Physiological Plant Pathology (1975) **5**, 45–50

Increased levels of cytokinin and indoleacetic acid in peach leaves infected with *Taphrina deformans*

I. Sziráki, E. Balázs and Z. Király

Department of Pathophysiology,
Research Institute for Plant Protection, Budapest, Hungary

(*Accepted for publication February 1974*)

Neoplastic tissues of curled peach leaves by *Taphrina deformans* exhibit increased cytokinin activity and contain increased levels of indole-3-acetic acid and tryptophan compared with healthy leaf tissues. Three, chromatographically similar, cytokinins are present both in healthy and infected leaves, but those in infected leaves are more active in the soybean callus test. Diseased leaf tissues contain an additional cytokinin which is not present in extracts from healthy leaves.

INTRODUCTION

As a result of infection of young peach leaves by the fungus *Taphrina deformans* neoplastic growth is initiated: the infected leaf cells increase in size and number and leaf form and structure change markedly. As a rule, the infected peach leaves become curled and increase in thickness.

There are several brief reports on the production of substances by the fungus having the properties of an auxin [*1*, *4*, *9*, *12*, *16*]. Sommer [*16*] has shown that a stimulation of cell division occurs in Alaska pea internodal sections by applying a substance(s) extractable from culture filtrates of *Taphrina deformans*. From the transfer ribonucleic acids of another *Taphrina* species (*T. betulina*) Kavoor & Klämbt [*7*] prepared cytokinins.

In this paper we demonstrate that purified extracts of diseased peach leaves show increased cytokinin activity compared with healthy leaves, and also the level of indoleacetic acid is slightly increased as a result of infection.

MATERIALS AND METHODS

Plant material

Peach leaves (*Persica vulgaris* Mill. cv. Elberta) naturally infected by *Taphrina deformans* (Berk.) Tul. and uninfected healthy leaves were harvested in early May from a few trees. The development of the disease was in the 3-week-stage following the appearance of the first symptoms.

Cytokinin extraction and bioassay

Leaf tissues were rinsed with tap water and blotted dry. Ten gram fresh weight samples were removed for dry weight determinations. Thirty-five gram fresh weight samples were macerated in a Waring Blendor with 70 ml 0·15 M-phosphate

buffer, pH 6·5 and then extracted with 250 ml of 96% ethanol for 12 h at 4 °C. The ethanol-tissue breis were filtered through Whatman No. 1 paper and then centrifuged at 5000 *g* for 15 min at 4 °C. The ethanolic extracts were concentrated to the aqueous phase under vacuum (approximately 70 ml). Samples were then centrifuged at 10 000 *g* for 15 min. The supernatants were adjusted to pH 9·0 with 1 N-NaOH and extracted with three equal volumes of petroleum ether (b.p. 60 to 80 °C) in separating funnels. The aqueous fraction was adjusted to pH 2·5 with 1 N-HCl and shaken with 3 equal volumes of ethyl acetate. Then the aqueous fraction was concentrated to 30 ml under vacuum at 35 °C, and passed through a column containing 100 ml of Dowex 50 W-X8 (H^+, 100 to 200 mesh) ion exchange resin. The column was washed with 100 ml of distilled water and then the active material was eluted with 75 ml of 4 N-NH_4OH; the ammonia was removed under vacuum. The water residue was adjusted to pH 7·8 with 1 N-NaOH and extracted in separatory funnels with 3 equal volumes of water saturated *n*-butanol, the active material going into the alcohol phase. The butanol layer was taken to dryness under vacuum and washed 5 times with distilled water and evaporated. Most of the butanol was removed by evaporation and the residue was dissolved in 90% ethanol, streaked onto Whatman No. 1 chromatography paper, and separated with *t*-butanol–concentrated NH_4OH–water (3 : 1 : 1, v/v/v). After drying, the chromatograms were divided into 10 equal strips and tested for cytokinin activity in the soybean callus bioassay.

Twenty millilitres of tissue culture basal medium [*10*] supplemented with agar and portions of chromatograms corresponding to specific R_F regions were added to each 50 ml Erlenmeyer flask. After adjusting the pH to 5·8 with NaOH, the medium was sterilized by heating twice at 100 °C for 45 min. A 1-day interval was allowed between heat treatments. Three callus explants (35 mg each) were transferred to each flask. Cultures were maintained at 27 °C for 28 days and then weighed.

For extraction and purification of cytokinins, the procedures of Király *et al.* [*6*], Miller [*11*] and van Staden *et al.* [*17*] were followed. The cytokinin bioassay with the soybean callus test was carried out according to the method of Miller [*10*] modified by Krasnuk *et al.* [*8*]. Data of tables and figures show the means for five experiments in three replicate samples. All the data are expressed on a dry weight basis, because the water content of infected, neoplastic tissue is always very high. Healthy leaves contained 31·22% dry material, while the infected ones only 17·83%.

Extraction and assay of indole compounds

Healthy and infected leaves were rinsed in tap water and blotted dry. Thirty-five gram fresh weight samples were extracted with 350 ml of 60% ethanol in a Waring Blendor for 10 min. The ethanol-tissue breis were centrifuged at 5000 *g* for 10 min at 4 °C. The supernatants were concentrated to 100 ml under vacuum at 40 °C. The concentrated extracts were adjusted to pH 2·0 with 1 N-HCl, and then extracted with three 50-ml volumes of diethyl ether in separatory funnels. The ether extracts were combined and shaken with three 50-ml volumes of 5% $NaHCO_3$ (w/v). The bicarbonate fractions were combined and adjusted to pH 2·0 with HCl, then re-extracted with three 50-ml volumes of diethyl ether. The ether fractions were

concentrated to dryness under an air stream. The residues were dissolved in 90% ethanol and line-loaded onto Whatman No. 1 chromatography paper. The chromatograms were developed with a solvent of isopropanol–concentrated NH_4OH–water (10 : 1 : 1, v/v/v) or with chloroform–ethyl acetate–formic acid (5 : 4 : 1, v/v/v). For identification of the separated indole compounds authentic samples were spotted as markers. The separated indole compounds on developed chromatograms were located and characterized by applying the Salkowski's reagent, and measured spectrophotometrically. The R_F regions from chromatograms corresponding to indole-3-acetic acid and tryptophan were eluted with ethanol and the absorbance of eluates were measured at 280 nm using a Unicam SP 800 spectrophotometer, and compared to known quantities of authentic compounds.

Regarding extraction of indole compounds, we slightly modified the procedure of Wiese & DeVay [*18*]. The spectrophotometric measurements were carried out according to Fletcher & Zalik [*3*]. The experiments were repeated in five cases. Data show the means for the five experiments, and expressed on a dry weight basis.

RESULTS AND DISCUSSION

In the course of extraction the u.v. absorption spectra of the isolated materials in the butanol fraction of extracts were measured between 210 and 320 nm (Fig. 1). The absorption maximum of the unchromatographed active material both from healthy and infected leaves was identical to that of the purine compounds [*8*, *13*]. It is also seen from the spectra that the optical density of the purine compounds extracted from the infected tissues is two times greater than that of the healthy tissues.

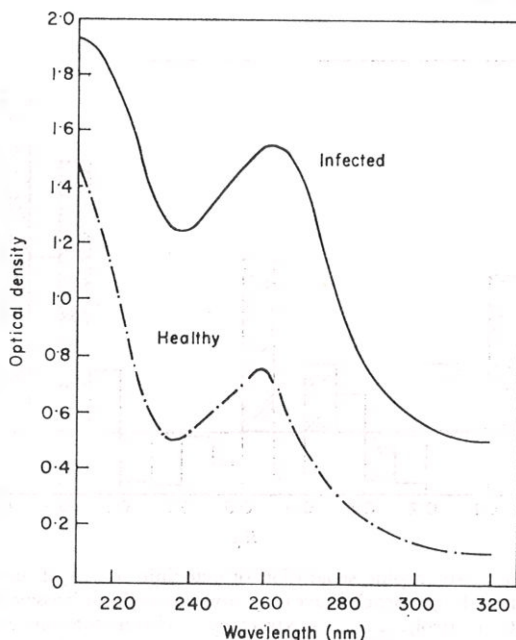

FIG. 1. Ultraviolet spectra of the unchromatographed active material from healthy and infected peach leaves, under neutral condition.

The extracts were further separated by paper chromatography. Then the chromatograms were divided into ten R_F regions, and the individual regions were incorporated into soybean medium for bioassaying the cytokinin activities. Following a 28-day incubation period, the fresh callus yields were weighed (Table 1). It is seen from this Table as well as from Fig. 2 that the isolated fraction represented a mixture of active materials. Maximum activity of the active material was at R_F 0·8 to 0·9 with both the healthy and infected leaves. In fact, the analysis of chromatograms revealed four peaks of cytokinin activity: at R_F regions 0·0 to 0·1, 0·4 to 0·5 and 0·7 to 0·8 in addition to the most active 0·8 to 0·9 region. The most significant

TABLE 1

Soybean callus bioassay of chromatograms of purified extracts of peach leaves, healthy and infected by T. deformans[a]

	Callus yield (mg/flask)									
R_F:	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0
Healthy	294	234	185	264	184	202	180	290	416	212
Infected	376**[b]	297	188	297	391**	219	188	483**	571*[c]	250
Control (without extract)	231									

[a] Purified extracts were chromatographed on Whatman No. 1 paper in *t*-butanol–conc. NH_4OH–water (3 : 1 : 1, v/v/v). Cultures were grown on basal medium supplemented with portions of chromatograms corresponding to R_F regions and maintained at 27 °C for 28 days. Average values of five experiments in three replicate samples.

[b] Statistical significance: ** $P = 0{\cdot}01$.

[c] Statistical significance: * $P = 0{\cdot}05$.

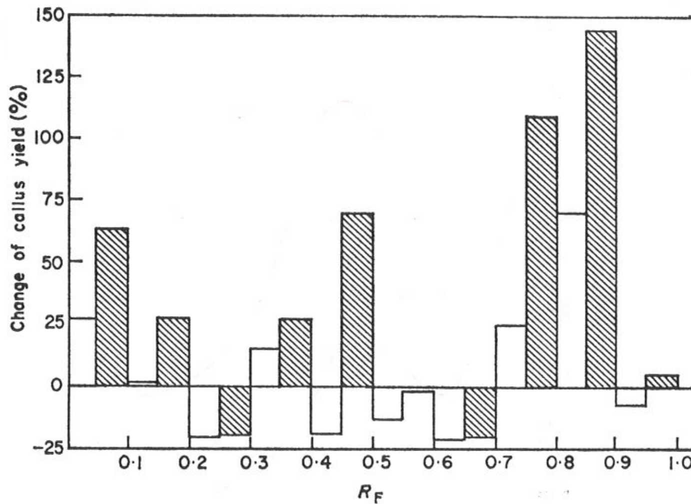

FIG. 2. Paper chromatographic separation of cytokinin activity from purified extracts of healthy (□) and infected (■) peach leaves by soybean callus bioassay (per cent change of callus yield over water control). Purified extracts were chromatographed on Whatman No. 1 paper in *t*-butanol–conc. NH_4OH–water (3 : 1 : 1, v/v/v). Cultures were grown on basal medium supplemented with portions of chromatograms corresponding to R_F regions. Cultures maintained at 27 °C for 28 days.

difference in activity between the healthy and infected leaves was found at R_F 0·4 to 0·5. When healthy leaves contained cytokinin activity at a given region, the infected leaves always exhibited higher activity at the corresponding region. The data obtained suggest that at least three active components were present in the isolated products, which were chromatographically similar both in healthy and infected leaves; those from the infected leaves being always more active (R_F regions 0·0 to 0·1, 0·7 to 0·8, 0·8 to 0·9). As a result of infection leaves contained a cytokinin (region 0·4 to 0·5) which was not found among the active components of healthy leaves.

Identifying indole compounds, we applied in the chromatographic studies authentic samples as follows: indole-3-acetic acid, indoleacetonitrile, 3-indoleacetamide, 5-hydroxy-indol-3-yl-acetic acid, indole-propionic acid, tryptophan. Only indole-3-acetic acid and tryptophan were located and characterized from plant materials by applying the Salkowski's reagent and the authentic indole compounds. In addition, two unknown spots were located on the chromatograms of both healthy and infected leaves; however, it was not possible to characterize them by using the above-mentioned authentic compounds available to us.

TABLE 2

Spectrophotometric estimation of contents of indole-3-acetic acid and tryptophan in healthy and Taphrina-*infected peach leaves on a dry weight basis*[a]

Compounds	µg/kg dry weight Healthy	Infected
Indole-3-acetic acid	107	194**[b]
Tryptophan	23	35**

[a] Compounds were located by spraying a portion of the chromatographic paper (Whatman No. 1) with the Salkowski's reagent. The corresponding R_F regions from unsprayed portion of the paper were eluted with ethanol and the absorbance of the eluate at 280 nm was measured spectrophotometrically and compared to known quantities of authentic compounds. Average values of five experiments.

[b] Statistical significance: ** $P = 0{\cdot}01$.

As is seen in Table 2 infected, neoplastic leaf tissues contained more indole-3-acetic acid than the healthy ones, and the content of the most important auxin precursor, the tryptophan, was also on a higher level in diseased tissues. The absorption values of both compounds at 280 nm were measured spectrophotometrically in ethanol extracts of paper strips of chromatograms corresponding to the R_F regions of indole-3-acetic acid and tryptophan. The change in concentration of tryptophan (54·5% increase) seems to be slight at the first glance. However, the seemingly slight increase in tryptophan content occurs at a time when increased protein synthesis, rather than breakdown, is characteristic for the diseased host tissues. It is probable that the increase in the auxin content of diseased tissues is the result of the production of this hormone by the pathogen. The increase of indole-3-acetic acid and its precursor in diseased peach leaves is similar to that shown by Kiermeyer [*5*] and Fehrmann [*2*] in other host–pathogen complexes [cf. *14*, *15*]. It remains to be shown whether the cytokinin increase in infected leaves is of pathogen or host origin, or both.

One can conclude from these data only that neoplastic tissues of peach leaves infected by *T. deformans* show increased cytokinin activity and increased indole-3-acetic acid and tryptophan content as compared to healthy leaf tissues. Three, chromatographically similar, cytokinins are present both in healthy and infected leaves, but those in infected leaves are more active in the soybean callus test. Infected tissues contain an additional cytokinin which is not present in extracts from healthy leaves. It is very probable that the striking morphological changes in infected peach leaves are in a cause-and-effect relation with the hormonal imbalance in tissues. Increased cell growth and water uptake may be related to the increased auxin content and the stimulated cell division and abnormal growth pattern of infected leaves could be a consequence of increased cytokinin activity.

We are grateful to Noémi Kiss for her technical assistance and Dr István Varga for providing some of the experimental materials.

REFERENCES

1. Grady, E. E. & Wolf, F. T. (1959). The production of indole-acetic acid by *Taphrina deformans* and *Dibotryon morbosum*. *Physiologia Plantarum* **12**, 526–533.
2. Fehrmann, H. (1965). Zum Auxin-Stoffwechsel phytophthorakranker Kartoffelknollen. *Naturwissenschaften* **52**, 481.
3. Fletcher, R. A. & Zalik, S. (1963). Quantitative spectrophotometric determination of indolyl-3-acetic acid. *Nature* **199**, 903–904.
4. Hirata, S. (1957). Studies on the phytohormone in the malformed portion of the diseased plants. 3. *Annals of the Phytopathological Society of Japan* **23**, 155–158.
5. Kiermeyer, O. (1958). Papierchoromatographische Untersuchungen über den Wuchsstoffgehalt von *Capsella bursa-pastoris* nach Infektion mit *Albugo candida* und *Peronospora parasitica*. *Osterreichische Botanische Zeitschrift* **105**, 518–528.
6. Király, Z., El Hammady, M. & Pozsár, B. I. (1967). Increased cytokinin activity of rust-infected bean and broad bean leaves. *Phytopathology* **57**, 93–94.
7. Kovoor, R. & Klämbt, D. (1968). Cytokinins in transfer ribonucleic acids. In *Biochemistry and Physiology of Plant Growth Substances*, Ed. by F. Wightman & G. Setterfield, pp. 57–60. Runge Press Ltd, Ottawa.
8. Krasnuk, M., Witham, F. H. & Tegley, J. R. (1971). Cytokinins extracted from Pinto bean fruit. *Plant Physiology* **48**, 320–324.
9. Link, G. K. K., Wilcox, H. W. & Eggers, V. (1938). Growth tests with extracts of *Erwinia amylovora*, *Phytomonas rhizogenes*, *Taphrina cerasi*, *T. deformans* and *Ustilago zeae*. *Phytopathology* **28**, 15.
10. Miller, C. D. (1965). Evidence for the natural occurrence of zeatin and derivatives: compounds from maize which promote cell division. *Proceedings of the National Academy of Sciences* (*Washington*) **54**, 1052–1058.
11. Miller, C. D. (1967). Zeatin and zeatin riboside from a mycorrhizal fungus. *Science* **157**, 1055–1056.
12. Perley, J. E. & Stove, B. B. (1966). On the ability of *Taphrina deformans* to produce indoleacetic acid from tryptophan by way of tryptamine. *Plant Physiology* **41**, 234–237.
13. Reddy, M. N. & Williams, P. H. (1970). Cytokinin activity in *Plasmodiophora brassicae*-infected cabbage tissue cultures. *Phytopathology* **60**, 1463–1465.
14. Sequeira, L. (1963). Growth regulators in plant disease. *Annual Review of Phytopathology* **1**, 5–30.
15. Sequeira, L. (1973). Hormone metabolism in diseased plants. *Annual Review of Plant Physiology* **24**, 353–380.
16. Sommer, N. F. (1961). Production by *Taphrina deformans* of substance stimulating cell elongation and division. *Physiologia Plantarum* **14**, 460–469.
17. van Staden, J., Webb, D. P. & Wareing, P. F. (1972). The effect of stratification on endogenous cytokinin levels in seeds of *Acer saccharum*. *Planta* **104**, 110–114.
18. Wiese, M. V. & DeVay, J. D. (1970). Growth regulator changes in cotton associated with defoliation caused by *Verticillium albo-atrum*. *Plant Physiology* **45**, 304–309.