GERMINATION AND STORAGE OF POLLEN

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Dit proefschrift met stellingen van

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landbouwkundig ingenieur, geboren te Amsterdam 20 Juni 1922, is goedgekeurd door de promotor, Dr Ir S. J. WELLENSIEK, hoogleraar in de tuinbouwplantenteelt.

De Rector Magnificus der Landbouwhogeschool,
W. F. EIJSVOOGEL

Wageningen, 25 April 1955

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR W. F. EIJSVOOGEL
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIÏNG,
DE WEG- EN WATERBOUWKUNDE EN DE
BOSBOUWARCHITECTUUR
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 20 MEI 1955 TE 16 UUR

DOOR

TIJS VISSER

H. VEENMAN & ZONEN – WAGENINGEN – 1955

Dit proefschrift verschijnt tevens in de Mededelingen van de Landbouwhogeschool 55(1) 1955, als Publicatie No 134 van het Laboratorium voor Tuinbouwplantenteelt.

Aan mijn Ouders

Aan Marleen

VOORWOORD

Gaarne neem ik deze gelegenheid te baat om allen, die tot mijn vorming en tot het welslagen van dit proefschrift bijdroegen, hartelijk te danken.

Vooral U, Hooggeleerde WELLENSIEK, ben ik grote dank verschuldigd. U hebt mij immers reeds als student opgewekt om in den vreemde de eerste schreden op het pad des onderzoekers te zetten. Later hebt U mij gewezen op een terrein van onderzoek waarvan de exploratie mede door U mogelijk gemaakt werd en waaruit deze studie is voortgekomen. Dat daarbij Uw leiding, gepaard gaande met Uw helder inzicht en vruchtbare critiek, mij steeds tot steun is geweest, heeft mij ten zeerste aan U verplicht. Ik ben U tevens bijzonder erkentelijk voor de grote mate van vrijheid die U mij bij mijn onderzoek hebt toegestaan. Het stemt mij tot grote dankbaarheid en ik acht mij bevoorrecht mijn wetenschappelijke opleiding van U te hebben mogen ontvangen.

Hooggeleerde EDELMAN, Uw boeiende bodemkundige colleges en Uw brede visie op problemen van velerlei aard hebben een blijvende indruk op mij gemaakt en zeer bijgedragen tot de verruiming van mijn inzichten. Ook U, Hooggeleerde SCHUFFELEN, ben ik oprecht dankbaar voor de prettige en degelijke opleiding die ik tijdens mijn studie aan de Landbouwhogeschool van U mocht ontvangen.

Hooggeleerde WASSINK, ik ben U zeer erkentelijk voor de leerzame tijd die ik op het Laboratorium voor Plantenphysiologisch Onderzoek heb mogen doorbrengen.

ZeerGeleerde Mejuffrouw GORTER, Uw levendige belangstelling en Uw waardevolle suggesties zijn voor mij van grote betekenis geweest. Dat U, Hooggeleerde HARRINGTON, WelEdelGestrenge FELIUS en ZeerGeleerde DOORENBOS, de moeite hebt willen nemen taalkundige en wetenschappelijke critiek op dit proefschrift te leveren, heb ik op hoge prijs gesteld.

Geachte Collegae, U allen wil ik gaarne danken voor de hartelijke sfeer waarin ik met U heb mogen samenwerken en zo menig persoonlijk gesprek en wetenschappelijke discussie heb mogen voeren. Aan de vele facetten van leven en werk, zoals die door U vertegenwoordigd worden, heb ik mij en naar ik hoop met vrucht, kunnen spiegelen. Uw beider assistentie, Mejuffrouw J. C. BAARS en Mevrouw A. C. ZIJLSTRA-SCHRAAGEN, heb ik zeer gewaardeerd. Dank zij Uw hulp en toewijding werd dit onderzoek zeer vergemakkelijkt en bespoedigd.

Het behoeft geen betoog, dat nog velen werkzaam op het Laboratorium voor Tuinbouwplantenteelt en daarbuiten nog een woord van dank toekomt. Van hen wil ik speciaal noemen de Heer R. SABARTE BELACORTU voor hulp in velerlei opzicht en de Heer J. W. GREVERS voor de uitmuntende verzorging van het teken- en fotomateriaal.

Deze studie werd mogelijk gemaakt door een subsidie van de Landbouw Organisatie T.N.O. Ik ben de Organisatie en in het bijzonder de WelEdelGestrenge Heer VAN DER GIESSEN hiervoor zeer erkentelijk.

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CHAPTER I

GENERAL INTRODUCTION

The maintenance of the vitality of pollen is of great importance in plant breeding by facilitating the crossing of plants which flower at different times or in different places of the world. The shipping of pollen is easier and simpler than the transport of living plants and in addition the sending of pollen is not hampered by strict regulations concerning the transmission of pests and diseases. Moreover, in plant breeding schemes with annual plants and in which the same pollen donors are used throughout, it would be useful if the pollen could be kept viable from one season to the next or longer. Thus, the growing of these donors could be omitted for one or more years. Apart from its importance for practical purposes, successful pollen storage is also indispensable in cases where the physiology of pollen is investigated.

In a great many references on pollen storage, the vitality of the pollen is expressed by the percentage of germination obtained in an artificial medium. The question arises what value can be attached to the germination percentage of the pollen as determined *in vitro* with regard to its ability to set fruit *in vivo*. The statements in literature relative to this question are not in accord. Some investigators (51, 73, 109) found no fruit set or a very poor one although the pollen was able to germinate to a moderate degree *in vitro*. Others (3, 91, 126) demonstrated that pollen which gave no germination or only a low percentage of germination was able to induce a fair fruit set. Apart from the disagreement as to the correlation between the germinability and fruit setting ability of different pollens, the literature also disagrees in several aspects with respect to the medium required for germination of the same pollen. For example good (optimal) germination with pollen of *Corylus avellana* has been obtained in water (78), 15–20% (92), 25% (13) and 35–50% (23) sugar solutions, respectively. Another controversy can be derived from literature with regard to the effect of boric acid on germination. Since the discovery of the stimulating effect of this compound by SCHMUCKER in 1932, it has been found to improve the germination of numerous pollens to a marked extent. Before this discovery, however, many investigators (1, 46, 74, 93) have found an excellent germination with pollen species, *e.g.* belonging to the genera *Pyrus* and *Prunus*, which in later investigations (10, 16, 102, 137) germinated optimally only when boric acid was added to the medium.

Presumably, such disagreements are explainable as lack of comparable conditions in different tests and/or on account of differences in the composition of pollen of the same species, but of different origin.

It is apparent that an insight into the physiology of the pollen in general would considerably aid an investigation into the optimal conditions for storage and germination of any pollen in particular. However, no up to date and comprehensive review and study on the physiology of pollen germination and storage is known to the author, the most recent ones being those of BRINK in 1924 on germination and of HOLMAN and BRUBAKER in 1926 on storage. For this reason, a review and discussion of the literature with regard to the germination and storage requirements of pollen has been undertaken. In addition an investigation into the germinability and longevity of pollen under divergent conditions was carried out, in order to include in one comprehensive study as many factors of physiological importance as was practical. Special attention has been paid to the role of boric acid in germination. All germination experiments were carried out with apple and pear pollen, the storage experiments with apple, pear, tomato, rhododendron and azalea pollen.

CHAPTER II

A LITERATURE REVIEW AND DISCUSSION ON THE BASIC FUNCTION OF THE GERMINATION MEDIUM

1. GENERAL

In the first place the pollen grain requires water for its germination (= the formation of the pollen tube). Consequently, the primary function of any medium is to supply water. Besides the necessity of a sufficient water supply, the question arises to which extent the germination depends on other substances, *e.g.* nutrients. A review of the literature on this subject reveals that the requirements of pollen with regard to its medium appear to be relatively simple. In the great majority of investigations in which hundreds of pollens (= pollen species) were tested, excellent germination was obtained in sugar media with or without a low percentage of agar or gelatine. Even water alone, either as fluid or as vapour, proved to be sufficient for a satisfactory germination of a number of pollens. It may be added that also other factors, like temperature and pH, appear to affect the germination significantly, while a great number of substances have been found to act either as 'stimulants' or 'inhibitors'. These investigations, however, will not be mentioned in this review. The germination of pollen will be discussed mainly with a view to the function of the basic components — water and sugar — of which the medium is composed. The germination media employed may be roughly divided into 3 groups: 1) germination in water, 2) germination in sugar solutions with or without agar or gelatine, 3) germination on solid substrata under limited humidity conditions.

1.1. *Germination in water*

VAN TIEGHEM (130, 1869[1])), LIDFORSS (78, 1896; 79, 1909) and somewhat

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[1]) In this and further citations the first number refers to the References on p. 64 and following, while the second number indicates the year of publication.

later JOST (66, 1907) have reported that pollen species of approximately 30 genera germinated readily in water without the occurrence of bursting. Also ADAMS (1, 1916), KNIGHT (72, 1917) and MARTIN *et al.* (84, 1918) with *Pyrus malus*, SCHOCH-BODMER (116, 1936) with *Corylus* and RIGHTER (104, 1939) with *Pinus* secured a fair germination in distilled water, while TISCHLER (131, 1917) with *Plantago*, DANIEL (38, 1952) with *Impatiens* and SAVELLI *et al.* (112, 1940) with *Nicotiana* obtained good pollen tube growth in water. PATON (94, 1921) even states that the germination of pollen of a *Lilium* species was optimal in tap water. Also WELLENSIEK found a good germination of tea (140, 1932) and cacao pollen (141, 1938) in water to which stigma secretion or part of a stigma had been added. COOPER (33, 1939) with *Carica*, SCHWARZENBACH (123, 1953) with *Cyclamen* and EHLERS (42, 1951) with several other pollens, while testing the influence of certain substances on the germination, used water as a basic medium. It should be mentioned, however, that in the latter investigations the germination percentages obtained in pure water were often low.

1.2. *Germination in sugar solutions*

Since in many instances water did not give satisfactory germination and bursting occurred, sugar solutions with or without agar or gelatine have been used in many investigations. The concentration of sugar employed in the germination media varies widely. As far as the addition of agar or gelatine is concerned, in no case less than 0.5% or more than 2% was added; in most cases agar instead of gelatine was used. MOLISCH (86, 1893) and PFUNDT (99, 1915) have given a list of the sugar concentrations required for the optimal germination of a great number of pollens. It is apparent from their data that the optimal sugar concentration for germination differs considerably among the different pollens. They also observed that quite a number of pollens were able to germinate in a very wide range indeed, *e.g.* MOLISCH reported that *Deutzia* and *Lilium* species germinated in sugar solutions containing from 1 to 40% of sugar. PFUNDT reported that many pollens are able to germinate in 0 to 40% sugar solutions. It should be noted, however, that both authors added 1% agar or gelatine to their media.

To give an impression of the varying concentrations of sugar which have been employed, a short summary is given of a number of references. The very extensive lists MOLISCH and PFUNDT gave of their germination trials will not be reported here, except for a few examples. For the sake of brevity the pollens are grouped under a sugar concentration range. In cases where more than one concentration had been used, the optimal concentration has been recorded.

a) Germination in sugar concentrations $\leq 10\%$

Without agar	With agar
Berberis (128), *Cyclamen* (128), *Fragaria* (1), *Impatiens* (13), *Lathyrus* (125), *Lilium* (19, 128), *Lupinus* (19, 24), *Philadelphus* (128), *Pinus* (60, 125), *Plantago* (21), *Prunus* (10, 74), *Pyrus* (1, 8, 10, 72, 74, 84, 137), *Rosa* (128), *Salix* (128), *Tradescantia* (125), *Tulipa* (19, 128).	*Amaryllis* (95), *Antirrhinum* (125), *Bryophyllum* (125), *Carica* (36, 133), *Cinchona* (98), *Hippeastrum* (25), *Lilium* (95, 96), *Melandrium* (13), *Picea* (62), *Pinus* (40, 62), *Pyrus* (124, 134), *Vinca* (27), *Vitis* (26).

Most of the above pollens were germinated in sugar concentrations near 10%.

b) Germination in sugar concentrations >10 ≤20%

Without agar	With agar
Aesculus (13), *Antirrhinum* (73), *Campanula* (58), *Coffea* (44), *Corylus* (92, 116), *Digitalis* (58), *Hypericum* (58), *Linaria* (58), *Medicago* (58), *Nicotiana* (111), *Phoenix* (3), *Plantago* (131), *Prunus* (60, 74, 93), *Pyrus* (23, 93), *Raphanus* (120), *Ribes* (1), *Rubus* (58), *Tropaeoleum* (58), *Vitis* (91, 144).	*Hevea* (39), *Nicotiana* (41), *Pistachia* (70, 126), *Prunus* (46, 70, 89), *Pyrus* (70, 89), *Vitis* (89), *Zea* (9, 73, 110).

Most of the above pollens were germinated in concentrations which lie nearer to 20% than to 10%.

c) Germination in sugar concentrations >20 ≤30%

Without agar	With agar
Berberis (13), *Brassica* (120), *Corylus* (13), *Forsythia* (84), *Lathyrus* (13, 29), *Matthiola* (139), *Pyrus* (77), *Primula* (13), *Rosa* (30), *Sinapis* (120).	*Beta* (68), *Lythrum* (43), *Saccharum* (110).

d) Germination in sugar concentrations >30%

Sugar solutions higher than 30% to which 1% gelatine or agar had been added, were employed by MOLISCH and PFUNDT. For example, optimal germination of *Dactylis* and *Lolium* species was obtained in 30–40% sugar solutions. Otherwise, not much use has been made in more recent studies of sugar solutions higher than 30% and if so, no agar or gelatine had been added. BERG (13, 1929) and TISCHLER (131, 1917) found optimal germination of *Ribes* and *Cornus* pollen in 40% sugar solution. TISCHLER found 35 to 50% for some *Cassia* species to be optimal. BRANSCHEIDT (23, 1930) only obtained germination of *Cornus*, *Helleborus* and *Cannabis* pollen in sugar concentrations as high as 70–80%; *Corylus* pollen germinated optimally in 80% sugar solution, while 35–50% sugar solutions were optimal for pollen tube growth.

1.3. *Germination on solid substrata*

In the above instances the water was taken up by the pollen from aqueous media. It has been found by MOLISCH that many pollens are also able to germinate on a coverslip in humid air. For some pollens, however, it is not so much the ability, but the necessity to germinate under such conditions which must be stressed. Their germination can be secured only under conditions of a 'limited' water supply.

For instance, good germination was obtained by JOST (66, 1907) with pollen of *Arrhenatherum* and *Dactylis* on soaked parchment paper, by ANTHONY *et al.* (6, 1920) with pollen of *Hordeum* and by FIRBAS (45, 1922) with pollen of *Triticum* and *Secale* on a coverslip and a limited humidity supply and by RENNER (103, 1919) with *Oenothera* pollen on dried stigma secretion and specific air humidity. These pollens did not germinate in sugar solutions with or without agar, while most grains burst. Likewise, WALDERDORF (139, 1924) with different pollens and KUHN (75, 1937) with *Matthiola* pollen found a greatly improved germination on pre-dried drops of 10% gelatine under specific humidity conditions. The sensitivity of these pollens to humidity is demonstrated by the fact that the above investigators and also BAIR *et al.* (9, 1941) with *Zea* pollen

observed that a saturated atmosphere was harmful to the pollen. It seems likely, that the pollens which are only able to germinate in very high sugar concentrations must be classified also as pollens being sensitive to an 'ample' water supply.

2. THE FUNCTION OF SUGAR IN GERMINATION

From the foregoing it is evident that the pollen germination depends among others on the rate at which water is released from the medium and taken up by the pollen, termed by the author as 'the diffusion rate of water'. The influence of the diffusion rate of water is especially apparent in those cases where germination could be affected under conditions of a limited water supply only. It follows also from the fact that the concentration of sugar necessary for a good germination varies widely for different pollens. Even assuming that the sugar functions as a nutrient, it cannot explain the noted divergence of sugar concentrations. It seems logical, therefore, to infer that the sugar, apart from its possible nutritive value, is necessary to create a certain osmotic value, thus controlling the diffusion rate of water. Also agar and gelatine, though not having osmotic properties, impede the movement of water due to their colloidal character.

Since BRINK in 1924 studied and discussed the function of the medium both in regard to its osmotic function and its function as a possible source of nutrition, these two factors have not had the attention they deserved in later years. As a matter of fact no comparatively recent investigation nor an up to date review is known to the author in which both these factors have been studied in conjunction. For that reason, the literature related to these subjects will be reviewed and discussed in some detail.

2.1. *Sugar as an osmotic agent*

Bursting. – A common and frequently observed feature of pollen cultures is the occurrence of bursting of pollen grains and pollen tubes. Since this happens often in media not quite adapted to good germination, it is of interest to know to what extent bursting depends on osmotic phenomena.

This question has been answered in the negative by VAN TIEGHEM (130, 1869), MOLISCH (86, 1893) and LIDFORSS (78, 1896), since no distinct relationship between the amount of bursting and the concentration of sugar was found. Also WADDINGTON (138, 1929) working with *Matthiola* pollen states that bursting is not influenced by the sugar concentration. This is, however, not quite true as it appears from his data that the amount of bursting in the highest concentration is 30% less than in the lower ones. In this connection it may be noted that KUHN (75, 1937) showed that sugar solutions are not very suitable media for *Matthiola* pollen (see p. 5). Most pollen grains burst in any sugar concentration, though less bursting occurs in the higher concentrations. That bursting is positively related to the diffusion rate of water, expecially for those pollens which are sensitive to an ample water supply, has been shown by ANTHONY *et al.* (6, 1920), KUHN (75, 1937), RENNER (103, 1919), SCHOCH-BODMER (116, 1936) and WALDERDORF (139, 1924). They noted much less bursting when the pollen was germinated on solid substrata under specific conditions of air humidity instead of in sugar solutions. Moreover, these investigators and others (9, 120) also found that bursting is inversely related to the osmotic value of the medium. That is to say, the amount of bursting de-

creases with increasing osmotic value of the medium. The greatest amount of bursting is to be found in water or saturated air, although under the latter conditions usually less bursting occurs than in water.

Germination. – There is plenty of evidence that the percentage of pollen germination is also related to the osmotic properties of the medium. It follows from the experiments of many authors (1, 75, 84, 107, 120, 132, 138, 139) that the relationship between the percentage of germination and osmotic value can be approximated by an optimum curve. The same holds true for the pollen tube growth (1, 75, 84, 116). It can be derived from the findings of ADAMS (1, 1916), BRINK (26, 1924), KUHN (75, 1937), MARTIN *et al.* (84, 1918), PFUNDT (99, 1915) and others that the 'optimum for germination' of many pollens is rather broad: *viz.*, a high germination percentage and long tubes could be obtained in a comparatively wide range of sugar concentrations.

The results of several authors show that the germination percentage was higher and the tubes longer *within the same period of time* as the sugar concentration was lower. Or, in other words, the *rates* of germination and tube growth increase as the osmotic pressure of the medium decreases. The relation between germination rate and osmotic pressure is also stressed by the findings of SCHOCH-BODMER (116, 1936) with *Corylus* pollen. She found that the period of time which elapsed before the germination started became shorter as the osmotic pressure was lower. Therefore, when both the percentage and rate of germination and the amount and rate of tube growth are considered it appears that a specific optimum (sugar concentration) for germination exists. The fact that in many instances water or relatively low sugar concentration are not found to be optimal for germination, is due to the bursting of grains and tubes under such conditions.

Agar or gelatine serves the same purpose as sugar, but the diffusion of water is in this instance restricted by the colloidal nature of these substances. This follows from experiments by ESSER (43, 1953), JOHNSON (62, 1943), KING *et al.* (70, 1938), KUHN (75, 1937), MOLISCH (86, 1893) and TISCHLER (131, 1917). They found that the addition of agar or gelatine to sugar solutions reduced the occurrence of bursting and improved the germination. For instance, TISCHLER showed that pollens of some *Cassia* species germinated readily in 2% agar media with, but also without sugar. Without agar, on the other hand, the sugar concentration had to be as high as 35 to 50% before bursting had decreased and germination was satisfactory.

2.2. *Sugar as a nutrient*

In nearly all references concerning the growth of pollen tubes either *in vitro* or *in vivo* it has been alleged that if the pollen tube is to attain a considerable length an outward source of nutrition must be present. On account of numerous experiments in which good germination was obtained in sugar solutions, it seems likely that if any major nutrient is involved it will be sugar. In those instances where germination media were made up with sugar, the sugar employed was in the majority of the experiments saccharose.

2.2.1. Germination *in vitro*

1) The fact that pollen tubes of *Pinus* (83, 86, 131), *Picea* (131), *Nymphea* (131) and *Zamia* (52) when growing in sugar solutions showed a visible accumulation of starch grains or showed starch that formerly had not been present,

has led the respective investigators to believe that intake of sugar took place. The more so, as MOLISCH (86, 1893) found that this phenomenon did not happen in saturated air. Likewise, from the observation that pollen tubes lose their starch more rapidly and grow less in water than in sugar solutions, BODMER (20, 1921) and others derive that sugar serves as a nutrient.

These phenomena with respect to starch, however, cannot be regarded as conclusive evidence. They may just as well be seen as an adaption of the metabolism of the pollens to their respective media because of differences in water supply. This view is substantiated by the fact that RENNER (103, 1919) and BODMER (20, 1921) observed that the presence of starch in *resting* pollen grains is related to the humidity conditions: the more humid the environment, the quicker starch disappears. It is also worthy of note that many investigators (21, 30, 84, 86, 103, 131) observed that resting pollen loses its starch with increasing age rather quickly. The 'starch pollens', however, did not germinate any better than pollens in which starch was no longer present (21, 103, 131). It may be further noted that some of the evidence is not consistent. Namely, TISCHLER (131, 1917), in contrast with the findings of BODMER, found that *Plantago* pollen germinated just as well in distilled water as in sugar solutions, while even the long tubes grown in water were not devoid of starch.

GREEN (53, 1894) also believed in the nutritive value of sugar, among others based on the observation that the enzyme activity of some pollens was found to be much higher in sugar solutions than in water. The author, though, would like to point out that the lesser enzyme activity of the pollen germinating in water may well be due to bursting.

2) The fact that many pollens will germinate and produce long tubes in sugar solutions of widely different concentrations suggested to BRINK (26, 1924) the conclusion 'that the cell membrane of the pollen grain and its tube become permeable to sugar and that the final result as far as osmotic pressure is concerned is the same as though the surrounding medium were water'. This statement is obviously founded on a half truth. The observation that pollens may germinate in varying concentrations of sugar is quite true, but not complete. It appeared namely in the foregoing (p. 7) that both the amount and the rate of germination and pollen tube growth are correlated with the osmotic value of the medium. And this dependency of the germination and growth rate on osmotic forces points rather to the impermeability of the cell membrane for sugar. For indeed, if sugar was taken up, thus adding to the original osmotic force of the cell sap, the growth rate would rather be constant instead of decrease with increasing sugar concentration. Moreover, many other pollens germinated much better on solid substrata containing no sugar than in sugar solutions, while the same behaviour with respect to osmotic forces was observed.

BRINK's observations (27, 1924) on the growth of pollen tubes of *Vinca* in sugar-agar media may be regarded as presumptive evidence in favour of non-nutrition. He noted that the tubes ceased to grow as soon as their reserves (fat) were exhausted, the maximum length attained by the tubes often being 5 mm or more. BRINK asserts that under conditions of culture *in vitro*, the potential length of the tube and the amount of food material initially present in the pollen grain seem to be causally related.

3) The fact that pollen tubes of a number of pollens when cultivated in sugar or in sugar-agar media have been found to become as long as or longer than the style which they would have had to transverse in nature, has been brought

forward by some authors (25, 117) as a strong indication in favour of the nutrition of the pollen tube. The following pollens were found to produce tubes *in vitro* of a sufficient length to reach the ovulum: *Chionodoxa* (27), *Gagea* (42), *Hippeastrum* (27, 66), *Impatiens* (14), *Muscari* (27), *Pachyphytum* (42), *Puschkinia* (27), *Pyrus* (72), *Ribes* (42), *Rumex* (20), *Scilla* (27), *Sedum* (42), *Vinca* (17, 25) and *Vitis* (22).

Again it can be said that these observations do not show that the sugar has been actually used for nutrition. It may well be that the presence of sugar is only essential for creating favourable osmotic conditions for germination. In this connection it must be noted that many pollens may germinate readily and produce tubes of considerable length in pure water or on substrata which do not contain sugar. For instance, MARTIN *et al.* (84, 1918) found that apple pollen germinated exceedingly well on a membrane soaked in water and produced tubes which were 2 mm long. No increase in growth was observed when the membrane was soaked in sugar solution instead of in water. Thus it follows, that the reserve materials of many pollens are sufficient to support an appreciable amount of growth. Actually positive evidence that pollens belonging to different genera may attain tubes of a length sufficient to affect fertilization without an outward source of nutrition has been recently forwarded by EHLERS (42, 1951). He found this to be the case for pollen of *Convallaria*, *Echeveria*, *Genista*, *Impatiens* (7 species), *Scilla*, *Tradescantia* (2 species), *Vicia* (2 species), *Vinca* and *Xanthosoma*. All these pollens were cultivated in destilled water to which only 10 or sometimes 100 ppm boric acid had been added. The presence of boron appeared to be essential, since without boron the tube growth in water was generally very much less. The addition of sugar, on the other hand, did not increase the average or maximum growth of tubes of the above pollens significantly. The tubes of some other pollens attained a sufficient length only on sugar-agar media. Nevertheless, EHLERS does not believe on account of several observations that in those cases the sugar serves as a nutrient.

2.2.2. Germination *in vivo*

1) CORRENS (34, 1889) tried to answer the question whether *Primula* pollen was nourished by the style with the aid of a calculation. He concluded from the calculated amount of reserves that without additional nutrition the thickness of the tube wall of small grains after the tube has reached the ovulum of long styled flowers would be thinner than can be realised in nature. EHLERS (42, 1951), however, on account of his own investigations, points out that the estimation of the swelling of the membrane by CORRENS is too low. In this connection it is worthy of note that SCHOCH-BODMER (116, 1936) observed that the intine is able to swell to a large extent.

2) BRINK (25, 1924) considers the fact that several authors (4, 31, 37, 52, 71) have found that the conducting tissue of the style contains amyloid materials, sugar and probably other carbohydrates as part of the evidence that the pollen grain derives nutrition from the style in the form of readily diffusible sugars. The same view is also held by GESSNER (49, 1948) following the observation that the gymnostemium of orchids contains abundant amounts of reserves. Although these findings may imply nutrition of the pollen tube, they can by no means be regarded as positive proof.

3) From pollination experiments with self sterile *Petunia's* STRAUB (127, 1947) concludes that the pollen is nourished by the conducting tissue. He observed

that the pollen tube growth after cross pollination preceded by self-pollination was normal, while the growth appeared to be inhibited when cross-pollination was preceded by cross-pollination. He assumed that cross-pollination leads to a quicker exhaustion of nutrient materials in the style than self-pollination. This hypothesis, however, cannot be regarded as conclusive evidence for nutrition, since the assumption of another mechanism could interpret these findings as well. Especially so, as nothing much is known about the chemical and physiological background of these phenomena.

4) SCHOCH-BODMER (117, 1945; 118, 1947) founded her belief in the nutrition of the pollen on phenomena connected with the growth of pollen tubes in the style of *Lythrum salicaria*. She observed that the tubes penetrate into the thick collenchyma walls of the conducting cells and dissolve this collenchyma during their growth. She presumes this to be due to the action of pectinase, an enzyme which has been found to be present in several pollens by PATON (94, 1921). It was also found that, though in some instances all pollen tubes together occupy as much as 40% of the volume of the conducting tissue, no increase of the style circumference could be measured. From these observations and from the fact that the conducting cells neighbouring the tubes were found to shrivel, she concludes that the pollen grain derives nourishment from the stylar tissue in the form of water, mineral salts and sugar.

Again the author cannot agree with SCHOCH-BODMER that the outcome of her experiments justify such positive conclusions, since no direct evidence is given that the dissolved materials, like pectin, are in fact taken up. For one thing the shriveling of the conducting cells may be exclusively due to loss of water necessary for the growth of the pollen tubes. The more so, as several investigators (103, 131, 139) have shown for a number of pollens, including *Lythrum salicaria*, that the osmotic pressure at which the pollen germinates readily, exceeds the osmotic pressure at which the cells of the style show plasmolysis.

5) Evidence pointing to a non-nutrition of the pollen tube has been forwarded by EHLERS (42, 1951). He observed that undernourished pollen of *Amaryllis* (presumably pollen from undernourished plants) produced pollen tubes in the style which were in several instances significantly shorter than those of normal pollen. Pollen tubes of *Tradescantia* pollen after having transversed different lengths of style and having completed their subsequent growth *in vitro* were found to attain the same maximal length. Since in both cases the maximum length reached by the tube is not affected by the style it seems very likely that the growth of the pollen tube depends exclusively on its own resources.

6) The non-nutrition of pollen tubes *in vivo* may be also derived from observations made by SCHWANITZ (120, 1942; 121, 1949; 122, 1950). He found that the 'colchicine-made' tetraploid forms of species of *Brassica*, *Sinapis*, *Rumex*, *Raphanus* and some other species were less fertile than the original diploid plants. According to SCHWANITZ the lesser metabolism (due to a relatively smaller size of the cell nuclei) of the tetraploid plants causes a poorer supply with reserve materials to the pollen. This circumstance is probably responsible for the poor vitality and decreased ability of the diploid pollen grains to affect fertilization. The observations made by ESSER (43, 1953) on the germination of *Lythrum* pollen can be likewise regarded, albeit on opposite grounds, as pointing to the non-nutrition of the pollen tube. He ascertained that in incompatible crossings diploid pollen tubes grew further into the style than haploid pollen

tubes (diploid pollen grains are larger than haploid ones). He also observed that the pollen tubes of the bigger haploid grains attained a greater length than those of the smaller grains. Since the diploid grains have a larger stock of reserves ESSER presumes that this accounts for the greater length of their tubes in incompatible crossings.

3. DISCUSSION

A. From the first part of the review it follows that the requirements of pollens with regard to their germination medium are very variable. Some pollen species are able to germinate well under conditions of an ample water supply, others can only germinate when the availability of water is restricted.

As a rule, the germination rate and the growth rate of the tubes appear to be positively correlated, because both increase with increasing diffusion rate of water (function of the osmotic and/or colloidal value of the medium). The bursting of pollen grains (or tubes) also increases with increasing diffusion rate of water. Because of this latter relationship the optimum for germination must be defined as that diffusion rate of water (osmotic/colloidal value) at which maximal germination is obtained within the shortest possible time and *without the occurrence of bursting*.

B. In this connection it is of interest to consider somewhat more closely the function which the osmotic pressure has in germination. SCHOCH-BODMER (116, 1936) concluded that the turgor pressure — as a function of the osmotic pressure — is largely responsible for the protrusion (germination in the strict sense) of the pollen tubes. The turgor pressure not only plays an important role in the *rate* of their subsequent growth, but may also affect their *length*. Whenever, as a consequence of a too high diffusion rate of water, more water is taken up than can be used for growth, small or no tubes will be formed, because bursting will occur due to a too high turgor pressure. The maximal diffusion rate of water (minimal osmotic pressure) at which good tube growth can be obtained depends on the maximum rate at which essentials for growth can be mobilised. At lower diffusion rates, if not too low, the rate of germination and tube growth will slow down in proportion to the diffusion rate of water.

The omittance of this latter feature in many experiments explains why so many pollens have been alleged to have a broad optimum. Namely, though the rate of germination decreases with decreasing osmotic pressure, maximal germination may be found within rather wide limits, if sufficient time for germination is allowed. A similar explanation holds true for the noted absence of a relationship between bursting and sugar concentration. It may be assumed that any factor that causes the arresting of the growth of pollen tubes at a certain moment, would automatically lead to bursting, since the water intake goes on. The time needed to reach the limit of tube growth will be longer and consequently bursting will occur later as the sugar concentration is higher. Therefore, if one waits too long with one's observations one may find that most tubes have burst, irrespective of the sugar concentration.

C. Eventually, it must be mentioned that many pollens can apparently adapt themselves to a wide range of osmotic pressures, while other pollens produce tubes only under conditions of a specific and limited 'water supply'. Presumably the turgor pressures the cell walls of the latter group of pollens are able to maintain are rather low.

D. From the second part of the survey it follows that none of the observations

with regard to exogenous nutrition of the pollen tube can be regarded as valid proof. On the other hand, both positive and good presumptive evidence has been brought forward in favour of non-exogenous-nutrition of the pollen tube. From this evidence it may be concluded that the growth of the tube of many different pollens, whether cultivated *in vivo* or *in vitro*, is independent of the presence of nutrients in the medium in the form of sugars. In those cases where sugar is required for optimal pollen germination it seems obvious that, on account of the relationship between the diffusion rate of water and germination, only its 'water-regulating' properties are involved. Since the evidence pointing to a non-nutrition of pollen concerns a considerable number of pollens belonging to different genera of various families, it is likely that in general the pollen tube is exclusively built up from the reserves of the pollen grain.

CHAPTER III

POLLEN GERMINATION AND FRUIT SET OF PEAR AND APPLE AS AFFECTED BY BORON APPLICATION

1. LITERATURE

SCHMUCKER (113, 1932) has been the first to discover the significance of boron in germination experiments. He observed that *Nymphea* pollen germinated readily in the secretion of the stigma (containing 1% glucose), but not in a pure glucose solution. He found this to be due to the fact that the secretion contained approximately 10 ppm B_2O_3 (113, 114). In 1935 SCHMUCKER (115) investigated the influence of boron on the germination of pollen of 40 plant species and found that pollen of 14 species showed a marked increase of germination percentage and pollen tube growth, while less or no bursting occurred in the presence of boric acid. A few years later BOBKO and ZERLING (19, 1938) ascertained the promoting influence of boric acid on the germination percentage and the growth of tubes of pollen of 25 different species. BLAHA (16, 1939) observed the same effect in germination trials with pollen of 85 varieties of *Prunus* species (*domestica*, *insititia* and *avium*) and 55 varieties of *Pyrus communis*.

Since then, many investigators have observed that small amounts of boric acid or other boron compounds added to the germination medium greatly improved the germination and diminished the amount of bursting of numerous pollens: COOPER (33, 1941), VASSILIEV (135, 1941), KUHN (76, 1943), ADDICOTT (2, 1943), ÖSTLIND (92, 1945), HUANG (66, 1948), MOEWUS (85, 1950), BATJER and THOMPSON (10, 1950), EHLERS (42, 1951), VISSER (137, 1951), CALVINO (30, 1951), GÄRTEL (47, 1952), ESSER (43, 1953), KATO (68, 1953) and REMY (102, 1953).

The concentration required for optimal germination was for the majority of pollens 10 ppm, in some cases between 50 and 100 ppm was found to be necessary, while a minority of pollens requires less than 10 ppm boric acid. There is no doubt that the influence of boric acid is not due to its acidity, since *e.g.* borax is just as effective (19, 42, 115, 135). Moreover, the small amount usually required for optimal germination scarcely lowers the pH (115, 92).

The germination of some hundreds of pollens belonging to the following genera was found to be improved upon addition of boric acid or other boron compounds:

Allium (60), *Aloë* (115), *Batrachium* (115), *Beta* (68), *Brassica* (120), *Bulbine* (115), *Campanula* (19), *Carica* (33), *Chelidonium* (115), *Corylus* (92), *Cucumis* (19), *Dicentra* (115), *Digitalis* (19), *Echeveria* (42), *Eschscholtzia* (19), *Euphea* (19), *Fabiana* (115), *Forsythia* (85), *Gagea* (42), *Galanthus* (42), *Genista* (42), *Ginkgo* (60), *Impatiens* (42, 115), *Lilium* (19, 60), *Lupinus* (19), *Lycopersicum* (19, 76, 135), *Lythrum* (43), *Mesembrianthemum* (115), *Milla* (2), *Mimulus* (19), *Narcissus* (19), *Nicotiana* (19), *Nymphea* (113, 114), *Papaver* (19; 42), *Petunia* (19, 115), *Pinellia* (115), *Pinus* (60), *Primula* (19), *Prunus* (10, 16, 60, 92, 102), *Pyrus* (7, 10, 16, 92, 137), *Rosa* (30), *Saintpaulia* (115), *Sempervivum* (115), *Sinapis* (120), *Solanum* (19), *Tradescantia* (42), *Tropaeoleum* (2), *Tulipa* (19), *Vicia* (19), *Vinca* (42), *Vitis* (47), *Xanthosoma* (42).

From the foregoing it appears that the germination of many pollens increases in the presence of boron. The germination of other pollens is apparently not so affected, since many investigators obtained an excellent germination of numerous pollens without boron.

In the literature almost no reference has been made to the factors on which the sensitivity of pollen to boron *in vitro* may depend. The present study was undertaken to ascertain what conditions determine the sensitivity of the pollen to boron. It was also tried to answer the question, whether and to what extent the degree of 'boron sensitivity' of the pollen is correlated with boron 'deficiency' (level) of the plant as determined by its fruit set.

2. THE INFLUENCE OF BORON AND SUGAR SUPPLY TO THE PLANT ON POLLEN GERMINATION

The fact that many pollens were found to be sensitive to boron and other pollens appear not to be affected by boron, is possibly explained on account of differences occurring in the boron levels of the plants grown in different places and/or seasons. Also differences in the germination techniques may be responsible for the lack of uniformity with regard to the response of the pollen to boron.

In order to find out if and to what extent the germination is affected by these two factors, the germination of pear pollen harvested from branches treated with boric acid solutions has been investigated. Since it has been found that boron may influence the carbohydrate metabolism (48, 56, 64) of plants, also sugar solutions have been supplied either alone or in combination with boric acid. Pears were chosen for these experiments, since their pollens – as is demonstrated by photo 1 – may be very sensitive to boron.

2.1. *Experiments with pear pollen originating from cut branches*

Material and methods. – Branches of Précoce de Trévoux and Clapp's Favourite were cut off at the end of February and put in glass jars with different concentrations (0, 1, 5, 10, 20 ppm) of boric acid in distilled water and 10 ppm superol. Superol (sulfa-oxy-chinoline) has been found to improve the growth of young seedlings of tomato and *Petunia* cultivated in nutrient solution (136). This is probably due to the prevention of contamination of the solution. The jars were placed in a glasshouse (16°–24° C). In the experiments with Précoce 4% of sugar had been added to all solutions, except to the control (water). In the experiment with Clapp's Favourite solutions both with and without 4% sugar were used. The solutions were renewed every 4–5 days. The unit of experiment consisted of 3 branches, each with 7–12 flower buds, per jar. The experiment with Clapp's was carried out in singular, that with Précoce in duplicate. In this latter experiment the one set of jars (I) was standing slightly warmer than the other (II) and consequently flowered 1–2 days earlier. Although the flower buds were in a stage of imposed dormancy at the time the branches were cut, they already flowered after approximately 3 weeks in the glasshouse. At that time the stamens were collected, dried at room temperature and stored at 4° C and 10% relative humidity.

In these experiments as well as in all the following ones the germination of the pollen was tested by means of a 'hanging-drop culture' in a Van Tieghem cell (130). The germination took place in distilled water with or without a certain percentage of sugar (which was in all experiments saccharose) at a temperature of 23° C. These media were slightly acid (pH: 6.5–6.7). The germination percentage was determined as the mean of the germination percentages found in 6 drops, in each of which 100 pollen grains were counted. Drops of about the same volume with approximately the same number of pollen grains were used in all experiments. Only grains which produced tubes longer than $\frac{1}{2}$ × grain diameter were recorded as having germinated.

Results. – The data obtained with Précoce and Clapp's pollen are recorded in table 1 and 2 respectively.

TABLE 1
The effect of boron and sugar supply to cut branches of Précoce de Trévoux on the germination of pollen in 10% sugar solution with and without 30 ppm H_3BO_3; germination time $3\frac{1}{2}$ hours.
% PT>5D = % tubes longer than 5× grain diameter;
S = 4% sugar; 1 B, 5 B, 10 B, 20 B = 1, 5, 10 and 20 ppm H_3BO_3 respectively;
*) difference with the control (H_2O) significant (P = 0.01).

1	2	3	4	5	6	7	8	9
Treatment of branches	Germination in 10% sugar				Germination in 10% sugar+30 ppm H_3BO_3			
	% germination		% PT>5D		% germination		% PT>5D	
	I	II	I	II	I	II	I	II
H_2O	4.3	0.3	31	0	61.8	61.7	89	75
S	3.8	0.2	30	0	77.7*	69.5	91	86
S+ 1 B . .	3.5	1.0	39	0	78.0*	75.8*	89	91
S+ 5 B . .	8.2*	1.2*	59*	0	78.8*	77.0*	93	94*
S+10 B . .	6.5	1.0	28	0	73.7	59.2	92	71
S+20 B . .	3.7	1.3	14	0	67.7	50.2*	88	53*

TABLE 2
The effect of boron and sugar supply to cut branches of Clapp's Favourite on the germination of the pollen in 10% sugar solution with and without 30 ppm H_3BO_3; germination time $3\frac{1}{2}$ hours.
All differences with the control (H_2O) were significant (P = 0.01).
Abbreviations as in table 1.

1	2	3	4	5
Treatment of branches	Germination in 10% sugar		Germination in 10% sugar+30 ppm H_3BO_3	
	% germination	% PT>5D	% germination	% PT>5D
H_2O	5.3	0	12.7	2
S	10.5	3	42.5	58
1 B	14.3	15	46.2	70
5 B	15.0	33	57.2	76
10 B	13.8	18	58.7	70
20 B	14.5	7	43.0	65
S+ 1 B . . .	10.2	12	49.8	64
S+ 5 B . . .	11.3	23	59.2	87
S+10 B . . .	13.0	12	79.1	96
S+20 B . . .	10.8	8	44.8	70

From the tables it can be derived:

1) The germination and tube growth of Précoce in sugar solutions *without* boron (table 1, columns 2, 3, 4) appear to have been stimulated by sugar and boron uptake in one instance only (S+5 B); pollen of Clapp's (table 2, columns 2, 3) profited by the sugar and/or boron supply to the branches in all instances. However, the germination is on the whole rather poor under these conditions.

2) The germination of Précoce (sample I) in sugar solution *with* boron improved significantly upon sugar supply alone (column 6); the germination of sample II only improved significantly after the supply of both sugar and boron (column 7). The influence of the supplied solutions on the tube growth is small.

The germination and tube growth of Clapp's pollen markedly increase after supply of sugar and boron to the branches, either alone or in combination (table 2, columns 4, 5). For instance, the pollen from branches supplied with a low concentration of boron (1 B) only, germinates as well as the pollen of branches supplied with sugar only (S). The germination is very much better in both instances (46.2 % and 42.5 % respectively) than that of the control (12.7 %). The best germination (79.1 %) is obtained with pollen originating from branches supplied both with sugar and boric acid (S+10 B).

It may be further noted that, apart from the 'control' pollen, no bursting occurred in the sugar solution with boron, while many pollen tubes were longer than 20 D after 3½ hours of germination in both varieties. The addition of boric acid concentrations higher than 10 ppm to the solution taken up by the branches proved to be harmful to the vitality of the pollen.

2.2. *Experiments with pear pollen originating from branches attached to the tree*

Material and methods. – In contrast with the foregoing experiments the solutions were not taken up by branches cut from the tree, but they were supplied to the branches on the tree in the orchard. This was done by giving the branch at its basal end a long cut in an upward direction. The thus loosened strip was put in a bottle containing the solution (see photo 2).

The experiment was carried out on branches of 4 pear varieties between the 18th and 20th March of 1954 when the buds began to show some activity. Solutions of 0, 1, 10, 100 and 1000 or 2000 ppm boric acid in distilled water with or without 4 % sugar were supplied to the branches. Since the bottles contained 50 ml of the solution and were not refilled, the respective amounts of boric acid supplied per branch were 0, 0.05, 0.5, 5, 50 or 100 mg. The solutions were completely taken up by the branches within 24 hours. The loosened strips of wood were fastened and coated after the removal of the bottles; the wounds were almost healed at flowering time.

The unit of experiment was one branch with 30–60 flowerbuds. The respective treatments were carried out on seperate branches (of approximately equal vigour, length and diameter) of one tree. The pear varieties used were Précoce de Trévoux, Conference, Beurré d'Anjou, Doyenné du Comice. The trees flowered at the end of April at which time the pollen was collected, dried at room temperature and stored.

Results. – A. Branches supplied with boric acid only. – The germination percentages of pollen originating from branches supplied with different concentrations (amounts) of boric acid are presented in figure 1, page 16.

From the germination percentages summarized in this figure it can be derived that:

The percentage of germination of pollen of Précoce, Conference and Anjou in sugar solution *without* boric acid is positively correlated with the amount of boron supplied to the branches in most instances. The germination percentage of Doyenné pollen is equally high, irrespective of the treatment of the branches.

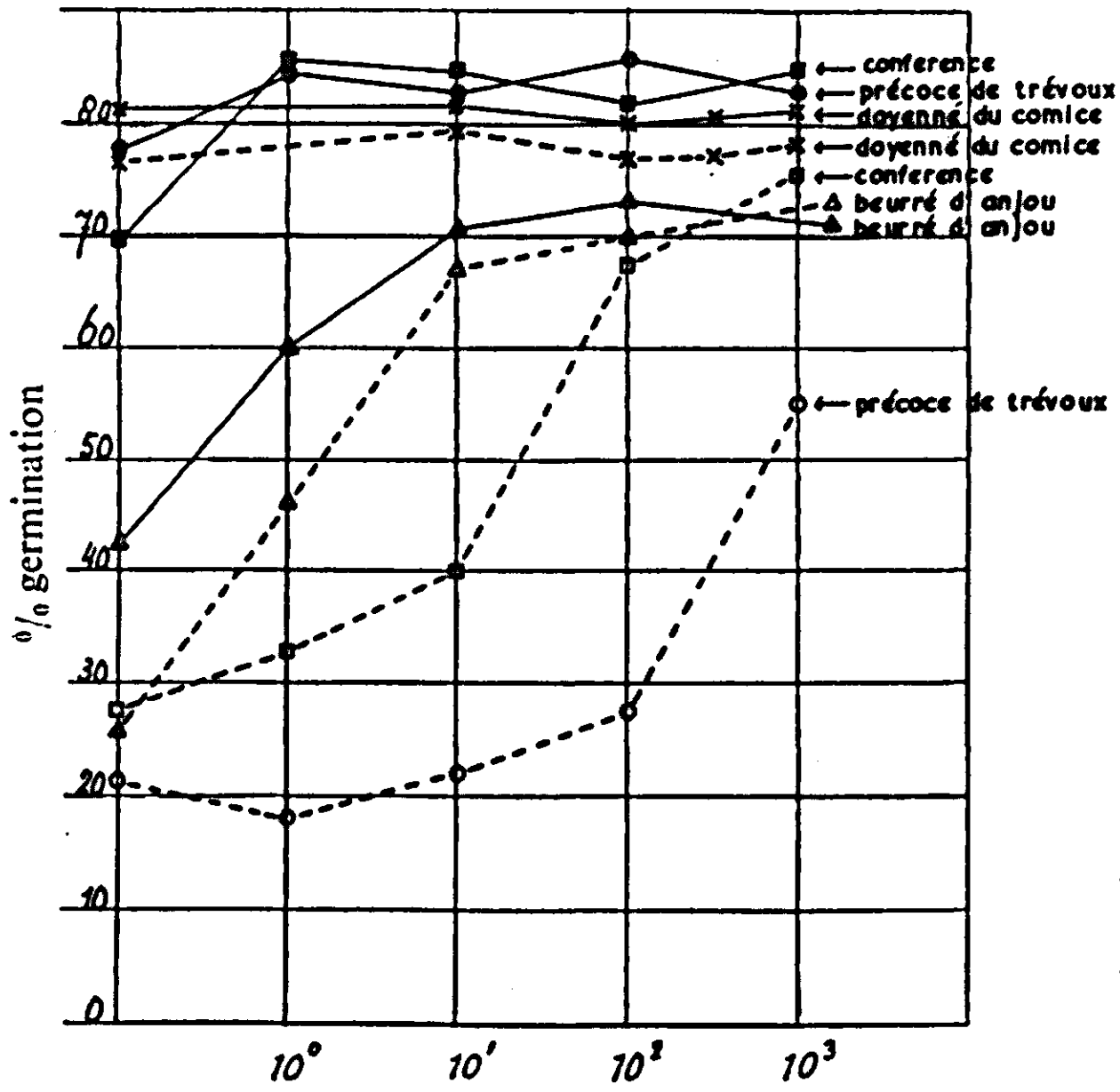

FIGURE 1.
The effect of boron supply to branches of pear trees on the germination of their pollen in 10% sugar solution with and without 30 ppm H_3BO_3; germination time $3\frac{1}{2}$ hours.

--- germination in medium without H_3BO_3,
—— germination in medium with H_3BO_3.

The percentage of germination of the pollen of 3 varieties obtained in 10% sugar solution *with* boric acid appears to be little affected by the amount of boron taken up by the branches. Hence, the vitality of the above pollens is approximately the same, irrespective of the pretreatment of the branches. The vitality of the Anjou pollen is apparently related to the supplied amount of boron.

It can be further remarked that the length the pollen tubes attain in sugar solution *without* boron is also directly related to the amount of boron taken up by the branches; this holds true for 3 tested varieties. For example, the percentages of tubes longer than 5D of pollen originating from branches with 0, 10, 100 and 1000 ppm boric acid respectively were: for Précoce 45, 47, 63 and 81%; for Conference 61, 70, 77 and 100% respectively. The percentages of burst grains and tubes decrease in both cases with increasing concentration of H_3BO_3 supplied to the branches. The percentage of burst Doyenné and Anjou pollen is small in all cases; the pollen tubes are longer than those of the other 2 varieties. The pollen tubes grown in sugar solution *with* boron were of approximately equal length, irrespective of the pretreatment of the branches; no bursting occurred.

The germination of the pollen from the control branches (with water) and that of pollen from the branches supplied with 1000 ppm boric acid has also been tested in *water* with and without boric acid.

From table 3 it can be noted that:

The germination percentage of pollen from branches supplied with 1000 ppm boric acid in water *without* boron is in all instances (columns 3, 5, 7, 9) higher than that of pollen from branches supplied with water only (columns 2, 4, 6, 8). The germination percentage of the '1000 B pollen' in water *with* boron is for Conference and Anjou twice as high as that of the '0 B pollen'. The differences

TABLE 3

The effect of boron supply to branches of pear trees on the germination of their pollen in water without and with 30 ppm H_3BO_3; germination time $3\frac{1}{2}$ hours.
0 B, 30 B, 1000 B = 0, 30 and 1000 ppm H_3BO_3 respectively.

1	2	3	4	5	6	7	8	9
Germination medium	Variety and % germination of pollen of branches supplied with							
	Conference		Précoce		Anjou		Doyenné	
	0 B	1000 B	0 B	1000 B	0 B	1000 B	0 B	1000 B
water+ 0 B	5.5	14.0	3.0	6.0	18.4	36.9	22.5	34.2
water+30 B	9.9	19.6	24.3	22.8	27.2	56.7	58.0	51.5

for the other 2 varieties are insignificant. The tubes of Anjou and Doyenné (1000 B) were fairly long, also in water without boron.

The germination of all pollen is stimulated by boron in the medium, but the 'degree of boron sensitivity' (ratio of the germination percentages obtained with and without boron respectively) of the control pollen, except Anjou, is much higher than that of the 1000 B pollen.

It is of interest to note that the germination of the 1000 B pollen in water is markedly affected by the adding of boron in contrast with its germination in 10% sugar solution (compare figure 1).

B. Branches supplied with boric acid and sugar. – The germination of pollen from branches supplied with solutions containing both boric acid and 4% sugar was compared with the germination of pollen from branches with boric acid only.

TABLE 4

The effect of boron and sugar supply to branches of pear trees on the germination of their pollen in water or 10% sugar solution without and with 30 ppm H_3BO_3; germination time $3\frac{1}{2}$ hours.
0 B, 10 B, 30 B, 100 B = 0, 10, 30 and 100 ppm H_3BO_3 respectively; –S and +S = no sugar or 4 % sugar supplied to the branches.

1	2	3	4	5	6	7	8
Variety	Germination medium	Germination percentage of pollen from branches supplied with					
		0 B – S	0 B+S	10 B – S	10 B+S	100 B – S	100 B+S
Précoce de Trévoux	water+ 0 B	3.0	2.5	4.3	6.3	7.3	8.3
	water+30 B	24.3	19.7	17.0	22.0	18.2	22.8
	10% sugar+ 0 B	21.3	15.3	22.0	17.3	27.7	34.0
	10% sugar+30 B	77.6	70.8	84.8	82.7	85.5	88.5
Conference	water+ 0 B	5.5	7.0	5.2	2.3	7.8	7.7
	water+30 B	9.9	11.3	20.0	18.1	18.8	24.4
	10% sugar+ 0 B	27.3	27.7	39.7	42.3	67.0	68.0
	10% sugar+30 B	69.6	84.0	84.6	80.3	81.6	81.0
Doyenné du Comice	water+ 0 B	22.5	36.4	–	–	23.0	38.4
	water+30 B	58.0	52.0	–	–	50.6	60.2
	10% sugar+ 0 B	79.3	78.0	–	–	76.5	80.7
	10% sugar+30 B	82.0	81.0	–	–	79.5	80.0

Table 4 shows that the germination percentage of pollen from branches +S (columns 4, 6, 8) is in 18 instances slightly higher and in the other 14 cases slightly lower than the germination percentage of pollen from branches –S (columns 3, 5, 7). These results imply that the supply of sugar, either alone or in combination with boron, to the branches had no significant effect on the germinability of the pollen. The data also demonstrate the influence of the composition of the germination medium on the germination.

3. THE INFLUENCE OF BORON SPRAYS ON THE FRUIT SET AS RELATED TO THE BORON SENSITIVITY OF THE POLLEN

When a pollen is found to be sensitive to boron *in vitro*, the question arises whether this phenomenon is a symptom of boron deficiency of the pollen as related to boron deficiency of the plant itself. Part of this question has been answered in the foregoing in which it was shown that a certain relationship exists between the degree of boron sensitivity of the pollen and the (artificially raised) boron level of the plant. Whether a correlation exists between the boron sensitivity of the pollen and actual boron deficiency of the plant as determined *e.g.* by its fruit set is not known. The author, therefore, investigated on the one hand the effect of boron sprays during bloom on the subsequent fruit set of apple and pear and on the other hand the degree of boron sensitivity of pollen originating from the treated trees, but collected before spraying. In this way it could be ascertained whether the degree of boron sensitivity of the pollen can serve as an indirect measure for the degree of boron 'deficiency' of the plant as indicated by the fruit set.

3.1. *Experiments with apple and pear in 1953.*

Material and methods. – Preliminary experiments were carried out with several pear and apple varieties in 1953. The unit of experiment consisted of the flower cluster thinned to 2 (apple) or 3 (pear) flowers which were emasculated and pollinated by hand just before they opened. The flowers were sprayed only once with water, water+70 ppm, water+140 ppm boric acid respectively immediately after pollination. Approximately 40 clusters were used per treatment per variety.

The experiments were carried out in an orchard at Wageningen (No. VI) and at Noordbroek in Groningen (No. VIII). In the former orchard 5 pear varieties were treated: Comtesse de Paris, Zwijndrechtse Wijnpeer, Clapp's Favourite, Doyenné du Comice, Sucré de Montluçon and 3 apple varieties: Yellow Transparent, James Grieve, Belle de Boskoop. The pears were cross-pollinated with one of the following pear pollens: Précoce de Trévoux, Doyenné du Comice, Comtesse de Paris, the apples with either James Grieve or Yellow Transparent. In the latter orchard 3 apple varieties were treated: Groninger Kroon, Zoete Kroon, Bramley's Seedling; they were pollinated by pollen of Sterreinette.

Results. – From germination experiments with pollen from the treated trees (of non-sprayed branches) and with pollen used for pollination, it can be derived that:

The germination percentage of the pear pollen determined in 10% sugar solution+30 ppm boric acid was much higher than that obtained in sugar solution without boric acid in most cases. The germination of the apple pollen in sugar solution was not affected by the presence of boron. The germination in water, however, could be considerably improved when 30 ppm boric acid was added.

The pollination experiments in orchard VI at Wageningen partly failed due to severe night frost after the fruit set. For that reason only the fruit set after

the first drop could be recorded. No night frost occurred in orchard VIII at Noordbroek; the set of mature fruits was recorded. The results are summarized in table 5.

TABLE 5
The effect of boron sprays on the fruit set of pear and apple.
0 B, 70 B, 140 B = 0, 70 and 140 ppm H_3BO_3 respectively.

Results summarized for	Total number of flowers sprayed with			Fruit set after spraying with		
	0 B	70 B	140 B	0 B	70 B	140 B
5 Pear varieties (VI) . .	561	568	576	55.4 %	57.0 %	58.2 %
3 Apple varieties (VI) .	254	250	262	31.5 %	32.8 %	33.9 %
3 Apple varieties (VIII) .	246	240	244	45.2 %	41.7 %	47.7 %

It follows from table 5 that the fruit set or harvest was very good in all cases. Some increase of the fruit set may be observed after spraying with boric acid, but none of the differences with the control is significant. The seed set of the fruits harvested in orchard VIII was neither significantly affected and amounted to 6.0, 5.7 and 5.7 seeds per fruit after spraying with 0, 70 and 140 ppm H_3BO_3 respectively.

3.2. *Experiments with pear in 1954.*

Material and methods. – A large scale experiment was carried out on pear trees in 8 different orchards in 1954. All these orchards were well managed, but were situated on different soils and in different parts of the country. The unit of experiment was one branch with a mean number of approximately 35 clusters (not thinned); all treatments were carried out on (seperate) branches of one tree. The branches were sprayed with 20, 100 and 500 ppm boric acid respectively. In a few cases the branches were sprayed with 100, 500 and 1500 ppm boric acid.

Each of the concentrations was applied 1×, 2× and 3× respectively (on different branches) at intervals of approximately 10 days. Hence, per tree 9 branches were treated, while 2 branches were left unsprayed and used as a control. The experiment was repeated on 7–10 trees per variety. The branches were sprayed for the first time when approximately half of the flowers were open; no emasculation or artificial pollination took place. The pollen of all treated varieties was collected from non-sprayed branches before the first spray was applied to prevent any possible influence on their germination *in vitro*.

In the following table the localities of the orchards and the varieties of trees which were treated are given:

TABLE 6
Location of orchards, their soil and the varieties treated with boron sprays.

Location	No.	Soil description	Treated varieties
Maasbracht	I	Loamy clay soil, deposited by the Meuse	Jeanne d'Arc, Doyenné du Comice
	II	Idem	Doyenné du Comice
Kesteren	III	Heavy clay soil in the subrecent river levees of the Rhine	Conference, Triomphe de Vienne
	IV	Idem	Conference, Doyenné du Comice
Wageningen	V	Heavy basin-clay soil, deposited by the Rhine	Conference, Doyenné du Comice
	VI	Idem	Conference, Comtesse de Paris, Beurré d'Anjou, Beurré Beucke
	VII	Moist humic fine-sandy soil	Conference, Doyenné du Comice
Noordbroek	VIII	Shallow peat reclamation soil	Conference, St. Rémy

TABLE 7

The germination of pear pollens in media with or without sugar and boric acid (germination time 3½ hours) and the effect of boron sprays on the fruit set of the varieties from which the pollen was gathered.

Number of		Variety	% Germination of pollen in				Number of treated		% Clusters set after June-drop after spraying with [3]			
experiment	orchard		H_2O	H_2O+B [2]	S	S+B	trees	clusters (total)	0 B	20 B	100 B	500 B
1	III	Conference	0	10.5	78.8	77.6	10	4058	26.4	29.7	27.4	32.5
2	IV	Conference	0	6.5	70.8	78.8	12	3243	37.3	29.8 [1]	32.2	40.1
3	V	Conference	2.5	7.6	80.3	82.6	7	1732	42.2	40.5	44.3	44.2
4	VI	Conference	1.6	4.3	26.0	70.0 [1]	4	955	24.6	22.5	24.3	31.3 [1]
5	VII	Conference	0	10.5	78.0	79.2	7	2249	21.2	19.4	18.7	20.9
6	VIII	Conference	0.8	8.6	33.0	46.6 [1]	10	3293	54.5	69.9 [1]	64.3	59.3
7	I	Doyenné d. C.	8.8	37.3	80.5	79.0	10	4187	13.4	14.8	14.3	q4.8
8	II	Doyenné d. C.	0.3	17.3	78.5	78.3	10	3251	37.3	31.1	31.2	23.2 [1]
9	IV	Doyenné d. C.	5.2	42.2	70.5	87.2 [1]	8	3992	0	0	0	0
10	V	Doyenné d. C.	7.3	40.8	88.5	87.8	9	4572	5.0	5.5	5.6	6.0
11	VII	Doyenné d. C.	3.6	25.2	76.8	75.3	8	2064	0	0	0	0
12	I	Jeanne d'Arc	1.3	12.7	42.0	79.0 [1]	10	2806	17.8	17.3	17.4	15.3
13	III	Triomphe d. V.	9.6	33.5	71.0	79.0	10	4828	16.7	17.5	14.9	16.5
14	VI	B. d'Anjou	1.0	16.2	67.2	72.8	4	2412	0	0	0	0
15	VI	B. Beucke	0	7.3	15.0	81.0 [1]	7	3083	31.3	28.3	33.0	35.4
16	VI	Comtesse d. P.	0	2.7	13.3	31.5 [1]	7	2201	14.7	11.4	8.1 [1]	11.1
17	VIII	St. Rémy	0	0.7	5.3	11.2 [1]	10	1820	62.4	66.1	52.9	66.0
								Mean	23.81 %	23.74 %	22.86 %	24.51 %

[1] Differences with the control (without boron) significant (P = 0.01 for germination; P = 0.05 for fruit set).
[2] *All* differences between germination in H_2O and in H_2O+B significant (P = 0.01).
[3] Experiments 2, 9 and 17 were sprayed with 100, 500 and 1500 ppm H_3BO_3 respectively, the others with concentrations as indicated in the table.

Results. – It is the author's experience that fruits of pear which survive the June-drop will practically all remain on the tree — apart from storms or pests — until they are harvested.

Therefore, only the fruit set after the June-drop has been recorded. Since no significant differences occurred between the fruit sets of branches sprayed 1 ×, 2 × or 3 × with the same concentration, these data have been added and the average percentages of fruit set obtained per concentration are given in table 7. The germination percentages of the pollen originating from the treated varieties have also been recorded in this table. Their germination was tested in water (H_2O), water+30 ppm H_3BO_3 (H_2O+B), 10% sugar solution (S) and 10% sugar solution+30 ppm H_3BO_3 (S+B) respectively. The percentages of fruit set of the different treated branches, determined as the mean from all fruit sets, irrespective of variety or orchard, are presented in table 8, in which the results with St. Rémy were excluded, since each concentration was sprayed only once.

TABLE 8
The effect of concentration and number of boric acid sprays on the average fruit set of all pear varieties treated.

	Control not sprayed	Sprayed with 20 ppm H_3BO_3			Sprayed with 100 ppm H_3BO_3			Sprayed with 500 ppm H_3BO_3		
		1 ×	2 ×	3 ×	1 ×	2 ×	3 ×	1 ×	2 ×	3 ×
Total clusters per treatment	8901	4459	4288	4476	4362	4654	4427	4272	4502	4525
Fruit set after June-drop	21.40 %	20.87 %	21.30 %	22.64 %	22.50 %	19.04 %	22.86 %	22.39 %	21.50 %	22.01 %

The following observations can be made with respect to the germination:

1) The percentages of germination obtained in water with boron are significantly higher than those obtained without boron in all instances.
2) The germination percentages obtained in 10% sugar solution with boron are significantly higher than those obtained without boron in 7 instances, the differences in the other cases are insignificant.
3) Considerably higher germination percentages are obtained in 10% sugar solutions than in water.
4) The degree of sensitivity of the pollen to boron is higher in water than in sugar solution.

With regard to the fruit set it can be derived:

1) In 3 experiments [9, 11, 14] no fruits set, either without or with boron sprays.
2) In 9 experiments [1, 3, 5, 7, 10, 12, 13, 15, 17] none of the differences is significant.
3) In 3 experiments [2, 8, 15] the fruit set of one of the treatments was significantly lower and in 2 experiments [4, 6] significantly higher than the fruit set of the control.
4) The mean percentages of fruit set (table 7 and 8) are practically the same, irrespective of the applied treatment.
5) When the differences between all separate treatments and their controls are compared, irrespective of their significance, it is found that the treated

branches give a higher yield than the control in 21 instances and a lower one also in 21 instances.

It can be concluded from the results that the effect of the boron sprays on the fruit set has been insignificant. The trees are apparently not deficient in boron; none of the trees showed visible symptoms of boron deficiency, neither at flowering time nor later in the season. It is not surprising, therefore, that — as is shown in table 7 — the degree of boron sensitivity of the pollen appears not to be related with the effect of boron sprays on the fruit set of trees from which the tested pollen originated.

4. DISCUSSION

A. From the experiments with pollen of *cut* branches of pears it can be concluded that the supply of these branches with sugar affects the germination of their pollen to a greater or lesser extent(tables 1, 2 on p. 14). This observation suggests that this carbohydrate is in the minimum under the given conditions. The significance of a sufficient carbohydrate supply can also be derived from experiments of WINKLER (143, 1926) with *Vitis* pollen. He found that the germinability of the pollen improved as a result of earlier development of the foliage and an increase in its area with less severe pruning.

The germination of the pollen was also found to be improved after the uptake of boron by the branches. It is of interest to note that the pollen germination could be stimulated by supplying either boron or sugar to the branches, while the best germination was observed with pollen originating from branches supplied with both boron and sugar. These observations suggest that boron plays a role in the carbohydrate metabolism of the plant. In this connection it may be mentioned that the experiments of GAUCH and DUGGER (1953, 48) with 'labelled' sugar provide direct evidence that boron accelerates and increases the transport of sugar (taken up by a leaf) in tomato and bean. The improvement of the germination of pollen originating from branches supplied with boron and sugar is in essence due to an increase of their vitality. Also BOBKO and ZERLING (19, 1938), in experiments with *Trifolium pratense*, found a markedly higher pollen vitality of plants fertilized with a boron compound than of plants deficient in boron. This infers that the vitality of the pollen is related to the boron level of the plant.

B. The vitality of the pollen from *attached* branches appeared not to be significantly affected by the boric acid supply for 3 of the 4 tested varieties (fig. 1, p. 16). The boron level of these varieties was apparently sufficient in itself to ensure a good vitality of the pollen. The germination of the pollens in sugar solution (and also in water; table 3, p. 17) without boron, on the other hand, was found to be directly related to the amount of boron taken up by the attached branches. Or in other words, *the germinability of the pollen is the same — within certain limits — whether the 'extra' boron is taken up from the tree beforehand or taken up by the pollen from its germination medium afterwards.* From the fact that the relative influence of boron on the germination *in vitro* was generally smaller as the amount of boric acid taken up by the branches was greater, it can be derived that *the degree of boron sensitivity of the pollen is inversely related to the boron level of the plant.* This statement is also corroborated by the observation of ANTLES (7, 1951). This investigator applied a fertilizer containing boron to a pear orchard during 4 successive years, because he found that the pollen originating from this orchard, though germinating

for 100% in sugar solution with boron, did not germinate at all without boron. He observed that the germination in a medium without boron increased successively each year, until in the fourth year of fertilizing a 100% germination was obtained *without* boron in the medium.

The sugar supplied to pear branches attached to the tree had no significant effect on the pollen germination (table 4, p. 17). The natural supply of carbohydrates is in all probability ample under those conditions.

C. With regard to the sensitivity of pollen to boron *in vitro*, it is of importance to note that this sensitivity not only depends on the boron level of the tree, but also on the properties of the medium used for germination. It appeared, namely, that the relative influence of boron on the pollen germination in water is much greater than the relative influence of boron on the germination in 10% sugar solution (table 7. p. 20). This was found to be true for all pollens tested. The same can be derived from experiments carried out by ESSER (43, 1951) with *Lythrum* pollen and by KATO (68, 1953) with *Beta* pollen. In both cases the addition of sugar and/or agar to the germination medium led to a marked decrease of the influence of boron on the germination. On account of the evidence presented in chapter II it is very unlikely that this difference in response to boron is due to sugar uptake by the pollen. It can be assumed, therefore, that the degree of sensitivity of the pollen to boron is related in some way to the osmotic (or colloidal) properties of the medium.

D. What happens *in vitro*, presumably also takes place *in vivo*, since it was found in a great number of pollination experiments carried out by the author, that 'boron sensitive' pollen is able to affect an excellent fruit set. Probably the osmotic or colloidal conditions on the stigma or in the style are such that no difficulties arise as to the germination of such pollens. Moreover, it has been shown in several instances that the boron content of pollen of normal plants is rather high (15, 18, 19). Even, if the boron level of the pollen is not high enough, the lacking amount of boron is quite possibly supplemented by the style. Namely, the styles of several analysed plants have been found to contain a relatively high amount of boron (15, 18, 19, 47). It was also found by SCHMUCKER (113, 1932) and GÄRTEL (47, 1952) with *Nymphea* and *Vitis* pollen respectively, that the stigma secretion contained a boric acid concentration equivalent to the concentration required for optimal germination *in vitro*.

E. With regard to the influence of boron sprays on the fruit set of apple and pear trees the results imply that the effect of spraying has been negligeable (tables 5, 7, 8; pp. 19, 20, 21). Nevertheless, all pollens originating from the sprayed trees (collected before spraying) appeared to be very sensitive to boron when germinated in water, while many pollens were also found to be sensitive to boron in sugar solution. Comparison of the fruit set of the treated varieties with the germinability of their pollens demonstrates, however, that the degree of the boron sensitivity of the pollen bears no relation to the effect of boron sprays on the fruit set. Hence, *the degree of boron sensitivity of the pollen, although related to the boron level of the tree, cannot serve as a measure for the degree of boron 'deficiency' or 'abundancy' as indicated by the fruit set.*

CHAPTER IV

THE ROLE OF BORON IN GERMINATION AND MUTUAL STIMULATION OF POLLEN GRAINS

1. RELATION BETWEEN GERMINATION AND ENVIRONMENTAL FACTORS

1.1. *Introduction*

From the literature review given in chapter II it appeared that the rate of water diffusion – per definition, the rate at which water is released by the medium into the pollen – is a determining factor in the germination of pollen in general. The effect of boric acid on germination also appears to be of a general nature, since so many pollens were found to be sensitive to boron *in vitro* (see chapter III p. 13). Experiments carried out with pollen of several pear varieties strongly indicated that the degree of boron sensitivity of the pollen *in vitro* is related to the boron level of the plant. It was also observed that the degree of boron sensitivity of the pollen is affected by the osmotic properties of the medium used for germination. However, what function boric acid has in germination has not yet been considered and few observations can be found in literature which throw any light on this subject. The author, therefore, has studied the influence of boric acid on germination as affected by certain environmental factors, in order to acquire some knowledge as to the role played by boron. The influence of the following factors was investigated:

a) Boric acid concentration.
b) Discontinuous boric acid supply.
c) Storage and pre-humidification.
d) Germination time.
e) Temperature.
f) Sugar concentration.

1.2. *The influence of boric acid concentration*

The majority of investigators (see literature review chapter III, p. 12) has not specially referred to the relation between germination and boric acid concentration, since they were mostly concerned with the finding of a suitable concentration for practical purposes. Therefore, this relationship has been investigated.

Material and methods: Pollen of 5 pear varieties: Beurré Superfin, Nouveau Poiteau, Clapp's Favourite, Conference, Doyenné du Comice and of 2 apple varieties: Sterreinette, Brabantse Bellefleur, was tested in a range of boric acid concentrations in 10% sugar solution. The concentrations used were: 0, 0.15, 0.35, 0.60, 1.2, 1.9, 2.9, 3.9, 5.8, 7.7, 11.6, 17.3, 38.5, 77, 150, 300, 600 and 1200 ppm. The germination technique employed in this paragraph and in the following ones is the same as has been used in chapter III (description on p. 14). The grains were recorded as germinated, when the tubes were longer than $\frac{1}{2}\times$ grain diameter.

Results. – With regard to the *apple pollen* it appeared that its germination was not affected by boron: the germination percentages were equally high (80–85%) and the tubes equally long, while no bursting occurred, irrespective of the boric acid concentration (0–300 ppm).

The germination of the *pear pollen*, on the other hand, was markedly stimulated by boric acid. It appears that the relationship between germination percentage and the boric acid concentration can be represented by a saturation

curve. The data obtained for 2 pear pollens and the curves they represent are recorded in figure 2. The results obtained for the pollen of the other pear varieties were very similar.

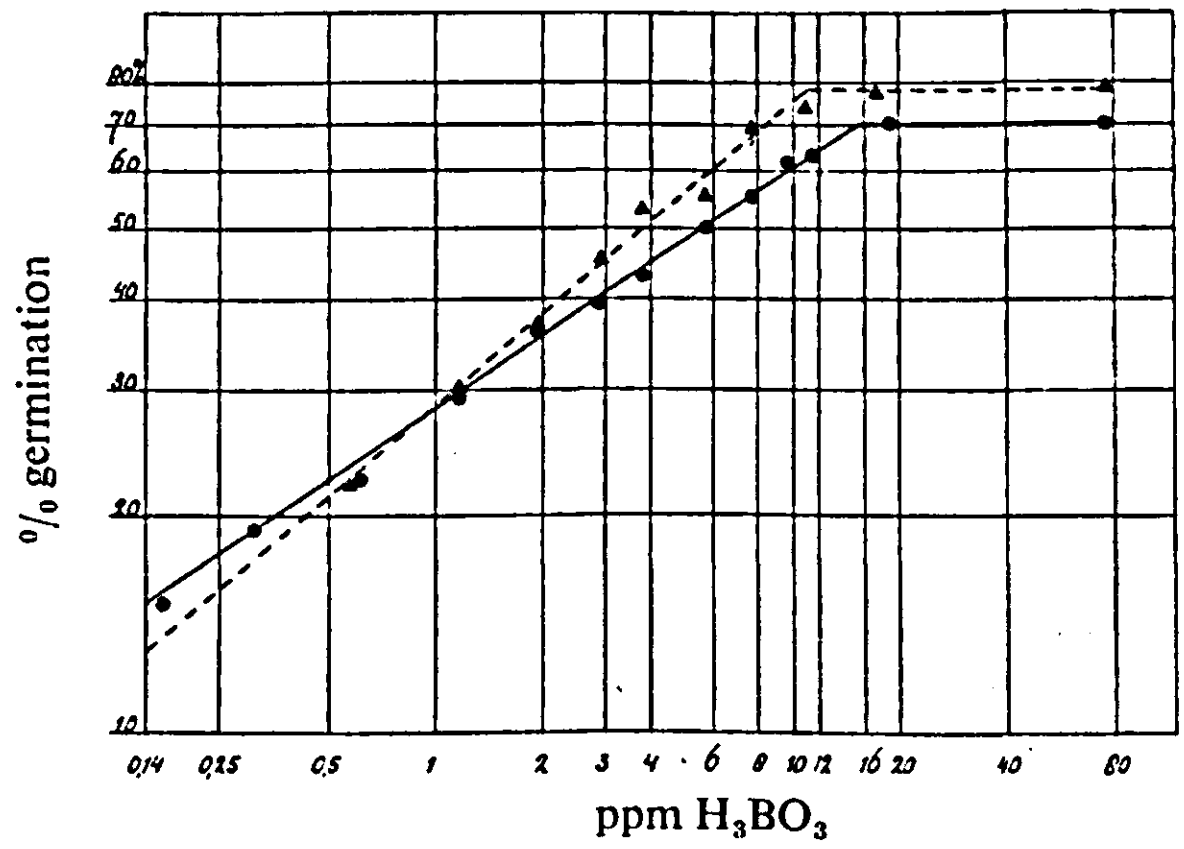

FIGURE 2. The relation between the germination of pear pollen and boric acid concentration in 10% sugar solution; germination time 3 hours.

▲—▲ Clapp's Favourite: $\log y = 0.34 \log x + \log 27.56$;
$\log \bar{y} = 1.5901 (\pm 0.0100)$.

●---● Conference: $\log y = 0.42 \log x + \log 28.36$;
$\log \bar{y} = 1.6563 (\pm 0.0153)$.

Figure 2 shows a linear relationship on double-logarithmical scale (statistically reliable, $P = 0.01$). The maximal germination percentage (the highest possible percentage under the given conditions) is reached between 12 and 16 ppm and is the same at 77 ppm boric acid.

The percentage of the pollen tubes longer than 5 × pollen diameter grown in 0, 0.60, 1.2, 1.9, 3.9, 7.7, 11.6 and 17.3 ppm were for Conference pollen 11, 18, 54, 76, 93, 100, 100 and 100 % respectively. The same holds true for the other pear pollens. Hence, the length of the tubes is directly related to the boric acid concentration. The percentage of bursting appeared to be inversely related to the boric acid concentration.

It is noteworthy that the very beginning of germination, after the grain has completed its swelling process, is characterized by the formation of a slight 'bulge' (at one of the germination pores). This phenomenon occurs, irrespective of the presence of boric acid. After that stage, however, it may depend on the presence of boric acid whether a distinct tube will protrude or bursting will occur.

Table 9 demonstrates that maximal germination and good tube growth can be obtained in concentrations many times higher than the minimum. For example, the germination of the apple and pear pollen in 77 and 300 ppm is almost the same as in 17 ppm boric acid. In 600 ppm mainly the tube growth appears to be inhibited, even in 1200 ppm boric acid most grains germinated, but the tubes remained very short. In the latter concentrations a number of grains did not burst, but shrunk to some extent, while their appearance had become darkish and granulated at the end of the experiment, indicating loss of vitality.

TABLE 9
The effect of high boric acid concentrations on the germination of pear and apple pollen; germination time 3 hours.
PT>5D = % pollen tubes longer than 5× pollen diameter.

Concentration H_3BO_3 in ppm	Doyenné du Comice		Sterreinette	
	% germination	PT>5D	% germination	PT>5D
0	27.0	21	81.0	90
17	88.6	100	80.0	96
77	79.6	97	78.0	89
300	74.0	90	75.7	87
600	70.0	16	71.0	9
1200	67.6	4	50.2	4

1.3. *The influence of a discontinuous boron supply*

The foregoing experiments imply that germination and tube growth of pear pollen depend on the amount of boron present in the medium. In this connection it would be of interest to know whether a continuous supply of boron is necessary to obtain a good germination. To that purpose experiments have been carried out in which boron was either removed from or added to the germination medium at a certain stage of germination.

1.3.1. *Removal of boric acid during germination*

Material and methods. – Pollen of Beurré Beucke and Clapp's Favourite was brought into drops of 10% sugar solution with 30 ppm boric acid. Half the number of drops was replaced by drops of the same solution (S+B→S+B), the other drops were replaced by drops of sugar solution without boron (S+B→S) 45 minutes after the beginning of the experiment. The replacement of the drops was done after absorbing the initial drops with strips of filterpaper.

Results. – From table 10 it follows that the germination percentage still increases (columns 3, 5, 9) during 90 minutes following the removal of the boron (S+B → S). In contrast with the replaced medium (S+B → S+B), however, no increase takes place thereafter (columns 9, 13).

TABLE 10
The effect of the removal of boric acid from the medium in the initial stage of germination on the subsequent germination of Beurré Beucke pollen.
T = time in minutes since the experiment began,
Ger = % germination, Bur = % of burst grains and tubes,
PT>5D = % pollen tubes longer than 5× pollen diameter.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Media	T	Ger	T	Ger	Bur	PT >5D	T	Ger	Bur	PT >5D	T	Ger	Bur	PT >5D
S (control) . .	45	7	90	17	45	0	–	–	–	–	150	15	60	0
S+B (control)	45	15	90	50	0	50	–	–	–	–	150	79	0	75
S+B → S . . .	45	8	90	45	20	30	135	57	35	35	150	58	40	40
S+B → S+B .	45	13	90	41	0	60	135	64	0	80	150	80	0	85

The growth of the tubes is also less as compared with the replaced medium (S+B →S+B; columns 7, 11, 15), while the percentage of burst grains and tubes gradually increases with time (columns 6, 10, 14). Apparently, the ger-

mination has profited by the presence of boron in the initial stage of germination, since the replaced sugar solution (S+B → S) gives better results than the non-replaced control (S). No significant differences exist between the germination in the replaced medium with boric acid (S+B→S+B) and that in the non-replaced control (S+B). In neither case did bursting occur. The experiment in which pollen of Clapp's Favourite was used gave similar results.

1.3.2. *Addition of boric acid during germination*

Material and methods. – Pollen of Clapp's Favourite was germinated in drops of a 10% sugar solution containing no boron. A small drop (⅓ of the usual amount) of sugar solution with 100 ppm boric acid was added after 40, 60, 80 and 100 minutes respectively. The controls contained either 30 ppm boric acid from start (+B), or had no boron (–B).

Results. – From table 11 it can be observed that the germination percentage at the end of the experiment (after 300 min.) is inversely related and the percentage of bursting directly related to the time at which boric acid was added. The germination percentage is as high as that of the control+B, when boric acid is added after 40 minutes. Apparently, a retarded addition of boron is not harmful to the germinability of the pollen grains when it occurs before the tubes begin to form (after approximately 55 minutes). The sum of the percentages germinated or burst grains is approximately constant at the end of the experiment, irrespective of the time boron was added. This suggests that whenever the grains are not able to produce tubes at a certain moment, they will burst (see also p. 25).

TABLE 11
The effect of addition of boric acid at different stages of germination on the subsequent germination of Clapp's Favourite pollen.

H_3BO_3 added after	Germination and bursting recorded					
	just before addition of H_3BO_3		after 150 minutes		after 300 minutes	
	% germination	% burst grains	% germination	% burst grains	% germination	% burst grains
0 minutes (control+B)	–	–	53.5	–	68.0	20
40 minutes	0	5	36.7	–	73.0	16
60 minutes	3.8	16	29.3	–	60.5	32
80 minutes	7.0	27	20.5	–	49.2	45
100 minutes	10.0	42	15.0	–	27.0	69
None added (control-B)	–	–	13.0	–	18.3	70

1.4. *The germinability of pollen as affected by storage and pre-humidification after storage*

A decreased rate and amount of germination and tube growth and an increase of bursting are the outward symptoms accompanying the ageing of stored pollen (137). Generally, the symptoms indicate a decreased vitality of the pollen.

With regard to the conditions for germination, PFUNDT (99, 1910) states that these have to be more exacting for (long) stored pollen than for freshly collected pollen. NEBEL *et al.* (89, 1936) found a marked 'revival' of too dryly stored apple pollen when it was exposed during 1 day to 80% relative humidity (pre-humidification) previous to germination. PFEIFFER (97, 1939) demonstrates that

Gladiolus pollen, stored at room temperature for some time, induced a much higher seed set when humidified prior to pollination as compared with non-humidified pollen. Striking results were obtained by DUFFIELD *et al.* (40, 1941) with *Pinus* pollen. From their data it can be derived that the relative influence of pre-humidification on the subsequent germination of pollen stored during 1 year, increases with decreasing storage humidity and increasing storage temperature. Presumably pre-humidification can be regarded as an adaptation of stored pollen to a medium not quite suitable for germination. Similar experiments with respect to the effect of pre-humidification on the germinability of stored pollen were carried out by the author. It was also investigated whether the boron sensitivity of the pollen is affected by the storage period.

Material and methods. – The experiments were done with pollen which had been stored at 10% relative humidity and 2–4° C during 7 months. In the first experiment pollen of 2 pear varieties and 2 apple species was humidified (exposed during one day at 80% relative humidity) and germinated in 10% sugar solution with 30 ppm boric acid.

In the second experiment Doyenné du Comice pollen from branches supplied with water and 1000 ppm boric acid respectively (see chapter III, p. 15) was pre-humidified and tested in sugar solution with or without boron.

In the third experiment pollen of 3 pear varieties, which showed a slight boron sensitivity only at the beginning of storage, was tested again after 7 months.

Results. – Table 12 shows that the germination of the pre-humidified pollen was better than that of the untreated pollen in all instances. The effect of pre-humidification on pollen with a low vitality (Comtesse, *Malus micromalus*)

TABLE 12
The influence of pre-humidification on the germination of 7 months old pear and apple pollen in 10% sugar solution with 30 ppm boric acid; germination time 4 hours.

Treatment of pollen	Précoce de Trévoux	Comtesse de Paris	Sterreinette	*Malus micromalus*
Control	56.1	1.0	56.2	3.7
Humidified	70.5	14.0	66.7	20.3

appears to be relatively greater than on pollen with a good vitality (Précoce, Sterreinette). The tubes of the treated pollen were longer than those of the control, while less bursting occurred.

TABLE 13
The influence of pre-humidification on the germination of 7 months old Doyenné du Comice pollen of differently treated branches. Germination in 10% sugar solution without (–B) and with 30 ppm boric acid (+B); germination time 3½ hours.
Ger = % germination, Bur = % bursting.

1	2	3	4	5	6	7	8	9

Treatment of pollen	Branches supplied with water				Branches supplied with 1000 ppm H_3BO_3			
	–B		+B		–B		+B	
	Ger	Bur	Ger	Bur	Ger	Bur	Ger	Bur
Control . .	40.8	78	83.5	0	69.8	10	78.0	0
Humidified	67.2	36	83.8	0	81.5	5	79.8	0

From table 13 it can be derived that the germination percentage in the *absence* of boron (columns 2, 6) increases and the percentage of bursting

(columns 3, 7) decreases after pre-humidification. The effect of the treatment on pollen from branches treated with 1000 ppm boric acid was relatively less than that on pollen from branches treated with water. The vitality of the Doyenné pollen was higher than that of the above pollens (table 12). This is illustrated by the fact that pre-humidification did not improve the germination of the former pollen in the *presence* of boron (columns 4, 8), while no bursting occurred (columns 5, 9). Apparently, pre-humidification reduces the boron sensitivity of the pollen.

TABLE 14
The influence of the storage period on the sensitivity of pear pollen to boron. Germination in 10% sugar solution without (–B) and with 30 ppm boric acid (+B); germination time $3\frac{1}{2}$ hours.

Period of storage	Précoce de Trévoux		Conference		Doyenné du Comice from branches supplied with			
					water		1000 ppm H_3BO_3	
	–B	+B	–B	+B	–B	+B	–B	+B
1–3 weeks	55.0	82.6	75.3	84.6	76.5	81.5	81.0	84.0
7 months	22.5	58.0	23.0	60.8	40.8	83.5	69.8	78.0

The data recorded in table 14 indicate that the addition of boron to the medium has a much greater effect on the germination of the pollen after a long storage period than on its germination shortly after its collection. Or in other words, the degree of boron sensitivity of the pollen increases with increasing age. It can be remarked that Doyenné pollen from branches supplied with boron is less sensitive to boron after storage than pollen from the control branches.

1.5. *Germination in relation with time*

The germination time, defined as the time interval after which the germination percentage is recorded, has an important function in germination tests (see chapter II, p. 11). This is especially true, if one wants to obtain an impression of differences between germination rates (percentage of germination per unit of time) as determined by factors, such as temperature and sugar concentration. For that reason, the relation between time and pollen germination in sugar solutions both with and without boric acid has been investigated.

Material and methods. – The experiments were carried out with pollen of Clapp's Favourite (collected in 1952 and 1953 respectively) and of Sterreinette. The pollen grains after having been brought into the drops were observed until germination started; the germination percentages were determined at intervals of 15 minutes from that moment. Instead of the usual 6 drops per test, 3 drops per test were used in each of which the germination percentage was determined from 2×50 pollen grains. The experiment was carried out at 18° and 23° C.

Results. – The germination data are recorded in figure 3. The relationship between the germination percentage and germination time appears to be practically linear for the 3 tested pollens, since the mathematical errors were insignificant ($P = 0.01$). The horizontal stretches of the curves are not determined by the formulae, but indicate that no further increase of the germination took place.

It can be further derived from figure 3 that the first tubes of pollen of the same variety appear at approximately the same time, irrespective of the pres-

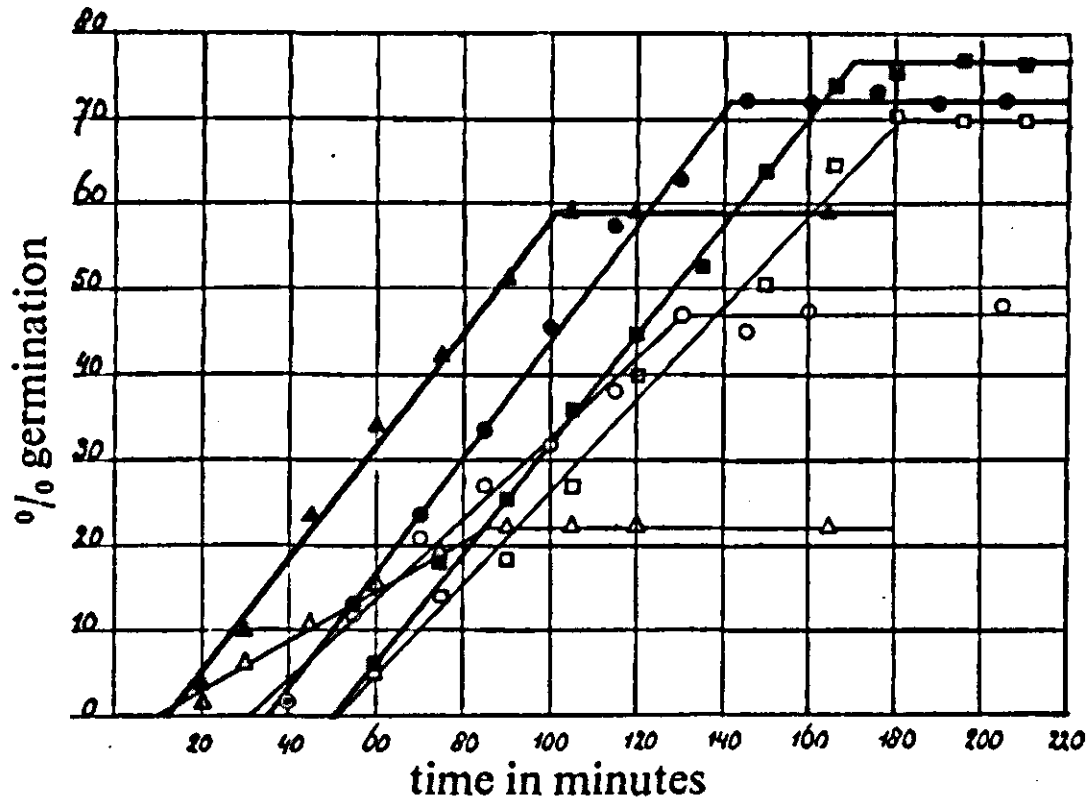

FIGURE 3. The relation between germination percentage and germination time in 10% sugar solution without (–B) and with H_3BO_3 (+B); germination temperature 23° C.

Clapp's Favourite (harvested 1952)	△—△ (–B);	$y = 0.29\,x - 3.10$,	$\bar{y} = 12.3 \pm 0.77\%$
	▲—▲ (+B);	$y = 0.66\,x - 8.03$,	$\bar{y} = 31.9 \pm 2.79\%$
Clapp's Favourite (harvested 1953)	○—○ (–B);	$y = 0.47\,x - 14.5$,	$\bar{y} = 25.5 \pm 1.54\%$
	●—● (+B);	$y = 0.68\,x - 23.7$,	$\bar{y} = 38.7 \pm 1.62\%$
Sterreinette	□—□ (–B);	$y = 0.54\,x - 27.7$,	$\bar{y} = 41.1 \pm 2.14\%$
	■—■ (+B);	$y = 0.64\,x - 31.9$,	$\bar{y} = 40.0 \pm 0.93\%$

Clapp's 1952 was germinated in 17 ppm, Clapp's 1953 and Sterreinette in 35 ppm H_3BO_3.

ence of boron. The maximal percentage of germination in the presence of boron as compared to its absence is reached about 10 minutes later in the case of pear and 10 minutes earlier in the case of apple.

The rate of germination and the maximal germination of the pear pollen is markedly higher with boron than without, the apple pollen is much less affected. The germination rates, as represented by the tangents of the curves, are approximately the same in the presence of boron for all 3 tested varieties: for Clapp's 1952, Clapp's 1953 and Sterreinette 0.66, 0.68 and 0.64 respectively.

With respect to the occurrence of bursting of the pear pollen, it can be said that the amount of bursting in the medium without boron increases with increasing germination time. No bursting occurred with boron. It is worthy of note that the number of germinated grains in the presence of boron approximately equals the number of germinated and burst grains in the absence of boron after any germination time (see p. 27). The apple pollen did not burst.

The experiment was repeated with Clapp's pollen at 18 °C, giving qualitatively the same results as at 23 °C. The germination rate and the relative influence of boron on the germination rate, however, were less, while a longer time (5–5½ hours) was required to reach the maximal germination percentage. The results of both these experiments and preliminary experiments not reported herein, infer that the times necessary to reach the maximal germination percentages with and without boric acid differ less as the temperature is higher.

With a view to the following experiments in which stress will be laid upon the relative influence of boric acid on the germination rate, it is of importance to regard the relation between time and germination somewhat more closely.

The relative influence of boron on the germination rate can be represented in the above experiments by the ratio of the tangents of the respective 'curves'

obtained with and without boron. It is not necessary, however, to know the tangents (hence, the entire curve) in order to determine the relative influence of boron on the germination rate. Namely, since the curves are practically linear and their origins the same, irrespective of the presence of boron, the relative influence of boron on the germination rate can also be represented by any ratio of the germination percentages determined with and without boron at any moment within the period of time the germination starts and reaches its maximum. At relatively high temperatures ($\leq$ 23 °C) the relative influence of boron on the germination rate can also be approximated by the ratio of the maximal germination percentages. In this case only a small error is made, because of the fact that the maximal germination percentages are reached at almost the same time.

1.6. *Germination and temperature*

With regard to the relation between germination and temperature it follows from experiments done by ROBERTS *et al.* (107, 1948) with pollen of several apple varieties that this relationship can be represented by an optimum curve. The optimum for germination and pollen tube growth was found to lie between 20 ° and 30 °C in many investigations, *e.g.*: *Vitis:* 30 °C (143), *Impatiens:* 20 °C (13), *Antirrhinum:* 25 °C (13, 125), *Bryophyllum:* 25 °C (125), *Pyrus Malus:* 20–24 °C (107). It may be remarked that an excellent germination can be recorded at suboptimal temperatures if only the germination time is long enough. For example, ADAMS (1, 1916) found with apple pollen 90–100 % germination at 14 °C after 24 hours. ÖSTLIND (92, 1945) even found 10–36 % germination for 4 pear varieties after 69 hours at 2 °C, while 2 pear varieties gave no less than 90–100 % germination after 119 hours at 2 °C.

Since no reference could be found in literature about the influence of boron on the germination at different temperatures, the author investigated the germination in sugar solution with and without boric acid at a range of temperatures.

Material and methods. – Pollen of 3 pear varieties: Clapp's Favourite, Nouveau Poiteau, Beurré Superfin and of 2 apple varieties: Sterreinette, Brabantse Bellefleur, was germinated in 10 % sugar solution with and without 20 or 70 ppm boric acid. The pear pollen was tested at 10°, 16°, 19°, 22°, 25°, 28° and 30° C, the apple pollen at 16°, 23° and 29° C. Germination was recorded after 4 hours, which is long enough to reach the maximal percentage of germination at an optimal temperature (±23° C), and again after 24 hours.

Results.

TABLE 15

The effect of boric acid on the germination percentage of apple pollen at different temperatures in 10 % sugar solution without (–B) and with 70 ppm H_3BO_3 (+B); germination time 4 hours and 24 hours (figures in parenthesis).

Medium	Sterreinette			Brabantse Bellefleur		
	16° C	23° C	29° C	16° C	23° C	29° C
–B	1.0 (69.7)	73.5	38.3	0.0 (19)	89.3	24.3
+B	1.0 (71.7)	78.5	79.0	0.0 (19)	86.5	70.7

It follows from table 15 that the germination of the apple pollen is not significantly affected by boric acid at 16 ° and 23 °C. The germination percentages at 29 °C, however, are in the absence of boron approximately half

or less than half of those obtained with boron. Apple pollen is apparently only sensitive to boron at relatively high temperatures. The germination percentages recorded at 16 °C had increased to a greater or lesser extent after 24 hours of germination. Those recorded at 23 °C and 29 °C did not increase after an extension of the germination time to 24 hours.

The germination percentages determined for the pear pollen of 3 varieties are shown in figure 4.

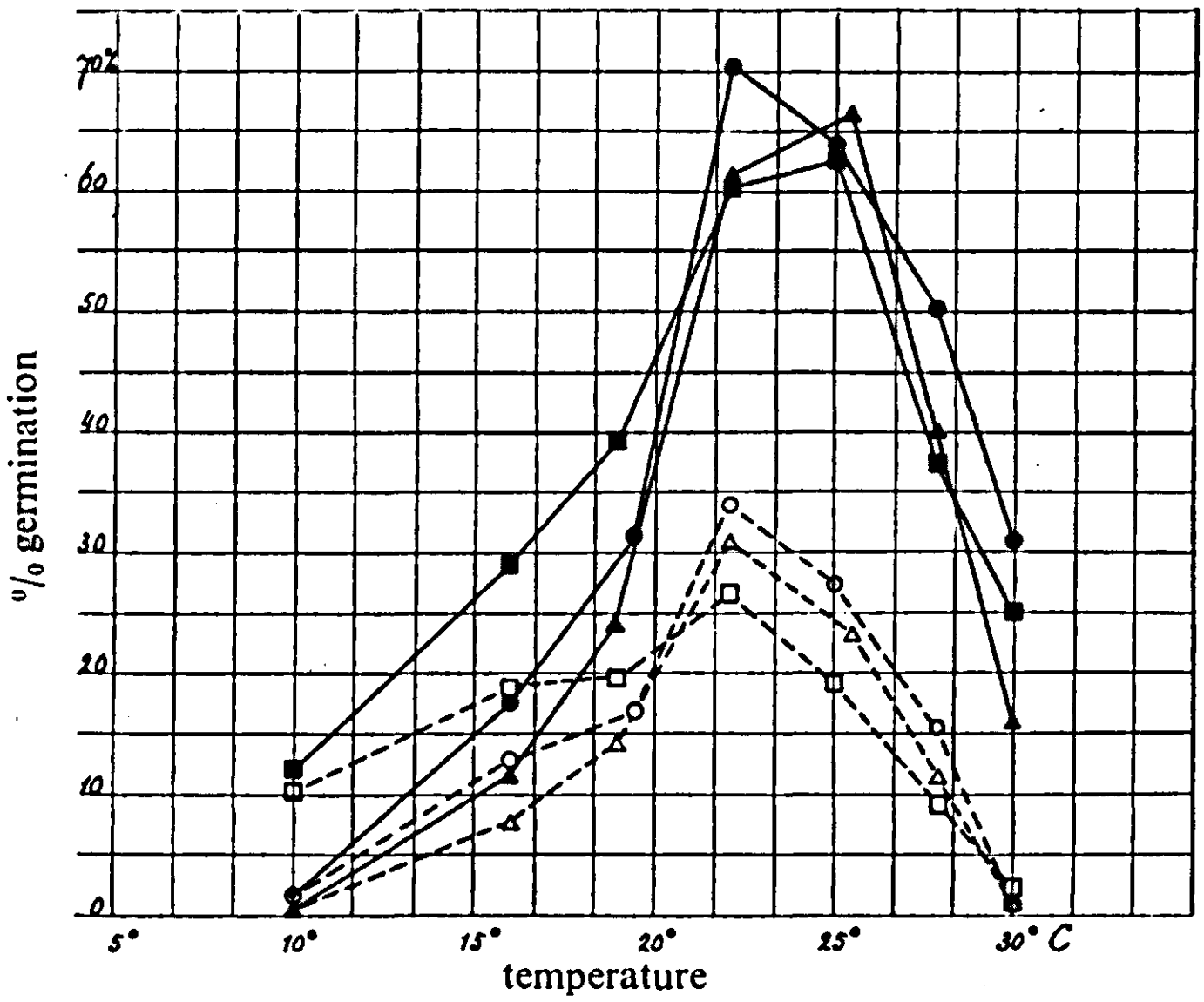

FIGURE 4. The effect of boric acid on the germination of pear pollens at increasing temperature. Germination in 10% sugar solution without and with 20 or 70 ppm H_3BO_3; germination time 4 hours.

Clapp's Favourite: □---□ without H_3BO_3; ■—■ with 20 ppm H_3BO_3.
Nouveau Poiteau: △---△ without H_3BO_3; ▲—▲ with 70 ppm H_3BO_3.
Beurré Superfin: ○---○ without H_3BO_3; ●—● with 70 ppm H_3BO_3.

1) From figure 4 it can be seen that the relation between temperature and germination can be represented by an optimum curve for all 3 pollens tested, both with and without boric acid. The curves determined in the presence of boron, however, lie at a much higher level than those determined in the absence of boron. A high percentage of germination is only obtained in the medium with boron. The optimal temperature for germination tends to be higher in the presence than in the absence of boron. It can be noted that pear pollen appears to be sensitive to boron at much lower temperatures than apple pollen.

2) What has been said for the germination percentage also holds true for the pollen tube growth after 4 hours of germination. An example is given in table 16.

Table 16 also demonstrates a positive correlation between germination percentage and pollen tube growth.

3) In the presence of boron few grains and tubes had burst at 22–25 °C or lower temperatures within 4 hours of germination. At 28° and 30 °C very many grains and tubes had burst. In the solutions without boron almost no bursting occurred at temperatures below 19 °C, while above 19 °C the amount of bursting markedly increased with the temperature.

TABLE 16
The effect of boric acid on the germination and pollen tube growth of Clapp's Favourite at increasing temperature. Germination in 10% sugar solution without (–B) and with 20 ppm H_3BO_3 (+B).

	Medium	10° C	16° C	19° C	22° C	25° C	28° C	30° C
% Germination .	–B	10.2	18:7	19.9	26.8	19.3	7.7	2.3
	+B	12.2	29.2	39.7	60.5	63.2	42.5	25.0
% Tubes > 5 × grain diameter	–B	0	0	5	53	41	9	0
	+B	0	42	71	96	97	50	33

4) The germination percentages at superoptimal temperatures were found to be unaltered after 24 hours of germination, while the germination percentages at suboptimal temperatures increased to a greater or lesser extent. For example, the germination of Superfin pollen at 16 °C increased from 12.5 (–B) and 17.6% (+B) to 34.4 (–B) and 53.8% (+B); of Poiteau from 7.8 (–B) and 11.8% (+B) to 43.3 (–B) and 61.0% (+B). The germination percentage in the absence of boron eventually becomes as high as or higher at 16 °C than at 23 °C. No bursting occurred at 10° and 16 °C, irrespective of the presence of boron, while at higher temperatures the majority of grains and tubes had burst.

The ratios of the germination percentages obtained with or without boron, presenting the relative influence of boric acid on the germination: $\frac{+B\%}{-B\%}$, are recorded in figure 5(p. 34). These ratios, however, also give a fair approximation of the relative influence of boron on the *germination rate*, on account of the foregoing reasoning with respect to the linear relation between time and germination (see p. 30).

The relationship between the relative influence of boron on the germination rate and the temperature is represented by a statistically reliable ($P = 0.01$) curve of the type: $\log y = ax+b$.

From figure 5 it follows that the relative influence of boron on the germination rate increases with increasing temperature and is approximately the same for the three tested pear pollens. From the formulae of the 'curves' it was calculated that the Q_{10} varied between 1.90 and 1.95. That is to say: an increase of the temperature with 10 °C increases the rate of germination in the presence of boric acid nearly twice as much as it increases the germination rate in the absence of boric acid.

1.7. *Germination and sugar concentration*

The literature review (chapter II) showed that a definite relationship exists between germination and the osmotic and/or colloidal value of the medium. It further appeared from germination experiments in water and in 10% sugar solution (chapter III) that the relative influence of boron on the germination of pollen, termed 'the degree of boron sensitivity of pollen' in chapter III, is also related to the osmotic properties of the medium. Since water and only one sugar concentration had been used as media in these experiments, a more extensive investigation was carried out with a range of sugar concentrations.

Material and methods. – The germination of the pear pollens: Clapp's Favourite, Précoce de Trévoux and of the apple pollen Sterreinette was tested in solutions of 0, 2.5, 5,

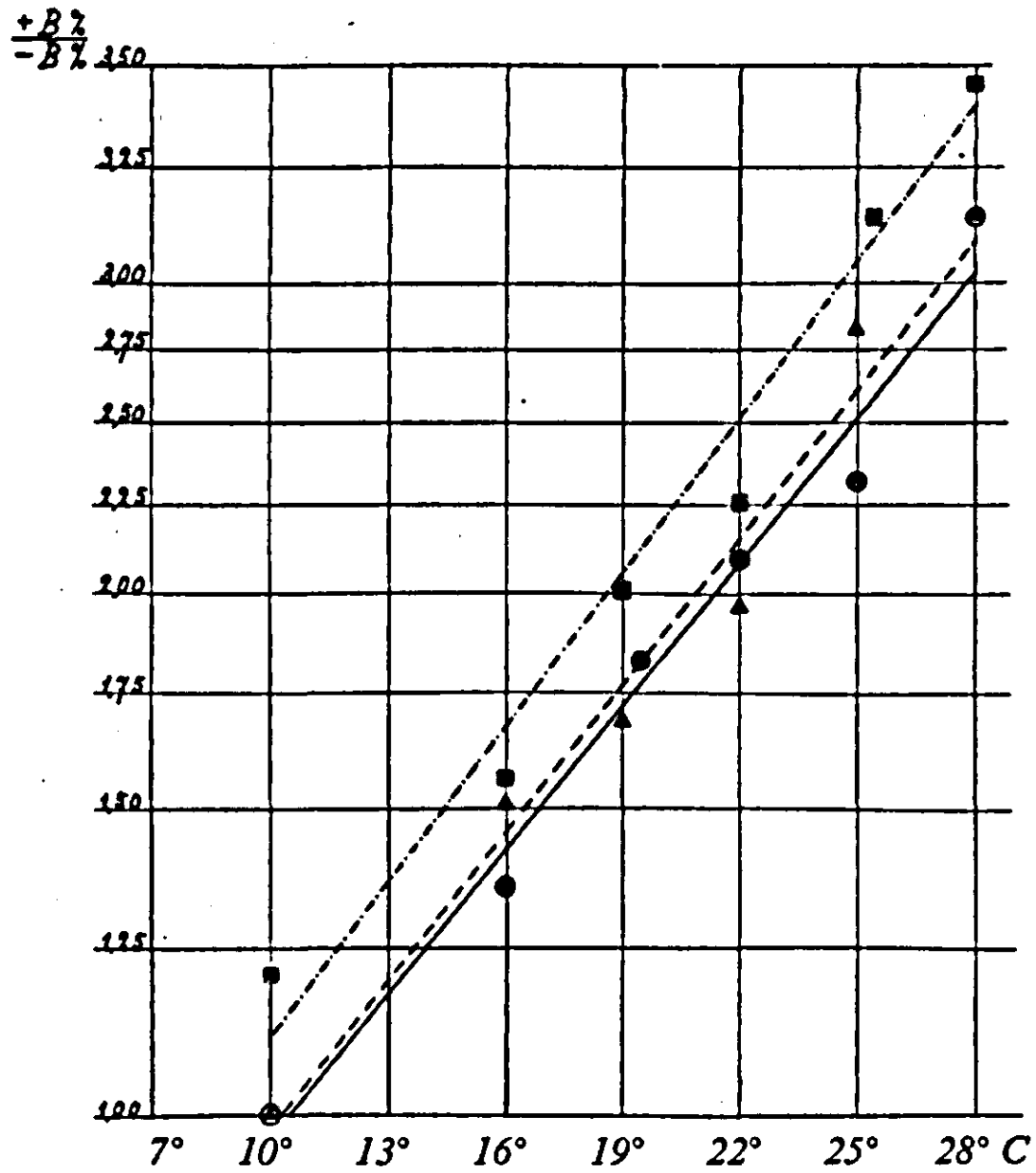

FIGURE 5. The relative influence of boric acid on the germination rate of pear pollen at increasing temperature.

■·–·–·–·■ Clapp's Favourite: $\log y = 0.0295\,x - 0.2466$;
$\log \bar{y} = 0.3394\ (\pm 0.0195)$;
$Q_{10} = 1.95$

▲–––––▲ Nouveau Poiteau: $\log y = 0.0283\,x - 0.2900$;
$\log \bar{y} = 0.2657\ (\pm 0.0238)$;
$Q_{10} = 1.91$

●———● Beurré Superfin: $\log y = 0.0278\,x - 0.2931$;
$\log \bar{y} = 0.2788\ (\pm 0.0231)$;
$Q_{10} = 1.90$

7.5, 10, 15, 20, 25 and 30% sugar with and without 30 ppm boric acid respectively. The germination was recorded after 4 hours – which allows for ample time to reach a maximal percentage of germination under optimal conditions – and again after 24 hours.

Results. – The percentages of germinated and burst grains are given in figure 6.

1) With regard to *apple* pollen it can be derived from figure 6 that the percentages of germination obtained in solutions with an increasing sugar concentration represent an optimum curve. Some bursting occurred in water, but none in the sugar solutions. The germination of the apple pollen is only significantly improved by boric acid when the pollen is germinated in water, the influence of boron in sugar solutions is negligeable. With regard to the growth of the tubes a positive correlation exists with the germination percentage.

The germination curve determined after 24 hours has an optimum traject. Namely, the germination percentages in sugar solutions ranging between $2\frac{1}{2}$ and 20% of sugar are approximately equally high ($\pm 80\%$). The germination in 25% and 30% sugar being 2% and 0% respectively after 4 hours, had increased to 63% and 53% respectively after 24 hours of germination, irrespective of the presence of boron.

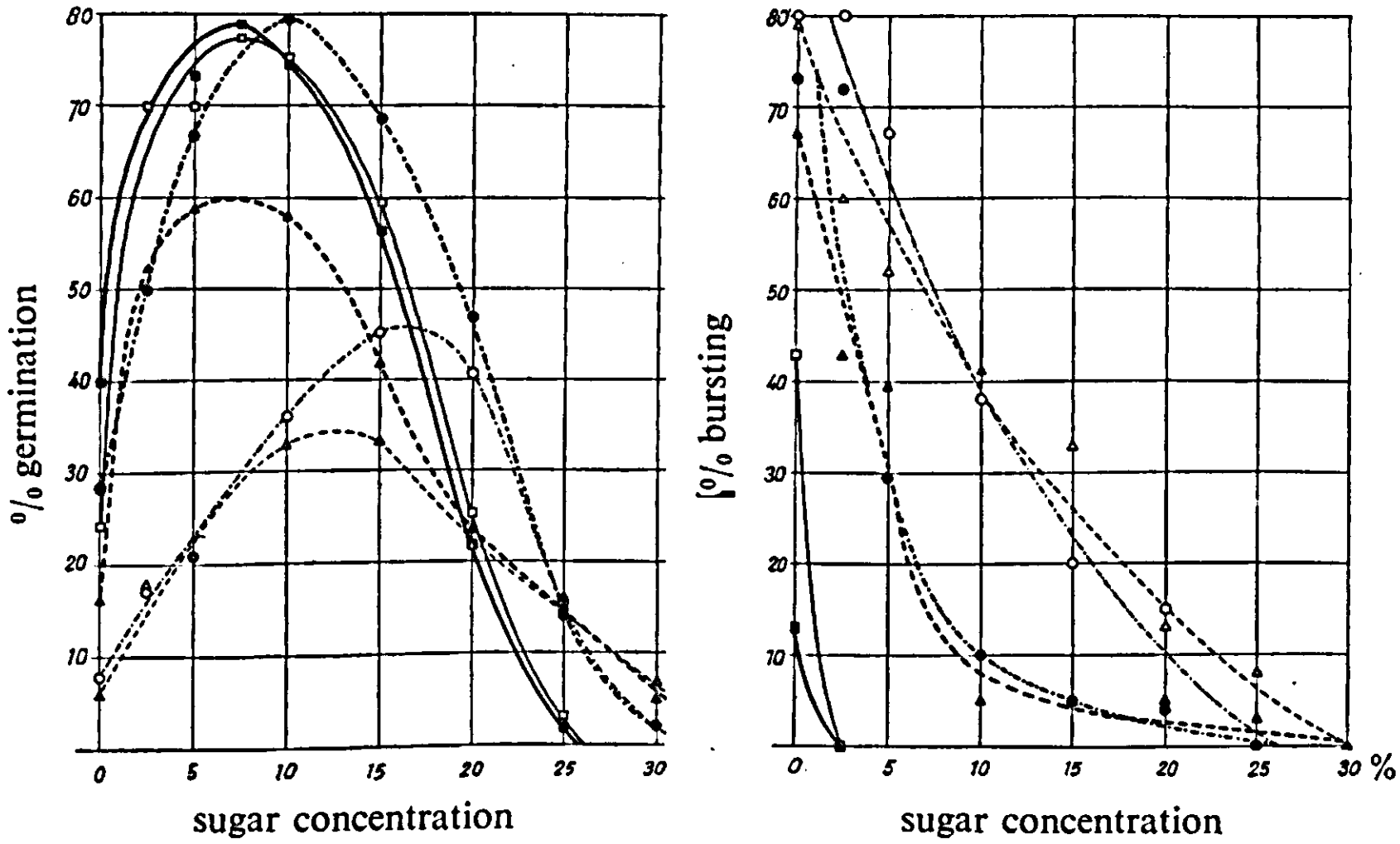

FIGURE 6. The effect of boric acid on the germination and bursting of apple and pear pollen at increasing sugar concentrations with and without 30 ppm H_3BO_3.

Sterreinette: □——□ without H_3BO_3; ■——■ with H_3BO_3.
Précoce de Trévoux: △---△ without H_3BO_3; ▲——▲ with H_3BO_3.
Clapp's Favourite: ○-·-·○ without H_3BO_3; ●—·—·● with H_3BO_3.

2) With regard to *pear* pollen figure 6 shows that the curves are qualitatively the same as the curves found for apple pollen. In contrast to apple pollen, however, the germination of the pear pollen is markedly promoted by boric acid in the lower sugar concentrations. The germination percentages found in sugar concentrations as high as or higher than 20% are approximately the same with and without boron. With respect to the pollen tubes it can be stated that their growth is correlated with the germination percentage.

The amount of bursting appears to be inversely related to the sugar concentration both with and without boric acid, but decreases more quickly in the presence than in the absence of boron. In this connection it can be remarked that the sum of germinated and burst grains is approximately the same in the same sugar concentration, irrespective of the presence of boron. This agrees with observations made in § 1.3 (p. 27) and § 1.5 (p. 30).

The optima for germination in the solutions without boron are found at a higher sugar concentration than in the solutions with boron. This shifting of the optimum is presumably due to the fact that the bursting of grains is not sufficiently diminished until the higher sugar concentrations are reached. This enables more pollen grains to produce tubes.

The germination percentages recorded in optimal or suboptimal sugar concentrations had not altered after 24 hours of germination. The germination percentages in superoptimal sugar concentrations increased to an extent inversely related to the sugar concentrations and were better with than without boron, also in sugar solutions higher than 20%. Much bursting had occurred in all sugar concentrations, except in the highest, after 24 hours of germination.

In order to show more clearly the effect of boric acid on the germination in relation to the sugar concentration, the curves representing the relative effect of boron on the germination — as determined by the ratios of the ger-

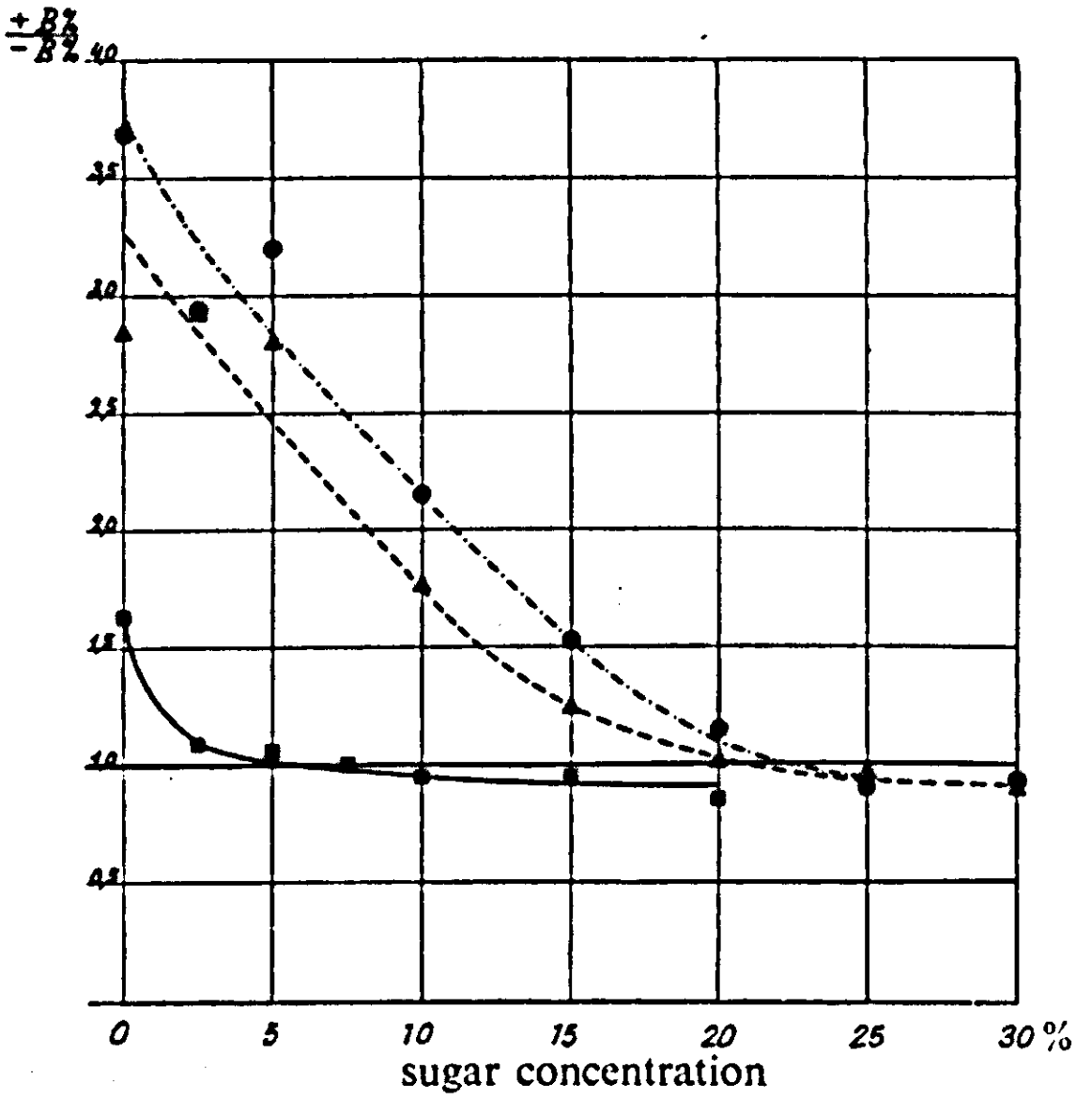

FIGURE 7.
The relative influence of boric acid on the germination rate of pear and apple pollen at increasing sugar concentration.

■——■ Sterreinette;
●-·-·-● Clapp's Favourite;
▲----▲ Précoce de Trévoux.

mination percentages with and without boron: $\frac{+B\,\%}{-B\,\%}$ — are shown in figure 7.

The curves not only represent the relative influence of boron on the germination percentages recorded at a certain moment, but presumably approximate the relative influence of boron on the *rate of germination*. There is, namely, no reason to believe that the specific relationship between time and germination with respect to boron, as has been found in 10% sugar solution (see fig. 3, p. 30), does not exist in lower or higher sugar concentrations. It may be assumed that the ratios derived from germination percentages obtained in superoptimal sugar concentrations directly represent the relative influence of boric acid on the germination rate, while the ratios of the 'maximal' percentages obtained in optimal or suboptimal concentrations give a fair approximation.

Turning to figure 7 it is evident that apple pollen is only markedly affected by boron when the germination takes place in water. With regard to the pear pollen, the figure shows clearly that an inverse relationship exists between the relative influence of boron on pollen germination — or boron sensitivity of the pollen — and the sugar concentration of the medium.

2. MUTUAL STIMULATION OF POLLEN GRAINS IN GERMINATION

2.1. *Introduction*

Apart from the marked influence of boric acid on germination, there is yet another factor of general importance. Namely, the density of sowing, or in other words the number of pollen grains per drop, has a striking effect on both the germination percentage and tube growth. That is to say, a great number of grains gives better germination results than a small number of grains per drop. This has been observed by: ADDICOTT (2, 1943), BEAMS *et al.* (11, 1947), BECK *et al.* (12, 1941), BRINK (28, 1924), HOLUBINSKI (58, 1945), KUHN (75,

1937), REMY (102, 1953), SAVELLI (111, 1940), SAVELLI *et al.* (112, 1940) and VISSER (137, 1951). The described phenomenon, termed 'the mutual stimulation of pollen grains in germination', was found to occur with pollen of plant species belonging to the following genera: *Campanula* (58), *Cucumis* (28), *Datura* (58), *Digitalis* (58), *Gladiolus* (112), *Helianthus* (58), *Humulus* (58), *Hypericum* (58), *Linaria* (58), *Lycopersicum* (58), *Lythrum* (28), *Matthiola* (75), *Medicago* (58), *Milla* (2), *Nicotiana* (111, 112), *Oenothera* (58), *Papaver* (58), *Prunus* (102), *Pyrus* (137), *Rubus* (58), *Tanacetum* (58), *Tropaeolum* (2, 58), *Vinca* (11, 28).

Since the mentioned phenomenon has been found to occur with pollen of all species which were tested, it can be assumed that the mutual stimulation is of a general nature.

It is not unlikely that what happens *in vitro* also occurs *in vivo*. In fact, own experiments with apple and pear have shown that pollen mixtures containing 98–99 % dead pollen and 1–2 % living pollen still gave a moderate fruit set, providing comparatively large amounts were used for pollination. LOBANOV (1951, 81) found that pollination with large amounts of pollen, covering the stigma completely, gave an increase of fruit and seed set in intervarietal crosses.

The phenomenon of mutual stimulation has been attributed to the promoting influence of a substance diffusing from the pollen cell into the medium. That the diffusing substance is not necessarily linked with the metabolism of the *germinating* pollen grain appears from the fact that pollen extracts have been found to stimulate the germination (23, 75, 138). The germination of certain pollens improved when grown in extracts of pollen of other species (22, 23, 137). In this connection the experiments in which pollens of 2 different species were germinated in one drop together are also of interest. For example, BRINK (28, 1924) found a marked stimulation of the germination of *Cucumis* pollen when cultivated in 'singles' among 'quartets' of *Vinca* pollen as compared with the control. BRANSCHEIDT (22, 1929; 23, 1930), testing several pollens in combination, observed in some cases a mutual promoting or inhibiting influence, in other cases one pollen was promoted and the other inhibited or indifferent. The number of grains per drop, however, was not taken into account in these experiments.

On the other hand, SAVELLI *et al.* (112, 1940) with *Nicotiana* and *Gladiolus* pollen found that a small number of the one pollen germinating together with a great number of the other pollen gave exactly the same results as if they had been germinated apart; no mutual stimulation occurred. Apparently, the diffusing substances in question are specific in some cases, but non-specific in many other instances.

On account of the fact that the influence of boric acid on pollen germination is very similar to the influence of an assumed substance originating in the pollen cell and because of the general nature of both phenomena, it seemed of interest to investigate whether they are interrelated.

2.2. *The relation between germination and number of grains per drop*

In none of the reviewed experiments boric acid was present in the germination medium, so that mutual stimulation theoretically can be due to boric acid diffusing from the grain. For that reason, the germination in drops with different amounts of grains has been studied in a medium both with and without boric acid.

Material and methods. – Different numbers of pollen grains were brought into drops of 10% sugar solution with or without 30 ppm boric acid. The drops were weighed on a self-registering balance beforehand: weight of drops between 5 and 7 mg. The number of grains per 10^{-3} ml solution was calculated from the weight of the drop and the total number of grains it contained.

One experiment was carried out with pollen of Clapp's Favourite and Précoce de Trévoux in 10% sugar solution with boric acid (A).

A second experiment was done with Conference pollen originating from branches supplied with water or 1000 ppm boric acid (see chapter III) and germinated in 10% sugar solution with and without boric acid (B). The germination percentages in this and the former experiment were recorded after 3½ hours.

A third and similar experiment was carried out with Sterreinette pollen, but in this case the germination percentages were recorded at intervals of 10–15 minutes until the maximal percentage was reached. In this case the germination of 6 quantities of pollen (each in duplo) was tested (C).

Results. – A. The data on germination in drops with increasing numbers of grains are given in figure 8.

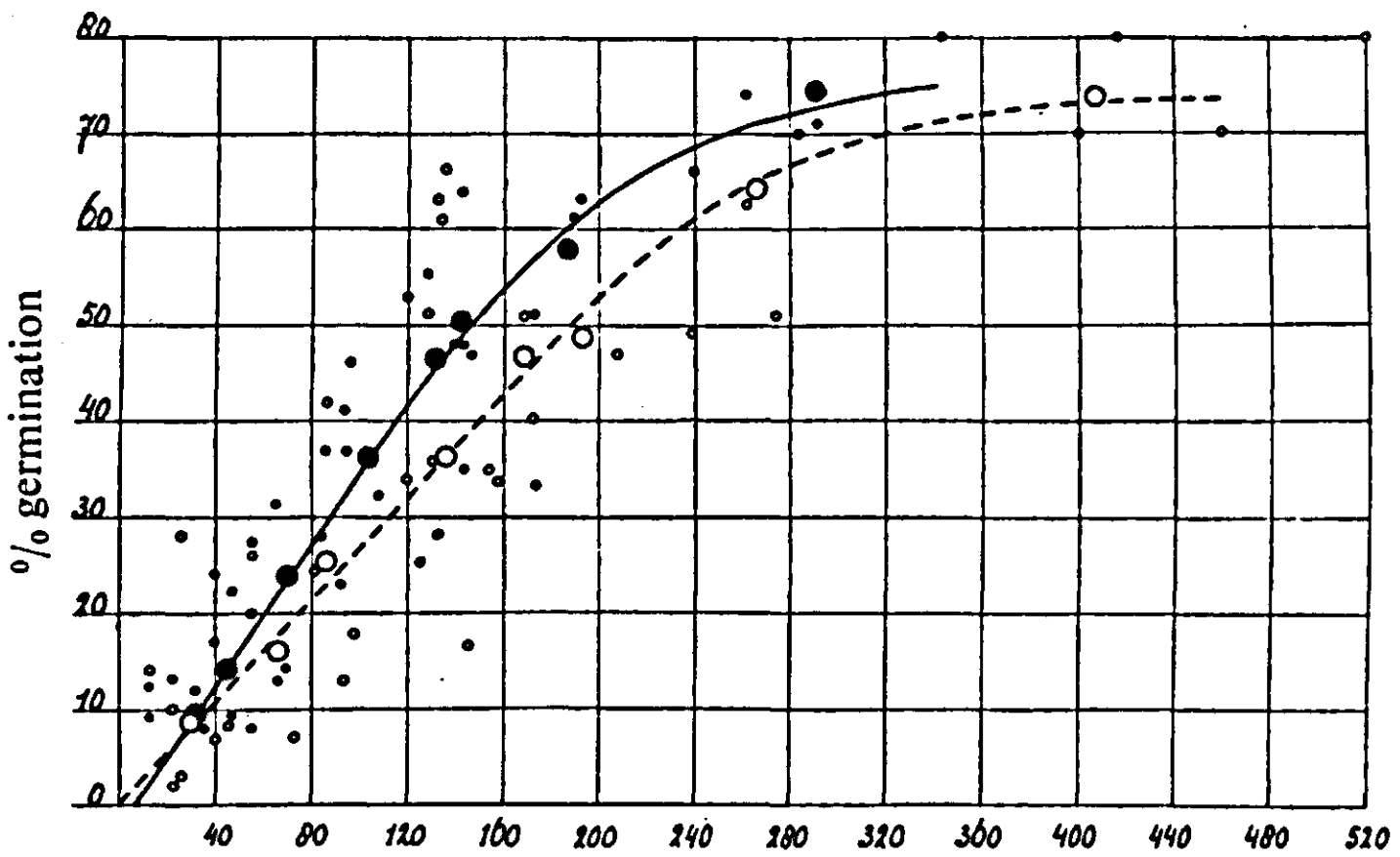

FIGURE 8. The relation between germination and number of pollen grains per 10^{-3} ml solution. Germination in 10% sugar + 30 ppm H_3BO_3; germination time 3½ hours.
——— Clapp's Favourite: • observations; ● means.
– – – – Précoce de Trévoux: ∘ observations; ○ means.

From figure 8 it can be derived that the relationship between the germination percentage and the number of grains per unit of volume is approximated by a saturation curve which is practically linear for the greater part. Since a surplus of boric acid is present, the promoting substance diffusing from the pollen cell cannot be identical with boric acid.

B. The data on the germination of Conference pollen from different 'branches' in relation to the number of grains and boric acid are given in figure 9.

From figure 9 it follows that the curves representing the germination of the pollens in sugar solution *with* boron are approximately identical, whether or not boric acid had been supplied to the *branches*. The maximal germination percentage is nearly the same for both pollens and is reached at about 140 grains per 10^{-3} ml. The curves representing the germination of the respective pollens in sugar solution *without* boron are very different. The pollen from branches supplied with water germinated very poorly and already reached its

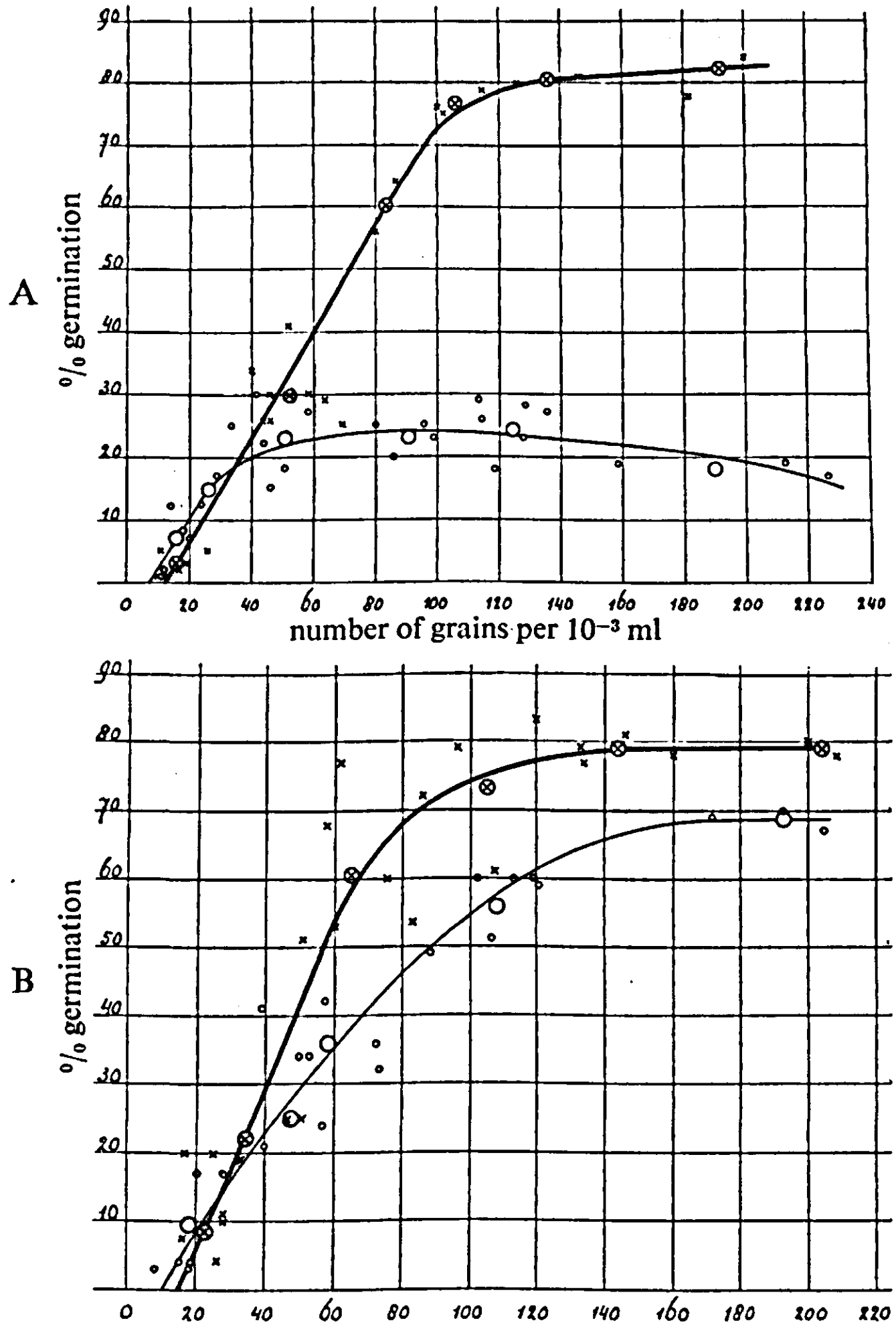

FIGURE 9. The relation between germination of Conference pollen and number of pollen grains per 10^{-3} ml solution; germination time $3\frac{1}{2}$ hours. Pollen from branches supplied with with water (A) and 1000 ppm H_3BO_3 (B) respectively.
——— germination in 10 % sugar: ∘ observations, O means.
——— germination in 10 % sugar + 30 ppm H_3BO_3: × observations, ⊗ means.

maximum at about 50 grains per 10^{-3} ml. The germination of pollen from branches supplied with boric acid is markedly better than that of the former pollen, but is still less as compared with its germination in the presence of boron.

C. With regard to the experiment with apple pollen in which the germination was recorded at regular intervals, it appeared again that the relation between percentage and time of germination is linear (see also fig. 3, p. 30). The formulae expressing this relationship, as affected by the number of grains per drop, are given in table 17. The average and maximal germination percentages together with the times after which the germination begins and reaches its maximum, are also recorded in this table.

TABLE 17
The relation between germination percentage and germination time as affected by the number of pollen grains per 10^{-3} ml solution. Germination of Sterreinette pollen in 10% sugar + 30 ppm H_3BO_3.

Grains per 10^{-3} ml	Formula of "curves"	% Germination		Time in minutes after which germination	
		mean ($\bar{y}$)	maximal	begins	ends
14	$y = 0.26x - 16.2$	19.6 ± 1.74	38.5	62	210
40	$y = 0.31x - 16.3$	25.8 ± 2.53	47.0	53	204
85	$y = 0.45x - 21.9$	33.1 ± 3.50	72.0	49	209
120	$y = 0.47x - 19.4$	36.2 ± 2.93	77.0	41	205
145	$y = 0.56x - 22.7$	43.8 ± 4.15	82.5	41	188
300	$y = 0.61x - 29.0$	37.8 ± 3.92	81.0	48	180

From table 17 it follows that the average and maximal percentage of germination and the germination rate, as represented by the tangent of the curve, increase with increasing numbers of grains per drop. The time after which the germination begins and reaches its maximum decreases with increasing numbers of grains per drop.

A positive correlation between the germination percentage and tube growth was found to exist in all 3 experiments. Little or no bursting occurred in the presence of boron. However, in drops containing a relatively small amount of pollen a number of grains became darkish after some time, indicating loss of vitality.

2.3. *The influence of pollen extracts on germination*

It has been shown in the foregoing paragraph that the diffusing substance is not identical with boric acid. On the other hand, it appeared that the mutual stimulation 'increased' upon addition of boric acid. It seemed of interest, therefore, to study the influence of pollen extracts also in the absence and presence of boron.

Material and methods. – The extracts were made as follows: the desired amount of pollen grains was brought into a certain volume of 10% sugar solution. This mixture was constantly shaken during 45 minutes after which the pollen grains were removed by filtration. In most instances a standard extract was made of 20 mg pollen grains per ml 10% sugar solution (20 mg/ml), from which the less concentrated 'extracts' were obtained by dilution with 10% sugar solution. Their concentration was also expressed in mg/ml. In cases where the test pollen was germinated in extracts containing boric acid, the boric acid was added to the extract after removal of the extracted pollen. If dead pollen was used for extracts, the pollen was killed by keeping it at 60–70° C for 1–2 days.

Pollen of Doyenné du Comice, Conference and Sterreinette was used for germination. Extracts were made of viable and dead pollen of Brabantse Bellefleur and Clapp's Favourite in one experiment (A), of viable pollen of Doyenné du Comice and Clapp's Favourite in a second experiment (B) and of viable pollen of Conference in a third experiment (C).

The experiments were carried out with a small number (100–200) and/or with a normal number (1000–1500) of grains per drop.

Results. – A. The data obtained on the germination of Conference pollen in extracts of viable and dead apple and pear pollen are recorded in figure 10; normal numbers of grains per drop were employed.

From figure 10 it can be derived:

1) The relationship between germination percentage and concentration of extract can be expressed by an optimum curve with a rather broad optimum in 3 instances. The germination of the pollen in the extracts was stimulated to

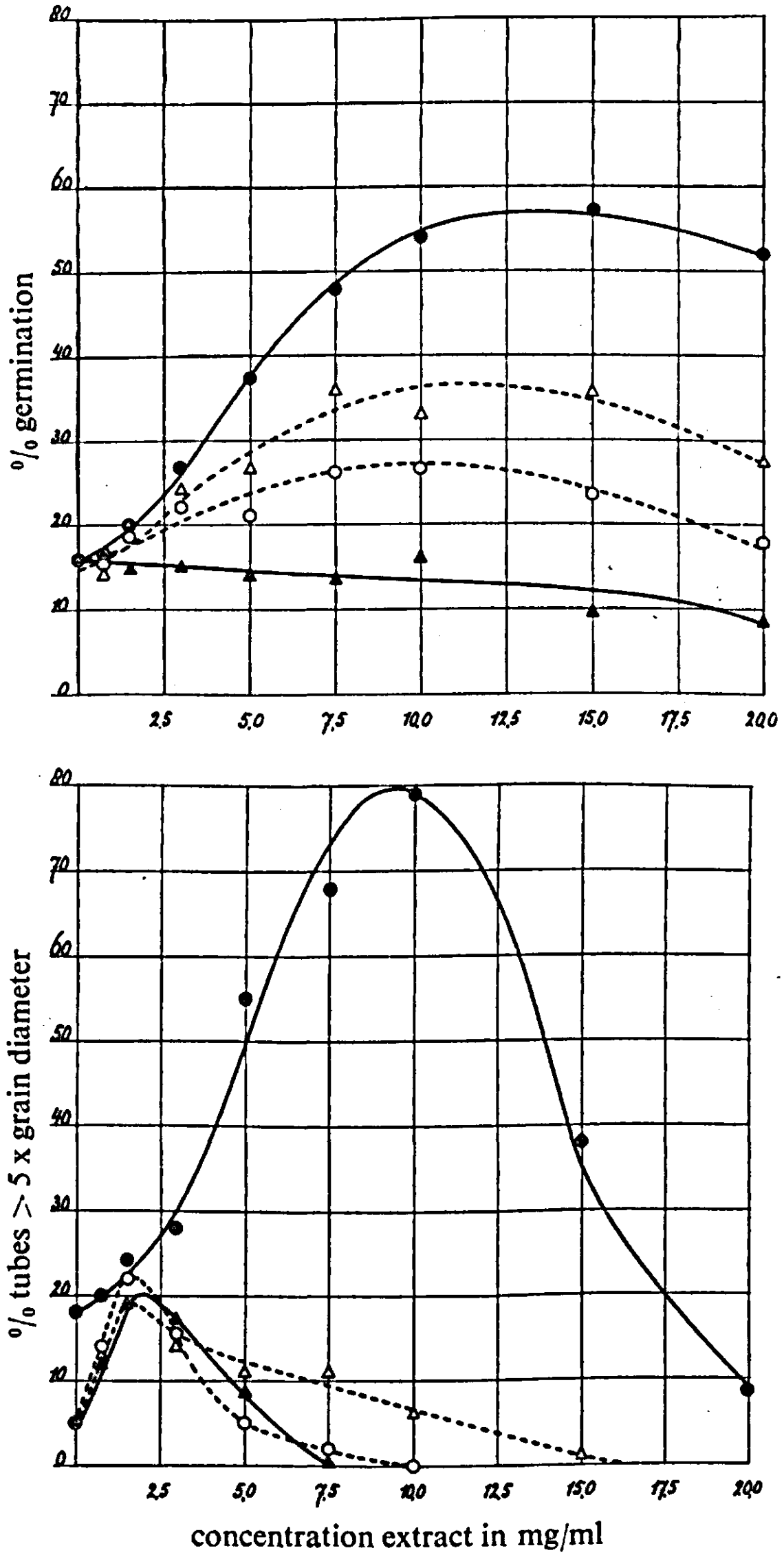

FIGURE 10.
The influence of pollen extracts in 10% sugar concentration on the germination (above) and pollen tube growth (below) of Conference pollen; germination time 3½ hours.

Germination, respectively pollen tube growth in extract of:
△—△ dead pear pollen;
▲—▲ viable pear pollen;
○—○ dead apple pollen;
●—● viable apple pollen.

a greater or lesser extent as compared with the germination in a pure 10% sugar solution (16% germination), but is in no case as high as that in the sugar solution to which boron had been added (72% germination). No bursting occurred in the latter medium but many grains and tubes burst in the extracts. The extract of the viable apple pollen gave the best, the extract of the viable pear pollen the poorest germination results. The germination in the latter extract is even lower than that of the control –B. This is probably connected with the fact that most of the *extracted* grains burst during extraction, in contrast with the other extracted pollens. The fact that the higher concentrations of all extracts impair the germination (and tube growth) suggest that, apart from a

promoting substance, also substances are extracted which quantitatively and/or qualitatively inhibit the germination.

2) With regard to the development of the tubes it is evident that their protrusion (germination in the strict sense) is much less affected by the concentrations of the extract than their further growth. The optima for growth are much more outspoken and have shifted to a lower concentration of extract than the optima for germination. The pollen tube growth in extract of viable apple pollen is markedly better than that in the 3 other extracts, while the optimum lies at a much higher concentration (± 10 mg/ml) than the optimum in the other extracts (± 1.50 mg/ml). In the control +B all tubes became longer than 5 × pollen diameter, in the control –B none of the tubes reached this length.

B. The germination data of Doyenné du Comice pollen in extracts of viable Doyenné du Comice and Clapp's Favourite pollen are reported in table 18; normal numbers of pollen grains per drop were used. The pollens used for extraction did not burst during this treatment.

TABLE 18
The influence of pollen extracts in 10% sugar solution without (–B) and with 70 ppm H_3BO_3 (+B) on the germination of Doyenné du Comice pollen; germination time 3½ hours.
Ger = % germination, Bur = % bursting,
PT>10D = % pollen tubes longer than 10 × pollen diameter.

1		2	3	4	5	6	7	8	9	10
Concentration of extracts →		0 mg/ml (control)			7 mg/ml			15 mg/ml		
Germination in extracts of ↓		Ger	Bur	PT>10D	Ger	Bur	PT>10D	Ger	Bur	PT>10D
Doyenné	–B	35.2	78	11	68.5	15	76	65.0	22	35
	+B	82.3	9	100	78.7	17	84	72.7	17	63
Clapp's	–B	35.2	78	11	56.0	48	45	58.5	41	20
	+B	82.3	9	100	76.8	14	91	71.8	13	83

The data recorded in table 18 indicate a marked increase of the germination (columns 5, 8) and tube growth (columns 7, 10) and a decrease of the amount of bursting (columns 6, 9) in both extracts *without* boron as compared with the germination in sugar solution without boron (columns 2, 3, 4). In the *presence* of boron, however, the pollen germinates as well or even better in a pure sugar solution than in the pollen extracts. The 15 mg/ml extract slightly inhibits the germination. An 20 mg/ml extract (+B) used in another experiment was found to decrease the germination even more.

In order to find out whether extracts containing boron also have a stimulating effect on the germination, both small and normal numbers of grains per drop have been employed; the results are recorded in table 19.

Table 19 shows again that the influence of both extracts on the germination is negligeable when normal numbers of grains are present in the drop. Only the tube growth was slightly promoted. On the other hand, the germination and tube growth of small numbers of pollen grains are considerably enhanced by the extracts.

C. The foregoing experiments showed that the germination of pear pollen is promoted by extracts of different pear pollens and also by extracts of apple

pollen. In table 20 data are given on the influence of an extract of pear pollen (Conference) on the germination of apple pollen.

TABLE 19
The influence of pollen extracts (7 mg/ml) in 10% sugar solution + 70 ppm H_3BO_3 on the germination of Doyenné du Comice and Conference pollen; germination time $3\frac{1}{2}$ hours.
PT>10D = % pollen tubes longer than 10× pollen diameter.

Variety	Germination medium	Small numbers/drop		Normal numbers/drop	
		% germination	PT>10D	% germination	PT>10D
Doyenné du Comice	Control (S+B)	43.6	6	79.8	86
	Doyenné extract	57.3	18	79.0	91
	Clapp's extract	55.2	70	78.8	91
Conference	Control (S+B)	29.8	18	70.3	71
	Doyenné extract	42.0	25	68.2	92
	Clapp's extract	56.0	35	70.0	100

TABLE 20
The influence of pear pollen extract (10 mg/ml) in 10% sugar solution without (–B) and with 35 ppm H_3BO_3 (+B) on the germination of Sterreinette pollen; germination time $4\frac{1}{2}$ hours.
PT>5D = % pollen tubes longer than 5 × pollen diameter.

Number of grains/drop	Medium	Control		Extract	
		% germination	PT>5D	% germination	PT>5D
Normal	S–B	50.0	25	60.3	10
Normal	S+B	72.5	92	71.3	84
Small	S+B	13.2	28	70.5	87

From table 20 it can be derived that the germination of normal numbers of grains per drop in the absence of boron is slightly promoted by the extract. The differences in the presence of boron are insignificant. The effect of the extract, however, is very striking indeed, when a small number of grains per drop was employed. The germination without extract is very poor, but the germination in the extract equals that of the control (normal numbers +B) without extract.

In table 21 data are given on the influence of the above extract (with boric acid) after it had been *heated* during 10 minutes at 100 °C. Only small numbers of grains per drop were used in this experiment.

TABLE 21
The influence of a heated pear pollen extract on the germination of small numbers of grains per drop of Doyenné du Comice and Sterreinette pollen; germination time $4\frac{1}{2}$ hours.
PT>5D = % pollen tubes longer than 5 × pollen diameter.

Variety	Control+B		Extract+B	
	% germination	PT>5D	% germination	PT>5D
Doyenné du Comice . .	30.3	85	91.2	100
Sterreinette	18.6	36	66.7	65

Table 21 demonstrates that the extract has retained its activity after having been heated, since the germination and tube growth of both pear and apple pollen very markedly increased as compared with the control.

3. DISCUSSION

A. 1) Considering the results obtained with increasing boric acid concentrations (§ 1.2), it appears that germination and tube growth are directly and bursting is inversely related to the concentration. Since it was found that boric acid is not needed until the moment that the tubes begin to form (table 11, p. 27), it can be concluded that boric acid is specifically required for the *growth of the pollen tube*. That is to say, the very beginning of the germination, when the grains form a 'bulge', does not depend on boron, but is caused by the turgor pressure (chapter II, p. 11). However, since germination is more than bulging alone, namely the protrusion of *distinct tubes* ($>\frac{1}{2}$ D in the author's experiments), it is understandable why boric acid may be found to increase the germination, *viz.*, because its presence determines whether the pollen will form visible tubes or will burst in the bulging stage. In general, bursting is brought about by impeded tube growth and is in essence an osmotic phenomenon: when lack of boron or an exhaustion of boron prevents or arrests the tube elongation in an early or later stage, bursting will occur as a consequence of the continued water uptake.

The necessity of a continuous and ample supply of boric acid implies that it is inactivated in the course of the elongation process.

2) A remarkable phenomenon to be observed in germination tests is the great tolerance of the pollen to high boric acid concentrations. The experiments of SCHMUCKER (115, 1935) with several pollens and GÄRTEL (47, 1952) with *Vitis* pollen show that optimal germination and tube growth can be obtained in a wide range of boric acid concentrations: between 10–100 and 10–150 ppm respectively. EHLERS (42, 1951) found that *Galanthus* pollen germinated in water with 1000 ppm boric acid and produced tubes which were 2 mm long. The present author also found a great tolerance, since optimal germination occurred in a range of 17–300 ppm, while even in 1200 ppm boric acid germination took place (table 9, p. 26).

Generally, boric acid concentrations higher than 10 ppm, when added to nutrient solutions, prove to be toxic to plants. Pollen, however, is not poisoned in concentrations which are 30 or even100 times higher.

B. 1) From the experiments in which the relationship between germination and time was investigated (§ 1.5), it appears that boric acid increases the germination rate, but does not affect the time at which the germination starts. It was also established in several instances (pp. 27, 30, 35) that the percentage of germinated grains (+possibly burst grains) in the presence of boron tallies with the sum of the percentages germinated and burst grains in the absence of boron. Hence, when bursting is considered as an alternative of germination, the rate at which the grains 'germinate' is the same, irrespective of the presence of boron. These observations infer that the rate of water uptake by the pollen is not affected by boric acid.

2) The ratio of the germination percentages obtained in the same medium with and without boron respectively, defined as the 'boron sensitivity of the pollen' in chapter III, was shown to represent 'the relative influence of boron on the *germination rate*' (see p. 30). This relative influence on the germination rate appeared to increase both with increasing temperature (fig. 5, p. 34) and with decreasing sugar concentration (fig. 7, p. 36). Since germination rate and growth rate are positively correlated (chapter II, p. 11), the above relationship

can also be expressed as follows: the boron sensitivity of the pollen *in vitro* is directly related to the rate at which the pollen tubes *have to* elongate (as determined by sugar concentration and temperature of the medium). This leads to the following conception: when the temperature is lowered or the sugar concentration raised, an equilibrium may be reached at which the growth rate is so far limited that the resources of the pollen itself suffice to meet the boron requirements of the growing tube. At higher temperatures or lower sugar concentrations no tube growth may take place, resulting in bursting, because the requirements for growth per unit of time exceed the supply of endogenous boron per unit of time. Under such conditions an outside source of boric acid is apparently needed to enable the tubes to elongate.

C. 1) The curves representing the relative influence of boron on the germination rate with respect to temperature (fig. 5, p. 34) have a Q_{10} of nearly 2. This implies that the determining reaction in which boric acid is involved is of a chemical nature. On account of the favourable effect of boric acid on tube growth it can be assumed that *a complex of boric acid and an unknown substance acts as a 'growth promotor' in tube elongation.*

2) From the experiments reported in chapter III, it was derived that the germinability of the pollen is the same, whether the boron is taken up via the tree beforehand, or taken up by the pollen from the medium afterwards. It also appeared that the degree of boron sensitivity of the pollen is inversely related to the boron level of the plant. It is suggested, therefore, that the substance in question is partly present in a free form, termed X, and in a complex form with boric acid, termed XB; X has no germination properties, while XB has. The proportion of X and XB is determined by the boron level of the plant. On account of the above observations the following view can be held with respect to the increase of germination and tube growth upon addition of boric acid: *Whenever the growth rate of the tube (as determined by sugar concentration and temperature) exceeds the supply of the complex compound from endogenous sources, this supply can be supplemented by addition of boric acid, thus leading to additional complex formation with the available free compound.*

D. 1) The experiments with an increasing number of grains per drop (§ 2.2) and those with pollen extracts (§ 2.3) imply that a substance (or substances) with 'germination-promoting' properties diffuses from the grains. The substance involved in the mutual stimulation of grains has been proved to be neither boric acid, because the phenomenon also occurs in its presence (fig. 8, p. 38), nor an enzyme, because of its thermostability (table 21, p. 43). The pollen extracts appeared to exhibit the same stimulating influence on germination as boric acid (fig. 10, p. 41; table 18, p. 42) and also improved the germination when mutual stimulation is not optimal, namely, when drops with too few grains were used (tables 19, 20 on p. 43).

2) The analogous effects of boric acid and of the substance(s) involved in mutual stimulation of pollen grains can be explained by the following hypothesis:

Both in boric acid stimulation and in mutual stimulation the same substances are involved: a substance X and a substance XB (see C 2). Only the latter has germination-promoting properties and is a complex of X with boric acid. This hypothesis is supported by the following:

a. It is a fair assumption that storage of the pollen under dry conditions decreases the mobility of substances essential for growth, such as XB, hence, not enough may be released for the formation of tubes. The fact that the

germination of stored pollen can be improved by *either* pre-humidification *or* by addition of boric acid (table 13, p. 18) suggests that the same substance(s) acts in both boric acid stimulation and mutual stimulation. Pre-humidification would increase the water content of the pollen beforehand and consequently the mobility of essentials for growth, including XB. On the other hand, the addition of boric acid would lead, through additional complex formation with the available X, to an increase of XB only and would not enhance the availability of other substances. Thus, if another substance than XB would act in mutual stimulation, this substance would presumably become just as well limiting after storage of the pollen, notwithstanding the presence of boron. Consequently, the germination would remain low, unless the number of grains per drop is raised. However, the fact that the same number of grains was used, irrespective of pollen age, and the fact that boric acid has been found to raise the germination to the same or higher level than pre-humidification infers that XB (X) is also involved in mutual stimulation.

b. Also, the increasing boron sensitivity of the pollen with increasing storage time (table 14, p. 29) is explainable on account of the need for additional formation of XB when the availability of XB from endogenous sources decreases. The fact that the addition of boron may raise the germination to its original level, indicates again that only XB is the limiting factor. This is stressed by the observation that pollen from branches supplied with boron is less sensitive to boron after storage (because the initial XB content is higher) than pollen from the control branches.

Presuming that another substance than XB would act in mutual stimulation, it would seem likely that the diffusability of this substance is impeded after storage, thus limiting germination. However, this does not happen in actuality, providing boric acid is present, implying that the phenomena in question are both based on XB (X).

c. The hypothesis in question gives a simple explanation why the mutual stimulation of pollen grains becomes more 'effective' in the presence of boric acid or when the pollen has a high boron level (fig. 9, p. 39), *viz.* because a greater quantity of the promoting complex is present.

d. The literature shows that pollen germination in general is stimulated by boric acid, which infers that boric acid reacts with substances of an identical or related structure in different pollens. This tallies with the fact that the substance which is active in mutual stimulation has been found to be non-specific in several instances.

The observation that pollen was found to tolerate very high boric acid concentrations, suggests that boric acid does not enter the pollen at all or at a very slow rate. On account of this fact and because the *diffusion* of a substance is the determining factor in the mutual stimulation of grains, it may be assumed that the added boric acid reacts with the 'free' substance X, either in the medium after it has diffused or at the surface of the pollen before diffusion.

Summing up the foregoing, it seems evident that *the same mechanism is involved in the stimulation by boric acid and in the mutual stimulation of pollen grains in germination.*

Finally, it can be remarked that the substance X is possibly a glucoside, since KUHN (76, 1943) and MOEWUS (85, 1950) found that pollen (*Crocus*, *Forsythia*) contained certain quercetin glucosides which, in combination with boric acid, played a role in germination.

CHAPTER V

POLLEN STORAGE

1. LITERATURE

The oldest reference made to the handling of pollen concerns the pollen of the date palm. According to ZIRKLE (145, 1935), the translation of several business contracts dating from the Hammurabi period at 2000 B.C. shows that the male inflorescences of the date palm were an important article of commerce at that time. Still earlier (2400 B.C.) the male and female plants were cultivated apart, thus implying that artificial pollination (and transport of the pollen) had to be carried out. It is not exactly known how the vitality of this pollen was maintained, but it is likely that the longevity could be extended due to the low air humidity prevailing in these parts.

KÄMPFER (67, 1719) writes that date pollen, if kept in a dark place, is capable of fertilization the following year. This is confirmed by the findings of ALBERT (3, 1930) who found that date pollen could affect a moderate fruit set in the next season after storage at room temperature, although CRAWFORD (35, 1937) obtained no fruit set after 1 year of storage at room temperature. SWINGLE (129, 1904) states that the Arabs still make a practice of conserving, for use in the following year, a few bunches of staminate flowers, which are put in tight paper bags and kept in a dry cool place. This statement makes it fairly certain that the conservation of date pollen is being practised up to the present time.

A more or less systematic research on pollen longevity started at the end of the last century. Data on the longevity of pollen of more than 80 species stored at air dry conditions are given by MANGIN (83, 1886), RITTINGHAUS (106, 1886) and MOLISCH (86, 1893). It was found by GOFF (50, 1901), SANDSTEN (109, 1909) and ROEMER (108, 1915) that pollen of several species retained its vitality longer at low than at high temperature. KNOWLTON (73, 1922) who stored *Antirrhinum* pollen at 5 different temperatures concluded that the lower the storage temperature the longer the pollen remained viable. Both SANDSTEN and ROEMER observed a greater longevity when the pollen was stored under dry conditions, while also KELLERMAN (69, 1915) stated that *Citrus* pollen, after having been predried and shipped under vacuum, had a higher germination capacity than pollen transported otherwise.

The relation between relative humidity and pollen longevity was thoroughly investigated for the first time by PFUNDT (99, 1910). Pollen of no less than 140 species was stored at 17–22° C and relative humidities of 0, 30, 60 and 90 %. From these experiments it appears that the pollen of the large majority of species maintains its vitality best at low relative humidities (0 and 30 %). These results were confirmed by HOLMAN and BRUBAKER (57, 1926), who tested the pollen longevity of 52 other species at 18° C and a range of relative humidities. They also gave a review of literature and compiled the data on pollen longevity at air dry condition of 231 species belonging to 175 genera and 23 families.

Only pollen of *Typha latifolia* was found to remain viable for one year. Pollen of most other species had a much shorter longevity. Until that time the only other species, apart from date, in which pollen longevity was about 1 year are recorded by HORSFORD (59, 1918) with lily and by MANARESI (82, 1924) with apple, grape, pear and plum. Since then it has been shown that by means of the

combination of low temperature and a favourable relative humidity or with the aid of temperatures below zero alone it is possible to keep pollen viable from one season to the next.

With regard to storage temperatures between 0 and 10° C the following investigators found that numerous pollens, when kept at relative humidities between 10 and 50%, maintain their vitality for approximately one year or longer: NEBEL and RUTTLE (89, 1936), NEBEL (88, 1939) and KING and HESSE (70, 1938) with several species of fruit and other species; PFEIFFER (95, 1936; 96, 1938; 98, 1944) with *Lilium*, *Amaryllis* and *Cinchona*; NEWCOMER (90, 1939), with *Ginkgo*; DUFFIELD and SNOW (40, 1941) and JOHNSON (62, 1943) with some *Pinus* species, the latter also with *Picea* and *Quercus*; GOLLMICK (51, 1942) and OLMO (91, 1942) with *Vitis*; STONE *et al.* (126, 1943) with *Pistachia*.

In contrast to the relatively long life time of the pollen of the above species, the longevity of pollen of *Gramineae* appears to be short under all conditions. Low and even moderate relative humidities are as a rule harmful and pollen stored at 0–10° C remains viable only for a few days or 1–3 weeks at the most, provided the relative humidity is high (80–100%). This has been found by: PFUNDT (99, 1910) and ANDRONESCU (5, 1915) with several *Gramineae* and other species; FIRBAS (45, 1922) with rye and wheat; ANTHONY and HARLAN (6, 1920) and POPE (101, 1939) with barley; KNOWLTON (73, 1922), SARTORIS (110, 1942), JONES (65, 1948), BERGH (14, 1952), LIEFSTINGH (80, 1953) with maize; SARTORIS (110, 1942) with sugarcane; JONES (65, 1948) with buffalo grass. Also pollen of *Hevea* species (DIJKMAN, 39, 1938) was found to maintain its vitality best at high humidities (70–80%).

Apart from the relative humidity, the influence of certain other atmospheric conditions on pollen longevity have been studied also. KELLERMAN (69, 1915) in tests with *Citrus*, PFEIFFER (95, 1936) with *Lilium* and VISSER (138, 1951) with apple and pear found a favourable effect of reduced air pressure. On the other hand, ANTHONY *et al.* (6, 1920), with barley, KNOWLTON (73, 1922), with *Antirrhinum*, SARTORIS (110, 1942), with sugarcane, PFEIFFER (98, 1944), with *Cinchona* and STONE *et al.* (136, 1943) in most cases with *Pistachia* demonstrated that the pollen remained viable better at normal than at reduced air pressure. High percentages of carbon dioxide in the atmosphere have been found to increase the longevity of *Antirrhinum* (73) and apple pollen (7), while storage of *Antirrhinum* pollen in pure oxygen proved to be less favourable (73).

With regard to the effect of temperatures between –10 and –35° C, GOFF (50, 1901) and SANDSTEN (109, 1909) stated that the vitality of pollen of several fruit species was not impaired after a short exposure to temperatures considerably below zero. Also KNOWLTON (73, 1922) showed that *Antirrhinum* pollen kept its vitality longest at –18 to –30° C, even exposure of this pollen to –190° C for half an hour did not reduce its germinability. In later years many investigators have found that pollen could be stored at deep-freeze temperatures without controlled humidities for long periods just as well or with a smaller loss in vitality than at temperatures above zero and controlled humidity; PFEIFFER (95, 1936; 96, 1938) with *Lilium* and *Amaryllis*; CRAWFORD (35, 1937) with date; OLMO (91, 1942) with grape; ANTLES (7, 1951), GRIGGS *et al.* (54, 1953), USHIROZAWA *et al.* (134, 1951) with many fruit species. The latter found a slight germination even after 9 years! On the other hand, the vitality of maize pollen decreases quickly at deep-freeze temperatures as has been shown by KNOWLTON (73, 1922) and LIEFSTINGH (80, 1953). Dry ice storage (–55 to –60° C) has been

used also by GRIGGS *et al.* (55, 1950) and ANTLES (7, 1950) with favourable results for apple and pear pollen. Finally, storage at a temperature as low as –180° C (liquid air) has been attempted by BREDEMANN *et al.* (24, 1947) with *Lupinus* pollen, while VISSER (137, 1951) stored apple and pear pollen at –190° C (liquid oxygen). The pollen thus stored showed no significant loss in germinability after several months of storage.

As an appendix to the above review the newer data of importance have been tabulated, because this has not taken place since 1926 (HOLMAN and BRUBAKER). The storage conditions and the longevity of pollens of species of *one* genus appear to be approximately of the same order. This can be derived from investigations with the following genera of which pollen of 3 or more species had been tested: *Coffea* (44), *Hevea* (39), *Lilium* (95, 96), *Pinus* (40, 62), *Pistachia* (70, 126), *Prunus* (54, 70, 89), *Pyrus* (54, 70, 82, 89) and *Vitis* (51). Therefore when more than one species of the same genus or more varieties of one species had been investigated by one author, the average storage results will be given. Not all storage conditions will be mentioned, as a rule the most favourable ones will be tabulated only.

TABLE 22

A review of storage data on pollen of species of 34 genera; supplement to HOLMAN and BRUBAKER (57, 1926).

BS = before storage, AS = after storage, T = room temperature, H = relative humidity in desiccators, RA = stored at reduced air pressure, AT = stored in air tight containers and normal air pressure, NAT = stored in non air tight containers, N = normal fruit set, M = moderate fruit set, P = poor fruit set.

*) Data reported in extenso in the paragraphs following hereafter.

Genus	Temperature in °C	Storage conditions	Storage time in days	Germination % BS	Germination % AS	Fruit set	Reported by
Amaryllis	10	35 % H	214	–	64	–	95, 96
„	–11	NAT	214	–	35	–	95, 96
Azalea mollis	2	10 % H	168	80	2	–	*)
Betula lutea	T	25 % H	30	40	3	–	62
Buchloë dactyloides	4	90 % H	7–8	–	–	M	65
Carica papaya, C. quercifolia	1	10 % H	153	–	45/80	–	133
Cinchona ledgeriana	10	35–50 % H	365	45/65	5/10	–	98
Citrus spec.	2	25 % H	550	80	63	–	70
Coffea robusta, C. excelsa, C. conuga	T	over CaO	21–28	80	–	N	44
Cucurbita moschata	–17	NAT	30	98	98	N	55
Cydonia spec.	2 to 7	25 % H	550	50–60	46	–	70
Ginkgo biloba	7	over $CaCl_2$	365	–	–	N	90
Gladiolus spec.	10	50 % H	100	–	–	N	97
Hemerocallis fulva	3	10 % H	92	–	–	M–P	63
Hevea spec.	6	70–80 % H	19	90	37	–	39
Hordeum vulgare	10	30, 60, 90 % H	1	40	0	nil	6
„ „	2	humid	14	–	–	N	101
Lilium auratum, L. longiflorum, L. speciosum, L. phillippinense	10	35–65 % H	365	–	–	M	95, 96
	–5;5	RA	365	–	–	N	95, 96
	–11	NAT	365	–	–	M–N	95, 96
Lupinus polyphyllus	–190	AT	93	78	78	–	24
Lycopersicum esculentum	0	over $CaCl_2$	100–300	–	–	M-nil	87
„ „	2 to 4	10 % H	252	47	10	–	*)
„ „	–20	AT/RA	1132	47	63	N	*)
„ „	–190	AT	662	47	35	–	*)
Medicago sativa	–17	NAT	34	88	73	N	54
Olea spec.	–17	NAT	374	33	32	N	54

Genus	Temperature in °C	Storage conditions	Storage time in days	Germination % BS	Germination % AS	Fruit set	Reported by
Persea americana	5	over $CaCl_2$	153	–	viable	–	119
Phoenix spec.	T	AT	275	–	5	M	3
„ „	1	AT	275	–	87	N	3
Phoenix dactylifera, P. reclinata, P. sylvestris	–13	AT	365	–	–	N	35
Picea abies, P. glauca	T	15–35 % H	365	–	6	–	62
	2	10–75 % H	365	–	48	–	62
Pinus strobus, P. retinosa	T	25 % H	413	93	20	–	40
	4	25 % H	413	93	70	–	40
	0	50 % H	413	93	91	–	40
Pinus strobus. P. retinosa, P. sylvestris, P. banksiana	T	15–35 % H	365	90	70	–	62
	2	25–75 % H	365	90	90	–	62
Pinus ponderosa	–	–	365	–	viable	–	104
Pistachia atlantica	0	25 % H	550	–	30	–	70
Pistachia atlantica, P. chinensis, P. intergerrima, P. terebinthus, P.vera	–1	10.5–21.5 % H	365/730	55/95	5/55	N–M	126
Prunus armeniaca	–17	NAT	402	–	58	N	54
„ „	2	25 % H	550	49	26	–	70
„ „	2 to 8	50 % H	912	60/80	20/30	–	88, 89
Prunus avium (cerasus)	–17	NAT	410	53	37	N	54
„ „ „	0 to 2	25 % H	550	57	55	–	70
„ „ „	2 to 8	50 % H	1460	60	20	–	88, 89
Prunus communis	–17	NAT	346/1130	91	82/24	N	54
„ „	2	0 % H	550	70	53	–	70
Prunus domestica (insititia)	–17	NAT	435	69	60	N	54
„ „ „	0;7	25 % H	550	–	44	–	70
„ „ „	10 to 30	over $CaCl_2$	400	76	5	–	82
„ „ „	2 to 8	50 % H	1277	40/60	20	–	88, 89
Prunus hybrids	0 to 5	over $CaCl_2$	365	–	–	N	102
„ „	0 to 5	35 % H	153	–	20/60	–	102
Prunus persica	–17	NAT	415	–	62	N	54
„ „	0	50 % H	550	85	42	–	70
„ „	2 to 8	50 % H	1277	75	3	–	88, 89
Pyrus salicina	–17	NAT	439	40	35	N	54
Pyrus communis	–17	NAT	419	77	65	N	54
„ „	0	25 % H	550	–	80	–	70
„ „	10 to 30	over $CaCl_2$	400	55	3	–	82
„ „	2 to 8	50 % H	1277	–	5/20	P	88, 89
„ „	2 to 4	10 % H	662	66	42	–	*)
„ „	2 to 4	RA	1032	64	15	nil	*)
„ „	–20	RA/AT	1032	64	50	N	*)
„ „	–190	RA/AT	662	64	50	–	*)
„ „	–17 to –37	5 % H	3287	–	1	–	134
Pyrus phaeocarpa	–	–	550	61	46	–	70
Pyrus malus	–56	NAT	275	–	96	N	55
„ „	–17	NAT	385	92	64	N	54
„ „	10 to 30	over $CaCl_2$	400	93	7	–	82
„ „	2 to 8	50 % H	1461	70/80	20	P	88, 89
„ „	2 to 4	10 %/RA	673	76	70	N	*)
„ „	–20	RA;AT	673	76	63	N	*)
„ „	–190	RA/AT	673	76	68	N	*)
„ „	–17 to –37	5 % H	3287	–	3	P	134
Quercus coccinea	2	15–35 % H	365	–	43	–	62
Rhododendron catawbiense	2 to 4	AT	252	30	2	–	*)
„ „	–20	AT	662	30	25	–	*)
„ „	–190	AT	662	30	25	–	*)
Saccharum spontaneum	4	90–100 % H	8	90	70/90	N	110
Secale cereale	16 to 18	humid	1–5	–	–	N–P	45
Triticum vulgare	16 to 18	humid	½	–	–	nil	45
Vitis vinifera, V. riparia, V. rupestris, V. cinera	1	45 % H	365	–	–	P	51
Vitis vinifera	10	25 % H	365	43	10	N	91
„ „	2	25 % H	730	43	7	N	91
„ „	–12	28 % H	1461	43	12	N	91
Zea mais	5	80 % H	4	–	–	N	14
„ „	4	90 % H	6–7	–	–	M–P	65
„ „	T	50 % H	4	–	–	N	80
„ „	4	90–100 % H	8	90	60/70	N	110

2. EXPERIMENTAL DATA ON POLLEN LONGEVITY

The choice of the different pollens used in the storage experiments was determined by the fact that these pollens were often used in plant breeding or floral biological experiments at the 'Laboratorium voor Tuinbouwplantenteelt' at Wageningen: VERKERK with tomato, DOORENBOS with azalea and rhododendron and the author with apple and pear. Since the experiments started simultaneously and under the same storage conditions, the as yet unpublished results with tomato, azalea and rhododendron pollen have also been worked out by the author and will be reported below.

2.1. *Storage at controlled humidities and temperatures above zero*

Material and methods. – The pear, apple, azalea (*A. mollis*) and rhododendron (*R. catawbiense*) pollen was collected before anther dehiscence and stored with the anthers after drying at room temperature. The pollen must stay at this temperature as short as possible while drying in sunlight should be avoided, as sunlight has been found to impair the vitality to a considerable extent (142). The tomato pollen was gathered by means of the 'artificial bee' just after anther dehiscence and stored without previous drying. All pollen was stored (dark) in small open vials and kept in desiccators at relative humidities between 0% and 100% which were obtained with different concentrations of H_2SO_4 (32).

The germination tests were carried out by means of the hanging drop culture (Van Tieghem cell – 130) in 7 – 10% saccharose solution with 70 ppm boric acid. The germination percentage of apple and pear pollen was determined after 4–5 hours, of tomato pollen after 2–3 hours and of rhododendron and azalea pollen after 24 hours at 18–23° C by counting 100 pollen grains in each of 3–6 replications. Since in the earlier trials wide variation occurred between the number of pollens per drop, the germination percentage also varied (see p. 37). Under these conditions the highest germination percentage was considered to be a closer estimate of the actual germination capacity than the mean. Therefore, in most tables referring to the germination results the highest germination percentage only has been recorded.

Results. – A preliminary experiment that started in 1949 with pollen of several apple varieties was not conclusive, although very low and high humidities proved to be unfavourable. The experiment was repeated, therefore, in 1950 with pollen of the pear Clapp's Favourite and of the apples: Sterreinette, Lane's Prince Albert and Brabantse Bellefleur.

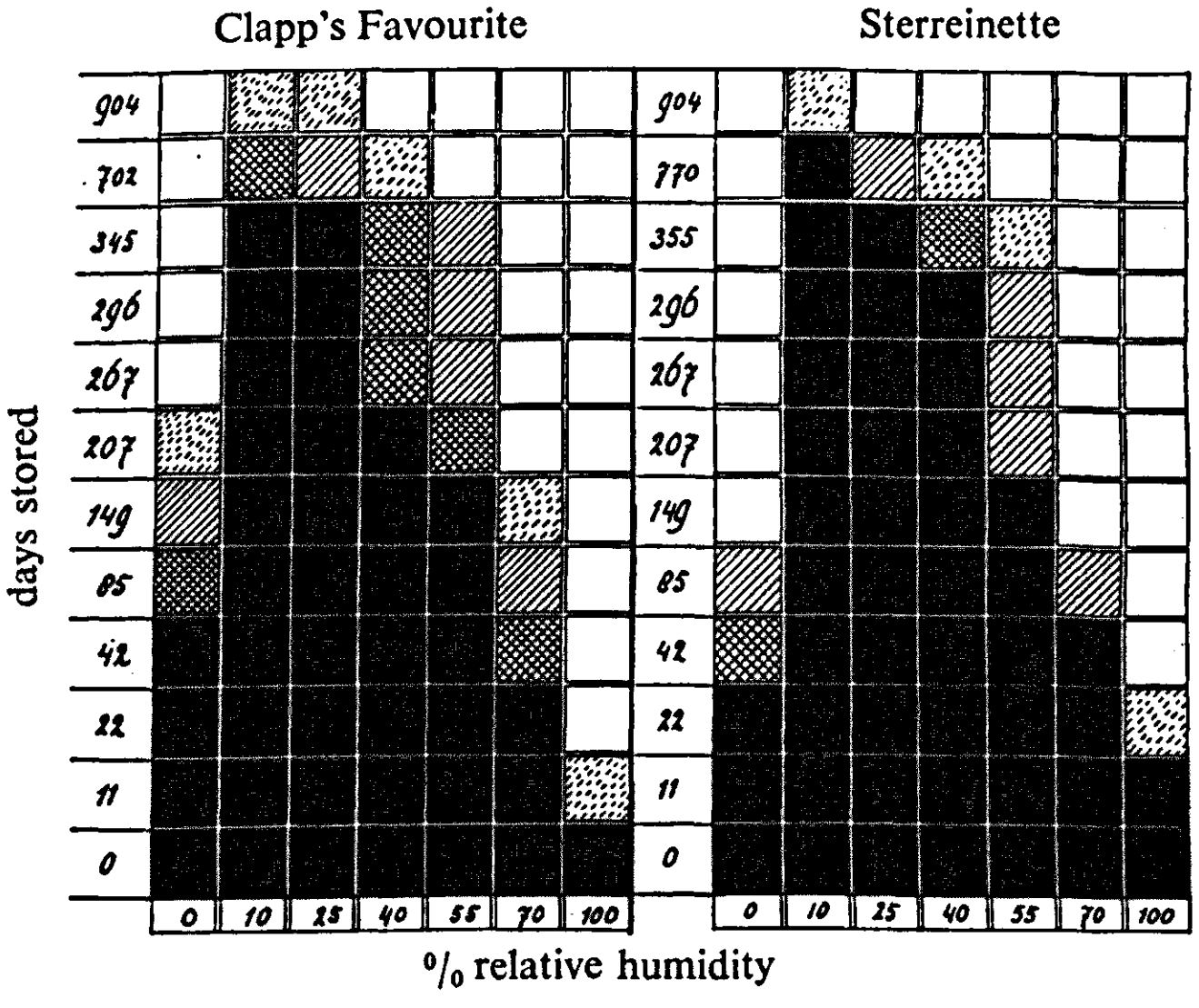

FIGURE 11.
The longevity of pear and apple pollen at 2°–4° C in relation to the relative humidity.

Germination percentage:

The results obtained with the pear variety and with one of the apple varieties are recorded in figure 11; the data on the two other apple varieties have been omitted, since they indicated a similar influence of the relative humidity.

It can be derived from figure 11 that the longevity of apple and pear pollen, stored between 10 and 100% relative humidity, decreases with increasing humidity. Storage at extremely dry conditions (0% relative humidity) is also unfavourable. The germination capacity is kept longest at 10% relative humidity, while storage at 25% humidity gave but slightly less favourable results. The pollen shows a moderate germination even after 2 years of storage under these conditions.

A second experiment has been carried out with pear and apple pollen collected in the spring of 1952, but in this case the pollen was stored at room temperature.

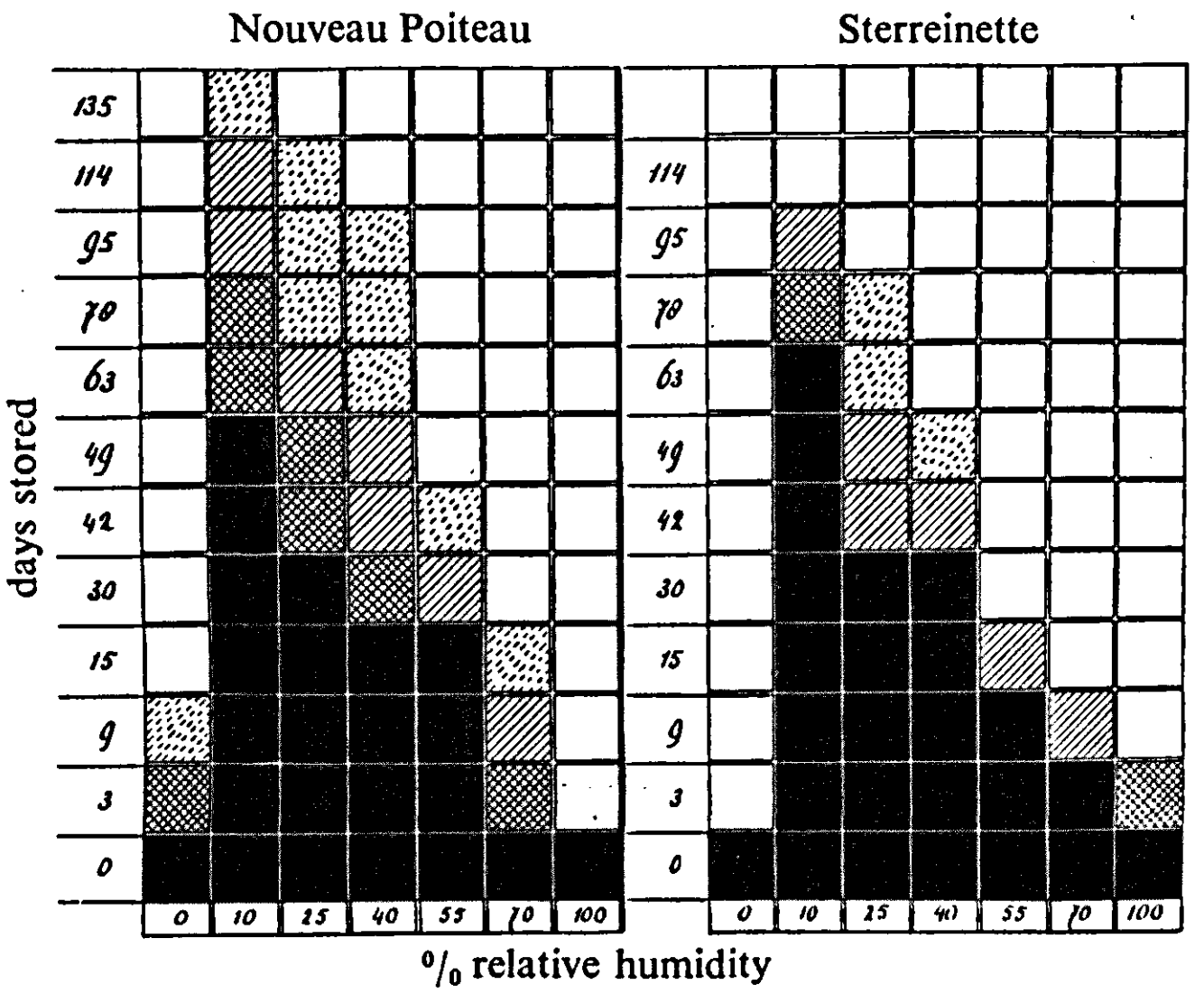

FIGURE 12.
The longevity of pear and apple pollen at 17–22° C in relation to the relative humidity.

From figure 12 it appears that the influence of the relative humidity on the vitality of stored pollen at room temperature is qualitatively the same as at a temperature near zero (fig. 11, p. 51). At the most favourable humidity (10%) the germination remains more than 60% for 7–9 weeks; the maximal longevity at these conditions is approximately 100–140 days. The effect of the temperature on the longevity is very striking as can be seen from comparison of figures 11 and 12: the pollen stored at 2–4 °C maintained its vitality at any relative humidity roughly 10 times as long as at 17–22 °C.

Pollen of azalea, rhododendron and tomato was collected in the early summer of 1949 and stored between 0 and 75% relative humidity; table 23 refers to the storage results.

From table 23 it can be derived that with regard to the relative humidity tomato, rhododendron and azalea pollen behave in a similar way as apple and pear pollen, while 10% relative humidity is optimal. It should be noted that the maximal longevity of these pollens at the above conditions is not much more than half a year, whereas the longevity of apple and pear pollen exceeds two years.

TABLE 23
The longevity of pollens stored at 2° C in relation to the relative humidity.

Species	Days of storage	Germination % after storage at humidities of					
		0%	10%	25%	35%	50%	75%
Tomato	0	55	55	55	55	55	55
	57	7	35	55	40	50	3
	108	0	55	45	55	45	0
	156	0	40	30	30	20	0
	180	0	8	–	2	–	0
Rhododendron	0	85	80	85	60	85	85
	39	3	50	60	65	50	10
	71	0	75	90	15	40	0
	124	0	50	50	20	0	0
	168	0	2	0	0	0	0
Azalea	0	10	35	20	50	45	30
	39	0	20	30	15	25	5
	71	0	10	10	10	30	0
	124	0	10	10	5	1	0
	168	0	9	0	0	0	0

2.2. *Storage at different air pressures and temperatures below zero*

Material and methods. – The pear (Clapp's Favourite) and apple (Sterreinette) pollen used in these experiments had been kept at 10% relative humidity and 2° C during 7–9 weeks after collection in the orchard. The tomato and rhododendron (*R. catawbiense*) pollen was gathered on the day the experiment started. One portion of the pear, apple and tomato pollen was put in small glass tubes which were sealed after 'freeze-drying' (at –25 to –55° C) and evacuation, giving an air pressure of 6 mm Hg and a very low humidity. These conditions were ascertained by means of a freeze-drying apparatus, used for the storage of bacterial and fungicidal spores. The second portion of these pollens, including rhododendron pollen, was put in glass tubes which were sealed at normal air pressure and room temperature. The third portion of the 4 pollens tested was kept in open vials with 10%, 25% and 40% relative humidity. The sealed tubes were stored at 19–22° C, 2–4° C, –20° C and –190° C (liquid oxygen), the open vials in desiccators at 2–4° C. The storage experiments with the different pollens started simultaneously at the end of June 1950.

Results. – The storage results as determined by the germination percentages are recorded in table 24.

This table shows that none of the tested pollens was able to maintain its vitality for long at room temperature (columns 6, 7). The initial germination capacity of all pollens has been maintained almost or entirely during 2 or 3 years at –20 °C and –190 °C (columns 10, 11, 12, 13). The tomato and rhododendron pollen lost their vitality nearly completely within 250 days at 2–4 °C (columns 8, 9). The germination percentages of apple and pear pollen stored during 2 years in the sealed tubes or at 10% humidity at 2–4 °C (columns 3, 9) do not differ much from those obtained after storage at –20 °C and –190 °C (columns 10, 11, 12, 13). The pollen tubes, however, were shorter after storage at the former conditions. The greater effectiveness of low temperature shows more clearly after 3 years of storage. The germination of the pear pollen stored at 2–4 °C (columns 8, 9) appears to be poor, while the germination of the pollen stored at –20 °C (columns 10, 11) had not altered significantly in the third year of storage.

Storage at low air pressure (columns 7, 9, 11, 13) gives in most instances somewhat better results than storage at normal air pressure (columns 6, 8, 10,

TABLE 24
The longevity of pollen in relation to different conditions of relative humidity, air pressure and temperature.
RH = relative humidity; NA = normal air pressure; RA = reduced air pressure;
[1]) too low germination temperature;
[2]) idem, second count after 24 hours of germination.

1	2	3	4	5	6	7	8	9	10	11	12	13
Species	Days of storage	%Germination after storage in desiccators 2–4° C			%Germination after storage in sealed glass tubes							
					19–22°C		2–4° C		–20° C		–190° C	
		10% RH	25% RH	40% RH	NA	RA	NA	RA	NA	RA	NA	RA
Pear	0	66	65	68	64	64	64	64	64	64	64	64
	42 [1])	25	20	20	16	25	22	20	22	36	25	20
	122	66	74	72	0	6	60	61	71	74	57	60
	254	63	69	55	0	0	56	54	70	75	61	58
	662 [2])	0 < 42	0 < 27	0 < 16	0 < 0	0 < 0	9 < 40	38 < 45	14 < 40	34 < 55	32 < 50	27 < 52
	1062				–	–	10	15	48	51	48	52
Apple	0	76	83	79	76	76	76	76	76	76	76	76
	42	71	84	84	46	61	84	75	85	81	74	68
	122	64	76	71	0	3	39	44	48	75	64	69
	254	67	80	68	0	0	34	40	74	71	71	66
	673 [2])	0 < 73	0 < 33	0 < 13	0 < 0	0 < 0	2 < 22	17 < 67	20 < 60	20 < 70	10 < 65	38 < 71
Tomato	0	47	47	47	47	47	47	47	47	47	47	47
	8 [1])	21	23	27	0	0	15	2	17	17	19	3
	42	40	45	42	0	0	33	1	34	19	58	15
	122	20	20	10	0	0	0	0	40	4	40	12
	252	10	3	0	0	0	0	5	40	25	45	20
	662	0	0	0	0	0	0	0	30	35	35	4
	1132	–	–	–	–	–	–	–	60	66	–	–
Rhododendron	0	30	30	30	30	–	30	–	30	–	30	–
	8	20	15	20	20	–	20	–	30	–	35	–
	42	25	20	15	0	–	15	–	30	–	35	–
	122 [1])	1	5	1	0	–	5	–	5	–	10	–
	252	0	0	0	0	–	2	–	25	–	20	–
	662	0	0	0	0	–	0	–	25	–	25	–

12) in the case of pear and apple, but in the case of tomato the effect of low air pressure storage was often less favourable. Storage at 2–4 °C in sealed tubes with a low or normal air pressure appears to have little or no advantage over storage in desiccators with an optimal relative humidity. It can be observed again that the longevity of apple and pear pollen greatly exceeds that of tomato and rhododendron pollen at temperatures above zero. Apparently, the vitality of the latter pollens can be only maintained to the next season when stored at temperatures below zero.

Apart from the behaviour of pollen stored under constant conditions, it was also investigated how the germinability of the pollen was affected when it was stored at a higher temperature following the storage at a considerably lower temperature.

In the first experiment, Clapp's Favourite pollen was kept at reduced or normal air pressure for 2 years at –190 °C and subsequently stored at 2–4 °C during 1 year. The germination of this pollen was 48% and 52% respectively after this 3-year period, as compared with 56% and 54% respectively for pollen stored under equal conditions of air pressure during 1 year at 2–4 °C

only. Hence, the storage properties of the pear pollen stored for 2 years at –190 °C are almost equal to those of freshly collected pollen.

In a second experiment, Sterreinette pollen that had been stored during 1 year at –20 °C was put in an incubator at 23 °C (humidity not controlled) together with freshly collected pollen. The germination percentages of both samples were tested at regular intervals; the results are given in figure 13.

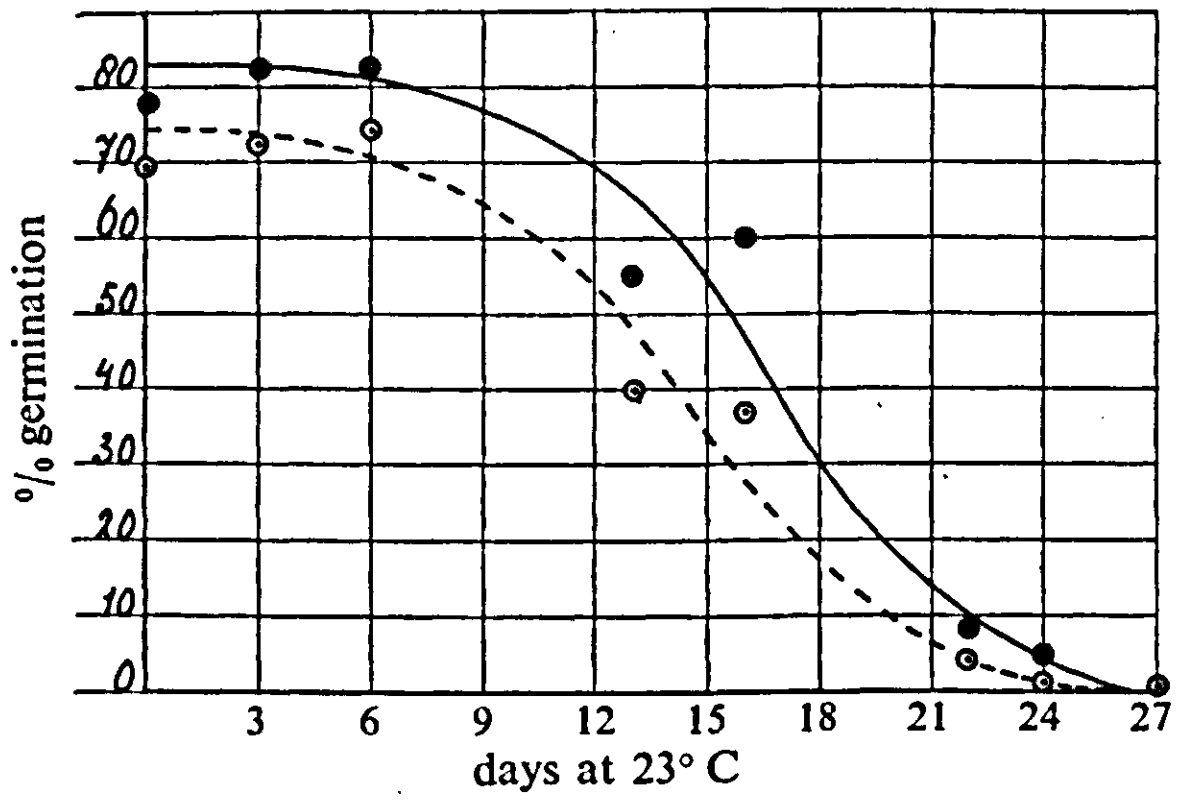

FIGURE 13.
The influence of a storage period at –20° C on the subsequent storage properties of Sterreinette pollen at 23° C.

●——● control: freshly collected;

⊙----⊙ previously stored at –20° C during 1 year.

From the above figure it appears that the rate at which the germinability decreases with time is for both pollens approximately the same. However, the germination percentage and the tube growth of the pollen previously stored at –20 °C were less in comparison with the control.

Both experiments indicate that the pollen is able to stand very considerable fluctuation in temperature without injurious after-effects.

2.3. *Fruit and seed set with stored pollen*

Material and methods. – The pollination experiments with apple and pear pollen were carried out with 20 or more clusters (each with 2 flowers) per test and per variety. The flowers were emasculated in the balloon stage and bagged after pollination. In the experiments with pollen stored in the desiccators the 'control flowers' were left untreated and pollinated by insects. In the experiment with pollen stored in the sealed tubes the control flowers were emasculated and pollinated with freshly collected pollen. Only a few (4–6) flowers were used in the tests with tomato pollen.

Results. – The fruit setting ability of the 3 apple pollens stored in desiccators (see fig. 11, p. 51) was tested on Yellow Transparent and Sterreinette. The mean germination of the pollens was 67%, 67%, 51% and 10% after 1 year of storage at 10%, 25%, 40% and 55% relative humidity respectively. The length of the tubes decreased with decreasing humidity.

The pollination results, recorded in figure 14, show that a distinct relationship exists between fruit and seed set and the relative humidity at which the pollen had been stored. The higher the humidity during storage, the less fruits and seeds were produced; no seeds set with pollen stored at 55% relative humidity. The 'control' gave the best set, but it must be noted that these flowers set under natural (and more favourable) conditions. The pollination experiments with the pear pollen after 1 year of storage were not conclusive due to a high set of parthenocarpic fruits, irrespective of the vitality of the pollen used for pollination.

The Sterreinette pollen stored under different conditions of air pressure and

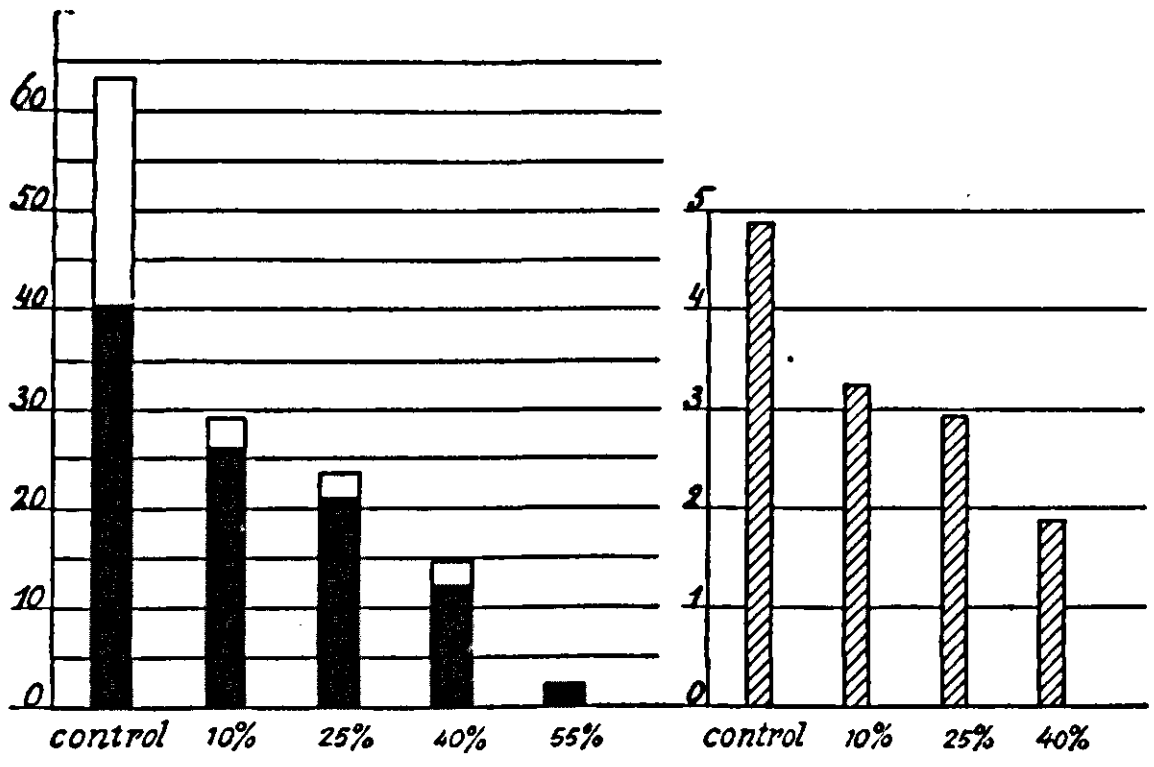

FIGURE 14.
The fruit setting ability of apple pollen after storage during 1 year at 2–4° C and different relative humidities.

temperature was tested on Cox's Orange Pippin and Golden Delicious after 2 years of storage. The germination percentages of the stored pollen samples, obtained 1 day before their use in the orchard, are recorded in table 24 on p. 54 (% germination after 673 days). The 'control flowers' were pollinated with 1 day old Sterreinette pollen, giving 78 % of germination. The pollination results are summarized in figure 15.

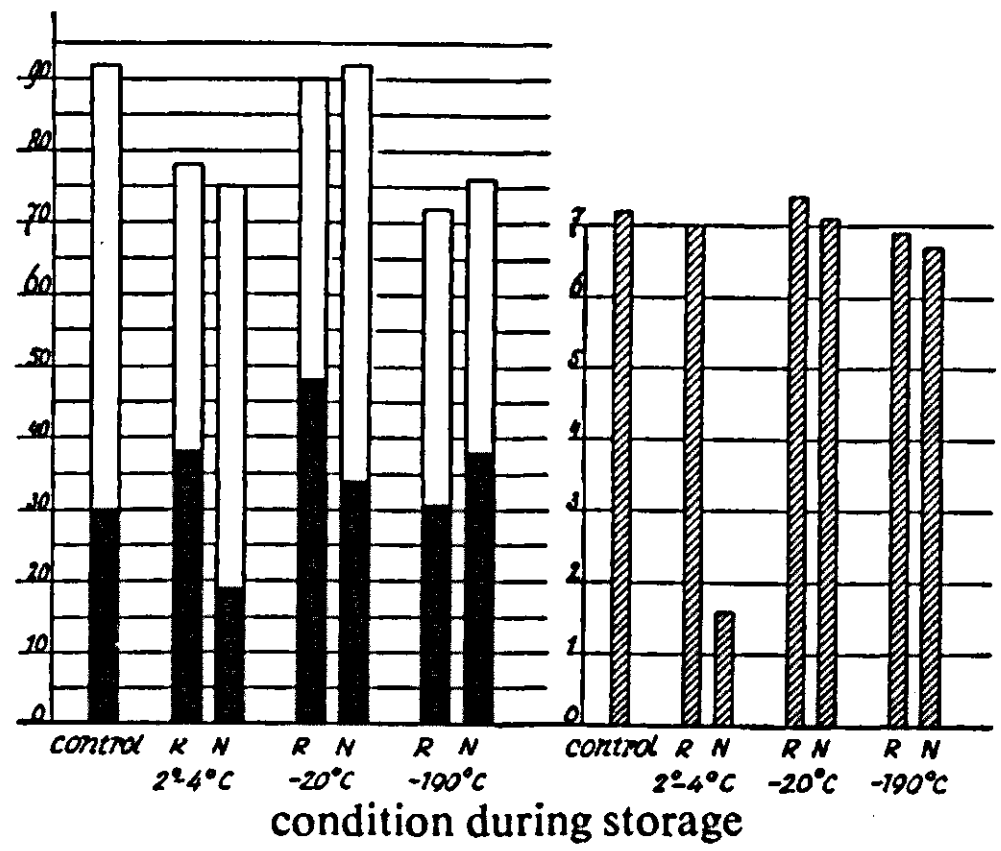

FIGURE 15.
The fruit setting ability of Sterreinette pollen after storage during 2 years at different conditions of air pressure and temperature.

R and N = reduced and normal air pressure respectively.

From figure 15 it can be derived that, as far as storage temperatures below zero are concerned, the fruit set and yield induced by the 2 year old pollen was as good as or better than the fruit set induced by the freshly collected pollen, while the seed sets did not differ significantly. The differences between reduced and normal air pressure are neither reliable. Also storage of the pollen at 2–4 °C, provided the air pressure had been reduced, gave equally good pollination results. Storage of the pollen at normal air pressure, did give fewer fruits and lowered the seed set markedly. This could be expected, since this pollen gave a germination of only 22 % and produced short tubes, while all other pollen samples germinated for more than 60 % and produced long tubes.

The Clapp's Favourite pollen that had been stored during 3 years in sealed tubes at 2–4 °C or at –20 °C was tested on the non-parthenocarpic pear variety André Desportes; the germination percentages are recorded in table 24 on p. 54.

Freshly collected pollen of Clapp's Favourite, giving 41 % of germination and producing long tubes, was used as a check. The pollen stored at 2–4 °C gave no fruit set at all, which is not surprising, since this pollen germinated for less than 20 % and formed short tubes only. The pollen stored at normal and reduced air pressure at –20 °C gave a fruit set of 47 % and 56 % respectively as compared to 71 % fruit set with freshly collected pollen. No mature fruits were harvested because of frost after fruit setting, but it can be assumed from the initial fruit set that the pollen was still able to affect a normal yield after 3 years of storage at –20 °C.

From the above pollination experiments the following relationship between the percentage of germination and fruit set can be approximated:

germination <20 % → fruit set poor to nil;
germination 20–40 % → fruit set poor to moderate;
germination 40–60 % → fruit set moderate to normal;
germination >60 % → fruit set normal.

The tomato pollen stored at different temperatures in sealed tubes has been tested after 8 and 42 days of storage; the germination percentages are recorded in table 24 on p. 54. In the first test all the pollens gave equally good results. Also in the second test the pollen — except the pollen stored at 19–22 °C and low air pressure — proved to be capable of inducing as good a fruit and seed set as freshly collected pollen. These tests show that tomato pollen stored at room temperature was still able to affect a fruit set, even though it failed to germinate in an artificial medium. After 3 years of storage only pollen stored at –20° was available for a pollination experiment (table 24, p. 54). This pollen produced 2 fruits with as many seeds per fruit as the 4 fruits formed after pollination with freshly collected pollen.

No pollination experiments were carried out with the stored rhododendron pollen, but it seems very probable from the results with apple, pear and tomato pollen that the fruit setting ability has been maintained during 2 years storage at –20 °C or at –190 °C.

5. DISCUSSION

When discussing pollen storage it is helpful to remember that its underlying principle is a very simple and general one, namely: the longevity of pollen depends on the extent to which its physiological activities can be reduced without damage being done to the organism.

A. It is not surprising, therefore, to note that the longevity of pollen increases with decreasing relative humidity, but in many pollens the water content cannot be reduced beyond a certain level without loss of vitality. In that case there is a definite optimal relative humidity. This means that at relative humidities lower than the optimum the pollen will lose its vitality sooner through an excessive loss of moisture. In case the relative humidity is higher, the life-time is shortened, because higher humidities allow greater physiological activity.

Other pollens, however, can be optimally stored at relative humidities near zero. Presumably, the 'water-holding capacity' of their colloidals is so great that their vitality is not impaired even under conditions of an extremely low humidity. For instance from experiments by PFUNDT (99, 1910) with 140 different pollens and by HOLMAN and BRUBAKER (57, 1926) with more than 50

different pollens it appears that about half of the pollens tested maintained their vitality equally well or even better over concentrated H_2SO_4 ($\pm$ 0.005% relative humidity) than at higher humidity (30%).

On the other hand, from experiments reported herein it can be derived that apple, azalea, pear, rhododendron and tomato pollen retain their vitality best at 10–25% relative humidity, while storage over H_2SO_4 proved to be distinctly harmful. In fact all newer references on storage (39, 40, 51, 62, 70, 96, 137), in which the longevity of pollen of approximately 50 different species or varieties was investigated at a range of humidities, demonstrate that a relative humidity near zero had an unfavourable effect on nearly all pollens tested. The optimal humidity was found to range between 10 and 50%. This remarkable contrast between older and newer data is illustrated by the fact that PFUNDT found a maximal longevity of 70 days with apple pollen when stored at room temperature over concentrated H_2SO_4, while the author found a complete loss of germination power within 3 days! Although the investigations of PFUNDT and HOLMAN *et al.* and those of later date concern mostly different pollens, it seems obvious that differences in storage and/or germination technique are responsible for this difference in behaviour. Apart from this controversy, it is evident from both the older and newer references that the large majority of pollens maintains its vitality best at low humidities.

In the foregoing, stress was laid upon pollens which show maximal longevity at rather low humidities. There is, however, a smaller group of pollens — *e.g.* graminaceous pollen (see p. 48) — which rapidly loses its vitality at dry conditions and which has to be stored at high relative humidities (80–100%). This means that a sufficient reduction of the moisture content of the pollen has been ruled out as one of the possibilities for reducing the physiological activity. As a consequence it is not remarkable that the longevity of such pollens has been found to be restricted even at temperatures near zero. The short life-time of this pollen, though, is undoubtedly also caused by their much weaker structure.

B. Apart from the use of low humidities, the lowering of the O_2 level and the raising of the CO_2 level have been attempted also as a means to increase pollen longevity. As has been reported in the literature review (p. 48) reduced air pressure proved in some cases to have favourable and in other cases unfavourable results. In the two cases where a high percentage of CO_2 was used in pollen storage favourable effects were observed. From the author's experiments it can be derived that apple and pear pollen stored at 2–4° C in sealed tubes retained their vitality better at reduced than at normal air pressure, but at temperatures below zero the differences were hardly significant. The results with tomato pollen are somewhat in favour of normal air pressure, but it is believed that the lower rate of germination of this pollen when kept at reduced air pressure is not so much due to this low pressure as to its drier condition (perhaps too dry) of the pollen thus stored. It should be noted that the humidity in the vacuum tubes must have been very low on account of the method employed in evacuation. It may well be that in a number of cases where reduced pressure gave a quicker decrease of vitality, the pollen has not been dry enough. Thus an anaerobic respiration may still have been possible with apparently harmful consequences for pollen vitality. It is doubtful if a low air pressure is of great value in pollen storage, since it appeared that apple and pear pollen stored in desiccators at low humidity conditions maintained their vitality just as well as pollen stored under vacuum conditions.

C. Another important factor in pollen storage is the temperature. It was established that pear and apple pollen stored at 2–4 °C (fig. 11, p. 51) maintained their vitality at any relative humidity approximately 10 times as long as at 17–22 °C (fig. 12, p. 52). At 2–4 °C tomato and rhododendron pollen have a longevity of about half a year (table 23, p. 53), but at –20 °C the longevity is more than 2 years (table 24, p. 54). With regard to temperatures below zero, only OLMO (91, 1942) investigated the influence of the relative humidity on the longevity. He found that the pollen when stored at –12 °C kept its vitality better at the lower (28%) than at the higher relative humidity (56%). Nevertheless, many other investigators, including the author, obtained favourable results without paying attention to the humidity during storage. Apparently, it becomes less important as the temperature decreases. It should be stated, however, that favourable results with storage at temperatures below zero can only be secured when the pollen itself has been sufficiently pre-dried and put in small sealed tubes before subjecting it to lower temperatures. Otherwise damage may occur through water condensation (subsequently ice formation) on the pollen surface which is harmful to the vitality (90), or the high moisture content of the pollen may lead to actual freezing damage.

Since drying is a *conditio sine qua non* for storage at temperatures below zero it can be assumed that all pollens which are able to maintain their vitality at a low moisture level can be stored at freezing temperatures. This assumption is supported by the fact that pollens belonging to different genera and which can be kept viable at dry conditions were found to profit from storage at temperatures below zero, *e.g.* pollen of: *Amaryllis*, *Antirrhinum*, *Lilium*, *Lupinus*, *Lycopersicum*, *Phoenix*, *Pistachia*, *Prunus*, *Pyrus*, *Rhododendron* and *Vitis*.

In this connection it is of interest to know whether graminaceous pollen can be subjected to temperatures below zero, because the high moisture content of the pollen (by necessity!) may lead to freezing damage. In fact temperatures of –17 °C or lower have been found to be unfavourable for storage of *Zea mais* pollen by KNOWLTON (73, 1922) and by LIEFSTINGH (80, 1953). In LIEFSTINGH's experiments, however, the pollen stored at –20 °C clotted, which indicates that it must have been quite moist before storage, while KNOWLTON does not mention the condition of the pollen. As no other data are available on the storage of such pollens below zero, it would be worth while to pay more attention to moderate freezing temperatures for storing. One would have to investigate on the one hand the degree to which such pollens can be dried without injury, while on the other hand it has to be determined which temperature below zero this partially dried pollen can withstand without freezing.

D. With regard to the influence of extremely low temperatures the scant information available concerns two pollens only (24, 73). Including these with the pollens referred to in this paper it can be stated that the vitality of pollen belonging to species of *Antirrhinum*, *Lupinus*, *Lycopersicum*, *Pyrus* (*malus* and *communis*) and *Rhododendron* is not impaired after shorter or longer exposure to temperatures of –180 °C or –190 °C. Since it appeared that the longevity of pollen increases with decreasing temperature, the question arises what significance a temperature as low as –190 °C has for the lengthening of pollen life. Theoretically it is a fair assumption that at –190 °C all physiological activities of the pollen have been reduced to nil, which implies that pollen stored at that temperature would retain its initial germination capacity for an indefinite period of time. As a matter of fact, both BREDEMANN *et al.* (24, 1947) with

Lupinus pollen stored at –180 °C for 3 months and the author with pear pollen which was stored at –190 °C for 2 years found that these pollens during subsequent storage in a cold store (2–4 °C) showed the same decrease in germinability as freshly collected pollen. It has been also shown by the present author that apple pollen germinated equally well and induced as good a fruit set after 2 years at –190 °C as freshly collected pollen. Evidently the time 'stands still' for pollen when stored at –190 °C. At the same time it appeared that a sudden increase in temperature of more than 190 °C does not injure the pollen at all. In contrast with storage at –190 °C, some biochemical activity is, apparently, still possible at –20 °C, since the storage properties of pollen thus stored are somewhat less than those of freshly collected pollen (fig. 13, p. 55). BREDEMANN *et al.* derived from experimental data that the germination power of the *Lupinus* pollen would remain unaltered for some millions of years when stored at –180 °C! Hence, by this method of storage the pollen, provided it is resistant, can be given 'eternal life'.

The fact that the pollens tested belong to species of 5 arbitrary genera and were found to be resistant, suggest strongly that a great number of other pollens can also be stored at –190 °C. It seems most likely that these will be pollens which are also 'drought resistant' (maximal longevity at low humidities), because these were shown to survive freezing temperatures.

CHAPTER VI

CONCLUSIONS

The germination of pollen grains and the growth of the pollen tubes *in vitro* appear to be greatly dependent on the osmotic (and/or colloidal) properties of the medium and on the temperature. Boric acid also plays an important role in the germination process, though its stimulating effect is not always apparent. Whether or not pollen is sensitive to boron depends primarily on the boron level of the plant from which the pollen originates and secondarily on the osmotic value and temperature of the medium. When the plant has a high boron level or when the osmotic value of the medium is relatively high and/or the temperature low, good pollen germination may also be obtained without boron. Part of the many conflicting findings in literature may be explained by these facts and by the phenomenon of mutual stimulation of pollen grains. The latter phenomenon can be a far greater cause of variance than non-uniformity of the pollen sample.

With respect to the stimulation of boric acid and the mutual stimulation of pollen grains the author has introduced the theory that in both phenomena the same compound is involved: *viz.* a complex of boric acid with an unknown substance, which complex has 'germination-promoting' properties. Both the stimulation by boric acid and mutual stimulation of grains are general phenomena. Also pollen or pollen extract have been found to stimulate the germination of pollen of other plant species or genera. Therefore, it seems likely that the compounds which react with boric acid have a related or identical structure among different species.

For practical purposes the following observations are of interest. It can be derived from data in the literature that the longevity of pollen is in general negatively correlated both with the relative humidity required for optimal

storage and with the minimum osmotic (and/or colloidal) value required for optimal germination. For instance, pollen of graminaceous species remains viable for a short time exclusively at a high relative humidity and requires a restricted 'water availability' (high osmotic values) to germinate. The opposite holds true for *e.g.* pollen of *Prunus* and *Pyrus* species. These have a far greater longevity, especially at low relative humidities, and are able to germinate at a comparatively high 'water availability' (low osmotic values). The above is also demonstrated after combining the storage and germination data given by PFUNDT (99, 1910); only data concerning pollens with long and short longevities are included in the following:

	26 Species with longevity >100 days	26 Species with longevity <15 days
Maximal longevity:		
At 0% relative humidity	21 pollens	6 pollens
At a higher humidity only	5 pollens	14 pollens
Independent of humidity	0 pollens	6 pollens
Mean (minimum) % of sugar in 1% agar required for optimal germination	10%	19%
Number of pollens which will germinate in 1% agar only	23	9

These examples show that a comparatively high humidity necessary for optimal storage not only goes with a short longevity, but usually also goes with a relatively high osmotic pressure required for optimal germination and *vice versa*. The properties of 'drought-resistant' pollen grains are apparently such that they are better able to withstand severe drying during storage without loss of vitality and during germination are better able to maintain high turgor pressures without bursting than non-drought-resistant pollen.

With regard to general application of pollen storage, it appears that the majority of the pollens has a maximal longevity at relatively low humidities and is consequently drought-resistant. The vitality of such pollens can be maintained for any length of time, if the storage temperature is only low enough. Of course, temperatures as low as –190 °C, though presumably guaranteeing 'ageless' pollen, are not very practical. Deep-freeze temperatures (*e.g.* –20 °C), however, have proved to give satisfactory results too, so that deep-freezing of pollen can be advised as a general and practical way of storing pollen until the next season or longer, provided the pollen is pre-dried and stored in small sealed tubes. Pollens which cannot withstand drying must be kept at high relative humidities and a temperature near zero.

SUMMARY

1. The knowledge of the conditions which determine the longevity of pollen and an insight into the factors which affect the germination of pollen *in vitro* can be of use in plant breeding work and flower biological research. For that reason, an investigation was undertaken into the storage and germination requirements of pollen, with special regard to apple and pear.

2. A review of literature has been given in which the significance of the

osmotic and colloidal properties of the medium and the function of sugar as a nutrient in pollen germination have been discussed. It was concluded:

2.1. The rates of germination and pollen tube growth are positively correlated, while bursting is negatively correlated with the rate of water diffusion into the pollen as determined by the osmotic and/or colloidal value of the medium.

2.2. There is no valid proof that sugar is taken up from the germination medium and used as a nutrient for pollen tube growth. On the other hand, presumptive and direct evidence has been forwarded in favour of non-exogenous-nutrition of the pollen tube by sugar, both *in vitro* and *in vivo*.

2.3. The effect of sugar on germination is in all probability exclusively due to its osmotic properties in aqueous solutions.

3. Pear pollen originating from cut and attached branches which were supplied with boric acid solutions of different concentration and/or 4% sugar was germinated in 10% sugar solution with or without boric acid. Furthermore, the influence of boric acid sprays during bloom on the subsequent fruit set of a number of apple and pear varieties was investigated. The pollen originating from these varieties (collected before spraying) was tested on its boron sensitivity in water and 10% sugar solution respectively. The following results were obtained:

3.1. Sugar and boron, either alone or in combination, supplied to cut branches improved the germinability of the pollen tested in sugar solution *with* boron significantly. The best germination was obtained with pollen from branches supplied both with 4% sugar and 5 or 10 ppm boric acid. The germination in sugar solution *without* boron was poor.

3.2. The germination percentage obtained in sugar solution *without* boron of pollen originating from the attached branches increased in proportion to the amount of boron supplied to the branches. The germinability of the pollen tested in sugar solution *with* boron was independent of the supplied amount of the pollen.

3.3. Almost all pollens appeared to be much more sensitive to boron when germinated in water than when germinated in 10% sugar solution.

3.4. The effect of the boron sprays on the fruit set was not significant in spite of the fact that the pollens originating from the treated trees were found to be more or less sensitive to boron *in vitro*.

4. Data are reported on the influence of environmental factors on the germination of apple and pear pollen with reference to boric acid. Besides, the mutual stimulation of pollen grains in germination and the effect of pollen extracts was investigated. The following observations were made:

4.1. The amount of boric acid present in the medium quantitatively determines the germination percentage and the length of the pollen tubes. The relation between germination percentage and boric acid concentration can be represented by a saturation curve.

4.2. The presence of boric acid is not required until the tubes protrude, but has to be continuously present from that moment on.

4.3. The degree of boron sensitivity of pollen increases with age, but decreases after 'pre-humidification'.

4.4. The percentage of germination is linearly related to the time of germination both with and without boric acid in the medium. The germination rate is higher in the presence than in the absence of boric acid.

4.5. Bursting appeared to be an 'alternative' of germination and occurs when the tube growth is impeded.

4.6. The relationship between germination and temperature on the one hand and between germination and sugar concentration on the other can be represented by an optimum curve in both cases.

4.7. The relative influence of boric acid on the germination rate is positively correlated with the temperature and negatively correlated with the sugar concentration. An increase in temperature of 10 °C approximately doubled the relative influence of boron on the germination rate ($Q_{10} \pm 2$).

4.8. The germination is positively correlated with the number of grains per drop, irrespective of the presence of boric acid. However, the mutual stimulation (with the same number of grains per drop) is more 'effective' when boric acid is added to the medium, or when pollen is used from branches with a relatively high boron level.

4.9. Pollen extracts made in 10% sugar solution both with and without boric acid stimulate the germination to a greater or lesser extent. Extract of apple pollen promotes the germination of pear pollen and *vice versa*. The extract does not lose its promoting influence after heating.

4.10 The literature referring to the influence of boric acid and the mutual stimulation of pollen grains on germination shows that both phenomena are of a general nature. With regard to these phenomena the following hypothesis was derived from the experimental results: *Both the stimulating effect of boric acid and the mutual stimulation of pollen grains on germination is due to a complex of boric acid and an unknown substance having germination-promoting properties.*

5. A review and discussion of the literature on pollen storage is given, including a table in which the newer data on the longevity of pollen of species belonging to 34 genera are compiled. Storage experiments were carried out with apple, pear, azalea, rhododendron and tomato pollen. These pollens were stored at different conditions of relative humidity (0, 10, 25, 40, 55, 70, 100%), temperature (17–22 °C, 2–4 °C, –20 °C, –190 °C) and air pressure (6 and 76 mm Hg). From this study it was derived:

5.1. The pollens tested maintained their vitality practically unimpaired at –20 °C and –190 °C for 2 to 3 years.

5.2. At 2–4° C only apple and pear pollen remained fairly viable at 10% relative humidity or at reduced air pressure during 2 years; the other pollens lost their vitality under these conditions within a year.

5.3. The great majority of pollens hitherto investigated, including those tested by the author, can be classified as 'drought-resistant'. This amounts to the fact that their longevity can be lengthened not only at low humidity conditions, but also at temperatures below zero, provided the pollen is pre-dried. In general, the longevity increases with decreasing temperature and is all but infinite at a storage temperature as low as –190 °C.

ACKNOWLEDGEMENTS

This investigation was made possible by a grant from the Organization for Applied Scientific Research (T.N.O.) and carried out under the direction of Prof. Dr Ir S. J. WELLENSIEK, to whom I am greatly indebted. My thanks are due to Dr Chre J. GORTER for her continued interest. I should also like to acknowledge the kind assistance given by Dr R. VAN DER VEEN and Dr K. T. WIERINGA in the application of certain storage techniques.

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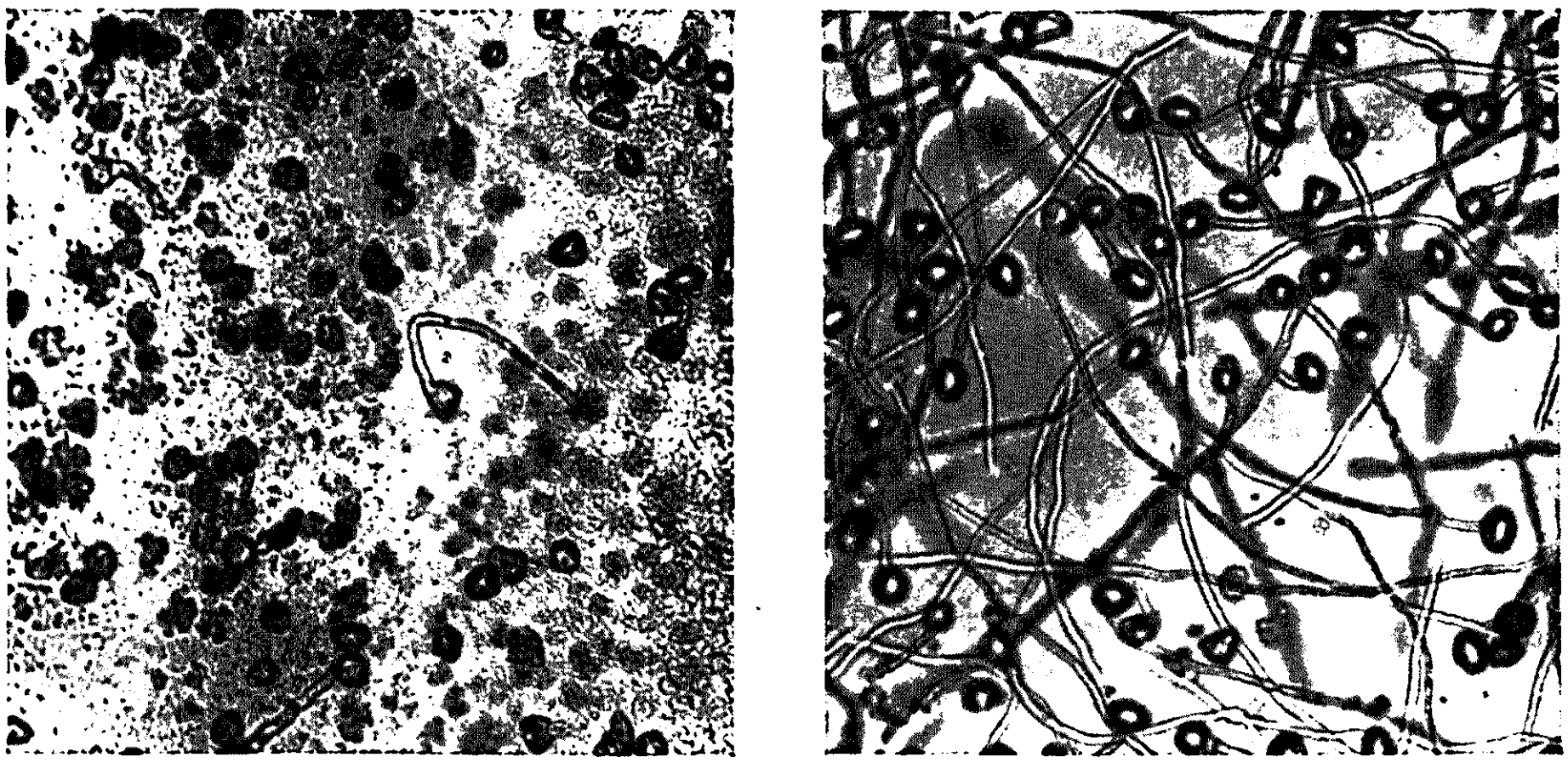

PHOTO 1. The germination of Conference pollen in 10 % sugar after 2½ hours at 23° C. Left: without boric acid; right: with 20 ppm boric acid

PHOTO 2. Method of supplying solutions to tree branches

STELLINGEN

I

De aan het kiemmedium van stuifmeel toegevoegde suiker is, met betrekking tot het kiemproces, uitsluitend werkzaam als osmotisch agens.

Dit proefschrift

II

Het verschijnsel, dat de bevordering van de kieming van stuifmeel als gevolg van de aanwezigheid van boorzuur in het kiemmedium met de temperatuur stijgt en met de saccharoseconcentratie daalt, kan niet verklaard worden uit het feit, dat boorzuur met saccharose een complex kan vormen.

Dit proefschrift

III

De conclusie van DE HAAS en SCHANDER, dat het endosperm van appelzaden een belangrijke rol zou spelen bij de „enzymatische en hormonale regulering" van het narijpings- en kiemproces, is niet houdbaar.

P. G. DE HAAS und H. SCHANDER, Z.schr. Pfl. zücht. 31 (3), 1952: 457–512

IV

De mate van parthenocarpe vruchtvorming wordt in eerste instantie bepaald door de levensduur en groeistofproductie van de onbevruchte zaadknop.

V

De betekenis van de natuurlijke geschiktheid van de grond voor tuinbouwteelten wordt overschat.

VI

De omstandigheid, dat het waterkerend vermogen van de dijken langs de grote rivieren plaatselijk verschillend is, heeft niet alleen waterloopkundige en waterbouwkundige oorzaken, doch is mede voortgevloeid uit de natuurlijke opbouw van de riviergronden.

VII

Ten aanzien van de op de zandgronden heersende agrarische situatie, moet, behalve met de door MARIS gegeven richtlijnen, om economische redenen ook rekening gehouden worden met de mogelijkheid tot bebossing.

A. MARIS, Diss. Aspecten kleine-boeren vraagstuk op zandgronden, 1951

VIII

De beschikbaarheid van organische phosphorverbindingen voor planten, gekweekt in voedingsoplossingen, kan geen uitsluitsel geven omtrent de beschikbaarheid van zulke verbindingen voor planten, gekweekt in grond.

H. T. ROGERS, R. W. PEARSON and W. H. PIERRE, Proc. Soil Sc. Soc. Am. 5, 1940: 285–292

Dissertatie T. Visser,
Wageningen 1955.