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The effect of salicylic acid and acetylsalicylic acid on red pigment formation in mechanically wounded scales of *Hippeastrum* x *hybr.* hort. and on the growth and development of *Phoma narcissi*

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Summary

Various organs of *Hippeastrum* infected by *Phoma narcissi*, infested with mite, *Steneotarsonemus laticeps* or mechanically wounded, produce red pigment on the surface of injured tissues. The aim of the present work was to study the effect of salicylic (SA) and acetylsalicylic acids (ASA) (inhibitors of biosynthesis of jasmonates and ethylene) on red pigment formation in wounded scales of bulbs of *Hippeastrum* and on the mycelium growth of *P. narcissi in vivo* and *in vitro*. SA and ASA at a concentration of 1 and 2 mM partially inhibited the formation of red pigment in wounded scales, first of all in first 2 days after treatment. The growth and development of *P. narcissi* on basal plate and scales of longitudinally cut *Hippeastrum* bulb treated with SA and ASA (1 and 2 mM) was similar as in control. SA (50 $\mu g \cdot cm^{-3}$) and ASA (250 $\mu g \cdot cm^{-3}$) inhibited the mycelium growth of *P. narcissi* on PDA medium, and concentration of 1000 $\mu g \cdot cm^{-3}$ of both almost totally inhibited the mycelium growth of the pathogen. Inhibitory effect of SA and ASA on the formation of red pigment in wounded scales of *Hippeastrum* may be caused by lowered biosynthesis and accumulation of jasmonates.

Key words: *Hippeastrum*, *Phoma narcissi*, salicylic acid, acetylsalicylic acid, red pigment, wounding, mycelium growth

INTRODUCTION

Various organs of *Hippeastrum* infected by *Phoma narcissi* infested by mite, *Steneotarsonemus laticeps* or mechanically wounded produce red pigment on the surface of injured tissues (Saniewska 1998). After mechanical injuries, when intensive reddish colouration is developed on scales and basal plate of *Hippeastrum*, the fungus *P. narcissi* does not induce disease symptoms (Saniewska 1992). The chemical nature of red pigment evoked by mechanical damage is not fully determined but probably it belongs to class of oxidized flavan(s) (Wink and Lehmann 1996; Saniewska and Budzianowski 1997). Hormonal control of the induction of the red pigment and its biosynthesis mechanism in wounded tissue of *Hippeastrum* is unknown.

Rapid increase in endogenous levels of jasmonates, mainly jasmonic acid (JA), was observed after mechanical wounding of different plant organs, pathogen infection, insect attack, as well as under osmotic stress conditions (Creelman and Mullet 1995). Wounding induces an expression of defense-related genes involved in plant responses to a pathogen or insect attack, what causes the accumulation of specific proteins and secondary metabolites (Blechert et al. 1995; Saniewski and Czapski 1999).

It is well known that salicylic acid (SA) and acetylsalicylic acid (ASA) inhibit biosynthesis of jasmonic acid by blocking the conversion of 13S-hydroperoxy linolenic acid to 12-oxo-phytodienoic acid (Peña-Cortes et al. 1993). SA and ASA inhibit also synthesis of proteinase inhibitor induced by wounding, or oligouronides, linolenic acid, JA, and systemin (Doherty et al. 1988; Peña-Cortes et al. 1993; Doares et al. 1995). It has been shown that SA inhibits ethylene production in numerous plant species (Leslie and Romani 1989).

The aim of the present work was to study the effect of salicylic acid and acetylsalicylic acid, on red pigment formation in wounded scales of *Hippeastrum* bulbs and on the growth and development of *Phoma narcissi in vivo* and *in vitro*.

MATERIAL AND METHODS

Hippeastrum x hybr. hort. 'Jan' was used for the experiments. The stock culture of *Phoma narcissi* was maintained on potato-dextrose-agar (PDA) slants at 25°C in the dark. Salicylic acid (SA) and acetylsalicylic acid (ASA) were purchased from Sigma-Aldrich Chemicals.

The effect of salicylic acid and acetylsalicylic acids on the red pigment accumulation in wounded scales of *Hippeastrum*. The scales were cut into small pieces, (dimension ca. 4 x 4 mm) and dipped in SA and ASA at concentration of 1, 2 and 3 mM during 2 h or in water (control). Then the tissue samples were drained off and incubated in closed Petri dishes at 20–25°C placed in chamber with maintained high humidity. Analyses of red pigment level were carried out after 34.58 and 83 h of incubation. The pigment was extracted with 90% methanol and the extracts absorbance was measured at 495 nm.

The development of *P. narcissi* on the scales and basal plate tissues treated with salicylic or acetylsalicylic acid. The bulbs of *Hippeastrum* were vertically cut into halves. The basal plate tissues and scales were dipped directly after cutting in 1 mM SA, or in ASA at concentration 1, 2 and 3 mM during 2 hr and then inoculated with a 5-day-old culture of *P. narcissi* immediately or 4 days after cutting of the bulbs.

***In vitro* growth of *P. narcissi* in the presence of salicylic or acetylsalicylic acid**. SA and ASA at final concentration 25, 50, 100, 250, 500, 1000 and 1250 $\mu g \cdot cm^{-3}$in medium were used. These compounds were dissolved in distilled and sterilized water and added to potato-dextrose-agar (PDA-Merck) after sterilization at temperature of about 50°C using 0.22 μm filter unit (Millex-GV). Five mm diameter plugs were taken from 7-day-old culture of *P. narcissi*, and placed in the middle of 90 mm Petri dishes containing PDA medium, supplemented earlier with tested compounds. Control plates contained the culture growing on pure PDA, without any additions. Five Petri dishes were used as an experimental unit and the trial was repeated twice. Incubation was conducted in darkness at 25°C. After 6 days of the process a diameter of fungal colonies was measured in two perpendicular directions.

The data was subjected to an analysis of variance and Duncan's multiple range test at 5% of significance was used for means separation.

RESULTS AND DISCUSSION

Salicylic acid (SA) at a concentration of 1 mM and acetylsalicylic acid (ASA) at a concentration of 1 and 2 mM substantially inhibited the formation of red pigment in wounded scales, mostly in first 2 days after treatment (Table 1). It is possible that the inhibitory effect of SA and ASA on red pigment formation in wounded scales of *Hippeastrum* bulbs may be an effect of lowered biosynthesis of jasmonates.

Table 1

The effect of salicylic acid and acetylsalicylic acid on red pigment level in wounded scales of *Hippeastrum* bulbs

Treatments	Absorbance at 495 nm after incubation time (hours)		
	34	58	83
Control (water)	0.112 a	0.291 a	0.555 a
SA 1 mM	0.031 b	0.085 c	0.306 c
ASA 1 mM	0.031 b	0.188 b	0.485 ab
ASA 2 mM	0.022 b	0.143 b	0.407 b

Means in columns followed by the same letters are not significantly different at 5% level

The growth and development of *P. narcissi* on basal plate and scales of vertically cut *Hippeastrum* bulb and immediately treated with SA and ASA (1 and 2 mM) were similar as in control (Table 2). The disease symptoms caused by *P. narcissi* inoculated on basal plate and scales of *Hippeastrum* bulbs contained red pigment (4 days after cutting) were much lower than in appropriate tissues infested with the pathogen right after cutting of bulbs (Table 3, Photo 1). Treatment with SA and ASA immediately after cutting of *Hippeastrum* bulbs and inoculation with *P. narcissi* after reddish colouration (4 days after cutting) caused similar disease symptoms on scales and basal plate as in the untreated cut bulbs.

Table 2

The effect of salicylic acid and acetylsalicylic acid on development of *Phoma narcissi* disease in basal plate and scales of *Hippeastrum* bulbs (plant tissues were inoculated immediately after cutting)

Treatments	Length of necrosis (mm) after days				Depth of necrosis (mm) 7 days after inoculation	
	3		6		basal plate	scales
	basal plate	scales	basal plate	scales		
Control	15.0 a	20.0 a	20.0 a	27.5 ab	10.0 a	14.2 d
SA 1 mM	13.3 a	17.5 a	20.0 a	25.0 a	7.5 a	11.0 c
ASA 1 mM	15.0 a	20.0 a	20.0 a	25.0 a	9.0 a	10.0 bc
ASA 2 mM	15.0 a	18.7 a	25.0 b	27.5 ab	9.2 a	9.3 b
ASA 3 mM	13.0 a	17.5 a	20.0 a	30.0 b	7.5 a	7.5 a

Means in columns followed by the same letters are not significantly different at 5 % level

Table 3

The effect of salicylic acid and acetylsalicylic acid on development of *Phoma narcissi* in basal plate and scales of *Hippeastrum* bulb (plant tissues were inoculated 4 days after cutting)

Treatments	Length of necrosis (mm) after days of incubation				Depth of necrosis (mm) 7 days after incubation	
	3		6		basal plate	scales
	basal plate	scales	basal plate	scales		
Inoculation carried out immediately after cutting: Control	10.5 b	20.0 d	13.7 c	24.5 c	10.0 c	12.7 d
Inoculation carried out 4 days after cutting: Control	1.25 a	6.5 c	3.5 a	11.5 a	2.5 a	3.5 a
SA 1 mM	1.25 a	1.7 b	6.0 a	10.2 a	3.0 a	5.0 ab
ASA 1 mM	1.25 a	7.0 c	7.5 a	14.8 b	4.0 b	6.5 b
ASA 2 mM	3.0 a	7.1 c	9.0 b	16.3 b	4.5 b	8.1 c
ASA 3 mM	2.5 a	0.0 a	6.7 ab	10.3 a	3.0 a	6.0 b

Means in columns followed by the same letters are not significantly different at 5 % level

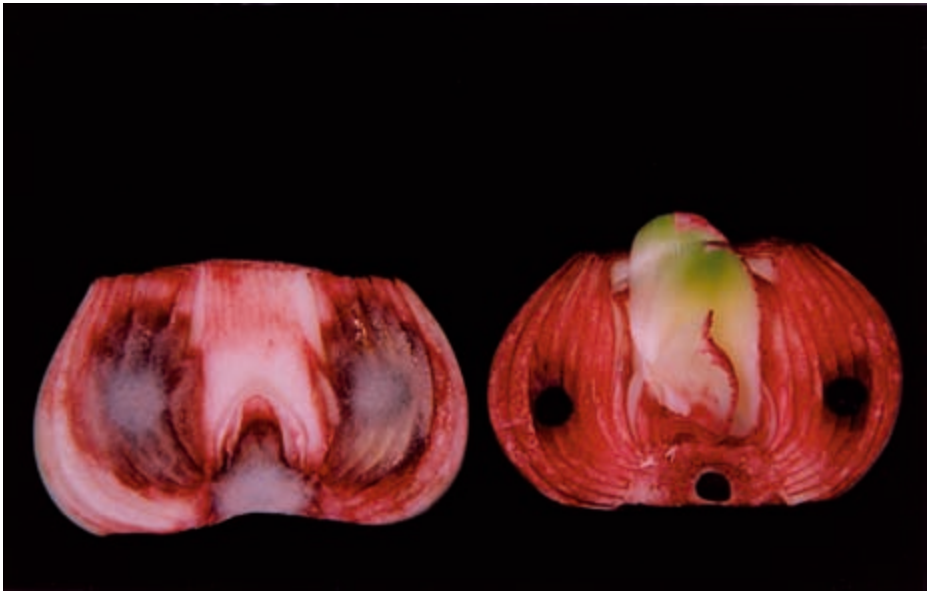

Photo 1 Development of *Phoma narcissi* on basal plate and scales of *Hippeastrum* bulb inoculated directly after cutting (on the left) – development of mycelium can be observed and 4 days later after reddish colouration (on the right) – small disease symptoms can be observed

Salicylic and acetylsalicylic acids showed strong inhibitory effect on the mycelium growth of *P. narcissi*. After 6 days of incubation with SA (50 μg·cm^{-3}) and ASA (250 μg·cm^{-3}) growth of *P. narcissi* was decreased ca. 40% in comparison to the control. Both compounds at high concentration (1000 μg·cm^{-3}) almost totally inhibited the mycelium growth of the pathogen (Fig. 1A,B).

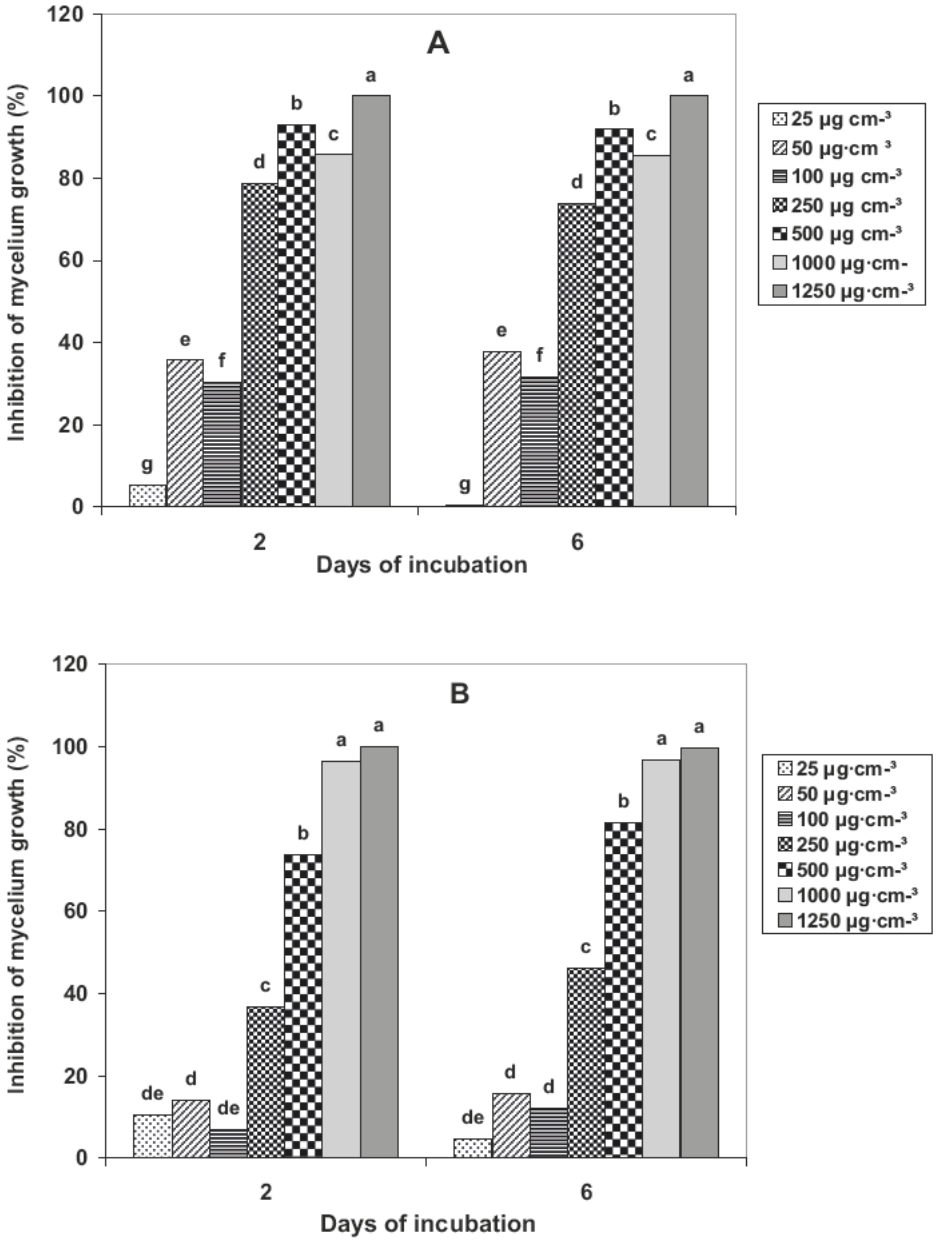

Fig. 1. The effect of salicylic acid (A) and acetylsalicylic acid (B) on the mycelium growth of *Phoma narcissi*

During studies *in vitro* Amborabé et al. (2002) showed that fungistatic and fungicidal properties against *Eutypa lata* depended on salicylic acid concentration: the fungicidal effect was found at 2 mM or higher concentrations of SA. According to the studies SA caused different modifications in the structural organization of mycelium *E. lata*, as for instance in cell wall, mitochondria, vacuole and nucleus.

Salicylic acid (SA) is assumed to be an endogenous signal molecule responsible for inducing the systemic acquired resistance (SAR) reactions in plants and exogenous application of SA can make a plant more resistant to a fungal infection (Raskin 1992; Malamy and Klessig 1992; Horvath and Chua 1994; Vernooij et al. 1994; Vallad and Goodman 2004). Exogenous SA treatment mimics certain aspects of pathogen infection, namely the ability to activate pathogenesis-related (PR) proteins (Malamy and Klessig 1992). Pretreatment of pea plants with salicylic acid and chlorosalicylic acid enhanced the induction of superoxide dismutase (SOD) and peroxidase after subsequent infection with *Ascochyta pisi* (Kuźniak et al. 1995).

In rare cases, SA probably suppresses certain resistance mechanism by blocking the biosynthesis of jasmonates which control specific proteins and secondary metabolites formation (Ponchet et al. 1983; Doherty et al. 1988; Niemann and Baayen 1989; Peña-Cortes et al. 1993).

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Wpływ kwasu salicylowego i kwasu acetylosalicylowego na tworzenie się czerwonego barwnika w mechanicznie uszkodzonych łuskach *Hippeastrum* x *hybr*. hort. i na wzrost i rozwój *Phoma narcissi*

Streszczenie

Różne organy *Hippeastrum* zainfekowane przez grzyb *Phoma narcissi*, w następstwie żerowania roztocza *Steneotarsonemus laticeps* lub uszkodzone mechanicznie, wytwarzają na powierzchni uszkodzonych tkanek czerwony barwnik. Celem badań było poznanie wpływu kwasów salicylowego (SA) i acetylosalicylowego (ASA) (inhibitorów biosyntezy jasmonianów i biosyntezy etylenu) na tworzenie się czerwonego barwnika w uszkodzonych łuskach cebuli *Hippeastrum* i wzrost grzybni *P. narcissi*. Kwas salicylowy i kwas acetylosalicylowy wykazały hamujący wpływ na tworzenie się czerwonego barwnika w uszkodzonych łuskach, zwłaszcza w pierwszych 2 dniach po traktowaniu. Wzrost i rozwój grzybni *P. narcissi* na piętce i łuskach przekrojonych wzdłuż cebuli *Hippeastrum* i traktowanych SA i ASA w stężeniu 1 i 2 mM był podobny jak w kontroli. Kwas salicylowy w stężeniu 50 $\mu g \cdot cm^{-3}$, oraz kwas acetylosalicylowy w stężeniu 250 $\mu g \cdot cm^{-3}$ ograniczały wzrost grzybni *P. narcissi* na pożywce PDA; w stężeniu 1000 $\mu g \cdot cm^{-3}$ wystąpiło niemal całkowite zahamowanie wzrostu patogena. Hamujący wpływ SA i ASA na tworzenie się czerwonego barwnika w uszkodzonych łuskach *Hippeastrum* może być spowodowany obniżeniem biosyntezy i akumulacji jasmonianów.

VACAT