

# Stimulation of Wound Reactions in Potato Tubers by Thiabendazole

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Z. Naturforsch. 36 c, 115–121 (1981); received October 10, 1980

*Solanum tuberosum* L., Wound Reactions, Ethane Production, Lipid Peroxidation, Thiabendazole

Thiabendazole (TBZ), an antifungal and antihelmintic benzimidazole derivative, after 6 min treatment with a 0.1 mM aqueous solution enhances CO<sub>2</sub> release in intact potato tubers for up to two hours. There are also indications for a reduced water permeability of isolated periderm layers from TBZ – treated potato tubers. Potato slices (ca. 3 mm thick) or discs (with ca. 5 mm diameter) from potato slices show enhanced ethane formation after TBZ – treatment exhibiting maximal rates ca. two hours after treatment, indicating enhanced unsaturated fatty acid peroxidation. This lipid peroxidation is not enhanced by TBZ in potato homogenates or in isolated potato mitochondria. The results of this communication might partly explain macroscopically observable, preservative effects of treatment of freshly harvested potatoes by a stimulation of certain metabolic responses after mechanical wounding.

## Introduction

Wounding of plant tissues causes a series of changes and inductions of new biochemical pathways of the cellular metabolism. These changes include an increased respiration, enhancement of certain oxidations and peroxidations, ethylene formation, *de novo* – synthesis or release from inactive precursors of antiparasitic substances (often designated as “phytoalexins”) and finally suberization and lignification of wound surfaces [1–3]. In contrast to fruits which mainly show increased ethylene formation and less ethane production after wounding, certain storage organs as potato tubers evolve more ethane than ethylene. Ethane seems to be derived from peroxidised linolenic acid while ethylene comes from methionine via S-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid [4–7]. Ethylene formation, on the other hand, seems to depend on intact but “irritated” tissue [3] whereas ethane production is maximal in homogenized [8], decompartmentalized [9, 10] or intoxicated plant [11] or animal [12] tissues. Thiabendazole, a fungicidal [13–15] and antihelmintic [16] benzimidazole derivative exhibits preservative effects on potato tubers during storage. This is indicated by bearing

less symptoms of senescence and deterioration as smell, shrinkage, colour changes, rotting and others. The above effect of TBZ is probably mostly due to the mentioned antiparasitic and antinecrotrophic activities. In the present communication we wish to report that part of the preservative effects of TBZ on stored potato tubers might also be due to an increased wound response, *e.g.* lipid peroxidation. Periderm perforations and scratches freshly set by mechanical harvesting might thus be rendered less accessible for parasites by toxic products, known to be produced during lipid peroxidation [1, 2, 17].

## Materials and Methods

Water loss of half potato tubers was determined by weighing. Respiration (*e.g.* CO<sub>2</sub> production) by intact potato tubers was determined by absorption spectrometry (URAS) and calculated according to the following equation:

$$P_M = M_{CO_2} \times \frac{P \times 10^{-6}}{RT} \times V/M \times \frac{\Delta CO_2}{\text{ppm}},$$

where:

$$P_M = \text{mg CO}_2 \text{ turnover/g FW,}$$

$$M_{CO_2} = 4.4 \times 10^4 \text{ mg,}$$

$$p = 1 \text{ atm,}$$

$$R = \text{gas constant (0.082),}$$

$$T = 293 \text{ K,}$$

$$V = 90 \text{ l} \times \text{h}^{-1},$$

$$M = \text{FW of potato tubers in g,}$$

$$\Delta CO_2 = \text{spectrometer readings (cf. [18]).}$$

Water permeability of isolated potato periderm layers was determined as recently described by Schönherr and Schmidt [19]. Microscopic observa-

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**Abbreviations:** TBZ, thiabendazole, 2-(4-thiazolyl)-benzimidazole; FW, fresh weight.

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0341-0382/81/0100-0115 \$ 01.00/0



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tions of ca. 3  $\mu\text{m}$  thick wound cell layers were done after fixation of tissue cubes with acrolein and imbedding in glycolmethacrylate, followed by regressive colouring with toluidine blue at pH 4.0.

Ethane and ethylene were determined gaschromatographically as described [10]. TBZ-treatments were performed as described in the tables and figures. Potato homogenates were prepared and mitochondria were isolated from potato tubers as recently described [20, 21].

In the experiments shown in Table III and in Fig. 1 and 2, potato tubers from the harvest 1979, stored during the winter (3 to 6 month), and a water-soluble, neutral TBZ-formulation were used, whereas in the following experiments (Tables VI and VII, Fig. 3 to 5) freshly harvested (1980) potatoes were used. Note the differences in ethane production!

## Results

### Respiration of whole potato tubers

After TBZ-treatment of 9 to 12 potato tubers (kept in 0.1 mM aqueous TBZ solution for 6 min), the initial rate of  $\text{CO}_2$  release from intact tubers is increased as compared to control tubers (Fig. 1a);

maximal differences are visible ca. 2 h after treatment (Fig. 1b). This result was obtained in three independent experiments.

### Microscopic observations

After TBZ-treatment, the phellogen activity appeared to be enhanced; the periderm layer after 168 h storage seemed to be thicker in TBZ-treated wound surfaces as compared to stored, untreated controls (data not shown). There was no light-microscopically detectable indication for a suberization of TBZ-treated wound surfaces.

### Water permeabilities

I. Water losses of half potato tubers are not decreased after TBZ-treatment of tuber halves as indicated by identical weight losses of treated and untreated tuber halves over a time period of three days after cutting and TBZ-treatment (Table I).

II. The water permeabilities of untreated and TBZ-treated native tuber periderm pieces were investigated with isolated periderm layers. After tuber treatment (6 min in 0.1 mM TBZ solution or in water) and storage at 8 °C for 192 h, periderm discs

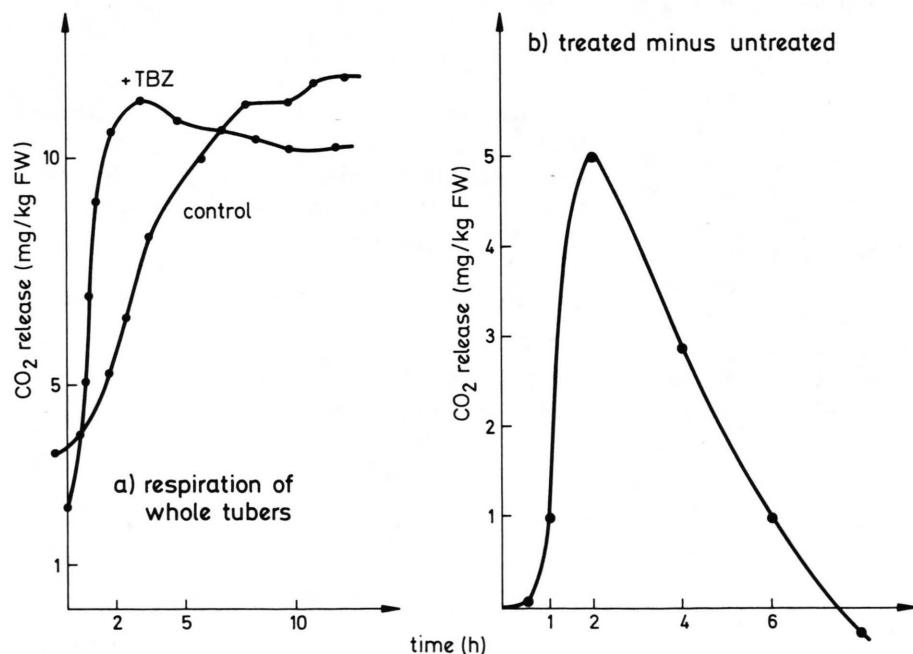


Fig. 1. Effect of TBZ-treatment on  $\text{CO}_2$  release by intact potato tubers. For experimental conditions see materials and methods. a)  $\text{CO}_2$ -release; b)  $\Delta\text{CO}_2$ -release: TBZ-treated minus control.

Table I. Comparison of the weight losses of water- and TBZ-treated potato tuber halves. Starting weights: 624.36 g (water treated controls) 637.60 g (TBZ-treated).

Time [days]	Weight losses			
	Water treated		TBZ-treated	
	[g]	[%]	[g]	[%]
1	4.85	16	5.04	16
2	7.56	24	7.68	24
3	10.30	33	10.38	33

The weighing experiment was done with 20 tuber halves and repeated 4 times with essentially identical results.

Table II. Comparison of water permeation through water-treated and TBZ-treated potato tuber periderm layers. For experimental conditions see Materials and Methods and text.

Treat- ment	Time [h]	$\bar{x} P_{tr}$	$s_{\bar{x}} \times t_{0.05}$	Interval of reliability
water	0	$2.289 \times 10^{-6}$	$9.84 \times 10^{-7}$	$1.31 \times 10^{-6} - 3.27 \times 10^{-6}$
water	192	$2.753 \times 10^{-6}$	$9.16 \times 10^{-7}$	$1.84 \times 10^{-6} - 3.67 \times 10^{-6}$
TBZ	192	$1.286 \times 10^{-6}$	$1.26 \times 10^{-6}$	$2.60 \times 10^{-8} - 2.55 \times 10^{-6}$

$\bar{x} P_{tr}$ , means of the permeation coefficients of transpiration.  $s_{\bar{x}}$ , average deviation from means.

were cut out from treated and control tubers. The periderm was isolated from the storage tissue by digestion with 4% pectinase and 0.4% cellulase at pH 4.0 and stored at 4 °C until use in 0.01 M MES buffer pH 6.0 containing 0.01 M CaCl<sub>2</sub>. Permeability coefficients were determined in the system water-periderm-vapour at at 25 °C and a rel. humidity of 78.5% (see ref. [19]). From the permeability coefficients and the water potential gradients between the two chambers, the transpiration rate was calculated. This transpiration rate indicates the amount of water (cm<sup>3</sup>) permeating per cm<sup>2</sup> surface within the time interval of 1 sec. For the selected experimental parameters ( $T = 25$  °C; rel. humidity 78.5%;  $\Delta \psi = -340$  bar) we obtained values of ca.  $4.5 \times 10^{-7}$  (cm<sup>3</sup>/cm<sup>2</sup> · s) corresponding to a transpiration rate of ca. 16.2 ml H<sub>2</sub>O per m<sup>2</sup> and sec. Periderm layers without "eyes" were used throughout these experiments. For each experiment, 2 to 3 periderm pieces from 5 different potato tubers were investigated. The results of the measurements of the transpiration rates are shown in Table II. TBZ-treatment reduces the average water permeability of potato periderm. Since the intervals of reliability are crossing, the

above numbers do not reflect a very high statistical significance, however.

#### Lipid peroxidation, measured as ethane production

As recently reported [10, 21–24] ethane production can be used as a reliable indicator of lipid peroxidation both *in vivo* and *in vitro* (see also refs. [6, 11, and 12]). Since potato tissue produces large

Table III. Effect of TBZ on ethane formation by potato discs, cut from potato slices. 15 discs with a diameter of approx. 5 mm were cut out from approx. 3 mm thick potato slices (cork borer). Only the outer region of the slices (ca. 1 to 3 mm distant from the periderm) was used. The discs (and also whole slices) were incubated at 22 °C for 2 h with or without 0.1 mM TBZ-solutions in buffers of the indicated pH-values. After the incubations in vessels sealed with serum rubber stoppers, 1 ml gas was withdrawn with a hypodermic syringe from the headspace of the incubations and analyzed gaschromatographically [10, 21].

Buffer	Experiment No.	Ethane formed [pmol/g FW]	
		control	TBZ-treated
0.2 M phosphate pH 7.2	1	3.8	7.1
	2	0.1	3.5
	3	3.1	9.4
0.2 M acetate pH 5.5	4	28.5	42.4
	5	13.9	21.5
	6	47.2	92.2

Table IV. Effect of TBZ on ethane formation by potato homogenates. For experimental conditions see Materials and Methods and Table III.

pH	Ethane formed [pmol/g homogenate]	
	control	+ 0.1 mM TBZ
7.2	803.5	376.5
5.5	1017.6	1055.3

The numbers represent means of 5 parallel determinations.

Table V. Effect of linolenic acid (lin) and TBZ on ethane formation by isolated potato mitochondria. Isolated mitochondria (refs. [20, 21]) were incubated for 1 h at 22 °C in 0.2 M acetate buffer pH 5.5.

Additions	Ethane formed [nmol/g dry weight]
none	0.5
0.1 mM TBZ	0.24
5 µmol lin	2.84
5 µmol lin + 0.1 mM TBZ	2.95

amounts of ethane after cutting or homogenization [21] we investigated the effect of TBZ-treatments of ethane formation by potato tissue.

As shown in Table III and Fig. 2 a, at both pH 7.2 and 5.5 TBZ stimulates ethane formation by potato discs whereas ethylene formation remains unchanged. This stimulation is not observed in potato homogenates (Table IV) or with isolated potato mitochondria (Fig. 2 b). The lack of effect of TBZ in homogenates or isolated mitochondria is probably not due to a substrate limitation (linolenic acid) in these preparations: addition of linolenic acid as the precursor of ethane [21] does not restore the TBZ effect (Table V). The above results indicate that the stimulation by TBZ of ethane formation in potato tissue seems not to be due to an enzyme activation or a detergent-like ef-

fect, but rather to a "phytoeffector" or "plant hormone-like" induction as further outlined below.

As shown in Fig. 3, different formulations of TBZ (SD 74 O 823 Ax, weakly acidic and SD 74 O 709, acidic) in buffers adjusting pH values of 7.2 and 5.1, respectively show stimulations of ethane production at 1% (Fig. 3 a) and 0.4% (Fig. 3 b).

The time course of ethane formation by potato slices shows an increase up to 6 h, where the increase of ethane in closed vessels follows a line known for "saturation type kinetics". If the potato discs are preincubated in air for different times and the vessels are closed for ethane accumulation for 1 h it becomes evident that maximal rates of TBZ-stimulated ethane production are observed ca. 2 h after treatment, suggesting an inductive process mediated by TBZ (Fig. 4).

Table VI. Effect of propylgallate on TBZ-enhanced ethane formation by potato slices. For experimental conditions see Table III.

Propylgallate added:	none	$10^{-6}$ M	$10^{-5}$ M	$10^{-4}$ M	$5 \times 10^{-4}$ M	$10^{-3}$ M	$5 \times 10^{-3}$ M
Ethane formed [pmol/g FW] pH 5.5	140	140	280	190	37	48	55

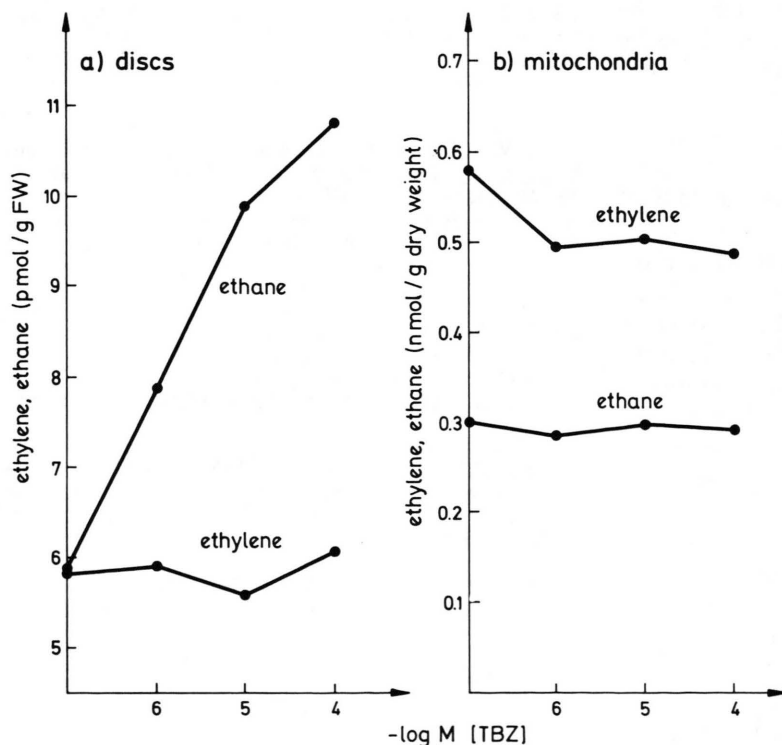


Fig. 2. Comparison of ethane and ethylene formation by potato discs and isolated mitochondria in dependence of different TBZ-concentrations. For experimental conditions see Table III.

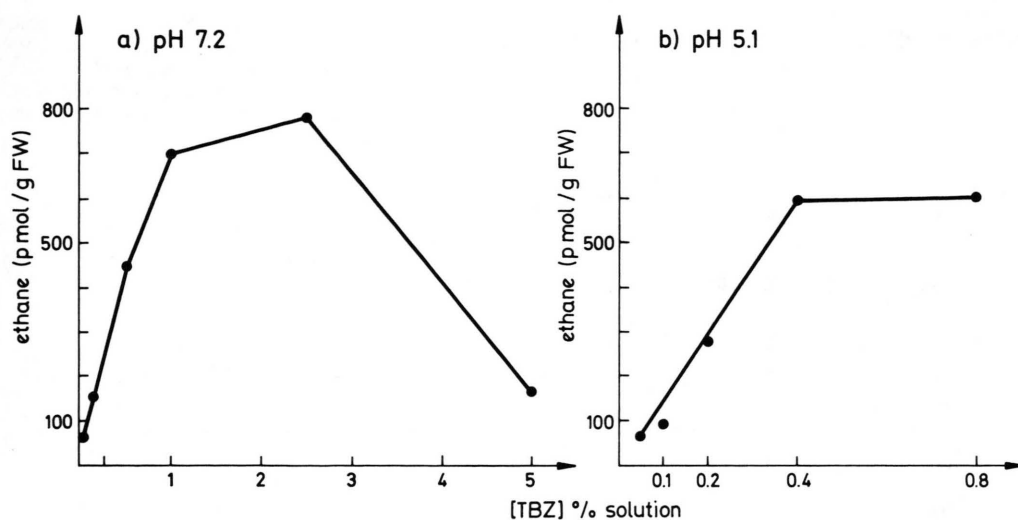


Fig. 3. Comparison of the effects of TBZ on ethane production by potato slices at pH 7.2 and pH 5.1. For experimental conditions see Table III. Formulation SD 74 O 832: slightly acidic, contains 8-hydroxyquinoline. Formulation SD 74 O 709: acidic, contains glycolic acid. pH 7.2: adjusted with phosphate buffer; pH 5.1: in acetate buffer, both 0.2 M.

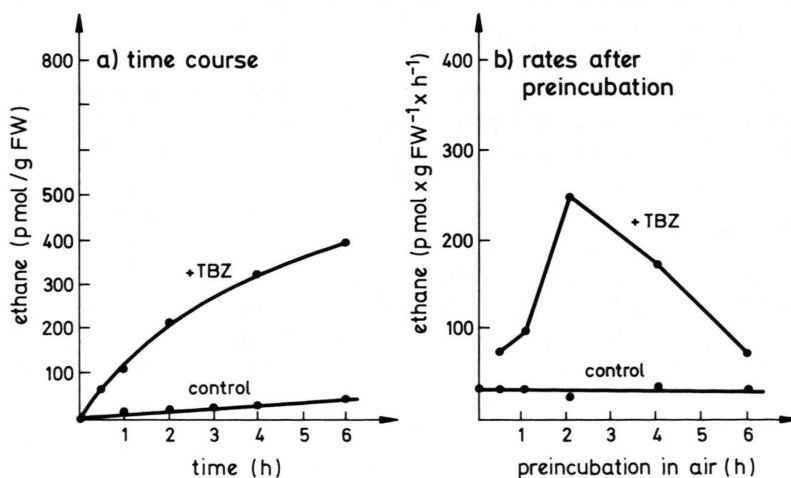
TBZ-induced ethane formation is further enhanced by low concentrations (0.01 to 0.1 mM) and inhibited by higher concentrations (0.5 to 5 mM) of the radical scavenger, propylgallate (Table VI).

The capacity of ethane production is not uniformly distributed throughout the potato tuber tissue, as shown in Table VII: discs from the outer region, obtained from slice-tissue in ca. 1 mm distance from the periderm, show the highest ethane production, followed by the center-disc. The inner (starch storage tissue) region, obtained from slice-tissue in ca.

Table VII. Ethane production by potato discs cut from different regions of potato slices. Discs with 5 mm diameter were cut out (cork borer) from the outer region (ca. 2 cm distant from the periderm) and from the center of the slices (3 mm thick). 15 discs were used for the incubations in 0.2 M acetate buffer pH 5.5 for 2 h. The numbers represent the mean of 5 different experiments:

	Discs from region:		
	outer	inner	center
Ethane formed [pmol/g FW]	1096	155	363

Fig. 4: Time course of TBZ-enhanced ethane formation by potato slices. Potato slices (3 mm thick) were treated with 0.4% TBZ for 6 min. Formulation SD 74 O 709 (acidic), adjusted to pH 4.8 was used. a) closed vessels; ethane was determined after the indicated times; b) after the indicated times of preincubation in air, the vessels were closed for 1 h for ethane accumulation.





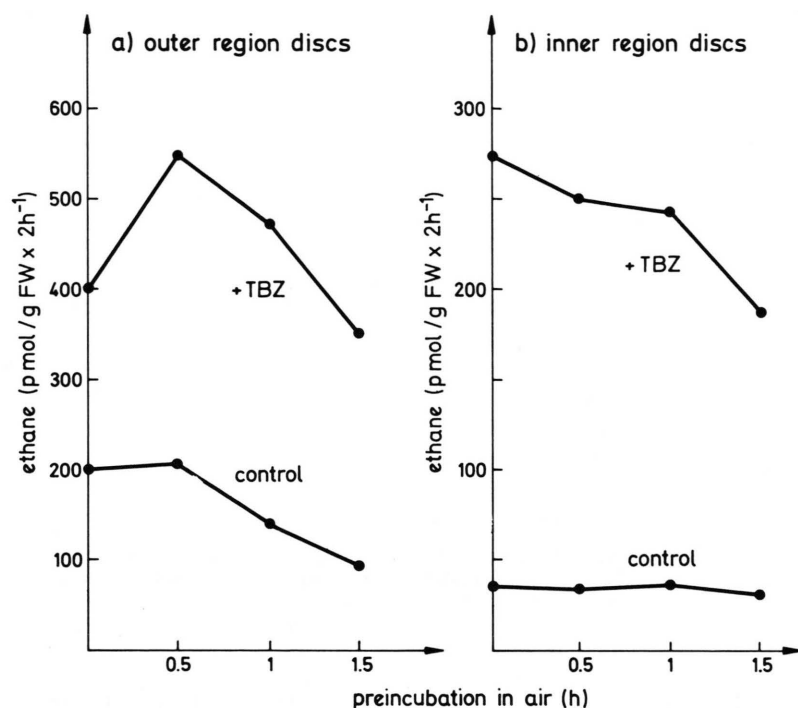


Fig. 5. Comparison of TBZ-enhanced ethane formation by potato discs from the outer region of potato slices with disc from the inner region of potato slices. For experimental conditions see Table III.

2 cm distance from the periderm, show the lowest ethane production.

Discs from either the inner or the outer region show also different response to TBZ-treatment: ethane formation by discs from the outer region is stimulated by ca. a factor of 2 while ethane formation by discs from the inner region is stimulated by a factor of ca. 8 to 10. The ethane production by discs from the inner region in the presence of TBZ approach the rates measured for discs from the outer region in the absence of TBZ. The effect of preincubation in air in the presence of TBZ is also different in discs from the inner and from the outer region: the induction-effect of ethane formation after preincubation in air in the presence of TBZ is only expressed in the outer region (Fig. 5).

## Discussion

The systemic fungicide Thiabendazole (TBZ), besides its antifungal activities might also exhibit preservative effects on stored potato tubers by decreasing the water permeability of the potato periderm (Table II). Besides this, ethane formation as a characteristic phenomenon accompanying lipid peroxidation [8–12, 21] is also increased in potato

tissue after TBZ-treatment. Since only potato tissue, and neither potato homogenates nor isolated mitochondria show this TBZ stimulation, this reaction does not seem to be due to a stimulation of ethane producing enzyme(s) but rather to an activity of TBZ comparable to certain “phytoeffectors” or “plant hormone-like” factors. This conclusion is supported by the finding that after a 2 h preincubation in air the highest rates of ethane formation seem to be induced by TBZ-treatment (Fig. 4).

A faster increase of initial CO<sub>2</sub> release is also observed after TBZ-treatment of whole potato tubers (Fig. 1).

It is known that freshly wounded or sliced potato tissue exhibits increased respiration at the expense of predominately lipids as substrates, derived from membrane degradation under the catalysis of acylhydrolases [25–27] and subsequent  $\alpha$ -oxidation or peroxidation by lipoxygenase(s) [28, 1]. Our findings support the view that TBZ-treatment activates a process connected with the mobilization and/or the oxidation of unsaturated fatty acids in potato tubers after wounding. Oxygen activation in context with lipid metabolism seems to play an important role in wound healing of plants:  $\omega$ -hydroxylation plays an important role in the conversion of fatty acids into

suberin of the wound periderm [1, 29], and "singlet oxygen-like factors" [30] besides other highly reactive derivatives [17] of potential toxicity to infesting parasites (*e.g.* aldehydes, hydroperoxides, alkoxy- and peroxy-radicals) may prevent colonization of the wounds. Lipid peroxidation has been reported to be involved in the natural "hypersensitive" reaction, responsible for the formation of necrotic lesions [31] and for leaf yellowing [32] after viral infections.

We thus might conclude from the presented results that TBZ-treatment of wounded potato tissue induces reactions similar to the hypersensitive re-

sponse which eventually might contribute in preventing infections of wounded potato tissue and also possibly reduce water losses.

#### Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. We are also greatly indebted to Dr. A. Eder and Prof. Dr. J. Schönherr for performing the CO<sub>2</sub> measurements, for the microscopic observations and for the determinations of water permeabilities.

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