Effect of 2,4,5-Trichlorophenol on Hyphal Membrane Potentials in Rhythmic Mutants of *Neurospora crassa*

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**Abstract**

The uncoupler 2,4,5-trichlorophenol (TCP) was used to test for differences in maintaining the hyphal membrane potentials in the wild type and rhythmic mutants of *Neurospora crassa*. Trichlorophenol (0.1 mmol·l⁻¹) resulted in a depolarization of 93 mV (wild type) and 144 mV in the mutant clock°. A total recovery was achieved in both strains after washing out the uncoupler. The circadian conidiation mutant band was more sensitive than the other two strains and showed two different reaction patterns: one with delayed reaction, total breakdown and without recovery; the other one with nearly immediate reaction, slow but not entirely total decline and partial recovery. These differences are discussed in their relation to the circadian rhythm of conidiation.

**Materials and Methods**

**Strains:** The following strains of *Neurospora crassa* Shear et Dodge were used in the experiments:

- STA4 (No 262 FGSC) wild type;
- band (bd) (No 1858 FGSC) circadian conidiation;
- clock (cl) (No 1166 FGSC) periodic alteration of branching pattern.

**Cultures:** Cultures were inoculated from stock cultures to solid media containing 2 % agar and 3 % malt extract. The media were covered with scratched and sterilized cellophane (type 600 PIO, Kalle, Darmstadt, W.Germany) and incubated at 25°C for 15 h (STA4), 20 h (band) or 24 h (clock).

**Measurements:** For measurements, pieces of 7 x 20 mm² were cut out from the cellophane and transferred into a chamber according to Gradmann and Slayman (1975), where the mycelium attached to the cellophane was bathed in a standard solution (see below). Measurements were made using conventional capillary microelectrodes filled with 3 mol·l⁻¹ KCl. The potential difference was measured by a high-impedance electrometer amplifier model 610 C Keithley instruments, connected to a Metrawatt recorder.

**Solutions:** The standard solution for recording the potential difference contained 10 mmol·l⁻¹ CaCl₂ and 133 mmol·l⁻¹ sucrose (Slayman and Slayman 1968). About 10 min after insertion of the electrode, i.e., after having reached a constant reading of the potential difference, the standard solution was substituted by the same solution containing in addition 0.1 mmol·l⁻¹ 2,4,5-trichlorophenol (TCP); EGA-Chemie, Steinheim, Germany.

**Results**

In Fig. 1a the typical reaction of the wild...
type after addition of 0.1 mmol·l⁻¹ trichlorophenol to the bathing medium is seen. The wild strain shows an immediately starting depolarization of about 93 mV (Table 1). Fig.2 gives evidence that the effect depends on the applied concentration of trichlorophenol. After removing the inhibitor, a total recovery of the potential is achieved.

The mutant band which differs from the wild type by its circadian conidiation, contrasts to this reaction pattern by a lag phase of about 8 min between the arrival of the uncoupler in the cuvette and the start of a visible reaction (Fig. 1b and Table 1). The reaction itself is very fast, in most cases it lasts less than 1 min. The final level differs by 172 mV (mean) from the original resting potential. Since the residual value results from a mere diffusion potential, this reaction leads to a total break-down of the membrane potential.

In some cases a slower reaction is found, which also results in a very low residual potential, but not in a total breakdown (Table 1). The differences between these two reaction patterns of band are not yet completely understood, presumably they are due to different circadian phases.

Marked differences between the wild strain and band are also found in the recovery after removing the trichlorophenol solution. No recovery was found after the typical "fast reaction" with its total breakdown. After the "low-speed reaction" a partial but not complete recovery was observed in but two cases.

To test whether the described alterations of the trichlorophenol effect are typical for the mutant band (and thus for its circadian conidiation pattern), another rhythmic mutant, the strain clock was included. It shows a periodical hyphal branching (zonating) which is neither circadian nor synchronized to environmental cycles. The PD alteration after addition of 0.1 mmol·l⁻¹ trichlorophenol is quite similar to that observed in the wild type, the difference is a longer decline (36 min instead of 24 min) and a resulting larger depolarization (144 mV as compared to 93 mV in the wild type; see Table 1). After replacing the trichlorophenol solution by the original bathing medium, a total recovery is observed. Thus, the overall pattern of clock resembles that of the wild type.

### Table 1: Mean resting potentials and reactions after application of 0.1 mmol·l⁻¹ TCP in hyphae of the listed rhythmic strains of Neurospora crassa.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Resting PD (mV)</th>
<th>Depolarization in TCP (mV)</th>
<th>Start of reaction after TCP addition (min)</th>
<th>Time between start and maximal reaction (min)</th>
<th>Recovery after TCP treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA4 (wild type)</td>
<td>-198±6</td>
<td>93±9</td>
<td>0.4±0.2</td>
<td>24.0±4.0</td>
<td>total</td>
</tr>
<tr>
<td>clock</td>
<td>-213±10</td>
<td>144±13</td>
<td>0.8±0.3</td>
<td>36.0±7.0</td>
<td>total</td>
</tr>
<tr>
<td>band</td>
<td>-186±8</td>
<td>172±8</td>
<td>7.7±1.4</td>
<td>0.6±0.2</td>
<td>no</td>
</tr>
<tr>
<td>-211±10</td>
<td>134±25</td>
<td>1.7±0.5</td>
<td>18.2±2.8</td>
<td>partial</td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 1: Resting potential difference (PD) and the typical reaction after application of 0.1 mmol·l⁻¹ TCP in the wild strain STA4 (a) and in the mutant band (b) of Neurospora crassa. The arrow marks the arrival of the TCP-solution in the chamber used for the measurements.](image)

![Fig. 2: Correlation between applied concentrations of TCP and depolarization in the wild type STA4 of Neurospora crassa.](image)