THE EFFECTS OF CERTAIN FUNGICIDES ON THE VIABILITY OF HEVEA BUDS.

BY

R. P. N. NAPPER.

INTRODUCTION.

In view of the existing legislation with regard to plant quarantine measures in relation to the rubber tree in Malaya, the following experiment was planned to investigate the effects of certain common fungicides on the viability of buds. There are two possible methods of complying with such legislation, viz. disinfection in the country of origin, and disinfection at the port of entry. It was decided that this experiment should deal with the former possibility.

The methods of treatment used were such as would be compatible with normal estate practice, and the fungicides (with one exception) were well known mixtures or proprietary preparations familiar to estate managers and easily obtainable in this country.

EXPERIMENTAL DETAILS.

On the morning of each of seven consecutive week days, budwood of one clone, AVROS 50, was cut from Block 4 nursery at the Experiment Station, Sungei Buloh, waxed, treated, packed in a box, and stored in a cool dry place. At an interval of seven days after packing, to represent a period of transportation from one country to another, each box was returned to the nursery where it was opened, notes were made on the condition of the budsticks, the sticks were stripped, and 100 buddings were made from each treatment. When budding was finished the stripped sticks were rolled in sacking, repacked in the box, and taken to the laboratory for further examination. Sixteen days after budding each day's task was examined and a record made of the percentage number of successful buddings obtained from each treatment, while after a further interval of 23 days a second and final inspection was made.

With one exception the treatments consisted in immersing the budwood in liquid, water miscible disinfectants, in a small wooden tank ($3\frac{1}{2}$ ft. × 6 ins. × 6 ins. holding 5 gallons of liquid), waxed inside, and provided with false cross partitions at either end. These partitions stopped short of the bottom of the tank so that the ends of the budsticks could be inserted below them and the surface of the sticks kept completely immersed. One partition was movable.

Clone AVROS 50 yields 20—25 buds per yard and, in order to obtain at least 120 buds treated with the same concentration of fungicide, 6 yards of budwood were used for each treatment. Of these, 3 yards were washed in water as soon as the sticks were taken from the tank, 60 buds were stripped, and 50 buddings were made from each sub-treatment.

On each day 36 yards of budwood were used, comprising 24 yards of treated material and 12 yards of control, of which 6 yards were washed with water only while the remainder were not treated in any way. As each treatment was completed, and without allowing the budwood to dry, the sticks were rolled individually in damp sterilised coconut fibre and placed in layers in a packing case $3\frac{1}{2}$ ft. × 1 ft. × 1 ft. each treatment occupying one layer. The layers were separated by a thickness of fibre and the sticks were prevented from chafing by tightly packing the spaces at the ends and sides of the box. The cases were of the correct size to accommodate 36 yards of budwood and the packing was excellently done.

The treatments and sub-treatments used are given in Table I:—

TABLE I.

Box No.	Treatment No.	Fungicide.	Strength per cent.	Time of treatment in minutes	After treatment.
No. 1.	C. U.	Control			None
	C. W.	Control			Washed
	I. W.	Copper Sulphate	2	2	Washed
	I. U.	,, ,,	,,	,,	None
	II. W.	,, ,,	,,	5	Washed
	II. U.	,, ,,	,,	,,	None
	III. W.	,, ,,	5	2	Washed
	III. U.	,, ,,	,,	,,	None
	IV. W.	,, ,,	,,	5	Washed
	IV. U.	,, ,,	,,	,,	None
No. 2.	C. U.	Control			None
	C. W.	Control			Washed

TABLE I.—(contd.)

Box No.	Treatment No.	Fungicide.	Strength per cent.	Time of treatment in minutes.	After treatment.
	VII. W.	Solbar	1	2	Washed
	VII. U.	,,	,,	,,	None
	VIII. W.	,,	,,	5	Washed
	VIII. U.	,,	,,	,,	None
	IX. W.	,,	2	2	Washed
	IX. U.	,,	,,	,,	None
	X. W.	,,	,,	5	Washed
	X. U.	,,	,,	,,	None
No. 3.	C. U.	Control			None.
	C. W.	Control			Washed
	XI. W.	Lime-sulphur	1	2	Washed.
	XI. U.	,, ,,	,,	,,	None.
	XII. W.	,, ,,	,,	5	Washed.
	XII. U.	,, ,,	,,	,,	None.
	XIII. W.	,, ,,	5	2	Washed.
	XIII. U.	,, ,,	,,	,,	None.
	XIV. W.	,, ,,	,,	5	Washed.
	XV. U.	,, ,,	,,	,,	None.
No. 4.	C. U	Control.			None.
	C. W.	Control.			Washed
	V. W.	Sulfinette.	2	2	Washed.
	V. U.	,,	,,	,,	None.
	VI. W.	,,	,,	5	Washed.

TABLE I.—(contd.)

Box No.	Treatment No.	Fungicide.	Strength per cent.	Time of treatment in minutes	After treatment.
	VI. U.	,,	,,	,,	None.
	XV. W.	Liver of sulphur.	1	2	Washed.
	XV. U.	,,	,,	,,	None.
	XVI. W.	,,	,,	5	Washed.
	XVI. U	,,	,,	,,	None.
No. 5.	C. U.	Control.			None.
	C. W.	Control.			Washed
	XVII. W.	Ammonium polysulphide.	1	2	Washed.
	XVII. U.	,,	,,	,,	None.
	XVIII. W.	,,	,,	5	Washed.
	XVIII. U.	,,	,,	,,	None.
	XIX. W.	,,	,,	2	Washed.
	XIX. U.	,,	,,	,,	None.
	XX. W.	,,	,,	5	Washed.
	XX. U.	,,	,,	,,	None.
No. 6.	C. U.	Control.			None.
	C. W.	Control.			Washed
	XXIII. W.	Uspulun.	5	2	Washed.
	XXIII. U.	,,	,,	,,	None.
	XXIV. W.	,,	,,	5	Washed.
	XXIV. U.	,,	,,	,,	None.
	XXV. W.		1	2	Washed.

TABLE I.—(contd.)

Box No.	Treatment	Fungicide.	Strength per cent.	Time of treatment in minutes	After treatment.
	XXV. U.	,,	,,	,,	None.
	XVI. W.	,,	,,	5	Washed.
	XVI. U.	,,	,,	,,	None.
No. 7.	C.	Control packed in normal fibre.			
	XXI.	½ lb. Sulphur dust included in the packing fibre.			
	XXII.	1 lb. ,, ,, ,, ,, ,, ,, ,,			

The budding was performed by a gang of six Tamil budders, and one sub-treatment was dealt with at a time. The sticks were stripped by the three most experienced budders, and the bud slips distributed between them and the others in proportion to their individual rates of working. Each budder performed a constant proportion of the total number of buddings from each sub-treatment, and thereby the personal factor was eliminated from the results.

In the following sections a sub-treatment will be referred to as a "treatment".

Difficulties Encountered.

The experiment, as planned, should have given a set of results strictly comparable one with another through the controls, but in practice several difficulties arose which affect the significance of the results obtained.

In the first place the amount of budwood necessary (234 yards of one clone) was so large that the Botanical Division could not provide all brown wood; some of the sticks were too young, and at least half were in the green condition. This gave rise to two difficulties, (1) it was impossible to include budwood with the same range of maturity in each treatment, and (2), the green wood sprouted to a considerable extent after defoliation, and the sprouted buds were frequently damaged during cutting and transportation to the packing shed (neither of which operations could be personally supervised) and also during treatment, in spite of the utmost care. These injuries provided entrance for fungi, and also exposed the delicate inner tissues of the cortex to the action of the disinfectants, both of which factors may easily have led to the death of neighbouring buds.

The magnitude of the experiment gave rise to a further difficulty, for it necessitated the budding of 600 seedlings per day, i.e. 100 each per budder per day. Budding was not straightforward, as it had to be suspended after every 50 buds had been put on, while the sticks of the next treatment were stripped. The day's task could not be completed under 5 hours, and since at that period of the year rain commenced to fall as early at 12.30 p.m. nearly every day, budding had to commence before 7.30 a.m. At this hour, however, the nursery was rarely dry and on two mornings in particular (December 8th and 9th) the stems did not dry out before 8.30 a.m. A wet stem and wet canopy during budding is the source of many disadvantages, for drops of water carrying all sorts of undesirable organisms cannot be prevented from getting under the tongue of bark, while waxed cloths will not adhere to a wet stem, and firm binding is difficult to achieve.

The controls were budded first each day, so that any drop in successes due to early morning budding might reduce, rather than exaggerate, the significance of the differences between the results from the treated sticks and from the controls. The abnormally low results obtained from the controls on the two days mentioned above (as compared with controls on other days) may well have been due to this factor. It is notable that on each of the following days, the 4th, 5th, 6th and 10th of December, the percentage successes obtained from the controls was exceeded by that obtained from one or more of the treatments budded later in the morning. Although in a perfect experiment this would suggest that disinfection with certain concentrations of fungicides has a beneficial antiseptic action on the buds, the greater success is probably due solely to the fact that the controls were budded before the stocks were sufficiently dry.

A fact which became obvious when the boxes of treated material were opened, was that the wax used (Entwas) had too high a melting point, and that in every case some of the die-back at the ends of the budsticks could be attributed to injuries during waxing. Unfortunately the extent of this injury could not be estimated, for inspection of the controls showed that it varied considerably. It was much more severe on green sticks and apparently small changes in temperature of the wax had considerable influence on its lethal effect. Such small changes could not be avoided, as the wax had to be heated in an open pan over a wood fire and it was impossible to keep it at a constant temperature.

Yet another source of error lay in the fact that the stocks on which the buddings were performed were not uniform. It would of course have been impossible in a small section of an ordinary nursery to find 4,000 buddable stocks in the same state of development. On the whole the stocks were in excellent condition but some of the beds lay in areas known to be infected with *Fomes lignosus*. In these beds growth was backward and very irregular, and this fact

may have contributed considerably towards the low percentage successes recorded in many of them.

Another difficulty arose on the 3rd Dec. when both the budding of the original Box No. I treated with copper sulphate solution on Nov. 26th, and the treatment of Box No. 7, were due to take place. Budding was to have been carried out on Pilmoor Estate, by kind permission of the Manager, and was actually started there on the morning in question. The treatment of Box No. 7 had therefore to be left in the hands of the subordinate staff on the Experiment Station, with, as is shown later, unsatisfactory results. (For various reasons budding at Pilmoor Estate had to be abandoned; Box No. 1 was repeated after Box No. 7, and all subsequent budding was done at the Experiment Station, Sungei Buloh, on stocks provided by the Botanical Division in Block 4 nursery).

The results obtained, which are given below, should be interpreted in the light of these observations.

Results.

The condition of the budwood at the time of budding was judged from its general appearance, and from the distance to which the bark had died back at the upper ends of the sticks. As would be expected there was always more die-back at the upper ends than at the lower ends. The cambium in all cases had died back beyond the limit of dead bark, but in the field the amount of bark die-back was easier to record than the amount of cambium die-back, as it could be determined readily by cutting a shaving of bark from the end of a stick and determining the point at which the latex flowed freely.

No buds were stripped from the portions of the sticks bearing dead bark. The budders were left to choose the strippable buds themselves, and as they were interested in the number of successes, they were very careful to select only apparently healthy buds.

On each day, as the buds were stripped, the sticks were tied together in lots according to treatment, placed in a sack, repacked in the same box, and taken to the laboratory, where they were examined on the following day by Mr. Beeley, who has kindly furnished further information with regard to the condition of the budwood at the time of budding. The observations made are too detailed to be given at length, but when considered in conjunction with the observations made at the time of stripping, and with the budding results subsequently obtained, they lead to the following conclusions.

None of the treatments used caused visible disorganisation of the bud tissues at the time of budding, except those in Box No. 1, where all treatments stronger than the soaking in 2 per cent. solution of copper sulphate for 2 minutes produced rotting of the buds to a greater or less extent. None of the fungicides appeared to have

penetrated the cuticle of green sticks during the interval between treatment and budding, but copper sulphate rendered the cuticle dry and brittle. In the majority of cases a mottling of the surface of the green sticks was noted which was not caused by fungus attack, but was probably due to some reaction between the disinfectant and the substances of the cuticle.

The fungicide which appeared to cause least superficial damage to the budwood was ammonium polysulphide, and the surfaces of the green sticks treated with this disinfectant were in a bright green, healthy, unmottled condition. The fungicide which caused by far the most serious damage to the budwood was copper sulphate and, even at the weakest concentration, death of some buds occurred. It was noticed that penetration took place around the base of the buds, and that in some cases a still healthy bud could be found isolated in a patch of dying cortex. In the budwood treated with copper sulphate the sticks died back from the buds as well as from the waxed ends, but in all other cases die-back of sticks appeared to proceed via the cambium from the waxed ends.

Observations on the age of the budwood made at the same time explained some apparent anomalies in the results from the subsequent buddings. It is known that, when budwood is very pithy and the buds are prominent and leave a large protuberance on the wood after stripping, the buds are immature, and the successes obtained from such budwood will be low. Mr. Beeley made a special note when reporting on treatments XIX.U. & I.U. to the effect that the budwood in each case was in this condition, and this probably accounts for the abnormally low number of successes obtained from each of these treatments compared with the treatments described above and below them in their respective tables. These results were the most strikingly anomalous and could not be explained away as due entirely, either to the treatment, or to abnormal injury during waxing.

After allowing the stripped sticks to stand for a few days many kinds of mould appeared on them, especially species of Diplodia, Gloeosporium, and Fusarium. The sticks from the copper sulphate treatments were actually mouldy at the ends when stripped for budding, and in every case it was the sticks which had been most injured, either by waxing or subsequent disinfection, that developed moulds most rapidly, irrespective of treatment. There was a suggestion, however, that the controls, and especially the washed controls, developed moulds more quickly than did treated material in apparently the same condition, but otherwise the fungicidal action of the various disinfectants seemed to have disappeared during the interval between treatment and budding.

As this paper was written before the second inspection of buddings was made, only the results of the first inspection are discussed below. The numerical results of the second inspection are, however,

given in the following tables, and are discussed collectively in a final paragraph.

Box No. 1, Copper Sulphate.

This box was originally packed on November 26th, to be budded on December 3rd, but for various reasons had to be abandoned. It was repeated at the end of the experiment, after the treatment of Box No. 7.

TABLE II.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	Good, slight die-back of bark at the ends -	97	85
C. W.	,, ,, ,, ,, ,, ,, ,,	95	83
VII. W.	One green stick died back 8 ins ; remainder good -	81 (×)	74
VII. U.	Good ; 1—2 ins. of die back -	76 (×)	74
VIII. W.	One green stick died back 4 ins ; remainder good -	98	80
VIII. U.	One stick died back 4 ins ; remainder good -	90	84
IX. W.	Youngest stick died back 4 ins ; remainder good -	90	80
IX. U.	Green stick died back 6 ins ; remainder fairly good -	96	82
X. W.	Excellent ; ½ - 1 in. die-back only -	98	96
X. U.	,, ,, ,, ,,	92	86

Box No. 2, Solbar.

Treated, November 27th, 1930. Budded, December 4th, 1930. Opened and inspected, December 20th, 1930. Second inspection, January 12th, 1931.

The results marked with a cross must be explained first. Treatments VII. U. and VII. W., in that order, were the last to be budded, and while the budding of VII. U. was in progress a drizzle of rain started. It was hoped the rain would cease, but after putting on a few buds from VII. W. a heavy storm occurred which suspended budding operations until late in the afternoon. It is interesting to note that the successes obtained from VII. U., which was budded actually

during the rain (c.f. remarks on this subject in the previous section), are below those obtained in VII. W., which was not completed until after 6 p.m. when the bud slips had been cut and exposed for six hours.

There are no significant differences between the rest of the results, except perhaps between X.W. and X.U. The budwood in both instances was in excellent condition and hardly affected by the hot wax, so that the results may with some justification be considered to be due to the treatments alone.

The conclusion reached from this set of treatments is that Solbar does not injure buds even in as high a concentration as 2 per cent. for 5 minutes as long as the sticks are washed (at this concentration) immediately after removal from the dipping tank. If left unwashed, the fungicide at this strength may have a deleterious effect on the buds.

TABLE III.

Box No. 3, Lime-sulphur (B).

Treated, November 28th, 1930. Budded, December 5th, 1930. Opened and inspected, December 21st, 1930. Second inspection, January 13th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	3 sticks good ; the remainder died back up to 6 ins.	93	85
C. W.	Good ; 2 - 4 ins. die-back	99	95
XI. W.	Excellent ; ½ - 1 in. die-back	100	90
XI. U.	Very good, except on one stick with 6 ins. of die-back	98	94
XII. W.	Very good ; 1 in. die-back	100	92
XII. U.	Excellent ; ½ - 1 in. die-back	100	98
XIII. W.	Poor ; up to 6 ins. die-back	92	90
XIII. U.	Good ; 2 - 3 ins. die-back	96	96
XIV. W.	Fair ; 4 - 6 ins. die-back	90	82
XIV. U.	Good ; except on two sticks with 4 ins. die-back	82	78

These results, although perhaps not statistically significant, certainly suggest very strongly that the mixture of Lime-sulphur at

1 per cent. concentration of polysulphide sulphur for 2 minutes and 5 minutes has no effect on the viability of the buds, but that when a 5 per cent. concentration is substituted it causes increasing mortality of the buds.

Two figures in the table require some explanation. The unwashed control shows a considerably lower percentage success than some of the treatments, and this is probably due to the two facts, that it was budded before the nursery was sufficiently dry, and that three of the sticks had been severely injured during waxing, or had not been properly waxed. They were the youngest and greenest sticks.

Treatment XIII. W. also gave a lower number of successes than was obtained from both the immediately preceeding and succeeding treatments, but here again the condition of the budwood was poor and the die-back of the bark may easily have led to the death of the buds stripped near the ends.

TABLE IV.

Box No. 4, Sulfinette & Liver of Sulphur.

Treated, November 29th, 1930. Budded, December 6th, 1930. Opened and inspected, December 22nd, 1930. Second inspection, January 14th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	Good, except on two sticks with 4 ins. die-back.	95	78
C. W.	Poor ; one stick dead to 8 ins, the others to 6 ins.	85	78
V. W.	Fair; one stick dead to 6 ins, another to 4 ins.	86	84
V. U.	Fairly good ; 4 ins. die-back.	94	90
VI. W.	Good, except one stick with 4 ins. die-back.	96	90
VI. U.	Fairly good ; two sticks with 4 ins. die-back.	96	94
XV. W.	Fairly good ; one stick with 6 ins. die-back.	88	80
XV. U.	Fairly good ; two sticks with 4 ins. die-back.	94	82
XVI. W.	Very good ; 1 in. die-back.	100	92
XVI. U.	Good ; 1 - 2 ins. die-back.	90	80

The differences between these results are not significant, and all that can be determined from them is that none of the treatments had any notable deleterious effect on the budwood. The figures for the last two treatments, XVI.W. and XVI.U., may be exceptions, as in each case the budwood was in better condition than in any of the other treatments, and the failures recorded may be regarded as due only to the effect of Liver of Sulphur on the buds. If this assumption is justifiable then it may be concluded that treatment for 5 minutes in 1 per cent. Liver of Sulphur solution does injure buds unless the budwood is washed immediately after treatment.

TABLE V.

BOX NO. 5, AMMONIUM POLYSULPHIDE.

Treated, December 1st, 1930. Budded, December 8th, 1930. Opened and inspected, December 24th, 1930. Second inspection, January 16th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	Fair; sticks too green; many buds sprouted; difficult to find sufficient for 100 buddings. -	88	69
C. W.	Fair; Do.	96	83
XVII. W.	Very good, except one stick with 6 ins. dieback. -	94	92
XVII. U.	Good; 1 - 2 ins, die-back. -	92	86
XVIII. W.	Excellent. -	100	100
XVIII. U.	Excellent. -	98	92
XIX. W.	Good, except on one green stick with 8 ins. die-back; sufficient buds obtained from other sticks. -	100	100
XIX. U	Poor; one stick badly waxed died back 9 ins; others 4 ins. -	70	58
XX. W.	Good, except one stick badly waxed which died back 9 ins. -	92	88
XX. U.	Fairly good; 2 - 4 ins. die-back. -	88	80

There had been heavy rain early in the morning on which this box of material was budded, and the nursery was unusually wet until after the budding of the controls had been completed. This fact, coupled with the fact emphasized in the above table, viz. that the budwood was too young and that it was difficult to find 100 unsprouted sound buds, may well account for the comparatively few successes obtained from both the controls. It is interesting to note that the washed controls, in which the budwood was in precisely the same condition as in the unwashed controls, but which were budded after the nursery had dried considerably, gave a greater percentage success.

The other results are difficult to interpret, and in any case do not indicate any striking toxic action of ammonium polysulphide at the concentration employed. It is difficult to understand why the weakest treatments should give lower results than the treatments of medium intensity, unless the effect of the wet nursery was more prolonged than on other days. The low percentage success obtained in treatment XIX. U. is certainly anomalous, and may be correlated with the exceptionally poor condition of the budwood when unpacked; but since laboratory examination showed that the budwood was thin and full of pith, it was probably too immature to have given good results even under favourable circumstances.

The only figure which may be significant is the last one, obtained from the strongest treatment. The budwood was in quite good condition, and yet only 88 per cent. success was recorded.

The conclusion which must be reached is that ammonium polysulphide does no great harm to buds when they are exposed to strengths of 1 per cent. and 3 per cent. polysulphide sulphur for 2 minutes or 5 minutes, but that unless the budwood is washed after the strongest treatment (5 minutes in a 3 per cent. solution) there may be some mortality among the buds.

TABLE VI.

Box No. 6, USPULUN.

Treated, December 2nd, 1930. Budded, December 9th, 1930. Opened and inspected, December 25th, 1930. Second inspection, January 17th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	Very good, except one stick. -	81	69
C. W.	Poor ; 6 - 8 ins. die-back on all sticks.	79	66
XXIII. W.	Good ; 1 - 2 ins. die-back. -	80	62
XXIII. U.	Good ; „ „ -	80	66
XXIV. W.	Fairly good ; two sticks with 2 - 3 ins. die-back. -	94	84
XXIV. U.	Good ; 1 - 2 ins. die-back. -	84	64
XXV. W.	Very good ; 1 in. die-back. -	94	76
XXV. U.	Excellent. -	98	84
XXVI. W.	Excellent ; one stick, too young, gave only 12 unsprouted buds ; 24 taken from each of the others. -	92	82
XXVI. U.	Excellent. -	84	76

This box of material was budded in an area very heavily attacked by *Fomes lignosus*, and in the beds where the treatments in the upper half of the table were budded, the growth of the stocks was extraordinarily poor and irregular. The early morning was wet and subsequent cloudiness prevented quick drying; all these factors conspired to rob the results in the first half of the table of any significance. Unfortunately the controls were exposed to the most unfavourable conditions and suffered heavily.

The last three treatments were budded under favourable circumstances and, as the condition of the budwood was the same in each one and excellent in all, it is fair to assume that the bud mortality was proportional to the lethal effects of the Uspulun. If these latter results are significant, they indicate that treatment for 5 minutes in 1 per cent. Uspulun without subsequent washing will cause considerable

mortality among the buds, while even if the budwood is washed after such treatment some deaths will still occur. However, treatment for 5 minutes in 0.5 per cent. Uspulun even without washing has no deleterious effect.

TABLE VII.

Box No. 7, Sulphur Dust.

Treated, December 3rd, 1930. Budded, December 10th, 1930. Opened and inspected, December 26th, 1930. Second inspection, January 18th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C.	Good, except one stick with 6 ins. die-back. -	84	69
XXI.	One stick very good ; rest poor ; 4 - 8 ins. die-back. -	86	72
XXII.	One stick good, three excellent, two very poor with 6 - 8 ins. die-back. -	82	66

This is the box which unfortunately had to be left in the hands of the subordinate staff. The waxing was done very badly as is seen from the extreme variability of the condition of the bud sticks. However, each treatment and the control suffered alike, and the results indicate at least, that neither treatment had a markedly injurious effect on the budwood.

TABLE VIII.

BOX NO. 1, (REPEATED) COPPER SULPHATE.

Treated, December 5th, 1930. Budded, December 12th, 1930. Opened and inspected, December 28th, 1930. Second inspection, January 20th, 1931.

Treatment No.	Condition of Budwood at time of Budding.	Successes 1st inspection per cent.	Successes 2nd inspection per cent.
C. U.	Poor ; 6 - 9 ins. die-back.	72	50
C. W.	Poor ; Do.	72	41
I. W.	Fair ; 2 - 6 ins. die-back.	62	50
I. U.	Very poor ; especially on young sticks.	20	10
II. W.	Good on one stick, fair on the rest.	56	46
II. U.	Fair on two sticks, poor on one with 1 ft. die-back.	26	18
III. W.	Fair on older sticks ; 4 ins. die-back, poor on younger ones; 6 - 12 ins. die back, only 49 buds available.	46	37
III. U.	Fair on oldest stick, very poor on younger ones ; 6 - 12 ins. die-back, only 46 buds available.	30	20
IV. W.	Very poor indeed ; 1 - 2 ft. die-back, only 46 buds available.	30	15
IV. U.	Hopeless ; youngest stick dead to 2 ft. others to 1 - 2 ft; only 41 buds available.	12	0

In order that the preparation of this box of material could be personally supervised, it had to be treated and packed after the budding of Box No. 3 on December 5th. The budwood had to be cut in the morning, and was placed with its ends in water until packing could be commenced. The budwood must have suffered to some extent from drying out, but since the controls were waxed and packed last of all they should have suffered most.

The results indicate that the control had suffered considerably, but in spite of this the treated budwood gave in all cases lower percentage successes. The results are very striking and illustrate the soundness

of the method used in this investigation, for with one exception (I.U.) the mortality of the buds rose rapidly and regularly with the severity of the treatment until after an immersion for 5 minutes in 5 per cent. copper sulphate, only 12 per cent. of the buds remained viable. Besides giving confidence in the methods employed, these results also indicate how washing with water after treatment affects the severity of the injury to the buds. In every case it is seen that washing with water before packing, i.e. removing the disinfectant entirely from contact with the buds at the end of the specified time, placed the treatment in a much lower class for severity.

With regard to disinfection with copper sulphate, it may be concluded that all the treatments tried were too severe, and treatment with a 5 per cent. copper sulphate solution is quite out the question. Even immersion for 2 minutes in 2 per cent. copper sulphate with subsequent washing is shown to be deleterious, and the experiment ought to be repeated using 1 per cent. for 2 minutes and 5 minutes, and perhaps 0.5 per cent for the same periods of time.

The budwood was so seriously injured by treatments IV.U., IV. W., III.U., III. W., and I.U. (the anomalous result), that in spite of the presence of the copper sulphate a general growth of surface mycelium was seen on the sticks when the damp fibre was removed, while the dead portions were covered with growths of moulds.

The abnormally low result obtained from I.U. is further explained by the fact that laboratory examination showed the budwood to be thin and full of pith, and the buds too immature for successful budding.

The Second Inspection.

After an interval of 23 days from the first inspection, the bud patches were examined for the second time. It was not expected that the results obtained from this second inspection would be as significant as those obtained from the first, and they were intended to be corroborative only.

In the short interval between budding and the first inspection all the buds were under the same conditions, except with regard to the vigour of the stocks (as influenced by soil, diseases, etc.), but between the time of budding and the final inspection the bud patches were exposed to these differences in vigour for 39 days, and for the latter 23 days of that period lacked the protection afforded by the budding tapes from the effects of unequal exposure to light, heat and precipitation. Furthermore, some of the stocks were required by the Botanical Division for budding purposes, and in the beds where these buddings were made many of the original bud-patches were partially or completely covered by budding tape for periods of one to two weeks prior to the second examination.

The results were obtained too late to be discussed collaterally with those from the first examination, since this paper was prepared before the second examination was completed, but as their purpose was confirmatory only they may be considered now briefly as a whole.

The successes obtained from the second inspection were more irregular than those obtained from the first, and taken alone cannot be regarded as significant, but when considered in conjunction with the results from the first examination they indicate, in general, an exaggeration of the differences between the effects of the various treatments as there expressed. Treatments which produced high percentage successes at the first examination showed a high percentage still at the second; while those which caused considerable mortality among the buds 16 days after treatment, caused a very severe drop in viability at the end of 39 days.

The results of the second inspection do not demand any change in the conclusions reached from a consideration of those obtained at the first inspection.

The accompanying table, Table IX, summarises all the numerical results obtained, and is intended for easy reference only. It must be considered in conjunction with the previous sections of this paper.

TABLE IX.

Fungicide.		Successes (1st inspection) per cent.						Successes (2nd inspection) per cent.					
		2 Minutes.		5 Minutes.		Controls.		2 Minutes.		5 Minutes.		Controls.	
		W	U	W	U	W	U	W	U	W	U	W	U
Solbar	1 per cent	81	76	98	90	95	97	74	74	80	84	83	85
	2 ,, ,,	90	96	98	92			80	82	96	86		
Lime-sulphur (B)	1 ,, ,,	100	98	100	100	99	93	90	94	92	98	95	85
	5 ,, ,,	92	96	90	82			90	96	82	78		
Sulfinette	2 ,, ,,	86	94	96	96	85	95	84	90	90	94	78	78
Liver of Sulphur	1 ,, ,,	88	94	100	90			80	82	92	80		
Ammonium polysulphide	1 ,, ,,	94	92	100	98	96	88	92	86	100	92	83	69
	3 ,, ,,	100	70	92	88			100	58	88	80		
Uspulun	0.5 ,,	80	80	94	84	79	91	62	66	84	64	66	69
	1 ,, ,,	94	98	92	84			76	84	82	76		
Sulphur dust	½ lb.			86						72			
	1 lb.			82		84				66		69	
Copper sulphate	2 per cent	62	20	56	26	72	72	50	10	46	18	41	50
	5 ,, ,,	46	30	30	12			37	20	15	0		

Discussion.

Several points of practical importance arise from these results. In the first place the possibility is established of disinfecting budwood when being packed for export without causing any harm to the buds, as long as certain intensities of treaments are not exceeded. The fungicides employed are known to be toxic to fungi such as Phytophthora under laboratory conditions even at the lowest concentrations employed in this experiment, but whether they have any useful fungicidal action at the above mentioned limiting concentrations against fungi lying dormant under bud scales, or on the surface of the budsticks, is a matter for further investigation. The latter point could have been cleared up to some extent in the present instance if it had been possible to cut back the budded stocks, allow the buds to grow away normally, and to record the appearance of die-back fungi among the various treatments. This was not possible as the Botanical Division needed the stocks for further budding work, and when the buds had grown sufficiently to enable the second inspection to be made they were cut out.

It must be remembered that only one clone has been dealt with in the foregoing experiments and that no generalisation can be made to include all types of budwood. It is most probable that results of the same type would have been obtained no matter what clone had been used, but until experiments of a like nature have been performed on a range of clones these results must apply to clone AVROS 50.

Another important point that has been established is that the washing of budwood after disinfection merely has the effect of weakening the toxicity of the treatment towards dormant buds and that, as far as viability of buds is concerned, weaker and therefore cheaper solutions used without washing will produce the same results as are produced by stronger and more expensive solutions when the material is washed after treatment. If it is found in subsequent experiments that short exposures of dormant fungi to strong disinfectants are more effectively toxic than prolonged exposures to weak ones, then the process of washing budwood after treatment will have an important practical application. If, as is more likely, dormant fungi are found to be affected by disinfection in the same kind of way as dormant buds are affected, then washing with water after treatment will have no practical value.

The results also indicate to what extent damage can be done to budwood by faulty waxing, or the use of wax with a high melting point, for throughout the experiment there was a high correlation between the condition of the sticks—as judged by the amount of the die-back of the bark at the ends—and the percentage success of the buddings made from them. When budwood is being cut and exported commercially on a large scale the following recommendations may be made:—

1. A wax should be used with a melting point lower than that of Entwas, but high enough to prevent softening under air temperatures.
2. A special melting tank should be installed provided with an automatic temperature control. The prevention of loss to consignments of budwood through excessive heating during waxing will repay the cost of this extra equipment.

CONCLUSIONS.

The following are the greatest intensities at which the various fungicides under investigation may be used to disinfect budwood from clone AVROS 50 without any resultant injury to the buds.

SOLBAR. Immersion for 2 minutes in a 2 per cent solution, or for 5 minutes in a 2 per cent. solution if washed immediately with water.

LIME-SULPHUR (B). Immersion for 5 minutes in a 1 per cent. solution is safe but for 2 minutes in a 5 per cent. solution is too strong. Another experiment should be conducted using a 2 per cent. solution.

SULFINETTE. Results indefinite, but soaking for 5 minutes in a 2 per cent. solution has no serious effect. Experiments should be carried out using a 3 per cent. solution.

LIVER OF SULPHUR. Immersion for 2 minutes in a 1 per cent. solution, or for 5 minutes in a 1 per cent. solution if washed immediately afterwards in water.

AMMONIUM POLYSULPHIDE. Immerson for 2 minutes in a 3 per cent. (polysulphide sulphur) solution, or for 5 minutes in a 3 per cent. solution if washed afterwards with water.

USPULUN UNIVERSAL. Immersion for 2 minutes in a 1 per cent. solution.

SULPHUR DUST. Results indefinite, but apparently the inclusion of 1 lb. of sulphur dust in the fibre required to wrap 6 sticks had no serious effect on the buds. This series of treatments should be repeated using also $1\frac{1}{2}$ lbs. of sulphur to the same quantity of packing material.

COPPER SULPHATE. The limiting concentration was exceeded even in the weakest treatment. The experiment should be repeated using 1 per cent. and 0.5 per cent. solutions instead of those employed above.

ACKNOWLEDGEMENT.

The writer wishes to take this opportunity of expressing his indebtedness to Mr. C. E. T. Mann for invaluable assistance in all the problems encountered relative to budding, to Mr. F. Beeley for the inspection work carried out at the laboratory, and to Mr. A. R. Sanderson for suggesting the line of investigation and for continuous advice and suggestions during the course of the experiments.