BLUESTAINED CASE TIMBER AND MOULD ROTS IN HEN EGGS.

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SUMMARY.

It is demonstrated that mould rots in eggs can be artificially produced by infection with spores of a sap-staining fungus isolated from case timber.

Early treatment of eggs with the sodium salt of salicyl anilide was shown to be an effective preventive of decay in eggs caused by moulds under the conditions of the experiment but was ineffective against bacterial rots.

Trials involving various methods of handling showed that under normal export conditions bluestained hoop pine (Araucaria cunninghamii) timber has no effect on the quality of eggs when it is used as a case material.

INTRODUCTION.

In 1938 attention was called to the use of bluestained hoop pine timber in cases in which eggs were being packed for export overseas and fears were expressed that the mould responsible for the staining of the timber might cause rots in the eggs. If this proved to be so, it was likely that bluestained timber would be rejected for case-making, resulting in a loss to the timber trade and a higher-priced case due to the better grade timber which would be required.

INFECTION OF EGGS WITH TRICHODERMA LIGNORUM.

Isolations from affected timber supplied by the Queensland Egg Board revealed the presence of the fungi *Diplodia pinea* and *Trichoderma lignorum*. It has been demonstrated in England and America that various species of *Penicillium* are capable of causing black rots in eggs; and in America it was shown in one investigation that *Penicillium* and *Cladosporium* moulding developed only when unseasoned cases were used for storage and it was concluded that infection of the eggs might take place either before or during storage whenever moisture is present.

Keeping these facts in mind, it was decided to test the susceptibility of eggs to a strain of *Trichoderma lignorum* isolated from case timber. For this purpose, six fresh eggs were sprayed with spores of the fungus and held in a moist atmosphere. At the end of a fortnight, all eggs showed evidence of rots and *T. lignorum* was isolated from the spotted areas. In preliminary trials, it was found that in a saturated atmosphere infection did not readily occur but that with lower humidities, with enough moisture present to support fungus growth, infection occurred readily.

PREVENTION OF TRICHODERMA ROTS IN EGGS.

Salicyl Anilide as an Egg Preservative.

The literature on the prevention of egg deterioration by fungal infection was reviewed but there did not appear to be any practical treatment which was entirely satisfactory for eggs. In New Zealand, a satisfactory treatment for preventing mould in butter boxes and on cheeses had been used. This consisted in dipping the timber and the cheeses in a 0.1 solution of salicyl anilide (water-soluble) for 10 minutes and then drying.

It was thought worthwhile to try the effect of salicyl anilide on eggs and trials were accordingly initiated. The salicyl anilide used in this case was the commercial preparation "Shirlan AG," an insoluble form which is applied as a suspension in water. The concentration used was 0.5 per cent. by weight.

In a preliminary test it was found that dipping in "Shirlan AG" prevented infection in eggs subsequently sprayed with *Trichoderma lignorum* spores. The flavour of treated eggs was in no way different from that of normal eggs.

Following this test a larger scale experiment designed to test the efficacy of "Shirlan AG" as an egg preservative was initiated.

The experiment was conducted in two series. In one series the eggs were held in an export case at room temperature and examined at weekly intervals. In the other series, the case of eggs was held for six weeks (the approximate time of carriage to Great Britain) under cold storage conditions similar to those obtaining during transport overseas, and on removal were examined at weekly intervals for the appearance of rots.

Eggs Held at Room Temperature.

For the purpose of the room temperature holding experiment, 250 fresh eggs were obtained from the one farm in order to have a comparable series. On receipt, the eggs were mixed and then divided into lots of 50 to provide for 5 treatments. The treatments were as follows:—

1. Application of *Trichoderma* spores, then dipping in "Shirlan AG" on the same day.

2. Application of *Trichoderma* spores, then dipping in "Shirlan AG" 24 hours later.

3. Application of *Trichoderma* spores on receipt; no dipping.

4. Dipping in "Shirlan AG," followed by application of *Trichoderma* spores on the same day.

The *Trichoderma* spores were suspended in distilled water and sprayed on to the eggs with an atomizer. In treatment 1, the eggs were allowed to dry before dipping in "Shirlan AG." The dipping process consisted in washing each individual egg in a bath of "Shirlan AG" with the aid of a cottonwool wad.

After dipping, the eggs were allowed to dry on a rack and were then packed in clean cardboard egg containers and placed in the case. Where "Shirlan AG" was applied 24 hours after the spores (treatment 2), the eggs were kept moist in the interval in order to facilitate the action of any fungi. The control eggs were not washed at all.

All treatments were divided into two lots, each of 25 eggs, each lot was placed in a separate tray and the 10 trays were distributed at random in the case. The whole case was kept moist by means of spraying with sterile water once daily for three days.

After the commencement of the trial the eggs were candled at weekly intervals until the end of the experiment. The results were as shown in Table 1. From these results, it will be seen that the most prolific yield of rots was in treatment 3, in which *Trichoderma* spores were applied but no dipping was performed. There was no significant difference between treatments 2, 4 and 5, all of which, except 5, involved a treatment with "Shirlan AG." Treatment 1 had the lowest percentage of rots. The fact that the number of rots in the untreated eggs was similar to that in all the "Shirlan AG"-treated eggs was due to infection before treatment. Since the number of rots in the untreated eggs was slightly in excess of that for any "Shirlan AG" treatment, the conclusion was drawn that

Table 1.

NUMBER OF ROTS IN EGGS HELD AT ROOM TEMPERATURE.

Treatment.	Number of Rots each Week.							Total.	Percentage Rotten.
	1st	2nd	3rd	4th	5th	6th	7th		
1. Spores, then " Shirlan AG "	..	..	1	2	1	1	..	5	10
2. Spores, then " Shirlan AG " in 24 hours	..	..	2	4	2	3	1	12	24
3. Spores, no " Shirlan AG "	..	4	4	6	4	3	13	34	68
4. " Shirlan AG " then spores	..	..	2	3	2	3	1	11	22
5. Untreated	..	..	1	1	3	..	9	14	28

this excess was due to infections which had not developed enough to penetrate beyond reach of "Shirlan AG" at the time of the treatment. It follows that "Shirlan AG" was efficacious in preventing all mould rots when infection occurred after treatment or within 24 hours before treatment.

The organisms isolated from the rotted eggs were *Trichoderma lignorum* and *Clostridium* sp. Their percentage occurrence in the various treatments was:—

Treatment.	*Trichoderma.*	*Clostridium.*
	Per cent.	Per cent.
1	100	—
2	80	20
3	60	40
4	66	34
5	100	—

Eggs Held in Cold Store.

In order to test the efficacy of "Shirlan AG" under cold storage conditions, the experiment was repeated on similar lines but, after treatment, the eggs were held in a commercial cold store in Brisbane at a temperature of 34 degrees C. for six weeks and were examined weekly after removal from storage. The eggs were kept moist during the observation period by spraying with sterile water. It was noticeable that there was a copious condensation of moisture on the eggs and cases after removal from the cold room. In this experiment, the results of the weekly candlings were as shown in Table 2.

Table 2.

NUMBER OF ROTS IN EGGS HELD IN COLD STORE.

Treatment.	Number of Rots at Weekly Observations.					Total Rots.	Percentage Rots.
	1st	2nd	3rd	4th	5th		
1. Spores, then " Shirlan AG "	..	1	2	8	12	23	46
2. Spores, then "Shirlan AG" in 24 hours	..	..	2	4	19	25	50
3. Spores, no " Shirlan AG "	..	12	15	12	4	43	86
4. " Shirlan AG " then spores	..	5	6	7	7	25	50
5. Untreated	..	3	11	13	6	33	66

The results follow the same general trend as in the previous experiment except that no doubt owing to the moister conditions of storage the number of affected eggs was greater. The treatment involving spraying with *Trichoderma* spores alone gave the largest number of rots, the control was next highest and the treatments involving dipping had substantially fewer rots. The higher percentage in the control in this case was no doubt due to infection during the period of observation in the moist atmosphere. It will be seen that even under moist conditions "Shirlan AG" was able to control 36 per cent. to 40 per cent. of the subsequent infection. The isolations from affected eggs revealed the presence of *Trichoderma* and *Clostridium* again in the untreated eggs in the proportion of four *Trichoderma* to three *Clostridium* infections and of one *Trichoderma* to one *Clostridium* in the "Shirlan AG" treatments. No other bacterial or fungal isolates were found.

Eggs Treated on the Farm.

As a further test an experiment was carried out on a poultry farm at Noosa in south-eastern Queensland. The treatments were:—

A. Farm-washed (abrasive soap and water).

B. Washed with 0.5 per cent. "Shirlan AG" in water.

C. Unwashed.

Each treatment was applied to 108 fresh eggs. The "Shirlan AG" treatment consisted in soaking the eggs in the fungicide for approximately three minutes. The eggs after six weeks in cold storage were delivered to the laboratory for weekly examinations, with results as shown in Table 3.

Table 3.

NUMBER OF ROTS IN FARM-TREATED EGGS.

Treatment.	Number of Rotten Eggs at Weekly Inspections.							Total No. Rots.	Percentage Rotten.
	1st	2nd	3rd	4th	5th	6th	7th		
A. Farm washed	..	2	9	7	7	8	15	43	44
B. Washed with "Shirlan AG" in water ..	2	1	9	17	21	13	16	79	63·1
C. Unwashed	..	1	1	..	6	2	1	11	10·1

In this case the eggs were again kept moist by spraying with distilled water. It will be noted that the worst results in this experiment were obtained from the "Shirlan AG" treatment and the best from non-washing, with the farm-washing procedure intermediate between the two. Isolations from the eggs revealed the presence of five bacterial rots to every fungus rot. Only *Trichoderma lignorum* was found amongst the fungal rots and the bacteria were all bacilli.

It is considered that, in the concentration used, "Shirlan AG" has no effect on bacterial growth and the relatively long period of immersion in the washing water in the "Shirlan AG" treatment was instrumental in distributing the bacteria, originating in the excreta and dirt, over the surface of the eggs and into the pores of the shell, thus considerably increasing the percentage of rotten eggs. The ordinary farm-washed eggs, being wetted in the washing water for a shorter period than in the "Shirlan AG" treatment, consequently received less infection. The keeping qualities of the unwashed eggs were, in this case, outstanding.

SHELL POROSITY AND INFECTION.

In the course of the experiments described above it was decided to see whether there was any marked correlation between shell porosity and infection. In order to determine porosity, the eggs were placed in a gentian violet solution in water and held under a vacuum of $7\frac{1}{2}$ lb. for three minutes and then allowed to absorb the stain. Porosity was indicated on breaking by the blue spots on the interior of the shell. Susceptibility to infection appeared to be correlated with the number of spots. The size of the spots did not appear to have great significance. This is explained by the fact that the large spots were not indicative of large shell pores but of small clusters of pores of normal size.

CASE TIMBER AS A SOURCE OF MOULD CONTAMINATION.

Following on the series of experiments in which "Shirlan AG" was shown to be effective as a mould preventive, attention was given to the possibility

of *Trichoderma* infection of eggs being caused by bluestained timber in egg cases. An experiment involving the following treatments was conducted.

1. Bluestained cases; eggs treated with "Shirlan AG."
2. Bluestained cases; eggs untreated.
3. Bluestained cases; treated with "Shirlan AG"; eggs untreated.
4. Bluestained cases treated with "Shirlan AG"; eggs treated also.
5. Clean case treated with "Shirlan AG"; eggs treated also.
6. Clean case treated with "Shirlan AG"; eggs untreated.
7. Clean case untreated; eggs untreated.
8. Clean case treated with "Shirlan AG"; eggs treated 24 hours after collecting.

The type of case used was that known to the trade as the "aeroplane" case, and one case containing 100 eggs was used for each treatment. The cases were obtained freshly made from the case manufacturers, those for the bluestain treatments being made from particularly badly stained timber. The clean cases showed no evidence of sapstain. The fillers used in the cases were the standard type of strawboard export fillers. Clean, unused fillers were used in all the cases.

The "Shirlan AG"-treated cases were immersed for three minutes in a 0·5 suspension of the fungicide and then dried.

The eggs were obtained in the Brisbane district from a commercial egg-producing farm, with a good record as to the quality of eggs submitted to market and with the capacity to provide all the eggs required in two gatherings. The eggs were gathered on the farm and treated and packed on the same day, except for the last treatment, when the washing was carried out 24 hours after gathering.

The procedure carried out at treatment was to wash the eggs by hand in the "Shirlan AG" mixture instead of in water. The eggs were then allowed to dry on a draining rack before packing.

On the completion of treatment and packing, the eggs were stored in cold rooms at a temperature of 35·0 degrees F. for six weeks. After the completion of the cold storage period, the eggs were removed to the ordinary store rooms, where the temperature varied from 65 degrees to 70 degrees F. Examinations were conducted by the Queensland Egg Board's graders at weekly intervals for three weeks. The fourth and final observations were carried out by a Poultry Expert of the Queensland Department of Agriculture and Stock with the assistance of the writer. After the fourth examination, the experiment was concluded because under normal circumstances all eggs removed from cold storage should have been consumed before four weeks had elapsed.

The results noted at the weekly gradings and obtained from the grading charts supplied by the Queensland Egg Board, together with the results of the final examination, were as shown in Table 4.

Table 4.

WEEKLY CONDITION OF EGGS FOR FOUR WEEKS AFTER COLD STORAGE.

Treatment.	1					2					3					4				
Condition.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.
Weekly Inspection																				
First	94	2	4	..	..	98	..	2	..	..	95	..	4	..	..	96	1	3	..	..
Second ..	94	2	4	..	..	98	..	2	..	..	95	1	4	..	..	95	1	3	..	1
Third	93	1	5	..	1	98	..	2	..	..	93	1	4	..	1	94	1	2	..	2
Fourth ..	93	1	5	..	1	98	..	2	..	..	93	1	4	..	1	94	1	2	..	2

Treatment.	5					6					7					8				
Condition.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.	G1.	G3.	C.	B.S.	B.
Weekly Inspection																				
First	94	2	3	..	1	98	1	..	..	1	96	3	..	..	1	92	2	2	..	4
Second ..	93	2	4	..	1	98	1	..	..	1	96	3	..	..	1	89	2	5	..	4
Third	90	2	6	..	2	97	1	..	1	1	96	3	..	..	1	87	4	5	..	4
Fourth ..	90	2	6	..	2	97	1	..	1	1	96	3	..	..	1	87	4	5	..	4

G1=Grade 1; G3=Grade 3; C=Cracked; B.S.=Blood Spot; B=Bad.

NOTE.—Grade 1 eggs were good but could not be sold as fresh owing to deterioration due to ageing. They were suitable for culinary purposes.

From the figures supplied in the table, it will be seen that there was no significant difference between the eggs in any treatment. No mould rots were found in any of the eggs noted as bad; the majority of these had "stuck" yolks. Only two eggs, one in treatment 5 (third week) and one in treatment 1 (third week), had bacterial rots. The eggs relegated to grade 3 were placed there on account of the condition of "watery white" or "ruptured membrane." It will be seen that in the whole experiment only two eggs showed typical decay caused by bacteria and none caused by mould. From this it was concluded that blue-stained hoop pine cases, even when so badly stained as to be culled out at the case mill, had no effect on the keeping quality of eggs.

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