SOME ASPECTS OF SKIN SPOT (OOSPORA PUSTULANS) INFECTION OF THE POTATO CROP

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Some aspects of skin spot (Oospora pustulans) infection of the potato crop.

by

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INTRODUCTION

Historical

The first record of what was probably potato skin spot came from Scotland in the middle of the 19th. century, when a condition described as 'pock and blind eye' was noted. Symptoms appeared in early spring, the tuber surface becoming covered with small round brown spots or pimples, and the eyes failing to sprout. The disease was attributed to an insect (Johnston, 1845).

The same disease was described more fully in 1904 - 'potatoes covered with numerous bluish-black warts of about $\frac{1}{4}$" diameter. When thinly peeled, a dark brown patch was found below each wart. On dissecting these patches the cells of the potato were found to be permeated by threads of very fine mycelium'. The causal fungus was not, however, identified (Carruthers, 1904).

A few years later the name 'skin spot' was first used to describe the disease, and the fungus erroneously identified as *Spicaria solani* (Pethybridge, 1915). Later workers suggested *Spicaria nivea* (Milburn and Bessey, 1915), *Rhizoctonia crocorum* (O'Brien, 1919), *Phoma eupyrena*(Wollenweber, 1920), and *Spongospora subterranea* (Shapovalov, 1923) as possible causal organisms, and one worker considered bad storage conditions as being solely responsible for the disease (Gussow, 1918).

The most authoratative work was that of Owen (1919) who described the fungus fully and named it Oospora pustulans. Her work was later confirmed by further investigation and successful inoculation experiments (Millard and Burr, 1923).

The Causal Organism

The organism responsible for skin spot disease is Oospora pustulans Owen and Wakefield, a member of the Hyphomycetales (Fungi Imperfecti). The mycelium consists of narrow septate filaments, 2 - 4µ diameter, hyaline or pale brown in colour. Conidiophores are numerous, up to 250µ long, usually erect and repeatedly branched. The chains of conidia break up easily, the individual conidia being oval-cylindrical or cylindrical single-celled structures with rounded ends, 6 - 12µ by 2.0 - 2.5µ (Owen, 1919; Kharkova, 1961(a)). The production of dark coloured sclerotia, 49µ to 1mm. in diameter has been reported from U.S.S.R. (Kharkova, 1961(a)), but this work has not so far been confirmed.

The fungus grows well on a wide range of cooked vegetable media, but less successfully on uncooked plant tissue (Owen, 1919). Addition of sugar to the media is stated to increase the growth rate (Kharkova, 1961(b)).

Oospora pustulans has a relatively low optimum temperature for growth, generally regarded as being between 12° and 16°C (Owen, 1919; Salt, 1957; Kharkova, 1961(b)). Growth of fungal colonies may be accelerated by initial exposure to temperatures of -5° to $-12^{\circ}C$ for three days (Kharkova, 1961(b)). Growth ceases below $0^{\circ}C$ or above $24^{\circ}C$, but the fungus can survive temperatures as low as $-39^{\circ}C$ during the winter (Kharkova, 1961(b)).

As a result of this low optimum growth temperature of Oospora pustulans, skin spot disease is confined to the temperate and cooler regions of the world, having been reported from Tasmania, New Zealand, Canada, and Norway (Anon., 1950), Germany (Fuchs, 1954), U.S.S.R. (Hrobruih, 1953), Sweden (Emilsson, 1960) and U.S.A. (Folsom and Bonde, 1950).

Method and Time of Infection

Infection of tubers by Oospora pustulans occurs during the growth of tubers in the soil prior to maturation and lifting. Early lifting decreases the severity of the disease (Allen, 1957; Boyd, 1957). Penetration of the fungus occurs through lenticels and round the eyes, both these areas being unprotected by the corky periderm covering the rest of the tuber (Greeves and Muskett, 1939; Allen, 1957). Wounds and abrasions of the periderm also allow entry of the fungus and are potential infection sites (Fuchs, 1954; Boyd and Lennard, 1961(a); Nagdy, 1962). It has not been established, however, whether penetration can occur through the intact periderm of the tuber. A report from the U.S.S.R. suggests that infection might spread internally through the stolons (Kharkova, 1961(a)), but this has not been confirmed by other workers. Entry of the fungus through the stolon scar, however, is known to occur (Boyd, 1957).

Symptoms

External symptoms of skin spot do not become evident until at least two months after infection owing to the slow growth of the mycelium, and under field conditions in Britain recognisable pustules are not fully developed until February or March (Allen, 1957; Boyd and Lennard, 1961(a)). There appear on the tuber surface small dark coloured spots, 0.5 - 2 mm. in diameter, which may occur singly, or in aggregated groups covering a large area of the tuber. These spots may take the form of superficial flat lesions, definite raised pimples, or more occasionally depressed irregular lesions, but in no case are they accompanied by any general rotting of the tuber tissues. The form and size of lesion is reported to depend on the structure of the tuber periderm(Owen, 1919), and also on environmental conditions during the development of infection (Todd, 1963).

Effects on the Tuber, and Economic Importance

Under the skin spots the cells are dark brown with thickened walls, their contents entirely disorganised. Attack is usually limited to the outer layers of cells (1 - 2 mm.) by the development of a cork barrier which isolates the infected region from the healthy tissues of the tuber (Millard and Burr, 1923). When severely infected, the tubers become unsightly, but because of the superficial nature of the infection the affected periderm is easily peeled off and the disease is thus not regarded as being of serious economic importance on ware tubers. With the increasing demand for washed and pre-packed potatoes the situation may change, since skin spot disfigurements are then more obvious and badly infected tubers likely to be discarded.

Cases of deeper skin lesions have been reported. Treatment of stored ware tubers with iso-propyl phenyl carbamate (IPC) in order to delay sprouting, prevented the formation of the cork layer beneath the site of penetration and resulted in abnormally deep and extensive lesions which rendered the tubers unfit for sale (Ives, 1955). After the unusually wet autumn of 1960, some potato crops had to be left in the ground overwinter: examination of these crops the following spring showed extensive, deep skin spot lesions similar to those mentioned above, and several varieties of potato normally regarded as being fairly resistant to the disease were badly affected. Such severe symptoms probably developed as a result of the low winter temperatures to which these crops were subjected, the fungus being stimulated and the formation of protective cork cambium being retarded (Boyd and Lennard, 1962).

Infection of the tuber eye is of much greater importance economically than skin spotting, particularly with regard to the seed trade. Where eyes are infected the fungus may completely kill the bud tissues, or at least delay growth of the sprout. Increased eye infection is known to delay sprouting and crop emergence, thus reducing final yield: in severe cases a proportion of the tubers may have all eyes infected and fail to sprout, resulting in blanking and uneven emergence of the crop. It has also been suggested that skin spot, where it gives rise to blanking, may increase the proportion of ware and correspondingly reduce the proportion of seed tubers in the final crop, the overall effect being a reduction in the total number of tubers per plant (Boyd and Lennard, 1961(a)). Thus assessment of eye infection rather than surface infection gives a more accurate representation of potential loss from this disease (Boyd, 1957; Boyd and Lennard, 1961(a)). Oospora pustulans will also infect potato roots and stolons, causing brown lesions to develop in the cortex: it is not known whether such infection reduces yield (Hirst and Salt, 1956; Salt, 1957; Hirst et al, 1963).

Persistence and Transmission

The fungus seems able to persist saprophytically in the soil for some time, and may be indigenous in certain soils. How long it can survive saprophytically is not known (Owen, 1919; Anon., 1932; Salt, 1957; Hirst and Salt, 1956; Nagdy, 1962). The principal means of transmission of the disease, however, is by planting infected seed, more severely infected seed producing heavier infection in the resulting crop under any given set of environmental conditions (Boyd and Lennard, 1961(a)).

Seasonal Variation

The severity of skin spot infection varies markedly from season to season. Such variations have been shown to be closely related to rainfall and temperature conditions during the lifting period and in the initial storage period of the potato crop. From a consideration of the data over a period of thirty-four years, it appears that there is a close relationship between above average rainfall during the lifting period (end of September, and October) and severe outbreaks of skin spot, presumably because damper conditions encourage fungal growth and sporulation at a time when the tuber periderm is susceptible to penetration. Since *Oospora pustulans* has a low optimum growth temperature, there is also every possibility of increased disease incidence if the temperature during the period October - December is below average. This effect may also be partly due to the low temperatures retarding the metabolic activity in the tuber, and slowing down the formation of a cork cambium below the site where fungal penetration has occurred (Boyd and Lennard, 1962; 1963). A third factor which appears to influence seasonal variation in skin spot attack is the incidence of the disease on the planted seed, a high inoculum level tending to increase infection on the subsequent crop. Evidence suggests that if two or more of these criteria are satisfied, then a high incidence of skin spot is likely to develop by the following spring (Boyd and Lennard, 1964).

Varietal Susceptibility

There are considerable varietal differences in susceptibility to skin spot, and it is recognised that surface and eye infections are not necessarily related (Boyd and Lennard, 1961(a)). Of the more commonly grown varieties, Golden Wonder and Dunbar Rover are two of the most resistant, whereas Kerr's Pink, Craigs Royal and King Edward rank as highly susceptible. The variety King Edward is particularly liable to eye infection with resulting blanking and irregular emergence in crops (Boyd, 1954; 1957; Boyd and Lennard, 1961(a)). Recent experimental work has shown that varieties with a thicker periderm and a higher crude fibre content in the skin, tend to be more resistant to skin spot infection, probably because of the greater mechanical resistance they offer to penetration by the fungus (Nagdy, 1962).

Control

It is now well established that dipping tubers at lifting time in an organo-mercury solution provides the most effective control of the disease, and that any delay in treatment reduces the effectiveness of dipping (Greeves and Muskett, 1939; Foister, 1943; Boyd, 1957; Boyd, 1960). Washing tubers in order to remove surface fungal contamination will also substantially reduce infection, again treatment being most effective if carried out at lifting time (Boyd, 1957). In certain circumstances application of fungicides to tubers at planting time may reduce disease ratings (Salt, 1958).

Boxing of tubers at lifting time, which allows rapid drying out of the surface, tends to reduce infection below the level of that shown by potatoes stored over-winter in an outdoor clamp (Boyd, 1957; 1960). Earlier work on premature lifting of potatoes showed no reduction in skin spot (Greeves and Muskett, 1939), but later investigation suggests that some control may be possible by this method, and also that removal of the haulms one month before lifting may give a small measure of control (Boyd, 1957).

It would thus appear that any treatment which reduces the amount or viability of the fungus on the tuber surface at lifting time will reduce the final incidence of skin spot. Any delay in such treatment appears to allow further penetration to occur, and so reduces the effectiveness of the control measure.

Introduction to Experimental Work

The potato industry in Scotland is based on the annual production of healthy seed, the bulk of which is sent to English markets. Within the last few years higher costs of production, marketing and transport have increased the price of seed potatoes to such a level that English growers have become much more critical of the quality of Scots seed, and many have turned to Irish and foreign sources of supply. To maintain interest in this important trade it is therefore essential that Scottish growers make every effort to prevent poor quality seed reaching the market.

Skin spot is one disease which has caused numerous complaints from potential customers, partcularly after the severe outbreaks in the spring of 1961 and 1962, which caused considerable blanking and delayed emergence in potato crops. As previously stated, tubers become infected with the fungus at or before lifting, but no obvious symptoms become apparent until about February, and even then they may not be readily visible on unwashed tubers. Thus many consignments of seed are dispatched in apparently good condition and free from the disease, but by planting time are found to be severely infected.

Such a situation has led to the demand for an effective method of preventing the development of skin spot on seed tubers, and the experiments reported in this thesis are designed to investigate the possibility of controlling the incidence of the disease by both cultural and chemical methods. Three approaches to the problem have been investigated:

(a) the effect of varying the environment in which the tubers are stored (after artificial inoculation). Incidence of the disease was studied after storage at high and low humidity under three temperature conditions:

(1) Continuous low temperature

(2) Variable temperature as found in farm (bulk storage

(3) Continuous warm conditions

(b) chemical control of the fungus using an organo-mercury dip, and a comparison with the effect of boxing. Both treatments were carried out before lifting, at lifting, and 1, 3, 6, 9, and (in the second year) 12 weeks after lifting, and the subsequent development of skin spot compared with the degree of infection on untreated tubers.

(c) investigation into the effect of varying conditions at planting on the emergence of infected seed tubers, and on the infection which developed on the subsequent crop:

(1) by using two planting dates

(2) by disinfection of seed immediately prior to planting.

MATERIALS AND METHODS

The variety King Edward was used in all experimental work. Not only is it widely grown in Scotland as a seed crop (approximately 13,000 acres annually), but as previously mentioned it is particularly susceptible to skin spot and liable to suffer seriously from damage to the eyes. This is borne out by advisory experience, where by far the greatest number of cases of irregular emergence associated with skin spot refer to this variety (Boyd, 1957; Anon., 1963).

As economic loss due to skin spot is, at present, confined almost entirely to the seed trade, only seed size tubers were used in experimental work - i.e. tubers which would pass through a $2\frac{1}{4}$" mesh riddle, but not a $1\frac{1}{4}$" mesh, these sizes being commonly used in Scotland to select seed tubers.

Assessment of Surface Infection

Surface infection on tubers was assessed visually by separating the sample into five categories:

(a) Severe - $\frac{1}{4}$ or more of the tuber surface covered with skin spot pustules.

(b) Moderate - 1/10 to $\frac{1}{4}$ of surface affected.

(c) Slight - more than 10 pustules but less 1/10 surface area affected.

(d) Trace - less than 10 pustules present.

(e) Free

A Surface Infection Index (S.I.I.) was calculated by multiplying the number of tubers in each category by the average percentage surface area affected, i.e.

Severe (S)	x 62.5
Moderate (M)	x 17.5
Slight (L)	x 5
Trace (T)	x 1
Free (F)	x 0

This total was divided by the number of tubers examined, and multiplied by 100/62.5 to give a mean percentage area affected (Boyd, 1957).

i.e.

$$\text{Surface Infection Index} = \frac{1.6(62.5\ S + 17.5\ M + 5\ L + T)}{N}$$

Where N = total number of tubers in sample.

This method of assessing surface infection is useful in that it allows the degree of infection to be expressed as a single figure. It is not, however, without its drawbacks.

Tubers are easily separated into 'free', 'trace' or 'lower slight' categories, but difficulty can be experienced in deciding whether a tuber should fall into the 'upper slight' or 'lower moderate' classes. This problem also exists for tubers on the borderline of 'moderate' and 'severe' infection. In practice it was found that after a trial period, any sample would be given a very similar Infection Index if re-assessed after an interval of some weeks. Thus for any one operator, this method should be suitable for comparison of infection on different samples. It would not be so suitable for comparing samples assessed by different operators as each would tend to have his own limits for the various categories. Even if standard tubers are used to provide a standard of comparison the problem is not entirely solved, as the scattered and uneven distribution of the skin spot pustules makes such comparisons difficult.

It was therefore considered that although the figures used in this thesis for surface infection index are all of a similar standard and reflect with reasonable accuracy the actual infection present, care would have to be taken if these figures were compared with those of other workers. This criticism is not, of course, confined to this method of visual estimation of skin spot infection, but applies to any estimation relying on visual assessment of size or area, and it is probable that in practice it gives results quite accurate enough for field work.

One further criticism of this method of surface estimation concerns the calculation of the percentage surface infection on severely infected tubers. The assumption made is that tubers falling into this category will have between 25% and 100% of their surface covered with pustules. In fact, tubers with 100% surface infection are never found, and the majority appear to have less than 50% of the skin affected. This means that the figure for average percentage area affected (severe infection) of 62.5 tends to give an exaggerated reading for the overall surface infection index of a sample containing severely infected tubers. This does not apply to such an extent with the other infection categories, as their limits fall within much narrower ranges. In the experimental work for this thesis, it was found that very few tubers fell into the 'severe' category, and for this reason the results will not be unduly affected.

This method of visual surface infection estimation was used throughout the experimental work to maintain continuity with previous experiments, as far as experimental and assessment errors allow.

Assessment of Eye Infection

(1) Visual Examination

An eye was classed as infected if the damage due to skin spot appeared sufficient to kill all bud tissue. In some cases a sprout may in fact have developed, but it was considered that the delay in growth would prevent such an eye from making any contribution to the yield from that plant.

Tubers were separated into three categories:

(a) All eyes infected.

(b) Some eyes infected.

(c) No eyes infected.

An Eye Infection Index (E.I.I.) was calculated by multiplying the number of tubers in each category by the average percentage eyes infected, i.e.

All eyes infected (A) x 100

Some eyes infected (S) x 50

No eyes infected (N) x 0

This total was divided by the number of tubers ~~in~~ examined to give a mean percentage of eyes infected (Nagdy, 1962), i.e.

$$\text{Eye Infection Index} = \frac{(100\ A) + (50\ S)}{N} \times 100$$

Where N = total number of tubers in sample.

This method of calculating eye infection index assumes that all tubers in the 'some eyes' infected category had 50% of their eyes actually killed. This means that a sample with few infected eyes would have an over-estimated eye infection index, while a badly affected sample would show an under-estimated index. One method of improving this estimation would be to divide the tubers into more categories, perhaps taking into account the position of the eyes infected, as eyes in different locations on the tuber vary in their contribution to the final plant.

(2) Microscopic Examination

Work at Rothamsted has shown that tuber eyes may be infected with Oospora pustulans although no skin pustules or obvious damage to the eye is visible. The technique used was to excise the tuber eyes and incubate for five days at 18°C in a humid atmosphere. This treatment produced aerial conidiophores of the fungus which were easily identified under a microscope (Salt, 1957).

In this investigation, tubers for microscopic examination were first washed thoroughly in running water to remove any surface contamination. The eyes were then removed using a $\frac{3}{8}$" diameter cork borer, modified by fitting a spring loaded plunger to eject the plug. This plunger had a concave end to reduce damage to the tuber eye, and it ensured that an approximately equal depth of plug was removed each time. The irregular end of the plug was removed with a scalpel. Normally each plug carried one eye, but because of the close proximity of eyes at the rose end of the tuber, plugs from this area sometimes had two eyes. In this case each individual eye was assessed separately.

To maintain a humid atmosphere round each plug, they were incubated in plastic boxes on damp blotting paper, and to ensure no drying out occurred, a piece of soaked cotton wool was attached inside the lid of each box. The plugs were incubated at a temperature of 60° to 65°F (15 - 18°C) for seven days in darkness, then examined under a dissecting microscope. Each eye was classified into one of four categories:

(a)	Eye Alive	-	Oospora	pustulans	absent
(b)	Eye Dead	-	"	"	"
(c)	Eye Alive	-	"	"	present
(d)	Eye Dead	-	"	"	" .

The majority of eyes consisted of three buds, and were classed as alive if one or more of the buds present showed signs of active growth. *Oospora pustulans* was classified as present if conidiophores were visible on, or round the base of, one or more of the buds. In the case of dead eyes the fungus was classed as present if conidiophores developed on the site of the eye. The fact that a dead eye showed growth of *Oospora pustulans* is not conclusive proof that it was this fungus that caused the damage. However, if no other fungal pathogen was observed, it was assumed that *Oospora pustulans* was the most likely cause.

EXPERIMENTAL WORK

EXPERIMENT 1.

Investigation into the effect of various overwinter storage conditions on the development of skin spot on potato tubers.

A considerable volume of evidence has been accumulated suggesting that skin spot tends to be more serious when tubers are stored under cool, damp conditions. With the increasing use of bulk indoor storage and the installation of forced draught ventilation, it is possible to vary the temperature and humidity conditions in the store within quite wide limits. There is also a growing interest in the use of refrigeration equipment to cool potato stores with a view to reducing sprouting losses during the storage period. Present knowledge of the pattern of skin spot development suggests that such artificially cool conditions, if maintained during the early period of storage, may substantially increase the risk of damage to susceptible varieties by skin spot, particularly if the tubers are wet when taken into the store.

To investigate the effect of different storage conditions on the development of skin spot, artificially inoculated tubers were stored overwinter under various temperature and humidity levels, and the subsequent surface and eye infection levels assessed the following spring.

Pre-treatment of tubers

King Edward potatoes were lifted on 17 October, 1962, and seed size tubers riddled out and retained for the experiment. These tubers were washed, and dipped for ½ minute in 1% formalin to remove surface contamination, then laid out overnight to allow the formalin to evaporate, and inoculated by dipping for ½ minute in a spore suspension of _Oospora pustulans_ containing 1.3×10^6 spores per ml. 10 inoculated tubers were then placed in each of 60 small cardboard boxes.

Treatments

(a) Temperature

Because of a lack of adequate storage facilities, only three temperature conditions could be used, 20 boxes of tubers being kept at each temperature.

(i) Low temperature

To simulate conditions which might be found in an artificially cooled store, tubers were stored throughout the winter in a refrigerated room, the temperature of which was thermostatically controlled between 1.5° and 2.0°C (35° and 38°F).

(ii) Moderate temperature

Temperature conditions very similar to those found in a commercial bulk store were found in a building on one of the University Farms, the temperature of which fluctuated between 10° and 15.5°C (50° and 60°F) at the beginning of the storage period, falling to 3.5° to 10°C (38° - 50°F) between November and February, and rising again to 5.0° to 15.0°C (40° to 60°F) at the end of storage.

(iii) Warm conditions

The third temperature treatment was provided in a store room in the heated building of the School of Agriculture, where the temperature during storage averaged 15.0°C (60°F), fluctuating between 12° and 18°C (55° and 65°F).

(b) Humidity

Only two humidity conditions were possible at each storage temperature:

(i) Dry

10 boxes of tubers were allowed to dry out to the humidity level obtaining in each store. In no case was surface moisture observed on the tubers during storage.

(ii) Damp

The remaining 10 boxes of tubers in each store were soaked in water at the start of the experiment and kept damp for the remainder of the storage period by regularly spraying the outside of the boxes with water. These tubers would therefore be kept at almost 100% relative humidity for the duration of storage.

Results

All tubers were removed from storage on 29 March,

TABLE 1. Effect of various storage conditions on skin spot development on surface and eyes of tubers artificially inoculated with *Oospora pustulans*.

(A) Surface Infection

Temperature:	Low		Moderate		Warm		L.S.D.	
Humidity:	Dry	Damp	Dry	Damp	Dry	Damp	1%	5%
S.I.I:	2.64	7.99	3.61	6.92	0.29	0.80	1.92	1.44
	5.32		5.26		0.54		1.36	1.02
	Dry = 2.18			Damp = 5.24			1.12	0.84

(B) Eye Infection

Temperature:	Low		Moderate		Warm		L.S.D.	
Humidity:	Dry	Damp	Dry	Damp	Dry	Damp	1%	5%
E.I.I:	21.5	56.0	28.6	48.2	0.5	4.5	9.9	7.4
	38.8		38.4		2.5		7.0	5.2
	Dry = 16.9			Damp = 36.2			5.7	4.3

1963, and visual examination made to estimate surface and eye infection due to skin spot.

The results are shown in Table 1, and the figures show that both temperature and humidity have a profound effect on the development of surface and eye infections. There is no significant difference in the infection levels which developed under the continuous cold and moderate temperature conditions, but both produced much higher levels of infection than did warm storage. The effect of high humidity was to increase significantly infection levels at the lower temperatures, but there was no significant humidity effect under warm conditions. These conclusions apply to both surface and eye infections.

The reasons for the higher infection levels which developed under cool damp conditions have not been fully investigated, but it is probable that two factors are important. Firstly, the fungus Oospora pustulans shows active growth at low temperatures, and will develop even at 0°C (32°F). High humidity is essential for the germination of most fungal spores, and there is no reason to suppose that Oospora pustulans differs in this respect. Secondly, cool conditions will retard the drying-out of the tuber periderm and also the rate of suberization, allowing easier penetration of the fungus into the inner periderm layers. Once such penetration has occurred, low temperatures will slow down the normal reaction of the cork cambium in sealing off the site of fungal penetration, thus permitting more extensive damage to occur. It was noticeable that the lesions which developed at the lower temperatures, apart from being more frequent, tended to be larger and rather deeper. This form of lesion has previously been observed on tubers stored under similar cold conditions (Boyd and Lennard, 1962; Todd, 1963).

EXPERIMENT 2.

Investigation into the control of skin spot on potato tubers by (A) boxing and (B) disinfection and boxing, at intervals after lifting.

As previously mentioned it is now well established that disinfection of tubers immediately on lifting is highly effective in controlling skin spot, and that boxing of the tubers will also reduce the incidence of this disease, both treatments being most effective if carried out at lifting time (Greeves and Muskett, 1939; Foister, 1943; Boyd, 1957; 1960).

Unless expensive continuous dipping machines are available, disinfection of tubers with organo-mercury compounds demands first of all the manual separation of seed from ware (to prevent mercury contamination of the ware), boxing of seed, dipping, draining and subsequent stacking of the seed trays. This involves a high labour requirement and means that on most farms the work could not be carried out at lifting time. The alternative would be to store the potatoes in bulk on lifting, dressing out and treating the seed later in the autumn or early winter when more labour is generally available.

However, Greeves and Muskett (1939) and Boyd (1960) have shown that any such delay in boxing or disinfection reduces the effectiveness of these treatments. Subsequent to Boyd's work, the years 1961 and 1962 provided conditions leading to severe outbreaks of skin spot which revived interest in any measure which might give some degree of control. This experiment was therefore designed to determine how long after lifting disinfection and/or boxing could be delayed, whilst still providing an effective reduction in skin spot.

The experimental work was carried out at Highfield Farm, near North Berwick, East Lothian, over the two storage seasons 1962 - 63 and 1963 - 64. The stock of seed tubers in each year was obtained from fields of King Edward potatoes grown for seed purposes and subjected to similar cultural treatments. In both cases the fields had not carried potatoes for the previous six years. Data from the annual survey of skin spot infection (Boyd and Lennard, 1963; 1964) shows that the infection levels on the planted seed was of a very similar order in both years (Table 2).

TABLE 2. Levels of surface and eye infection on planted seed, the produce of which was used in Experiment 2.

	Skin spot infection on planted seed	
Growing Season	Surface Infection Index	Eye Infection Index
1962	20.4	58.0
1963	17.0	47.0

The potential inoculum in the soil at the beginning of each growing season would thus have been at comparable levels, and the total amount of skin spot which developed on the produce by the following spring would thus be dependant largely on the conditions to which the tubers and pathogen were exposed during the growing season and in storage.

The tubers were lifted by elevator digger and the experimental sample stored till required in a clamp containing approximately 30 cwt of tubers. Clamp storage tends to provide a damp environment suitable for the development of skin spot, and was therefore chosen in preference to bulk shed storage. The tubers were piled to a height of approximately 4 ft, covered with layers of wheat straw and earthed up, and there is no reason to suppose that conditions were very different from those encountered in typical farm clamps.

On selected dates the clamp was opened and the required quantity of seed size tubers riddled out into six standard chitting trays, each holding about 1/3 cwt of tubers. Three of these trays were dipped in the organo-mercury solution, allowed to drain, and stacked with the three other undipped trays in an open shed. The dip selected was a readily available proprietary organo-mercury compound (ethoxy ethyl mercuric chloride) used at the recommended rate of 1 lb to 20 gal water (450 ppm mercury) for a ½ minute dip. In order to simulate conditions which would probably be encountered on a farm, the tubers were not washed before dipping. After treatment all trays were covered with sacks to prevent greening of the tubers. Because of the conflicting data regarding early lifting of tubers and its effect on skin spot development (Greeves and Muskett, 1939; Allen, 1957; Boyd, 1957; Nagdy, 1962) six trays of seed tubers were hand lifted from the same fields some weeks before the main lifting date and subjected to the same treatments.

Treatments

See table 3.

Visual estimation of the degree of skin spot surface and eye infection which developed subsequent to each treatment was carried out within a fortnight of the final sampling date in each year (i.e. end of March).

For the purpose of these estimations, a random sample of 33 tubers was taken from two trays and 34 tubers from the third tray of each treatment, giving a total of 100 tubers per sample. The size of the sample was limited because of the time involved in the washing of tubers and carrying out the estimations.

Results

Fig. 1 shows the surface and eye infection indices for skin spot which developed subsequent to each treatment in the first year of the experiment, 1962 - 63: Fig. 2 shows the corresponding results for the 1963 - 64 season. Included in each figure is the range of readings obtained for every treatment. Table 4 gives this data in tabular form.

TABLE 3. Treatments used in the investigation into the control of skin spot on potato tubers by (A) boxing and (B) disinfection and boxing, at intervals after lifting.

1. Treatments

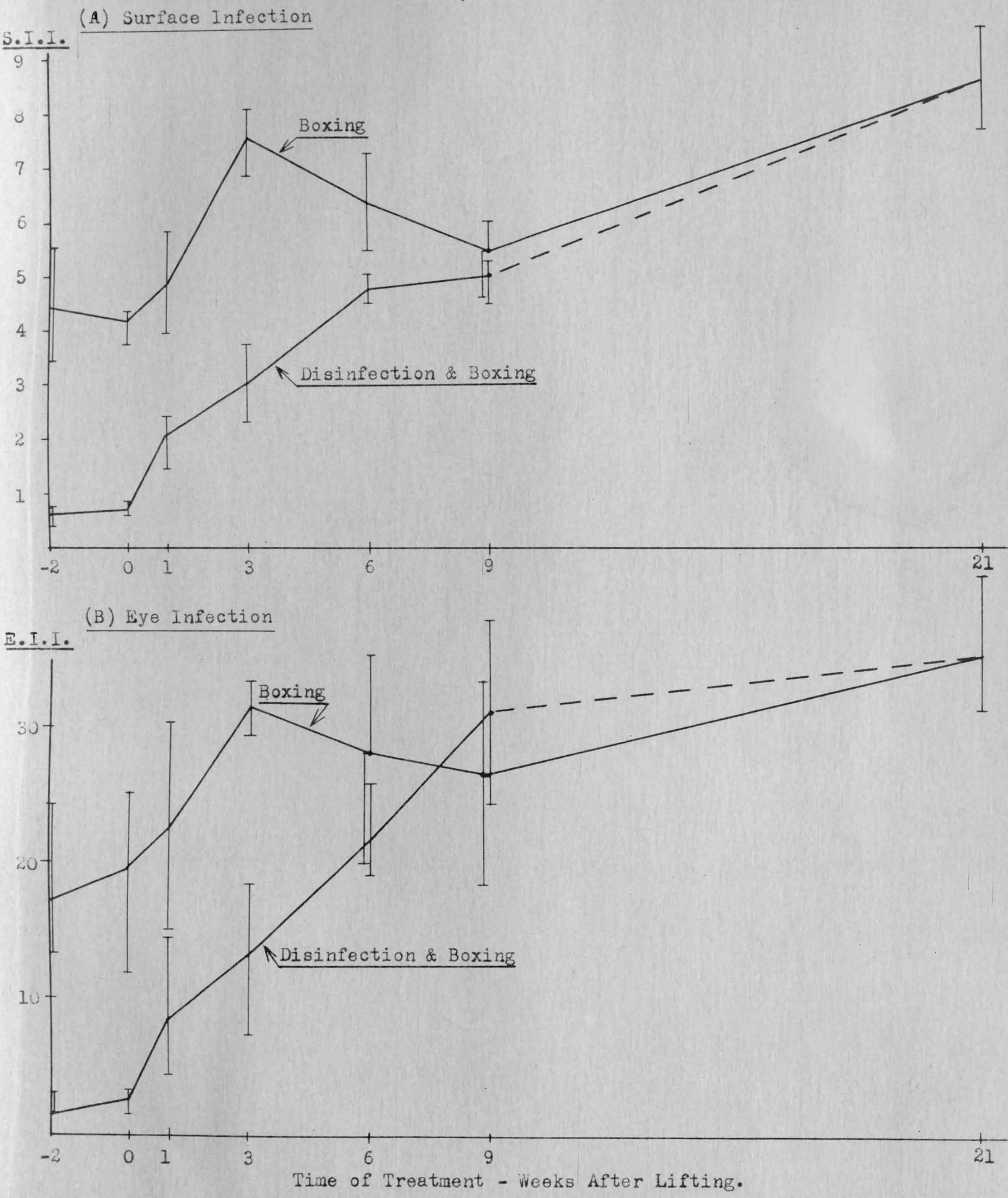
Treatment	1962 - 63			1963 - 64		
	Date of Treatment	Tuber Source	Weeks After Lifting	Date of Treatment	Tuber Source	Weeks After Lifting
1	1.10.62	Hand lifted	-2	27.9.63	Hand lifted	-5
2	16.10.62	Mechanically lifted	0	30.10.63	Mechanically lifted	0
3	23.10.62	From pit	1	6.11.63	From pit	1
4	6.11.62	" "	3	20.11.63	" "	3
5	28.11.62	" "	6	11.12.63	" "	6
6	18.12.62	" "	9	30.12.63	" "	9
7	Not carried out		-	22.1.64	" "	12
Control	14.3.63	From pit	21	27.3.64	" "	21

2. Sub-treatments - in each case the treatments were subdivided into tubers which were:

(A) Boxed only (three trays)

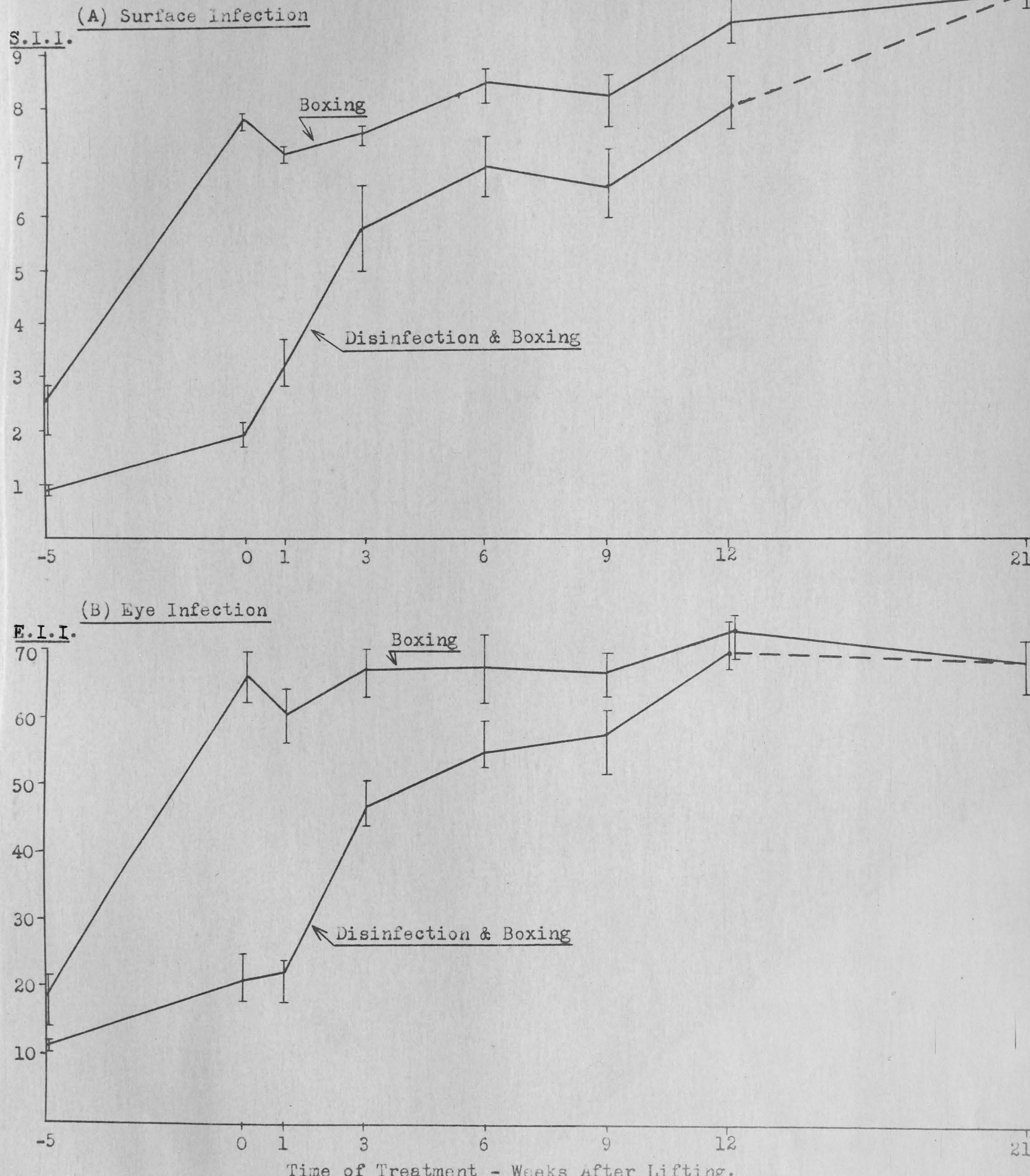
(B) Boxed and dipped for ½ min in an organo-mercury dip (three trays)

Fig. 1. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on resulting skin spot infection of King Edward tubers. Visual examination, 1962 - 63.



S.I.I. - Surface Infection Index
E.I.I. - Eye Infection Index

Fig. 2. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on resulting skin spot infection of King Edward tubers. Visual examination, 1963 - 64.



S.I.I. - Surface Infection Index
E.I.I. - Eye Infection Index

TABLE 4. Effect of (A) boxing and (B) disinfection and boxing, at intervals after lifting on the incidence of skin spot infection. Visual examination.

(1) Surface Infection Index

Treatment	Time of Treatment - Weeks After Lifting								L.S.D.	
1962 - 63	-2	0	1	3	6	9	12	Control	1%	5%
(A) Boxing	4.44	4.17	4.81	7.55	6.34	5.46	Not carried out	8.61	1.61	1.19
(B) Disinfection and Boxing	0.61	0.72	2.09	3.02	4.78	4.98		8.61		
1963 - 64	-5									
(A) Boxing	2.45	7.74	7.10	7.53	8.40	8.20	9.59	10.07	1.02	0.75
(B) Disinfection and Boxing	0.89	1.93	3.34	5.80	6.85	6.47	8.00	10.07		

TABLE 4. Effect of (A) boxing and (B) disinfection and boxing, at intervals after lifting (Continued) on the incidence of subsequent skin spot development. Visual examination.

(2) Eye Infection Index

Treatment	Time of Treatment - Weeks After Lifting								L.S.D.	
1962 - 63	-2	0	1	3	6	9	12	Control	1%	5%
(A) Boxing	17.0	19.4	22.6	31.5	27.9	26.5	Not carried out	33.0	12.8	9.4
(B) Disinfection and Boxing	1.8	2.5	8.6	13.1	21.5	31.0		33.0		
1963 - 64										
(A) Boxing	17.5	66.0	60.0	67.0	67.5	66.5	72.4	67.5	8.4	6.2
(B) Disinfection and Boxing	11.0	21.0	22.0	46.5	54.5	57.0	69.5	67.5		

Effect of Boxing Only (Sub-treatment A)

The results show that boxing tubers without disinfection did give some reduction in skin spot under certain conditions, but at no time was it as effective as the corresponding disinfection treatment, with the exception of treatment 6 (9 weeks after lifting) in 1962-63 when boxing alone gave slightly but not significantly less eye infection than disinfection (Figs. 1 and 2; Table 4). When tubers are boxed without dipping, the fungus remains potentially active and the amount of skin spot which develops is dependant not only on environmental conditions prior to boxing, but also on the conditions under which the boxes are then stored. The two principal factors involved are temperature and humidity. *Oospora pustulans*, as previously mentioned, tends to cause more damage at low temperatures (Boyd and Lennard, 1962), and so the development of skin spot on the boxed tubers was studied in relation to the temperature conditions during the storage period.

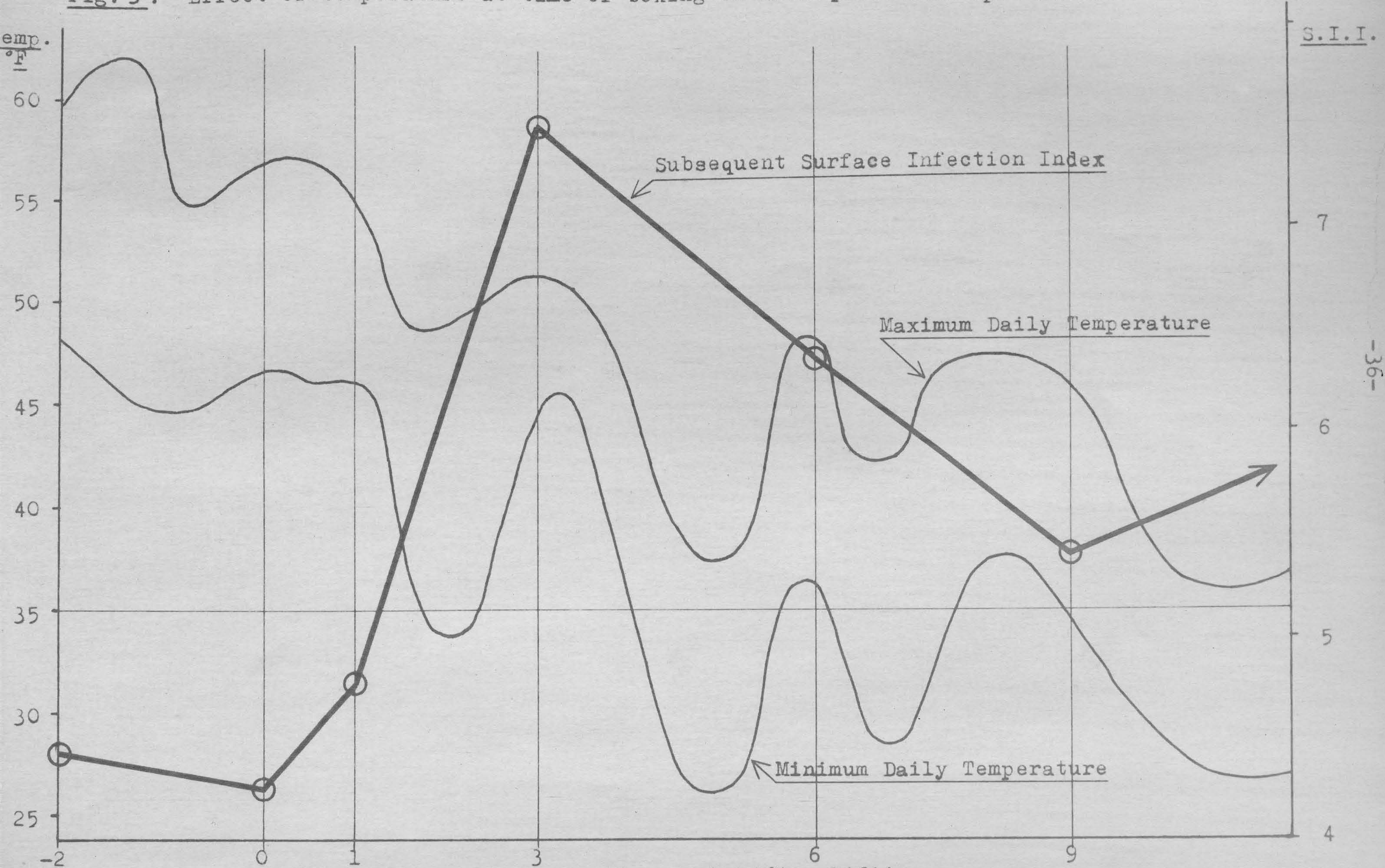
The site of the experiment was approximately midway between the weather stations at North Berwick and Haddington, and using data supplied by the Meteorological Office, the average of the maximum and minimum daily temperature readings at these two stations was calculated for October, November and December, and used as an estimate of the temperature at Highfield. As the boxed tubers were stored in a shed, the door of which remained open throughout the day, it was assumed that the temperature in the shed would follow closely the fluctuations in atmospheric temperature.

From the results shown in Figs. 3 and 4 it is evident that the temperature to which tubers were subjected subsequent to boxing were critical for the degree of control which the 'Boxing' treatment might afford. It is also considered that humidity conditions, particularly in the clamp prior to boxing, would affect the degree of control obtained. This is exemplified by the following conclusions:

a) Treatments 1, 2, and 3. In the 1962 - 63 season, boxing of tubers before, at or one week after lifting gave reasonable control of both surface and eye infection, reducing the surface infection index from approximately 8.5 (S.I.I. of the control) to approximately 4.5, and the eye infection index from about 35.0 (E.I.I. of the control) to 20.0. This can be accounted for partly by the drier conditions encountered on boxing, and also by the relatively mild temperature conditions (the average daily temperature never fell below 4.5°C (40°F)) prevailing for a period of at least four weeks after boxing (Fig.3).

b) Treatments 1, 2, and 3. In the following year, 1963 - 64, tubers boxed prior to the main lifting date again showed little subsequent development of skin spot (S.I.I. 2.5 as compared with S.I.I. 10 of the control: E.I.I. 17.5 compared with E.I.I. 67.5 of the control).

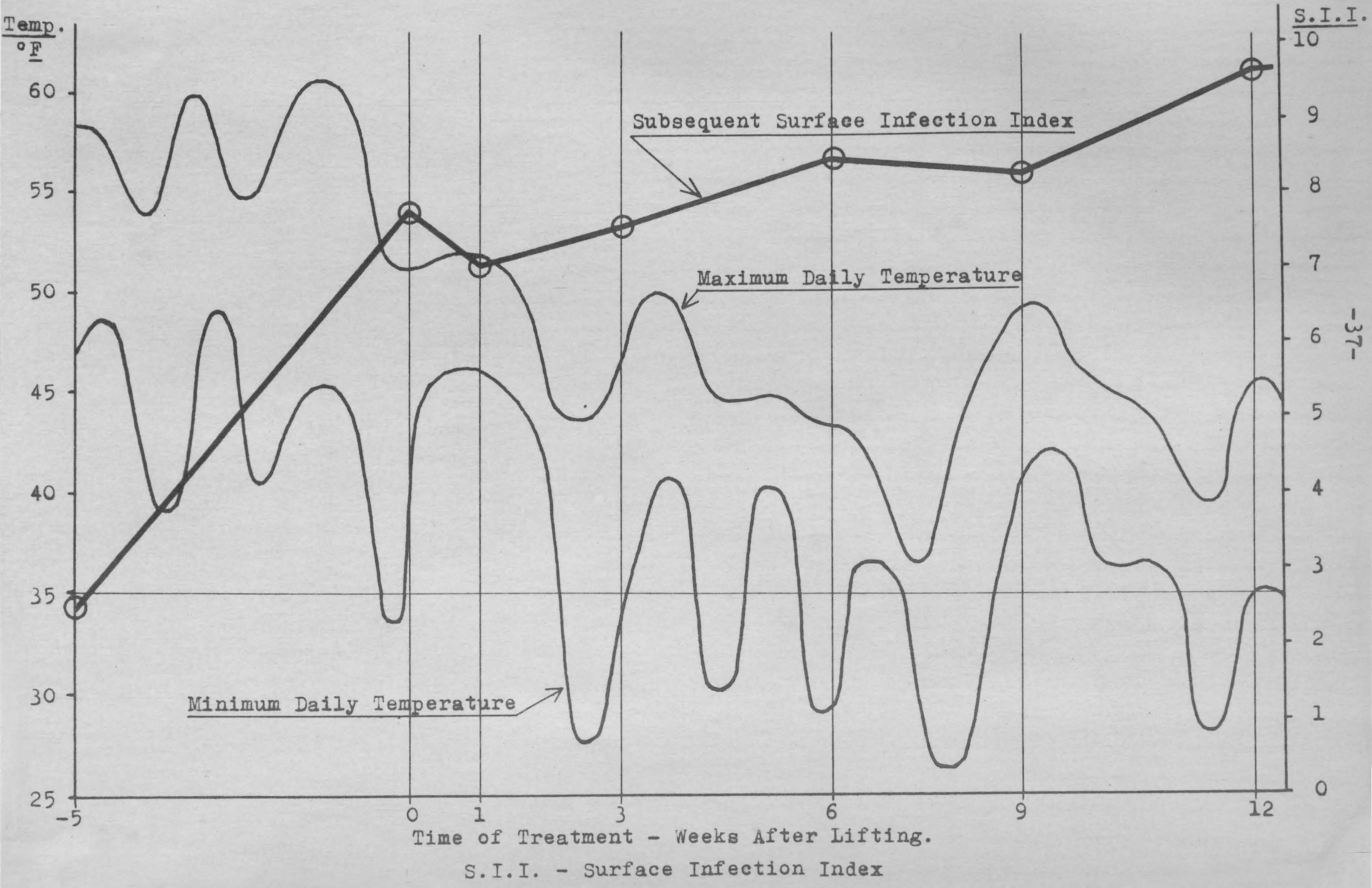
Fig. 3. Effect of temperature at time of boxing on subsequent skin spot infection, 1962 - 63.



Time of Treatment - Weeks After Lifting.

S.I.I. - Surface Infection Index

Fig. 4. Effect of temperature at time of boxing on subsequent skin spot infection, 1963 - 64.



Once again this can be explained by the reduced humidity in the boxes and the relatively warm conditions encountered after boxing. However, the tubers boxed at lifting and one week after lifting in this year showed substantially greater degrees of infection (S.I.I. 7.3 and E.I.I. 62.5) than similar treatments of the preceeding year, reflecting the much lower temperature (1.5°C; 35°F) to which they were subjected shortly after being boxed (Fig. 4).

c) Treatment 4. In both years boxing at three weeks after lifting resulted in very little control of the disease, as the average temperature after treatment was relatively low (at or below 4.5°C; 40°F)(Figs. 3 and 4).

d) Treatments 5 and 6. In 1962 - 63, boxing at 6 and 9 weeks after lifting was more effective than boxing at three weeks, since these tubers had dried out in the clamp, and had been insulated from the low air temperatures of the November cold spell. Despite the fact that these tubers were exposed, after boxing, to the cool conditions of the shed, the disease could apparently not develpp extensively because of the degree of maturation of the tuber periderm which had occurred in the clamp, making these tubers more resistant to fungal penetration.

e) Treatments 5, 6 and 7. Corresponding treatments in

the following

the following much wetter year, 1963 - 64 (including the additional boxing treatment at 12 weeks after lifting) showed very little reduction in skin spot infection compared to the control tubers, presumably due to the damper conditions observed in the experimental clamp during the later period of storage; the cold storage conditions after boxing encouraging fungal development on the less mature periderm.

It is noticeable that skin spot infection on the untreated tubers stored in the clamp until March (controls) was of a different level in each year. In 1963 - 64, the surface infection was slightly greater, and the eye infection markedly greater than in 1962 - 63.(Table 5). There was little difference in the infection levels on the seed tubers planted each spring, but the wetter weather conditions just prior to harvesting in 1963 and the damper conditions noted in the storage clamp probably account for the higher levels of infection in this year. It is not known why surface infection did not increase in proportion to eye infection.

TABLE 5. Levels of skin spot infection on planted seed and on harvested crop after storage overwinter in an outdoor clamp.

Year	Infection on planted seed S.I.I.	Infection on planted seed E.I.I.	Rainfall for 26 days prior to lifting	Resulting infection on control tubers S.I.I.	Resulting infection on control tubers E.I.I.
1962-63	20.4	58.0	0.84 in	8.6	35.0
1963-64	17.9	47.0	1.09 in	10.1	67.5

Effect of Disinfection and Boxing (Sub-treatment B)

In both years the results show that disinfection of tubers in an organo-mercury dip is highly effective in controlling the development of skin spot, provided such treatment is carried out before or at the main lifting date (Figs. 1 and 2; Table 4). In 1962 - 63 the untreated control sample removed from the clamp in March had a surface infection index of 8.5, compared with a surface infection index of 0.7 for tubers disinfected and boxed at lifting, a reduction in infection level of 92%. Corresponding figures for 1963 - 64 are 10.1 and 1.9, a reduction of 81%.

Eye infection was also greatly reduced in 1962 -63 by early disinfection (at or before lifting) from an eye infection index of 35.0 on the controls to 2.5 on those dipped at lifting, a reduction of 93%. In the following year, control of eye infection was not so marked, being from 67.5 to 21.0, a reduction of 69%.

It is assumed that disinfection will effectively kill all fungal spores and mycelium resting or germinating on the surface of the tuber, and that the mercury may also penetrate a short distance into the periderm. Any skin spot which subsequently developed would therefore be caused by fungal mycelium which had already penetrated below the reach of the disinfection. This may account for the rather greater skin and eye infections after the dipping treatment in 1963, as lifting time was two weeks later than in 1962, allowing more time for penetration of the fungus. This is borne out by the fact that the early lifting and disinfection and disinfection in 1963 - 64, five weeks before the main lifting date gave significantly less skin spot than the same treatment at the main lifting. Why the corresponding early lifting in 1962 - 63 did not likewise reduce infection cannot be explained, unless the drier conditions of this year had some effect on the rate of fungal penetration.

Results from both years for both surface and eye infections show a rapid increase in the level of skin spot which developed when disinfection was delayed after lifting. Delay of only one week produced significantly more infection of both surface and eyes in 1962 - 63, and surface infection in 1963 - 64. Eye infection in this second year showed no increase, and this result cannot be explained. It would seem that the action of lifting in some way stimulates the fungus to more rapid penetration of the tuber, possibly by allowing easier entry through damage and small abrasions caused by mechanical lifting and subsequent handling. In both years Treatment 6 (9 weeks after lifting) was approximately half as effective in reducing surface infection when compared with Treatment 1 (at lifting), and caused no significant reduction in eye infection in 1962 - 63, and only slight reduction of eye infection in 1963 - 64 (from E.I.I. 67.5 to 57.0). These figures suggest that the fungus may penetrate and damage the relatively unprotected eye tissues more readily than it penetrates the periderm, where there occurs a much thicker and more definite layer of suberised cells.

Effect of (A) boxing and (B) disinfection and boxing, at intervals after lifting: estimation of eye infection by microscopic examination.

Immediately after visual examination of skin spot had been carried out for this experiment, the three samples were bulked and a random sample of 20 tubers removed. These tubers were rinsed in running water and the eyes excised and incubated for microscopic examination as previously described. This method of eye examination has the advantage that it gives an indication of the number of eyes, alive or dead, carrying viable *Oospora pustulans* at time of planting (i.e. after storage throughout the winter).

Figs. 5 and 6, and Table 6 show the percentage of dead and alive eyes, with and without the fungus present.

Comparison with Figs. 1 and 2, and Table 4 shows that both methods of eye estimation reveal very similar patterns with regard to the total number of eyes killed by skin spot subsequent to each treatment. Taken over both years, the correlation between visual examination and microscopic examination of eye damage was found to be highly significant:

$$r = + 0.9206 \quad (n = 26), \quad P \ll 0.01.$$

In estimating the eye infection index there would therefore seem to be no advantage in using the longer and more complicated technique of excising the eyes and examining microscopically, except possibly with very

Fig. 5. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on subsequent skin spot eye infection.
Microscopic examination, 1962 - 63.

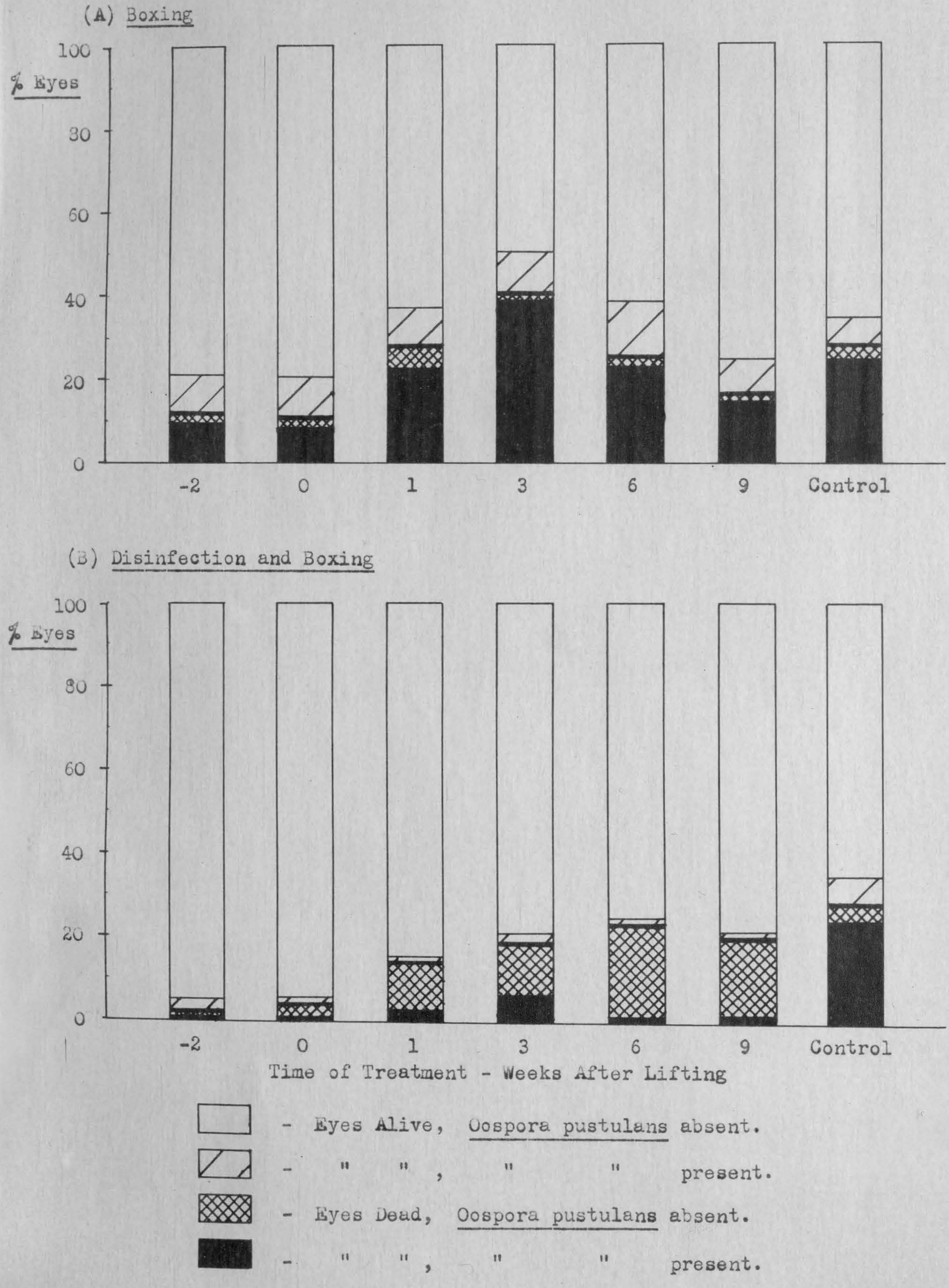


Fig. 6. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on subsequent skin spot infection. Microscopic examination, 1963 - 64.

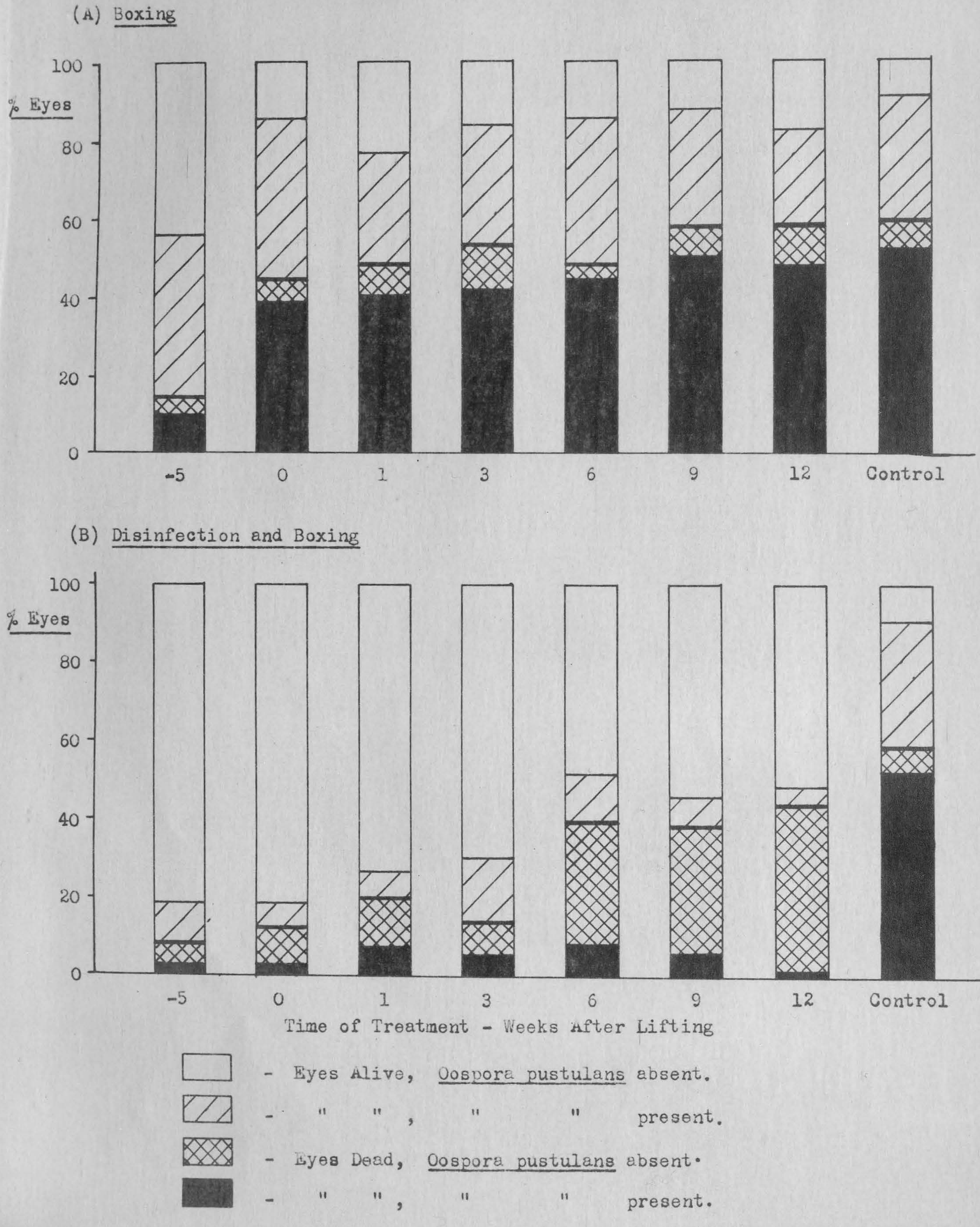


TABLE 6. Effect of (A) boxing and (B) disinfection and boxing, at intervals after lifting on subsequent eye infection by skin spot. Microscopic examination.

Treatment 1962 - 63	Time of Treatment Wks. after Lifting	Percentage of Eyes			
		Eyes Alive		Eyes Dead	
		Fungus* Absent	Fungus* Present	Fungus* Absent	Fungus* Present
(A) Boxing	-2	79	9	2	10
	0	79	9	2	9
	1	62	10	5	23
	3	49	11	1	39
	6	61	14	2	23
	9	76	9	1	15
	Control	66	6	3	25
(B) Disinfection & Boxing	-2	94	2	2	1
	0	94	1	4	1
	1	84	0	13	3
	3	79	2	12	7
	6	75	1	22	2
	9	78	1	19	2
	Control	66	6	3	25

* - Fungus = Oospora pustulans

All figures to nearest 1%.

TABLE 6. (Cont.) Effect of (A) boxing and (B) disinfection and boxing, at intervals after lifting on subsequent eye infection by skin spot. Microscopic examination.

Treatment 1963 - 64	Time of Treatment Wks.after Lifting	Percentage of Eyes				
		Eyes Alive		Eyes Dead		
		Fungus* Absent	Fungus* Present	Fungus* Absent	Fungus* Present	
(A) Boxing	-5	45	42	3	10	
	0	14	42	5	40	101%
	1	23	28	8	41	
	3	16	32	11	42	101%
	6	14	38	3	46	101%
	9	12	30	7	51	
	12	17	25	10	48	
	Control	9	31	7	53	
(B) Disin-fection & Boxing	-5	81	10	6	2	99%
	0	80	6	11	3	
	1	73	7	13	7	
	3	70	15	9	6	
	6	48	12	30	10	
	9	54	6	32	7	99%
	12	51	4	43	2	
	Control	9	31	7	53	

* Fungus = <u>Oospora pustulans</u>

All figures shown to nearest 1%.

low levels of skin spot infection. Microscopic examination is, however, of particular use in determining the presence or absence of sporulating Oospora pustulans on the eye tissues.

Figs. 7 and 8 use the same data to illustrate the effect of treatments on the survival of the fungus, and shows the total percentage eyes with viable Oospora pustulans against the total percentage of eyes without fungal growth. It can be seen that dipping tubers up to 9 weeks after lifting in 1962 - 63 was very effective in controlling the fungus, or at least inhibiting its ability to sporulate, samples from all dipping treatments having less than 10% of the eyes with viable Oospora pustulans present at the end of the storage period, compared with figures of between 19% and 45% for the corresponding boxing treatments (Fig. 7; Table 6). It thus seems possible that delayed disinfection, although not very effective in controlling skin spot symptoms on the tubers, may nevertheless considerably reduce the amount of fungus present on the tubers which would otherwise be added to the soil in the following year.

This is substantiated by the figures for 1963 - 64, when there was considerably more inoculum present on the harvested tubers, the 'boxing only' treatment showing 50% to 83% of the eyes carrying Oospora pustulans, whereas disinfected tubers had only 6% to 21% of their eyes infected (Fig. 8; Table 6).

It will be noted that in both years delaying

Fig. 7. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on the presence of Oospora pustulans on tuber eye tissue. Microscopic examination, 1962 - 63.

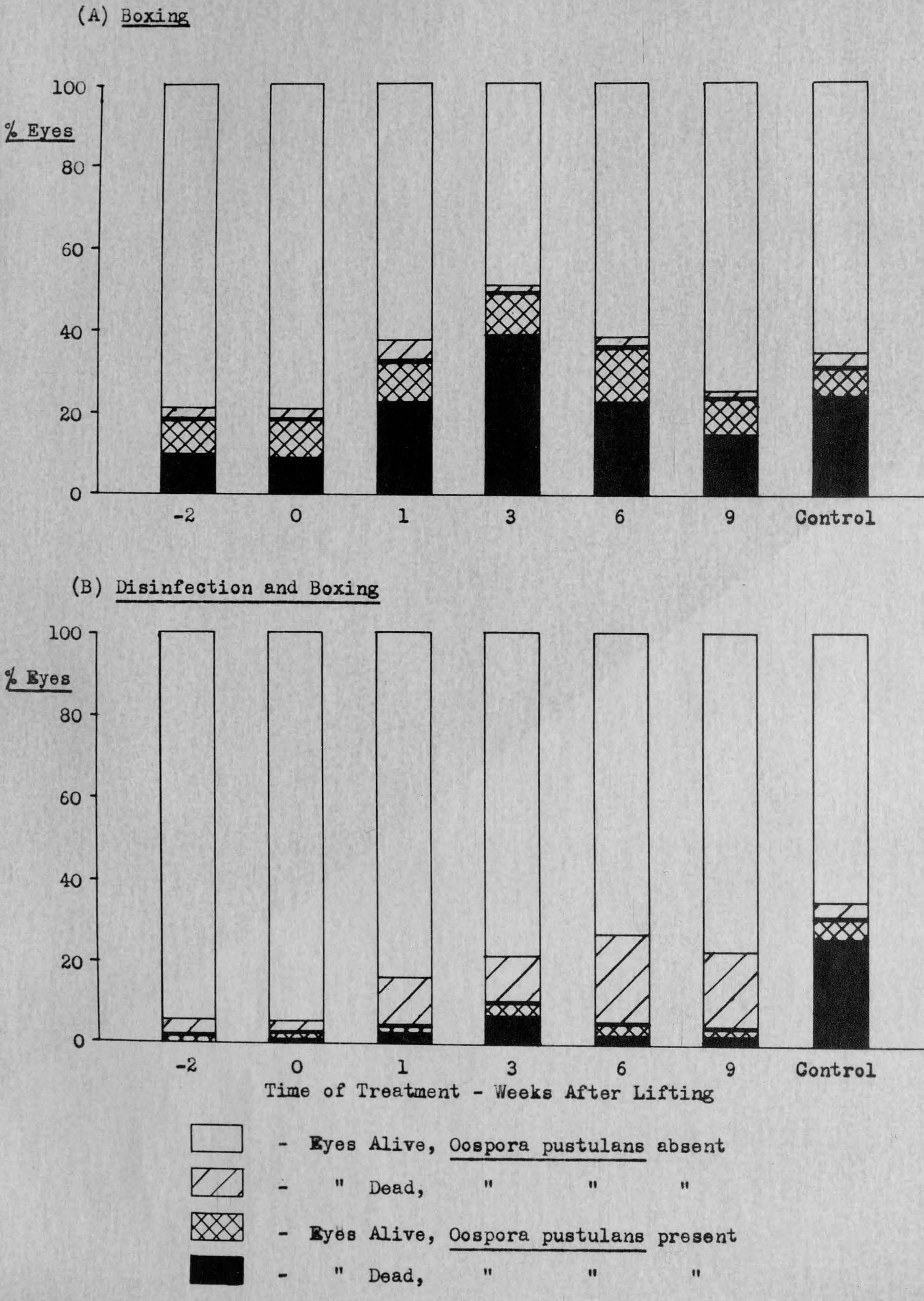
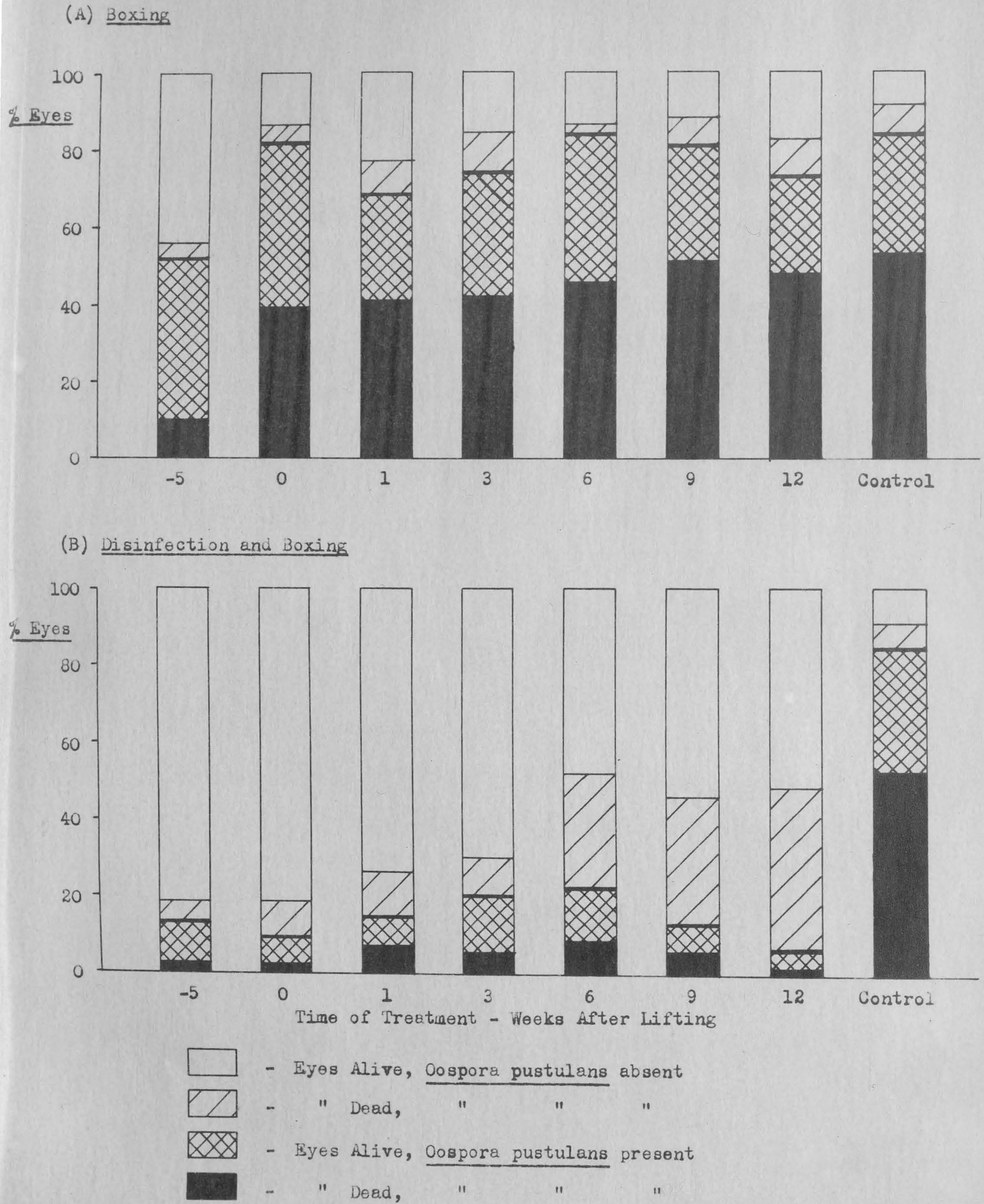


Fig. 8. Effect of (a) boxing and (b) disinfection and boxing, at intervals after lifting on the presence of <u>Oospora pustulans</u> on tuber eye tissue. Microscopic examination, 1963 - 64.



disinfection until 9 weeks after lifting resulted in a gradually increasing percentage of eyes found to be dead without <u>Oospora pustulans</u> present, when examined in March; from 4% to 18% in 1962 -,63 and from 10% to 33% in 1963 - 64 (Figs. 7 and 8). This would indicate that the fungus became active in the bud tissues at, or shortly after, lifting time (disinfection before lifting had negligible effect on the number of dead eyes), gradually killing an increasing number of the eyes. The organo-mercury dip effectively controlled the fungus, but in the later treatments much of the damage to the eye tissues must have already been done.

Progressive development of Oospora pustulans infection on tuber eyes during clamp storage.

In 1963 - 64 further microscopic eye examinations were made on tubers removed from the storage clamp at the times of the boxing and disinfection treatments, and thereafter at three-weekly intervals until March when the experiment terminated. These estimations were carried out immediately the sample had been taken, not as in the case of the other eye examinations which were done in March when all skin spot symptoms had fully developed. The purpose of these examinations was to give some indication of the progressive development of the fungus during the period of clamp storage. The results are shown in Fig. 9 and Table 7. The most noticeable feature is the steady increase in the percentage of dead eyes showing Oospora pustulans, from 5% at 6 weeks after lifting to 55% at 18 and 21 weeks after lifting. This supports the evidence obtained from the eye examination of the dipped tubers, suggesting that the fungus gradually kills an increasing number of eyes throughout the storage period, assuming that the store conditions are suitable for active fungal growth.

At each sampling date there was a small percentage (1 - 12%) of dead eyes which showed no sporulating Oospora pustulans. In some cases this was due to mechanical damage to the buds or to blight (Phytophthora infestans), but in the remainder no fungal or other

Fig. 9. Progressive development of Oospora pustulans infection on tuber eyes during pit storage.
Microscopic examination, 1963 - 64.

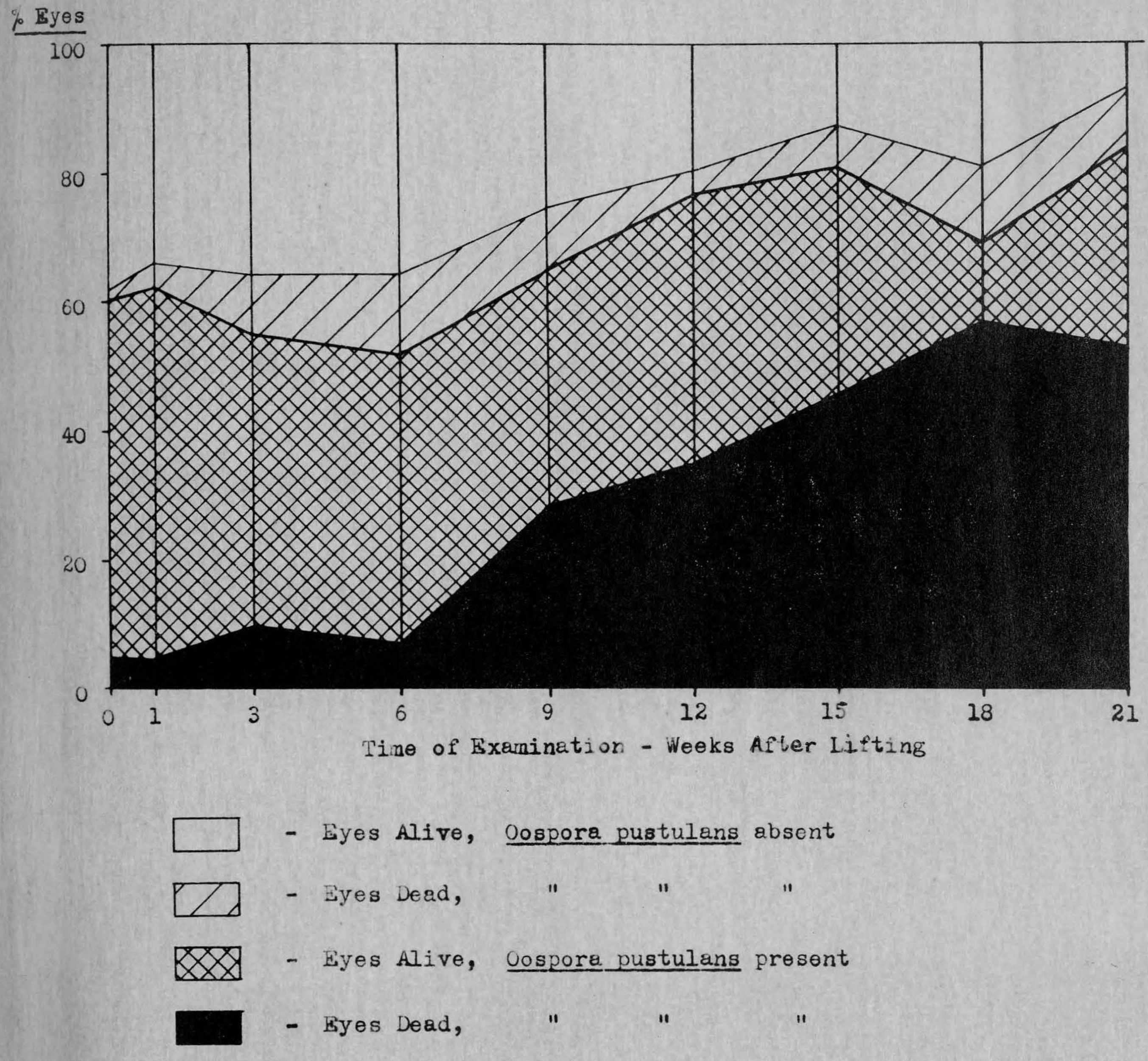


TABLE 7. Progressive development of *Oospora pustulans* infection on tuber eyes during clamp storage. Microscopic examination, 1963 - 64.

Time of Examination (Weeks after lifting)	Percentage of Eyes			
	Eyes Alive		Eyes Dead	
	Fungus* Absent	Fungus* Present	Fungus* Absent	Fungus* Present
0	39	54	1	5
1	34	57	3	5
3	38	45	8	9
6	37	45	12	6
9	24	36	10	29
12	20	41	3	36
15	13	35	6	46
18	19	11	13	57
21	9	31	7	53

* Fungus = *Oospora pustulans*

All figures to nearest 1%.

pathogen could be detected. It is possible that death may have been due to physiological causes or to the presence of a fungus which did not sporulate. In neither case, however, was the number of eyes in this category large enough to affect the general trend of the results.

With the increase in the number of dead eyes throughout the season there was a corresponding decrease in the number of live eyes. A decrease in the percentage of live eyes showing *Oospora pustulans* is to be expected as these eyes were gradually killed by the fungus. It was also noted, however, that the percentage of live eyes without *Oospora pustulans* also dropped during the storage period. The reason for this is difficult to explain, since it is generally believed that *Oospora pustulans* does not spread in storage, tubers becoming relatively resistant to the disease after the initial few weeks of storage.

The presence of conidia of *Oospora pustulans* in the air of a potato store has been reported from the U.S.S.R. (Kharkova, 1961(a)), and it was claimed that this would promote infection of tubers in the store. It would also appear from the current investigation that an increase in infection did occur during storage under the very damp conditions found in the clamp at Highfield during 1963 - 64, this high humidity being essential for the production of aerial conidia of the fungus. This might account for the much higher incidence of eye infection in the second season, 1963 - 64, compared with the 1962 - 63 season when the pit dried out within the first few weeks after lifting time.

One other explanation for the increase in total percentage eyes showing Oospora pustulans later in the season is that variation in storage conditions may alter the ability of the fungus to spore, and that a proportion of the live eyes examined early in the storage period may have been infected, but did not produce aerial conidiophores on incubation.

Effect of delayed boxing and disinfection treatments on the emergence of seed tubers, and on the final yield and skin spot development on the subsequent crop.

To investigate the effect of delayed boxing and disinfection of seed tubers on their rate of emergence, and also on the final yield of the subsequent crop and the level of skin spot which developed, tubers from the 1962 - 63 Highfield experiment were planted out in an experimental plot at Bush Estate, Midlothian. Because of the limited space available only nine of the thirteen treatments were used, as shown in Table 8.

TABLE 8. Treatments from boxing and disinfection experiment used to obtain data on yield and skin spot development on subsequent crop.

	Treatment	Time of Treatment
2 A	Boxed	at lifting
2 B	Disinfected and Boxed	
3 A	Boxed	1 week after lifting
3 B	Disinfected and Boxed	
4 A	Boxed	3 weeks after lifting
4 B	Disinfected and Boxed	
6 A	Boxed	9 weeks after lifting
6 B	Disinfected and Boxed	
Control	Tubers remained in pit overwinter	

All treatments were randomised in each of 4 replicates, each treatment replicate consisted of a 14 ft length of drill, in which were planted 15 tubers.

a) Emergence

The planting date was 2 May, 1962, and the first

TABLE 9. Effect of boxing and disinfection at intervals after lifting on the rate of emergence and yield obtained from the subsequent crop.

Treatment	Infection on planted seed		Average number of days to emergence	Total tuber emergence (Max. 60)	Average yield (lb)
	S.I.I.	E.I.I.			
2 A	4.18	19.5	38.4	60	21.6
2 B	0.72	2.5	40.0	60	20.6
3 A	4.80	22.5	41.8	60	16.1
3 B	2.10	8.5	41.2	59	16.8
4 A	7.56	31.5	40.9	59	18.0
4 B	3.01	13.0	40.6	59	18.8
6 A	5.46	26.5	38.8	60	17.4
6 B	4.98	31.0	43.5	58	17.5
Control	8.60	35.0	41.7	56	18.4
					(L.S.D. at 5% = 4.86)

tubers to emerge above ground level were recorded on 7 June. Emergence counts were thereafter made weekly until 12 July. The average number of days to emergence for each treatment replicate was calculated by dividing the sum of the number of days from planting to emergence for each tuber which produced growth, by the total number of emerged plants. The average number of days to emergence for each treatment planted, and the total number of tubers which emerged (out of a possible total of 4 x 15 = 60) is shown in Table 9.

These figures show that there was insufficient infection on any of the treatments to affect markedly the rate of emergence or the final number of emerged plants, although the tendency was for the control and later boxed treatment (6 B) to show a slower rate of emergence and lower final plant stand than the disinfection at lifting treatment (2 A). The intermediate treatments produced rather erratic results and no definite trend can be established.

b) Yield

The crop was lifted on 11 October, 1962, and the total produce from each treatment replicate weighed and placed in net bags which were then stored during the winter in an outside clamp in order to allow skin spot symptoms to develop.

The results, shown in Table 9, are not significant at the 1% level, and although there are significant differences at the 5% level, no general trend is obvious: under these conditions it would be wrong to conclude that the various disinfection and boxing treatments affected the final yields. It must be remembered, however, that the general level of infection even on the control tubers, was not of a high level, and it is probable that had this been greater and the early control measures equally effective, the resulting increase in the spread of infection levels would have been reflected in the emergence and yield figures.

c) Skin spot development on subsequent crop

The produce from the 1963 growing season was stored overwinter in an outside clamp, and estimations of skin spot infection which developed made in April, 1964. These results are summarised in Table 10, and may be outlined as follows:

a) Compared with the control, all treatments significantly reduced eye infection indices on the subsequent crop.

b) All disinfection treatments (B) gave significantly lower eye and surface infection indices than the control.

c) With the boxing treatment (A), only that carried out at lifting (2 A) reduced infection significantly.

d) In the case of treatments at lifting (2) and at 9 weeks after lifting (6), disinfection (B) was significantly more effective in reducing both surface

TABLE 10. Surface and eye infection indices on crops harvested from tubers boxed and disinfected at intervals after lifting.

Treatment in 1962	Infection on planted seed		Infection on harvested tubers	
	S.I.I.	E.I.I.	S.I.I.	E.I.I.
2 A	4.18	19.5	5.05	39.5
2 B	0.72	2.5	1.77	18.0
3 A	4.80	22.5	5.37	37.5
3 B	2.10	8.5	4.88	35.0
4 A	7.56	31.5	5.57	40.0
4 B	3.01	13.0	4.30	36.0
6 A	5.46	26.5	5.80	44.0
6 B	4.98	31.0	2.78	23.5
Control	8.60	35.0	7.80	59.5
			L.S.D. at 1% = 20.5 5% = 15.1	L.S.D. at 1% = 3.67 5% = 2.71

and eye infection than the boxing only treatment (A).

From these results it can be seen that the effect of treatments which reduce the incidence of skin spot on tubers will be reflected in the levels of infection which develop on the crops grown from these tubers, presumably because of the reduction in the amount of potential fungal inoculum which is available for stolon and tuber infection.

Calculation of correlation coefficients (r) from the data in Table 10 shows that:

a) Surface infection index on planted seed was significantly correlated with surface infection index on harvested tubers -

$$r = + 0.724, \quad P < 0.05 \quad (n = 5)$$

b) Eye infection index on planted seed was NOT significantly correlated with eye infection index on harvested tubers -

$$r = + 0.598$$

c) Surface infection index on planted seed was significantly correlated with eye infection index on harvested tubers -

$$r = + 0.757, \quad P < 0.05 \quad (n = 5)$$

These results suggest that surface infection supplies the greater part of the fungal inoculum to the soil at the infection levels found in this experiment, and that the potential surface and eye infection on the subsequent crop depends more on the degree of surface infection present on the seed than on the eye infection. If the individual replicates used in the disinfection experiment had been retained and grown on separately to provide values for the 'carry-over' infection, it would have increased the total number of comparable figures from 9 to 27, and given a more accurate estimate of the correlation coefficient.

EXPERIMENT 3(A)

Investigation into the effect of disinfection at planting time of skin spot infected tubers on the emergence, yield and infection levels on the subsequent crop.

The methods of controlling skin spot disease which have been described in the previous two experiments, where the emphasis has been on treatment as soon as possible after lifting, are inapplicable for the farmer who receives a badly infected stock of seed tubers in the spring for planting that year.

It has been shown that 'slight' infection of seed tubers will delay emergence, whilst 'severe' infection may, in addition, cause considerable blanking with subsequent reduction in yield, and an increase in the proportion of ware tubers (Boyd and Lennard, 1961(a)). Disinfection of infected seed tubers at planting time has been shown to reduce these effects, but it is very much less effective than disinfection at lifting time (Greeves and Muskett, 1939).

To investigate the effect of an organo-mercury dip at planting on the emergence, yield and skin spot development of a potato crop, an experiment was carried out at Bush Estate during the season 1963 - 64, with the following tuber categories and treatments:

Tuber Categories:

Free - tubers completely free from skin spot, by visual examination.

Moderate - tubers with 'moderate' surface infection. Because of the shortage of tubers in this year with high levels of skin spot infection, no account was taken of eye infection, and tubers with both 'all eyes' and 'some eyes' infected were planted at random.

Treatments:

(1) - tubers untreated.

(2) - tubers dipped for $\frac{1}{2}$ min in an organo-mercury solution two days before planting.

The four treatment categories were randomised within each of four replicates, each treatment replicate consisting of a 12 ft length of drill in which were planted 12 tubers. Date of planting was 30 May, 1963.

Results

a) Emergence

Emergence counts were taken weekly from 28 June to 26 July, and the average number of days from planting to final emergence calculated for each treatment replicate. It was considered that plants which emerged after 26 July could, from a practical standpoint, be regarded as blanks, since they remain very small and would make no contribution to the final yield.

TABLE 11. Effect of disinfection at planting on the number of plants emerged at two dates after planting.

Infection on planted tubers	Treatment at planting	Number of tubers emerged (maximum possible = 48)	
		28 June	26 July
Free	None	35 (73%)	48 (100%)
Free	Disinfected	38 (79%)	48 (100%)
Moderate	None	6 (12%)	30 (62%)
Moderate	Disinfected	0	26 (54%)

TABLE 12. Average number of days to emergence for tubers free from skin spot and with 'moderate' surface infection, both untreated and disinfected at planting.

Infection on planted tubers:	Free		Moderate		L.S.D. 1%
Treatment at planting:	None	Disinfected	None	Disinfected	
Average number of days to emergence:	29.4	28.0	38.1	41.4	N.S.
	28.7		39.8		4.26
	None = 33.8		Disinfected = 34.7		N.S.

Tables 11 and 12 show that the disinfection treatment did not significantly affect the rate of emergence or the final plant stand of either infection category, but the moderately infected tubers, as would be expected, showed considerably slower emergence and lower final plant count, this being due to the presence of infected eyes slowing, and in some cases completely preventing, sprout growth.

b) Yield

All tubers were lifted on 11 October and the total yield from each treatment replicate determined.

TABLE 13. Average yield from tubers untreated and disinfected at planting time.

Infection on planted tubers:	Free		Moderate		L.S.D. 1%
Treatment at planting:	None	Disinfected	None	Disinfected	
Average yield per replicate (lb):	12.6	13.8	5.4	4.4	N.S.
	13.2		4.9		3.35
	None = 9.0; Disinfected = 9.1				N.S.

The total yield of tubers per replicate, as shown in Table 13, reflects closely the data for rate of emergence, the disinfection treatment having no significant effect, but the moderately infacted tubers producing a much lower yield than tubers free from skin spot at planting.

c) Infection on harvested crop

After lifting and weighing, the tubers from each treatment replicate were placed in net bags and stored overwinter in an outdoor clamp to allow skin spot symptoms to develop. Visual estimation of surface and eye infection was carried out during April, 1964.

TABLE 14. Skin spot infection which developed on the produce from tubers untreated and disinfected at planting time.

(A) Surface Infection

Infection on planted tubers:	Free		Moderate		L.S.D.	
Treatment at planting:	None	Disinfected	None	Disinfected	1%	5%
Surface Infection Index:	0.75	0.51	3.33	1.31	N.S.	
	0.63		2.32		1.69	1.18
None = 2.04; Disinfected = 0.91;					1.69	1.18

(B) Eye Infection

Infection on planted tubers:	Free		Moderate		L.S.D.	
Treatment at planting:	None	Disinfected	None	Disinfected	1%	5%
Eye Infection Index:	5.0	5.0	25.9	8.4	17.1	11.9
	5.0		17.1		12.1	8.4
None = 15.4; Disinfected = 6.7					12.1	8.4

Figures for Surface and Eye Infection Indices are shown in Table 14, and may be summarised as follows:

(i) The produce from the moderately infected seed had significantly more surface and eye infection than the produce from 'free' seed.

(ii) The effect of disinfection at planting time was to reduce significantly the degree of eye infection on the produce from 'moderate' seed, but there was no reduction of eye infection on produce from 'free' seed, which was of a low order in any case.

(iii) Disinfection at planting did not significantly reduce surface infection on either 'moderate' or 'free' seed, but there did appear to be a trend in this direction, the results being almost significant at the 5% level.

In this experiment the effect of disinfection at planting time was thus confined to reducing infection levels on the produce, particularly the degree of eye infection. There was no effect on the emergence rate or yield of tubers. The action of the organo-mercury dip is probably to reduce sporulation of the fungus from the pustules on the seed tuber when it is planted in damp soil conditions, and hence to reduce the amount of inoculum which is liberated into the soil to infect the stolons and developing tubers of the new crop.

The infection which developed on the produce from 'free' tubers may have derived from Oospora pustulans already present in the soil, or liberated from neighbouring tubers. It is known, however, that tubers showing no visible skin spot symptoms may in fact be carrying the fungus, and the author has noted that 'free' tubers, even if dipped in organo-mercury two days before planting, may show a small percentage of eyes which produce actively sporulating Oospora pustulans when planted.

EXPERIMENT 3(B)

Investigation into the effect of planting skin spot infected tubers at two different dates on the emergence, yield and infection levels of the subsequent crop.

Unpublished work by Boyd has indicated that delaying the date of planting infected seed tubers may reduce the blanking in the subsequent crop and increase the rate of emergence. To investigate the effect of later planting of skin spot infected tubers on the emergence, yield and skin spot development of the subsequent crop, a plot experiment was laid down at Bush Estate during the 1963 - 64 season. The following tuber categories and treatments were used:

Tuber Categories:

(a) Free - tubers completely free from skin spot by visual estimation.

(b) Slight - tubers with 'slight' surface infection

(c) Moderate - tubers with 'moderate' surface infection

(d) Severe - tubers with 'severe' surface infection.

As in Experiment 3(A), a shortage of moderately and severely infected tubers meant that no account could be taken of eye infection, and tubers with both 'all' and 'some' eyes infected were planted at random. Tubers with 'slight' surface infection had both 'some' and 'none' of the eyes infected, as would be found in a field sample.

Treatments:

(a) Early planting - planted on 10 May, 1963.

(b) Late planting - planted on 30 May, 1963.

At the first planting, none of the tubers showed obvious sprouting, although growth could just be detected using a hand lens. Tubers for the second planting were stored from 10 May till required at room temperature (15.5°C; 60°F) in chitting trays exposed to daylight. At planting, the 'free' and 'slight' tubers carried sprouts averaging $\frac{3}{8}$" in length, the 'moderate' tubers showed sprout growth of about $\frac{1}{8}$", and the 'severe' tubers showed little sign of sprouting, although there were a few sprouts about $\frac{1}{8}$" long.

The eight treatments were randomised within each of four replicates, each treatment replicate consisting of a 15 ft length of drill in which was planted 15 tubers.

Results

a) Emergence

Emergence counts were taken at weekly intervals from 14 June to 26 July, and the average number of days to emergence calculated for each treatment replicate.

Table 15 shows that both the time of planting and the degree of infection had a significant effect on the average number of days from planting to emergence. There was no significant difference in the emergence rates of the moderately and severely infected tubers, but both

TABLE 15. Average number of days to emergence for tubers with various degrees of skin spot infection, planted on two different dates.

Infection on planted tubers:	Free		Slight		Moderate		Severe		L.S.D.	
Time of planting:	Early	Late	Early	Late	Early	Late	Early	Late	1%	5%
Average number of days to emergence:	39.5	20.8	47.2	21.9	51.3	32.8	48.1	37.1	6.71	4.93
	30.2		34.6		42.2		42.6		4.75	3.49
	Early = 46.5;			Late = 28.2					3.99	2.93

showed a significantly slower emergence rate than the 'slight' tubers. The slightly infected tubers took an average of 4.4 days longer to emerge than the 'free' tubers, a difference just not significant at the 5% level. As in experiment 3(A) these figures reflect the increased percentage of infected eyes on the more severely infected tubers compared with the 'slight' and 'free' tubers.

Within each of the four infection categories it will be noted that the early planted tubers took, on average, 18 days longer from planting to emergence than did the later tubers, planted after an interval of 20 days. Weather conditions during this interval were noticeably cool and damp, and these conditions encountered by the early planted tubers, whilst unsuitable for sprout growth, would seem ideal for the growth and sporulation of the Oospora pustulans present on infected eyes, and it might be expected that this planting would show a higher percentage of blanking in the final crop.

Table 16 shows the number of plants which had emerged at two dates after planting. As in experiment 3(A), plants which emerged after 26 July were regarded as blanks. Contrary to expectations, these figures show that 'severe' and 'moderate' tubers of the late planting resulted in considerably more blanking (50% and 87% respectively) than the corresponding categories of the early planting (33% and 47%). It may be that after the random sampling of tubers for each planting date, a higher proportion of tubers with all eyes infected was used for the later planting. No data on the level of eye infection was taken at planting time and it is difficult to suggest any alternative reason for this apparent anomaly.

TABLE 16. Effect of delayed planting of tubers infected with skin spot on the number of plants emerged on two dates after planting.

Infection on planted tubers	Time of planting	Number of tubers emerged (maximum possible = 60)	
		21 June	26 July
Free	Early	46 (77%)	60 (100%)
Slight	Early	22 (37%)	57 (95%)
Moderate	Early	8 (13%)	40 (67%)
Severe	Early	1 (2%)	32 (53%)
Free	Late	40 (67%)	59 (98%)
Slight	Late	40 (67%)	55 (92%)
Moderate	Late	2 (3%)	30 (50%)
Severe	Late	0	8 (13%)

b) Yield

All tubers were lifted on 11 October and the total yield from each treatment replicate noted. The results are shown in Table 17. As the tubers from both planting dates emerged at approximately the same time, it is unlikely that there was much variation in yield due to differences in the length of the growing season.

TABLE 17. Average yield per treatment replicate from tubers of various skin spot infection categories planted at two different dates.

Infection on planted Tubers:	Free		Slight		Moderate		Severe		L.S.D.	
Time of planting:	Early	Late	Early	Late	Early	Late	Early	Late	1%	5%
Average yield per treatment replicate:	17.5	17.0	16.0	16.2	10.0	6.1	8.2	1.8	4.2	3.1
	17.2		16.1		8.2		5.0		3.0	2.2
			Early = 12.9;		Late = 10.3				2.1	1.5

The 'free' and 'slight' categories showed no significant differences in yield, nor did the time of planting have any effect on the yield in the case of these two categories. 'Severe' infection significantly lowered yield compared with 'moderate' infection, which in turn showed a significantly lower yield than that from both 'slight' and 'free' tubers. Within the 'moderate' and 'severe' categories, yields from the later planting were significantly lower than those from the early planting.

It would appear that final yield depends principally on the percentage of blanking in each treatment, the lowest yields deriving from those treatments with the greatest number of blanks, and vice versa.

c) Infection on Harvested Crop

After lifting, tubers from each treatment replicate were placed in net bags and stored overwinter in an outdoor clamp to allow skin spot symptoms to develop. Visual estimation of surface and eye infection was carried out during April, 1964, and these figures are shown in Table 18.

It will be noted that results for the produce from severely infected tubers are not available. During the storage period the net bags used to separate the samples rotted badly, and a proportion of the tubers from each sample was lost. As the total produce from the severely infected tubers was already very limited, this loss

TABLE 18. Skin spot infection which developed on the produce from tubers of various infection categories planted at two different dates.

(A) Surface Infection

Infection on planted tubers:	Free		Slight		Moderate		L.S.D.	
Time of planting:	Early	Late	Early	Late	Early	Late	1%	5%
Surface Infection Index:	2.22	1.38	7.15	7.22	5.52	3.92	N.S.	
	1.80		7.19		4.72		2.39	1.73
	Early = 4.96;		Late = 4.18				N.S.	

(B) Eye Infection

Infection on planted tubers:	Free		Slight		Moderate		L.S.D.	
Time of planting:	Early	Late	Early	Late	Early	Late	1%	5%
Eye Infection Index:	23.0	15.0	48.5	48.0	43.5	34.2	N.S.	
	19.0,		48.2		38.9		17.7	12.8
	Early = 39.2		Late = 32.4				N.S.	

during storage left insufficient tubers to give an accurate estimation of the infection levels present.

The results available show that the infection which developed depended to a large extent on the degree of infection present on the planted seed, the produce from the 'free' tubers showing significantly less surface and eye infection than the produce from the 'moderate' or 'slight' seed. Surface infection on the 'slight' produce was significantly greater than that from the 'moderate' crop, although eye infection was not significantly different. No reason for this can be suggested.

Early and late planting gave no significant difference in infection levels on the produce, and although a slight trend towards lower infection after late planting of 'free' and 'moderate' seed is noticeable, it is not sufficient for any conclusions to be drawn from this experiment.

DISCUSSION

It has been known for many years that environmental conditions during the storage period can influence the amount of skin spot infection on potato tubers which develops by the following spring; Gussow (1918), although considering that no fungal pathogen was present, noting that the disease always occurred on tubers stored in badly ventilated (and presumably damp) cellars.

Comparison by Greeves and Muskett (1939) and Boyd (1957) of clamp storage, where conditions are normally damp at least for the first few weeks, and box storage where the tubers dry off rapidly, showed that the drier environment of the boxes allowed much less skin spot to develop. These results were confirmed in further work by Boyd (1960), who also noted that tubers boxed and stored at low temperatures developed more skin spot than those kept in warmer conditions. This effect of temperature was also noted by Nagdy (1962), although in some cases his results were not statistically significant.

A survey of the annual incidence of skin spot over the years 1927 to 1960 revealed that above average rainfall during the lifting period (leading to damp storage conditions), and below average temperature during the beginning of storage, shows a close relationship with above normal incidence of skin spot in the following year (Boyd and Lennard, 1962).

Such observations have led to the generally accepted conclusion that cool, damp storage will lead to maximum skin spot development, while warm dry conditions are least conducive to severe outbreaks of the disease.

The present work appears to be the first to compare directly dry and damp storage under various temperature conditions, using disinfected tubers inoculated with a spore suspension of Oospora pustulans in an attempt to provide a standard potential inoculum. The results obtained confirm the observations of previous workers. It was noticeable, however, that tubers stored under moderate temperature conditions (12.5°C (55°F) at the beginning of storage, falling to about 4.0°C (40°F) during the winter, rising again to some 10°C (50°F) at the end of storage), developed no less skin spot infection than those stored under continuous low temperature (1.5°C; 36°F). In the moderate temperature store, the temperature did not fall below 7°C (45°F) until mid-November (one month after lifting), and this suggests that the temperature after this time could still affect the final infection, or the initial warm period would have reduced infection levels below that found after continuous cold storage.

The influence of humidity and temperature on the host/parasite relationship is not yet fully understood. Low temperatures appear to permit active growth of the fungus and also reduce the ability of the host to resist penetration, although which factor is the more important is not known. Similarly, the effect of humidity is by no means fully understood.

It is obvious, however, that warm storage conditions (15.5°C; 60°F) will very markedly reduce the damage caused by skin spot, humidity having little effect at this temperature. It should be noted that as no control was possible over the levels of relative humidity in each store, the relative humidity of the 'dry' tubers under the warm conditions would be lower than that found in either the moderate or cold stores. Under continuous cold conditions (1.5°C; 36°F), humidity becomes an important factor, and if it can be kept to a minimum (e.g. by ventilation of bulk stores), the effect of the low temperature in promoting skin spot development is much reduced. Moderate temperature showed no better control than the continuous cold in the 1962 - 63 season, but it should be remembered that the winter of this year was very severe, and it is possible that in a more 'normal' season the temperature would have been rather higher, and the incidence of skin spot correspondingly reduced.

It would have been of interest to have obtained figures for the minimum temperature and maximum relative humidity during storage necessary to provide a reasonable degree of control of skin spot development. Unfortunately the ranges used in this experiment were not sufficiently numerous or closely defined to allow this to be done with accuracy, but the critical temperature would appear to be somewhere in the range 4.5° to 10°C (40° to 50°F).

The boxing treatments carried out in Experiment 2 confirmed that removal of tubers to a dry environment within 3 weeks of lifting may substantially reduce skin spot, provided the temperature remains relatively warm for a period of at least the first 4 weeks of box storage. Delaying boxing until 6 or more weeks after lifting gave relatively little control of the disease (as found by Boyd (1960)), and it would seem that under farm conditions 'boxing only' as a control for skin spot is unlikely to give satisfactory results unless carried out within 2 to 3 weeks of lifting, and the boxes subsequently stored in conditions where the temperature will not fall to low levels. Again, it is not possible to put an accurate figure on the critical temperature, but from Figs. 3 and 4 (pages 36 and 37) it would seem to be nearer 4.5°C (40°F) than 10°C (50°F).

Boxing relies for its control effect on the provision of an environment least suited to further fungal penetration and development. Disinfection kills spores or mycelium on or near the surface of the tuber, any subsequent skin spot which develops arising from mycelium already penetrated into the tuber periderm. Greeves and Muskett (1939), Foister (1943), and Boyd (1957; 1960) have shown that disinfection at lifting time is the most effective control measure for skin spot. In addition, Greeves and Muskett (1939) showed that disinfection at 2 and 4 weeks after lifting was almost as effective as this treatment at lifting, but that disinfection 8 and 16 weeks after lifting gave progressively less control, although even the '16 week' treatment gave a measurable reduction when compared with untreated tubers. However Boyd (1960) disinfecting tubers 6 weeks after lifting found a very much reduced control, while disinfection at 11 weeks gave no reduction in skin spot of any practical value.

The periodic disinfection in Experiment 2 confirmed the effectiveness of organo-mercury dips at lifting time as a control for skin spot. A delay of only 1 week with this treatment gave rise to a noticeable increase in infection, and a delay of 6 weeks reduced the effectiveness to a level unlikely to be of practical use. These figures tend to support the work of Boyd (1960) rather than that of Greeves and Muskett (1939), although the degree of control obtained by later dipping must depend to some extent on the storage conditions between lifting time and treatment, a factor which will vary from year to year.

The disinfection treatment carried out on early lifted tubers showed no marked reduction in skin spot compared with disinfection at the main lifting - the rapid increase in infection caused by a delay of 1 week after lifting indicates that the act of harvesting must in some way stimulate penetration of the fungus, possibly through damage to the tuber skin at lifting. The early lifted tubers which were boxed only showed no reduction in skin spot compared with tubers boxed at the main lifting in 1962 - 63, whereas the control by early boxing in 1963 - 64 was very marked. This effect was almost certainly one of temperature, warm conditions prevailing after both early liftings and after the main lifting in 1962 - 63, but cooler conditions after the main lifting in 1963 - 64. It is thus possible for early lifting to reduce skin spot infection (as suggested by Boyd, (1957)), but its effectiveness is probably more dependent on subsequent storage conditions than on the actual time of lifting.

The use of a technique first described by Salt (1957) involving the removal and incubation of tuber eyes to show the percentage infection with Oospora pustulans, revealed that tubers stored in a clamp overwinter showed a sudden and rapid increase in the number of dead eyes showing Oospora pustulans from 6 weeks after lifting, and confirms that any control measure attempted later than this time is certain to be less effective in controlling at least eye infection, than treatments at lifting. It was also noted that over the complete period of clamp storage, there was an increase of some 20% in the total number of eyes which showed the presence of Oospora pustulans. This may reflect a spread of the fungus in the very damp conditions of the experimental clamp in 1963 - 64, but further work would be necessary to confirm this. Undoubtedly the conditions in most clamps and all bulk stores would be considerably drier than those of the experimental clamp, and in practice the spread of Oospora pustulans, if it occurs, is probably uncommon, although spread in storage was also recorded in U.S.S.R. by

Kharkova (1961(a)).

A comparison of visual estimation of eye infection index (percentage of eyes killed by skin spot) and microscopic examination of tuber eyes, showed a very close relationship ,and it is considered that the former more rapid method is quite adequate for estimating the number of eyes killed by skin spot.

In the course of using the excised eye technique, fungi other than Oospora pustulans were noted round the eye tissues, and it may be that this method of eye examination would be useful for indicating the presence of pathogenic or other fungi. Fungi noted include: Rhizoctonia solani Kuhn or Corticium solani (Prill. & Delacr.) Bourd. & Galz.; Helminthosporium atrovirens (Harz) Mason & Hughes; Verticillium sp.; and various saprophytic moulds.

Planting out of tubers from the various boxing and disinfection treatments showed no significant differences in emergence rates or final yields, as the differences in infection levels were too restricted. Storage of the crop until the following year, however, revealed that the infection which developed was correlated with the surface infection on the planted tubers, the effect of the control measures 'carried-over' to the following crop. This agrees with the findings of Boyd and Lennard (1961(a)) who noted that the produce from severely infected seed developed more skin spot than produce from 'slight' infection category seed, both results suggesting that the infection present on the planted seed supplies the bulk of the fungal inoculum which causes infection on the new crop.

This is supported by results obtained from Experiment 3(B), where the 'moderate' and 'slight' seed gave significantly more surface and eye infection on the subsequent crop than did the 'free' seed. Admittedly the 'slight' seed gave more subsequent surface infection than the 'moderate' seed, but this is the only result which appears out of order, the eye infection showing no significant difference. It can also be seen from this experiment that tubers planted apparently free from skin spot produced crops with quite considerable skin spot infection. This is also apparent in Experiment 3(A), where the visually'free' tubers, even if dipped in an organo-mercury solution at planting time, showed skin spot on the produce. This is not necessarily an indication that the infection on the crop must have come from the soil, as it has been shown by Salt (1957) that tubers with no visible symptoms may in fact carry the fungus in the eye tissue or stolon scar, and the present author has noted that visually 'free' tubers, dipped in organo-mercury two days before planting, showed growth of Oospora pustulans conidiophores on the eyes at planting. It therefore seems unlikely that any tubers planted are completely free from skin spot, a fact which must throw doubt on the results of experimental work with apparently 'free' tubers planted on virgin land, and other observations, which indicated that Oospora pustulans was an inhabitant of certain soils (Anon., 1932; Boyd and

Lennard, 1961(a); Kharkova, 1961(a)).

The use of an organo-mercury dip at planting time did not increase the emergence rate nor yield of either 'free' or 'moderate' seed, but the final crop from the 'moderate' tubers showed a reduction of about 60% in the levels of surface and eye infection, compared with reductions of 80% to 90% when tubers were dipped at lifting. These figures compare very closely with those obtained by Greeves and Muskett (1939) when they compared disinfection at planting and at lifting. Disinfection with an organo-mercury solution at planting did not appear to affect emergence rate or final percentage of blanking, contrary to the findings of Boyd and Lennard (1963) who found that this treatment tended to retard the emergence of 'free' seed.

Delaying the date of planting infected tubers, in an attempt to provide conditions more suitable for rapid sprout growth away from the source of infection, and so reduce the number of delayed sprouts and possibly also the number of blanks, was found to have no beneficial effect in practice, and the later planting inexplicably increased the percentage of blanking with 'moderate' and 'severe' tubers causing a marked reduction in yield. There was also no reduction in infection levels which develpoed on the harvested crop, and from the results of this experiment, delayed planting could not be recommended as a method of reducing losses due to seed badly infected with skin spot.

During the course of this work, two observations were made which might be worthy of further study at some time:

a) During estimations of skin spot surface infection, it was noted in certain samples that sclerotia of the fungus Rhizoctonia solani were common on tubers with 'free' or 'trace' skin spot infection, but that sclerotia were almost never found on tubers with 'slight' 'moderate', or 'severe' skin spot infection, and it may be that these two fungi are mutually antagonistic.

b) When examining tubers in March from the 'boxed at lifting' treatment in Experiment 2, it was noted that some of the tubers round the edge of the seed trays had been greened by exposure to light. Comparison of these greened tubers with other unaffected tubers in the same tray showed that the greened in every case developed much less skin spot infection, and it is suggested that greening of tubers may produce a substance which will retard or prevent the development of Oospora pustulans in the tuber periderm.

SUMMARY

1. Temperature and humidity conditions during storage were found to have a very marked effect on the levels of surface and eye skin spot infection which developed on potato tubers artificially inoculated with the causal fungus, Oospora pustulans Owen & Wakef. Continuous warm conditions (15.5°C; 60°F) led to much less skin spot development than either continuous cold temperatures (1.5°C; 36°F), or fluctuating temperatures similar to those encountered in commercial bulk potato stores.(12.5°C (55°F) at the beginning of storage, falling to about 4.0°C (40°F) during the winter, rising again to some 10°C (50°F) at the end of storage). The effect of humidity was slight under warm storage conditions, but with fluctuating or continuous cold storage, high humidity increased damage caused by this disease.

2. Disinfection of tubers at lifting in an organo-mercury dip was shown to be the most effective means of controlling potato skin spot. If this treatment was delayed after lifting, its effectiveness was correspondingly reduced, and to obtain a worthwhile reduction in infection level, disinfection must be carried out within 6 weeks of lifting the tubers.

3. Boxing of tubers at lifting was also shown to give some measure of control over skin spot development,

but it was not as effective as the disinfection treatment. As with disinfection, delaying boxing after lifting reduced its ability to control the disease. The temperature to which the tubers were exposed after they were boxed was found to be critical, cold conditions favouring establishment of the pathogen and greatly reducing the effectiveness of boxing as a control measure.

4. A method of estimating eye infection caused by skin spot, involving the removal and incubation of the tuber eyes was compared with the more normal method of visual examination. The former method was found to be no more accurate as a means of assessing the number of eyes killed by the fungus, but it was useful for indicating the presence or absence of *Oospora pustulans*, particularly on live eyes. Using this technique, it was noted that there was an increase in the number of infected eyes during one storage season, suggesting that under very damp storage conditions, *Oospora pustulans* may spread in stores.

5. Disinfection of skin spot infected tubers at planting time in an prgano-mercury dip was not found to have any effect on the emergence rate or yield of the produce, but it did reduce the skin spot infection which developed on the subsequent crop.

6. Delaying the planting date of tubers infected with skin spot did not reduce the infection which developed on the subsequent crop, and in this work was found to increase the percentage of blanking and reduce the final yield from tubers with 'moderate' or 'severe' skin spot infection.

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