# Integrated Control of Bacterial Blotch in Israel

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Mushrooms have been commercially produced in Israel only on a small scale and there have been no reports on their common pathogens. But now *Pseudomonas tolaasii* (Paine), the causal agent of bacterial blotch of mushrooms, has been identified in Israel. The disease symptoms are well known, but the properties of the pathogen are confusing (9, 15). The main factor favouring infection is a free water film covering the mushroom caps (12). Water condensation is obtained when small differences between room and mushroom temperature are produced.

Chemical treatments for preventing bacterial blotch using chlorine, bromine, hexachlorophene and glutaraldehyde have not been satisfactory, whereas treatments with terramycin and sodium metabisulphate and biological control are still being tested (4, 7, 8, 9, 13, 14). Natural conditions for growing mushrooms are not encountered in Israel; most of the mushroom farms are in fully environmentally controlled rooms using artificial cooling.

The mushroom market in Israel consumes only white, fresh mushrooms, but not when covered with bacterial spots. Therefore, the disease causes relatively high losses to growers as compared to countries where mushrooms are canned (11).

## Growth conditions

All experiments were carried out in a modern champignon farm in Zikhron-Yaacov. Each growing room had an automatic control for cooling or heating and hand-controlled ventilation, circulation and misting.

The compost used was the so-called 'synthetic' composed of wheat straw and chicken manure manufactured by 'Kompit-Meona'. The champignon cultivar used was 'Somycel 53' (one of the most popular cultivars in Israel). Growth treatments were almost similar to those used in the Dutch mushroom farms (2). Chlorine was sprayed through the irrigation system.

Pieces from the upper parts of infected caps were cut with a sterile knife, sterilised with 1.0% NaCl for 1 min., washed 3 times with sterile saline (0.85% NaCl) and then placed on Petri dishes containing a King-B medium with penicillin and cycloheximide (KPC) (1) for the isolation of fluorescent pseudomonas. The cultures were incubated at 25°C for 24 hours. Colonies which produced a green fluorescent pigment were transferred to a medium containing either 0.5% ethanol or propylene-glycol (3). Colonies that developed after incubation were further identified by using the white line in agar test of Wong and Preece (15) and by observing disease development in inoculated mushrooms.

The pathogen was grown on nutrient broth (Difco) in a shaker at 25°C. The suspension was centifuged and the pellet resuspended in saline, to an absorbance of 0.1 units at 540 nm wavelengths. The mushroom caps were inoculated by carefully brushing with a small sterilised brush previously dipped in the bacterial suspension. The inoculated mushrooms were incubated at 20°C or 2°C in a humid chamber.

## Isolation of the pathogen

*Pseudomonas tolaasii* differs from other fluorescent pseudomonas only in some nutritional requirements for growth, i.e. it can use ethanol or propylene glycol as the sole carbon source (3). It was therefore possible to isolate the pathogen by following two steps: Fluorescent pseudomonas were first isolated in KPC medium and then streaked on propylene glycol or ethanol medium. They were further tested for production of a white line in agar (15). The colonies that developed were identified as the pathogen. Thus, they were separated from the fluorescent complex. Mushrooms were also inoculated with

fluorescent isolate that did not develop in propylene glycol or ethanol, with *Pseudomonas fluorescens* from the collection of our laboratory and with *P. tomato* (causal agent of bacterial speck of tomato). Only the pathogen produced symptoms within 24 hours (Fig. 1).

## Infection at low temperatures

Generally, mushroom growers store the collected mushrooms at 2–4°C in the refrigerator. Mushrooms showing no symptoms at picking time often developed symptoms after 48 hour storage. It was observed that when the refrigerator doors were opened several times on a warm day, the temperature increased and caused water condensation.

Symptom-free mushrooms were inoculated with a suspension of the pathogen and incubated in a humid chamber at 2°C. The same controls as before were used. All mushrooms covered with the pathogen showed light brown spots after 24 hours and became dark brown within 48 hours. They lost completely their market values, whereas the controls remained symptomless.

## Disease development and temperature changes

During the summer, air entered into the environment-controlled rooms where relative humidities are near the point of saturation, causing small increases in air temperature, and resulting in water condensation on the caps.

Experiments showing the effect of differences in temperature were carried out during the summer and winter of Israel. In summer, when hot air entered the room, disease appeared as a result of condensation whereas, in winter, mushrooms growing in the same room were generally clear of symptoms (Fig. 2). When the ventilation was lowered to the minimum during the hot hours (arrow in Fig. 2), the differences in temperature were reduced with a concomitant decrease in disease severity.

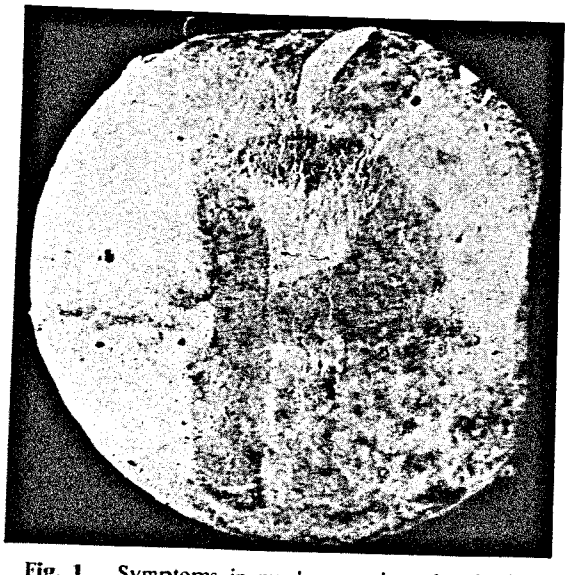

Fig. 1. Symptoms in mushrooms inoculated with *Pseudomonas tolaasii* isolated from infected caps. P — infection site.

## Prevention of disease caused by frequent irrigations

The cultivated mushrooms in Israel need to be irrigated nearly every day and, in the summer, even twice a day. As a result, the caps remain wet for long periods of time. This could be prevented by irrigating during the cool hours of the day in order to prevent the entrance of hot air. Afterwards, at the end of the irrigation, the circulation system and outside ventilation were both fully opened for 30–60 min. making possible the drying of the caps, without affecting casing soil moisture, Then, ventilation was lowered to a needed level. This simple treatment prevented disease spreading all over the room and maintained low infection levels on three separate rooms during the summer.

## Chlorine control

All experiments were done in beds 1.4 × 7.0 m, 3 beds for each treatment. Six days after the first cooling, volumes of 0.5 l/m² of 0.015% active chlorine were sprayed. The controls were irrigated with water. The plots were sprayed again two days before the second and third flush. Typical symptoms on caps were counted three times after each flush (Table 1).

## Integrated control: chemical and environmental

Integrated control was tested in two different rooms during the summer. Two chlorine treatments were given, and the amount of fresh air during the hot hours of the day was minimised. The beds were irrigated once a day and the caps were dried as described before.

Temp. °C

From everyday observations it was found that disease severity was very small (Fig. 3) and all mushrooms were sold on the fresh market.

Damage caused by *Pseudomonas tolaasii* to mushroom crops in Israel has been increasing lately. The organisms form part of the natural microflora of the growth beds (6). It was possible to isolate it from infected caps in Israel.

To summarise, small differences in temperature caused by essential ventilation of the cool rooms resulted in water condensation on the cap surface. Irrigations carried out when outside air temperatures prevailed and treatments for increasing yield by misting (2) also kept the caps wet for long periods of time, thus favouring infection.

Use of antibiotics, such as terramycin, which gave good control (14) is not permitted in Israel and control with chlorine has been reported to give unsatisfactory results (5). We have found that limited chlorine treatment (2–3 times at the picking period) together with environmental control and by drying the caps after irrigation, are able to decrease disease to a level of no economical importance. Also temperature in refrigerators where mushrooms are stored should be kept from changing by avoiding unnecessary opening or by using cooling rooms with constant temperature.

**Table 1**
**Effect of chlorine treatments on bacterial blotch in mushrooms**

| Treatment | Flush | % infection |
|---|---|---|
| Chlorine[1] | 1 | 0 a |
| | 2 | 0–1 a |
| | 3 | 1–2[2]a |
| Control | 1 | 2–3 a |
| | 2 | 5–8 b |
| | 3 | 8–12 b |

[1] Active chlorine 0.015% was sprayed at a rate of 0.5 l/m².

[2] Appearance of light symptoms was counted as positive. Experiments were carried out in three replicates (70 caps per replicate).

Numbers followed by different letters are significantly different at $P = 0.05$.

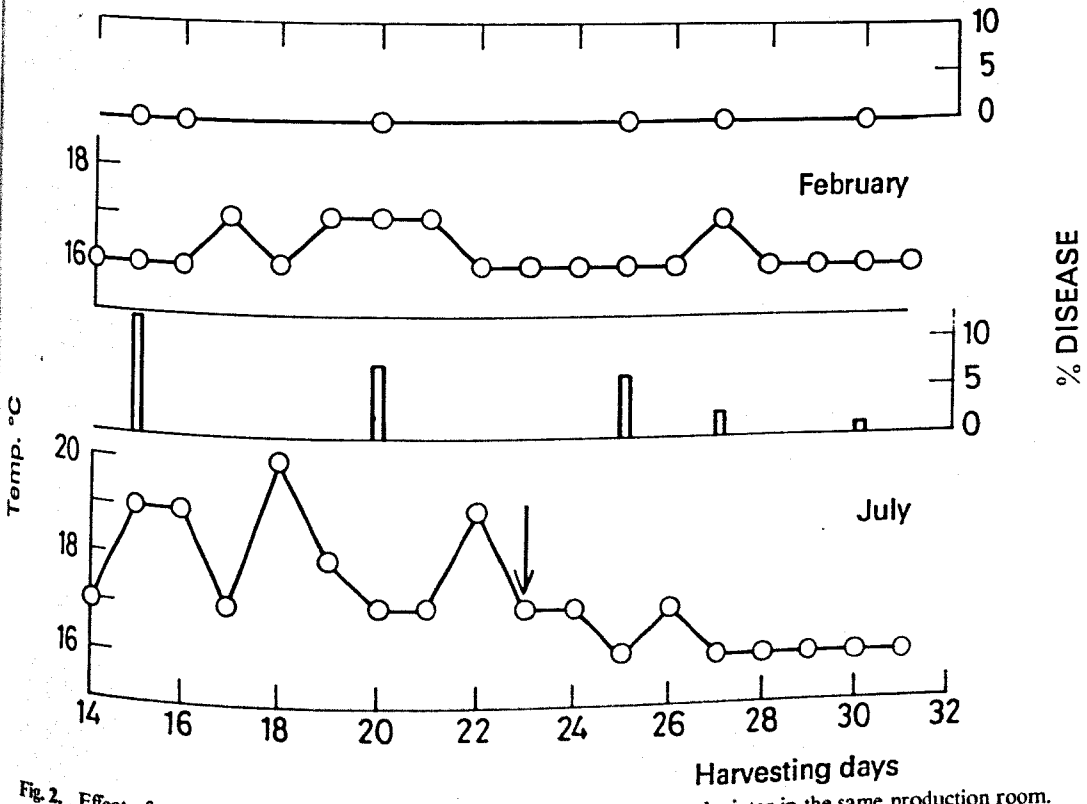


Fig. 2. Effect of temperature differences on disease severity in summer and winter in the same production room.

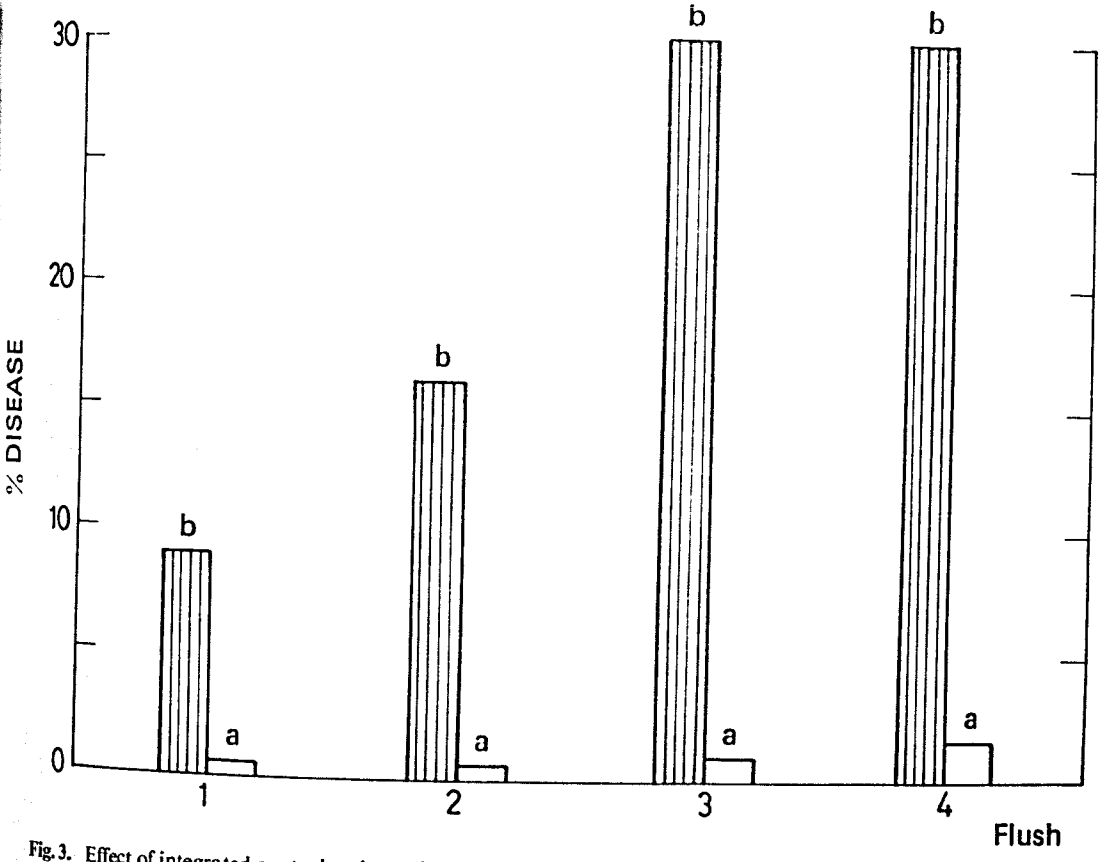


Fig. 3. Effect of integrated control on bacterial blotch severity in the summer. Two hundred caps were checked in each flush. Results are average of two rooms.

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