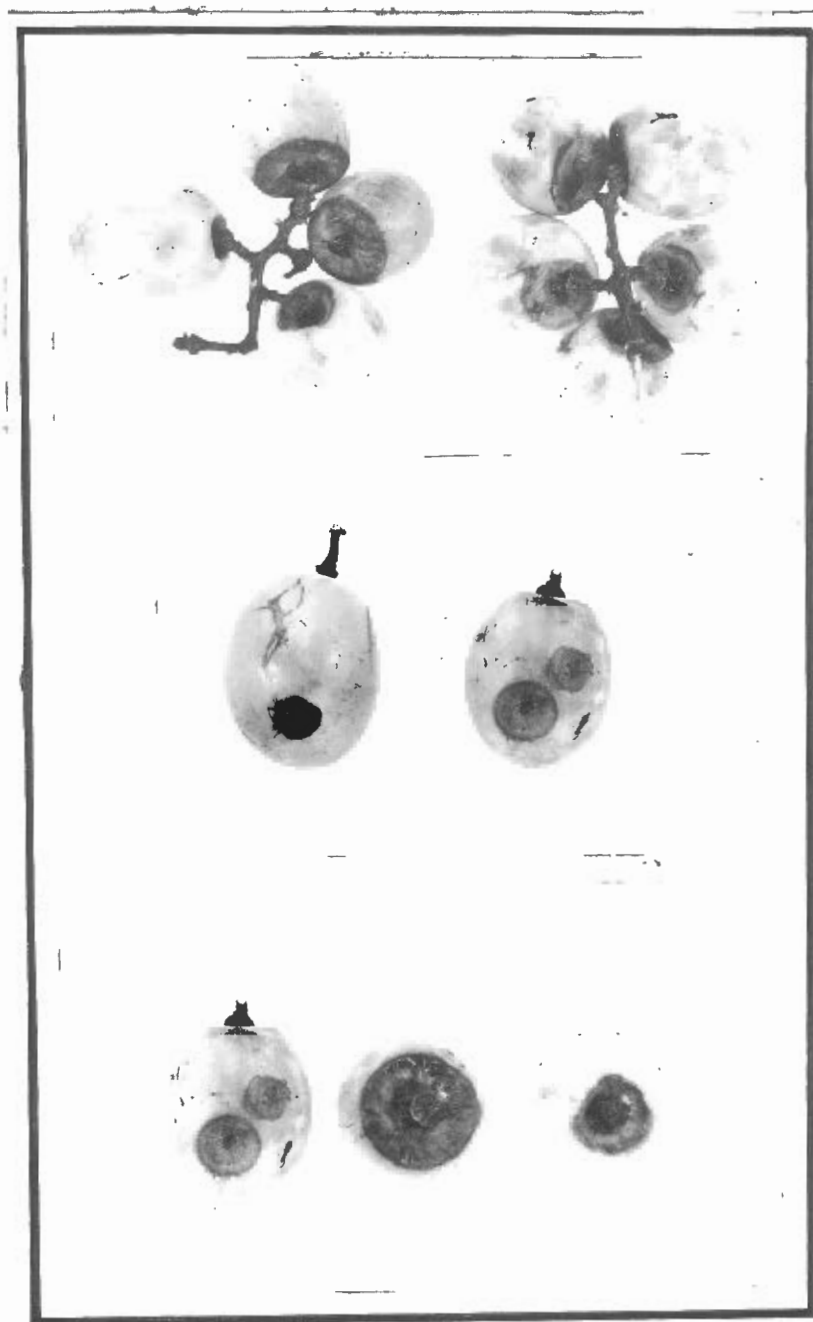


PLATE VII. - Formaldehyde injury to Raisin Blanc grapes (A and B); and at the pedicel ends and at punctures; and C-Cladosporium baccae infection at an injured spot.