Stimulatory and inhibitory effects of Glyceollin and Indole-3-acetic Acid (IAA) on sporulation and growth of Phytophthora megasperma f. sp. glycinea

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Stimulatory and inhibitory effects of Glyceollin and Indole-3-acetic Acid (IAA) on sporulation and growth of *Phytophthora megasperma* f. sp. *glycinea*

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Abstract

Kuhn H. and H. R. Hohl 1985. Stimulatory and inhibitory effects of glyceollin and indole-3-acetic acid (IAA) on sporulation and growth of *Phytophthora megasperma* f. sp. *glycinea.* Bot. Helv. 95: 301–310.

Glyceollin at either 10^{-9} or 10^{-7} M and indole-3-acetic acid (IAA) at 10^{-7} or 10^{-5} M promoted zoospore formation in all (glyceollin) or 2 (IAA) of the four strains of *Phytophthora megasperma* f. sp. *glycinea,* a pathogen of soybean tested. Glyceollin and IAA inhibited vegetative growth of the four strains above 2×10^{-5} M. In addition to its known inhibitory action at higher concentrations glyceollin but not IAA promoted growth of these strains *in vitro* at concentrations around 10^{-5} M.

Introduction

The phytoalexin glyceollin is known to inhibit the vegetative growth of the soybean pathogen *P. megasperma* f. sp. *glycinea* (Pmg) *in vitro* (Yoshikawa et al. 1978, Stössel 1983) and is widely considered as a main factor restricting growth of the pathogen in the incompatible host-parasite interaction (Klarman and Gerdemann 1963, Frank and Paxton 1970, Keen et al. 1971, Kuć 1972, Mansfield 1982, Yoshikawa et al. 1978, 1983, Keen 1981).

In the present study we report on stimulatory and inhibitory effects of glyceollin and of the plant hormone indole-3-acetic acid (IAA) on mycelial growth and on asexual reproduction of Pmg. The low stimulatory concentrations of glyceollin and possibly also of IAA present at very early stages of infection in the host tissue may be co-determining factors of the host-pathogen interaction and focus attention on the crucial first few hours of the infection.

Material and methods

P. megasperma (Drechs.) f. sp. *glycinea* (Kuan and Erwin) was obtained from Dr. N. T. Keen, Riverside, CA, U.S.A. Stock cultures of strains P900 (race 1), R1 (race 1), P892 (race 3) and R6 (race 6) were maintained on Borlotti bean agar at 21 °C in the dark (30 g of Borlotti beans were autoclaved for 8 min on 0.5 L of distilled water and then filtered through a nylon cloth. 15 g of

Difco agar were added to the filtrate which was filled up to one L with distilled water and reautoclaved).

Soybean cv. Harasoy and Harasoy 63 were obtained from Dr. J. D. Paxton, University of Illinois, Urbana, U.S.A. Seedlings were grown for 4 d on moist vermiculite in the dark at 21 °C.

Glyceollin was isolated and purified from 6 d old soybean plantlets of the cv. Harasoy 63 which had been inoculated 48 h earlier with the incompatible Pmg race R 1. Hypocotyl segments 10 mm long from the site of inoculation were extracted according to Yoshikawa et al. (1979) and the extracts chromatographed using the method of Keen (1978). The chromatographed material was eluted with ethanol and used for the tests. According to our own HPLC determinations glyceollins I, II, and III occurred in a ratio of 10 : 1 : 1.

Determination of glyceollin accumulation during compatible and incompatible interactions was done as described above at approximately 4 h intervals from the time of inoculation up to about 72 h (for P 900 to 96 h). Ten hypocotyl segments were used for each determination.

The effects of glyceollin and of IAA on vegetative growth were measured using agar-solidified and liquid growth media. For agar cultures the P-4 L medium of Hohl (1983) was used to which different concentrations of glyceollin (in 0.2% of ethanol) and of indole-3-acetic acid (Sigma Chemical Comp., in 0.2% ethanol) had been added aseptically after autoclaving. Mycelial plugs 6 mm in diameter were inoculated into the center of 6 cm plastic Petri dishes containing the agar medium and the cultures were incubated at 21 °C in the dark. The diameter of the mycelial colonies was measured after 6 d. For liquid cultures medium P-4 L was used without added agar. The medium to which different concentrations of glyceollin and of IAA had been added as described above was dispensed in aliquots of 100 ml into 500 ml Erlenmeyer flasks. The flasks were inoculated with 1 ml of a suspension of mycelium and incubated for 5 weeks at 21 °C in the dark whereupon the dry weight was determined. All determinations were done in triplicates.

For sporangium induction and zoospore release a method slightly modified from Eye et al. (1978) was used. Mycelial discs 6 mm in diameter were cut from the periphery of stock cultures and used to inoculate 9 cm plastic Petri dishes containing 20 ml of Borlotti agar medium diluted to ⅓ of its normal strength. After 5 d at 21 °C in the dark the plates were flooded with 7 ml of distilled water. 4 h later the liquid in the plates was changed 5 times at 50 min intervals, and the zoospores were harvested 16 h later and counted using a Neubauer hemocytometer.

To determine the effects of glyceollin and of auxins on zoospore formation, 5 d old agar cultures were flooded with 7 ml of distilled water containing different concentrations of these compounds in 0.2% of ethanol (IAA 10^{-7} M, pH 5.2; 10^{-5} M, pH 4.9); α-NAA 10^{-7} M, pH 5.2; 10^{-5} M, pH 4.9; β-NAA 10^{-7} M, pH 5.2; 10^{-5} M, pH 4.9; glyceollin 10^{-9}, 10^{-7}, 10^{-5} M, pH 5.7) and incubated at 21 °C for 14 h prior to the 5 changes with distilled water as described above. For controls the 5 d old cultures were treated with water containing 0.2% of ethanol.

Results

Fig. 1 combines the accumulation patterns of glyceollin of the 8 different interactions used in this study. In all compatible combinations levels of glyceollin reached after 8 h were roughly equal and about half those of incompatible interactions. Remarkably though, levels in both compatible and incompatible cases hardly differed from each other during the first 20 h.

Fig. 2 and 3 show the effect of different concentrations of glyceollin on colony growth and dry weight of 4 strains of Pmg. There was a remarkably uniform stimulation around 10^{-5} M in all 4 strains and in both the liquid and solid medium. Stimulation of colony growth occurred within a very narrow concentration range and was from 30–50%, whereas that of dry weight spanned a broader concentration range and was from 35–95%. At a concentration of about 2×10^{-4} M colony growth was completely inhibited while dry weight was little or not inhibited even at 10^{-4} M, the highest concentration tested.

In Fig. 4 the effect of different concentrations of IAA and α-NAA on colony growth of 4 strains of Pmg (R 1, P900, R6, and P892) are shown. Up to 10^{-5} M the two compounds did not or only slightly inhibit growth, whereas between 10^{-5}–2×10^{-4} M growth dropped to zero. While this inhibitory range was essentially the same as for glyceollin there was no stimulatory intermediate concentration range for the two auxins with the exception perhaps of strain R6, where a slight stimulation was observed with 10^{-5} M of IAA.

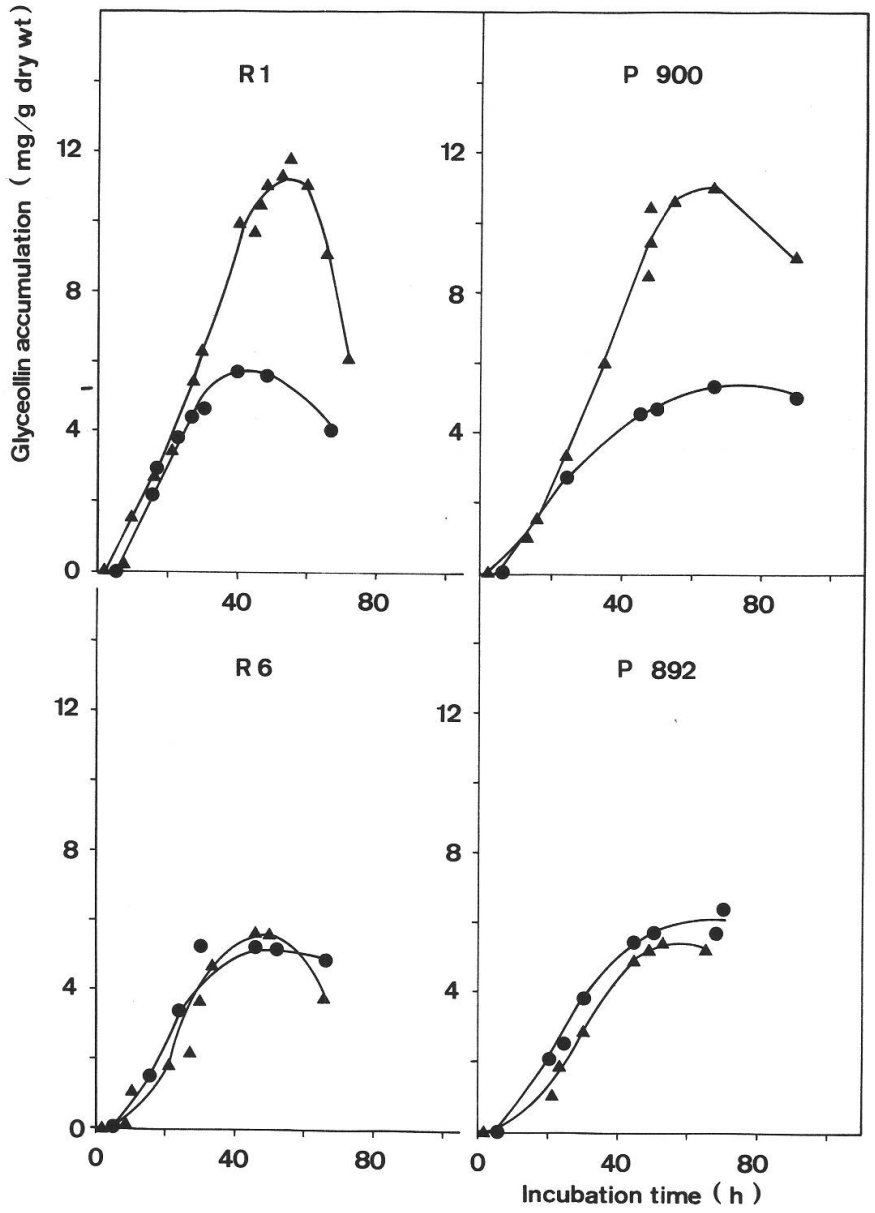

Fig. 1. Patterns of glyceollin accumulation in different host-parasite combinations. Soybean seedlings (H, H63) infected with either strain R 1, P900, P892, or R6 were incubated at 21 °C in the dark. ▲ Cv. Harasoy 63, incompatible with strains R 1 and P900. ● Cv. Harasoy, compatible with all four strains.

Fig. 5 summarizes the results obtained with glyceollin, IAA, α-NAA and β-NAA on zoosporogenesis of the same four strains of Pmg. Glyceollin proved stimulatory at either 10^{-9} or 10^{-7} M, and inhibitory at 10^{-5} M for all 4 strains tested. IAA markedly stimulated sporulation of strains R1 and P900 at 10^{-5} and 10^{-7} M respectively but hardly at all that of strains R6 and P892. It reduced sporulation of strain P900 at 10^{-5} M. α-NAA promoted sporulation in strain P900 at 10^{-7} and to a lesser degree in strain R1 at 10^{-5} M but reduced sporulation in all other cases. β-NAA did not stimulate sporulation in any situation tested and reduced it considerably in most instances. Overall stimulation of sporulation was much more pronounced with poorly sporulating strains (R1 and P900) than with those (R6 and P892) sporulating well in the presence of water only (see controls of Fig. 5).

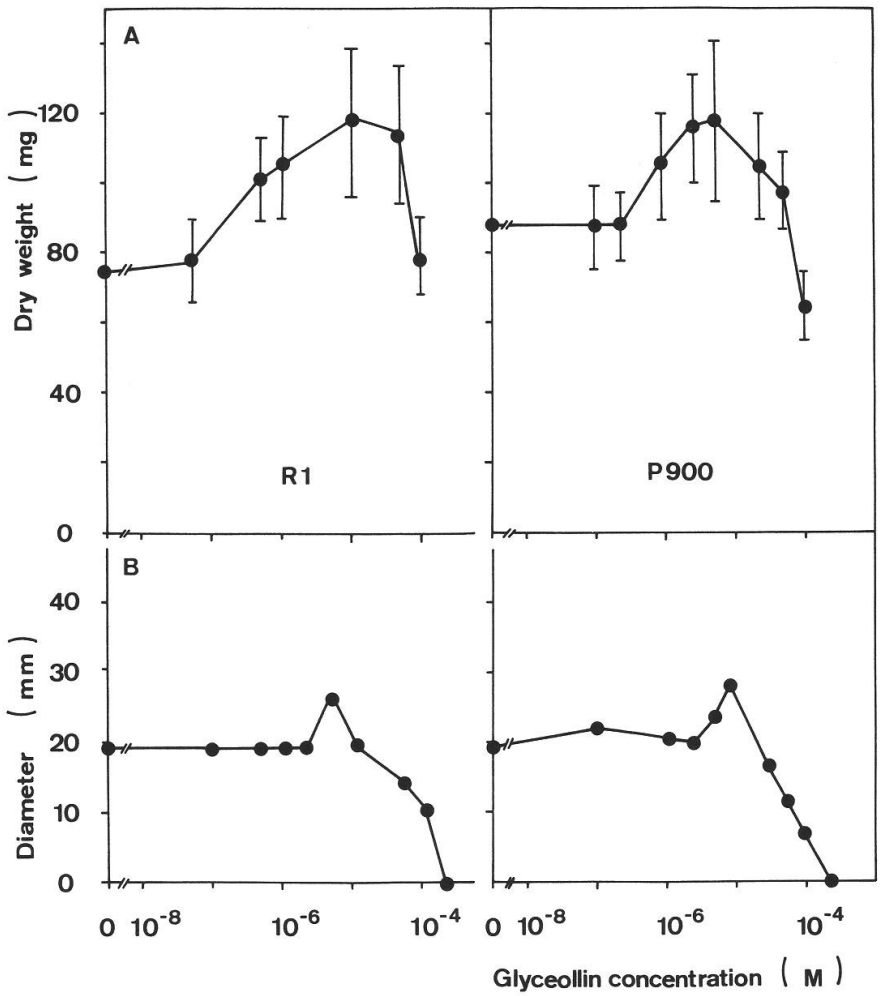

Fig. 2 and 3. Effect of glyceollin on vegetative growth of 4 strains of Pmg (fig. 2: strains R1, P900; fig. 3: R6, P892). (A) Mycelial dry weight after growth (5 weeks at 21 °C in the dark) on liquid medium containing different concentrations of glyceollin. (B) Diameter of growing colonies after 6 d at 21 °C in the dark on medium solidified with agar.

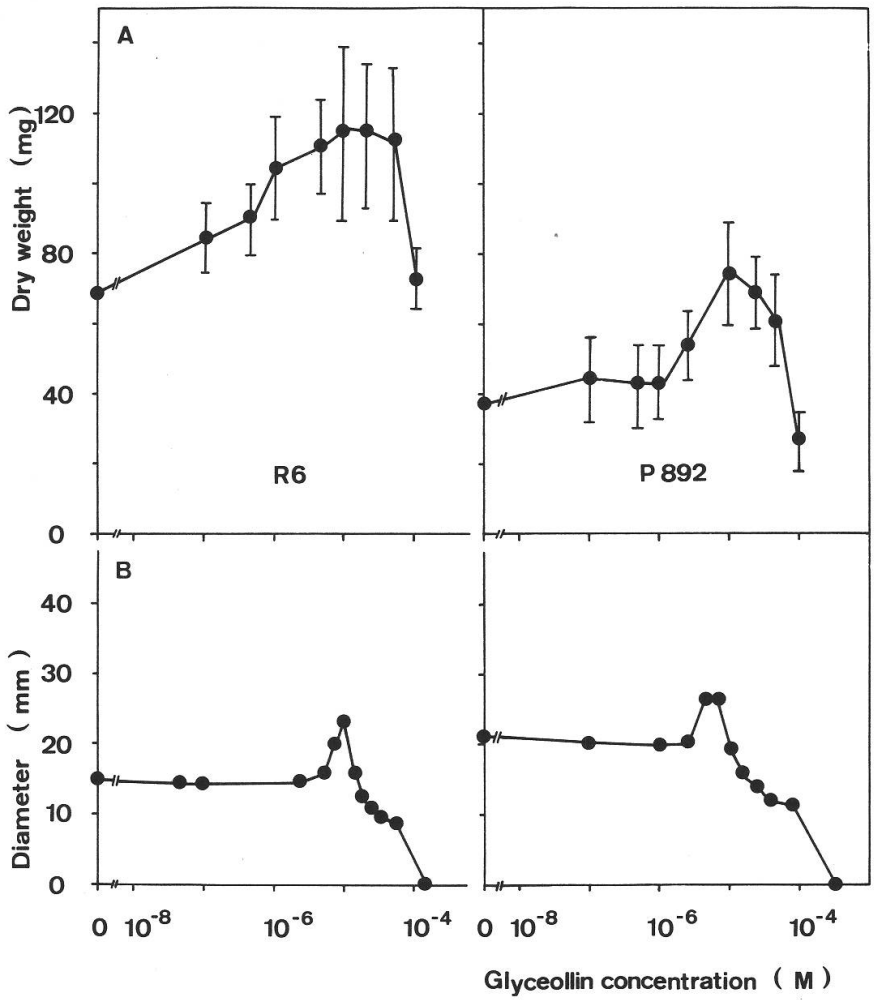

Fig. 3.

Discussion

This study has shown that two compounds present in the infected host tissue, glyceollin and IAA exhibit both inhibitory and stimulatory effects of growth and sporulation of this soybean pathogen. Possibly then, both types of effects play a role during host-pathogen interactions.

Concentrations of glyceollin promoting sporulation (10^{-7} or 10^{-9} M) are well below those inhibiting vegetative growth ($> 10^{-5}$ M) and essentially the same applies for IAA. α-NAA and β-NAA do in some instances behave differently from IAA in their effect on zoospore formation. Also, these substances in all likelihood act differently from sterols which have long been known to stimulate sporogenesis in pythiaceous fungi (Hendrix 1970). However, with the biochemical control mechanisms of zoosporogenesis almost totally unknown (Ribeiro 1983) it is futile to further speculate on the mode of action of this intriguing stimulation of zoospore formation by a plant auxin and a phytoalexin.

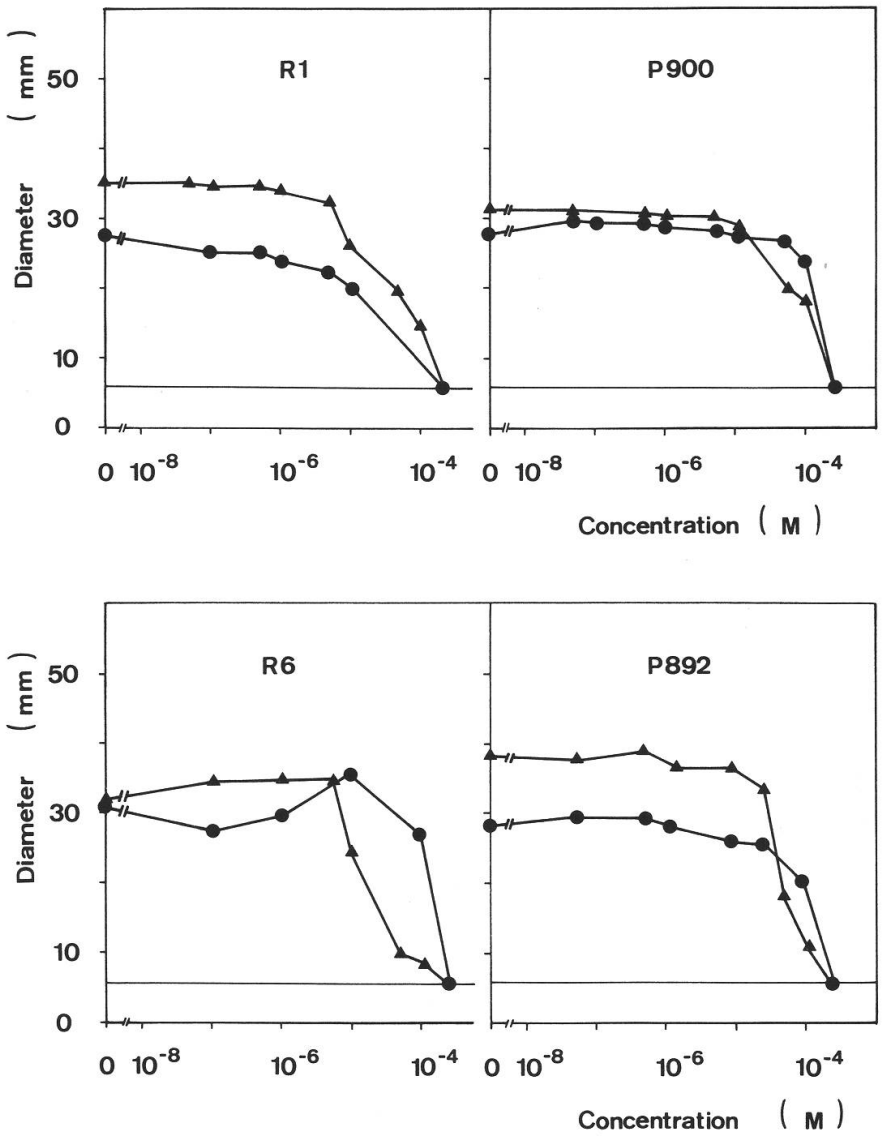

Fig. 4. Effect of IAA on mycelial growth. Diameter of colonies growing for 6 d at 21 °C in the dark on agar medium containing different concentrations of IAA. ● IAA, ▲ α-NAA, means of two experiments.

The observed stimulation of sporulation by glyceollin and by auxin is striking only in strains that have for some reason (e.g. extended in vitro cultivation) attained a low sporulation capacity. Possibly these two substances which are also present in vivo in the infected host tissue contribute to the well-known phenomenon of restoration of sporulation by passage through host tissue. Furthermore, sporulation is possibly stimulated at the edges of lesions where glyceollin concentrations are very low, a situation which would contribute to the spread of the pathogen. That IAA, too, might favor increased sporulation on the susceptible host is indicated by the fact that in soybean hypocotyls IAA concentrations of up to 2.6×10^{-7} M have been measured (Sweetser and Swartzfager 1978). Considering the observation that infected tissue often has elevated levels of

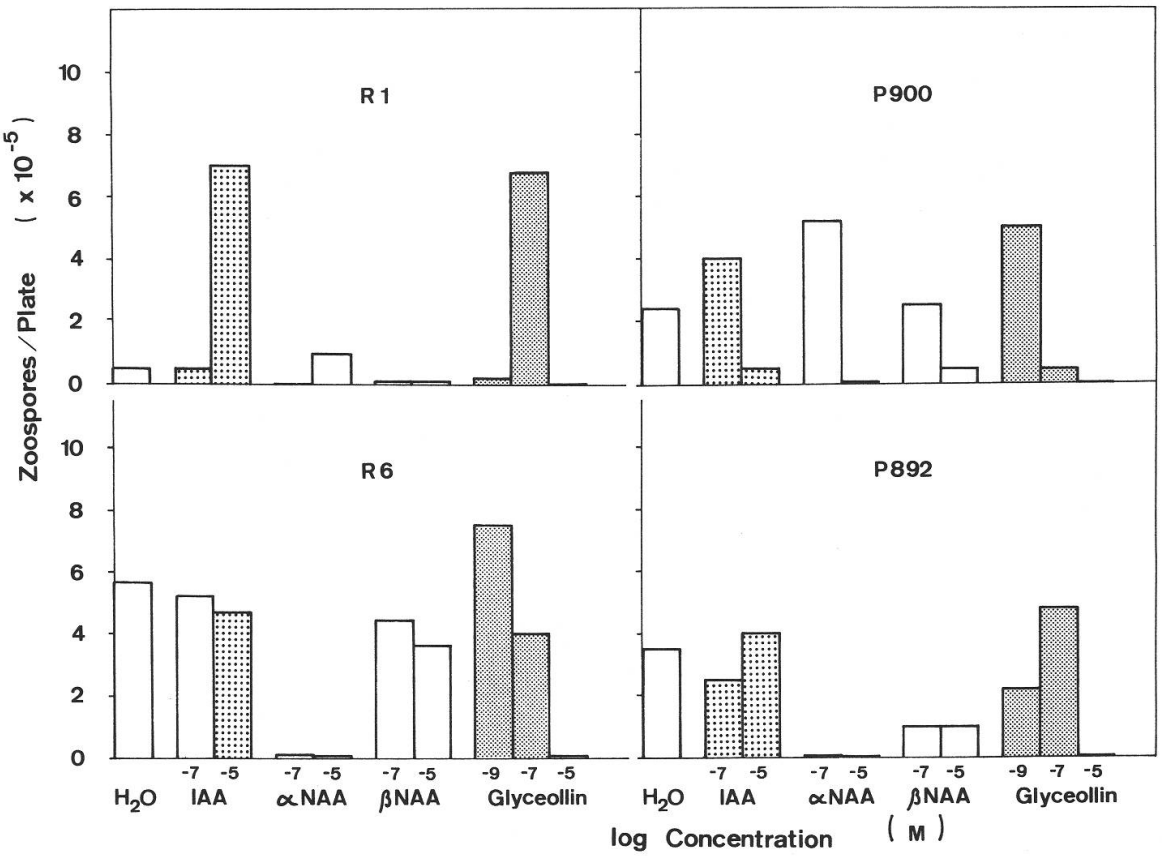

Fig. 5. Effect of glyceollin and of auxins on zoospore formation of strains R 1, P900, R6, and P892. The mycelium was first flooded with either H_2O, IAA, α-NAA, β-NAA, or glyceollin. After 14 h the solution was removed and the cultures flooded 5 times with distilled water at intervals of 50 min. Zoospore concentrations were determined 16 h later.

auxin (Gruen 1959) which is also the case in compatible soybean–Pmg interactions (Kuhn 1984) it is reasonable to suggest that IAA in infected soybean tissues may reach levels stimulatory to sporulation of the pathogen.

Stimulatory effects of the phytoalexin glyceollin on fungal growth and sporulation have not been reported before (Stössel 1983, Yoshikawa et al. 1978, Keen et al. 1971, Vaziri et al. 1983) most likely because these low concentrations were not tested. However, Vaziri et al. (1983) observed stimulation of zoospore production in *P. megasperma* f. sp. *medicaginis* by the phytoalexin medicarpin. This effect was restricted to *P. megasperma* isolates from alfalfa and was not observed in those from e.g. soybean or douglas fir.

Stimulation of fungal growth by auxins has been reviewed by Gruen (1959). Growth of oomycetes generally is not stimulated by this compound but higher concentrations may be inhibitory as reported for Pmg in the present study. Some studies (Grambow et al. 1977, Grambow and Müller 1978) suggest that possibly not IAA itself but some of its metabolites may influence fungal growth, at least in the rust species investigated. Very little is known about the mechanisms of these growth stimulations.

IAA not only stimulates sporangia production but also inhibits mycelial growth *in vitro* at higher concentrations (above 10^{-5} M). We may presume from published data (Sweetser and Swartzfager 1978) that these high concentrations are not reached in the soybean hypocotyls and thus do not directly contribute to inhibition of the pathogen in vivo. However, as has been discussed elsewhere (Kuhn 1984, Kuhn and Hohl, in preparation) possible interactions between the IAA metabolism and glyceollin and between IAA metabolites and the pathogen might influence the outcome of the infection process.

Although the concentration of glyceollin in the resistant host after 48 h reaches a level about twice that in the susceptible tissue our data indicate that during the critical early stages of infection this level rises to approximately the same degree in both incompatible and compatible interactions, a situation observed by others (e.g. Börner et al. 1983, Moesta et al. 1983 a, b). This implies that the overall rate of glyceollin accumulation per se does not fully account for the differential outcome of the infection process in compatible and incompatible situations.

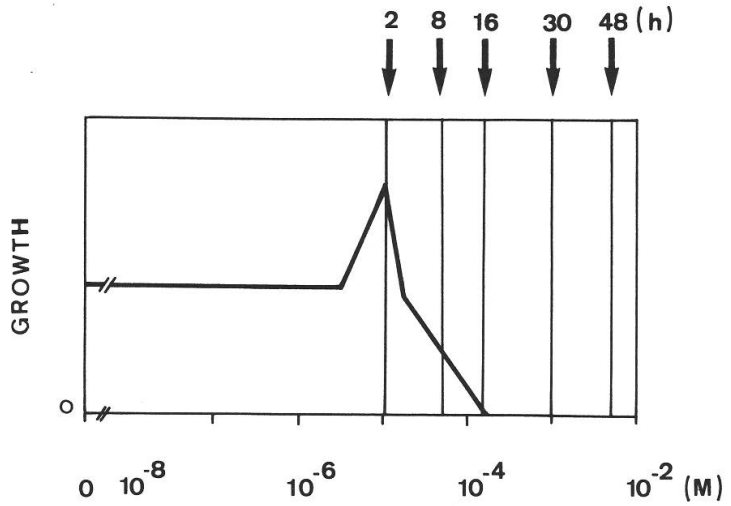

Fig. 6. Concentration-dependent effect of glyceollin on the vegetative growth of *Phytophthora megasperma* f. sp. *glycinea*. The thick solid curve shows the growth of the pathogen *in vitro* at different concentrations of glyceollin shown at the bottom. Top: approx. time (in h after inoculation) for the host tissue to reach the corresponding *in vivo* concentrations of glyceollin. The diagram illustrates that from 2–4 h after inoculation glyceollin levels in vivo are growth stimulating while after about 16 h growth is completely inhibited in compatible and incompatible interactions.

In Fig. 6 the concentrations of glyceollin reached during the early stages of infection are plotted against the corresponding effects these concentrations exert on the pathogen *in vitro*. The figure tries to emphasize 2 points: (1) During the initial few h of infection the glyceollin level is in the stimulatory range, and (2) about 8 h after infection the ED_{50} – value is reached in both types of interaction. Point 2 raises the question as to why the growth of the virulent pathogen is not inhibited by the inhibitory levels of glyceollin reached after about 8 h. Possible answers are tolerance or evasion. The first possibility is not supported by the study of Stössel (1983) nor by our own evidence since among the strains tested there are no distinct differences in sensitivity against gly-

ceollin. In addition fixed differences in sensitivity among strains of Pmg could not account for their differential behaviour since most races may be involved in both compatible and incompatible situations.

The second possibility, evasion, is a more likely alternative: The very low concentrations of glyceollin present during the first few hours of infection may effectively stimulate growth of the pathogen in the compatible situation thus allowing invasion of surrounding host tissue well in advance of the higher and toxic glyceollin levels building up behind. Accordingly, in the incompatible situation the avirulent race would be delayed in its early spread within the host tissue and get trapped in the inhibitory glyceollin concentrations. Factors which might accomplish this delay include a very rapid but highly localized increase in glyceollin concentration at the infection site (Hahn et al. 1985), the different types of penetration observed in susceptible and resistant hosts (Stössel et al. 1981a), papilla formation (Stössel et al. 1981b) or the presence of germ tube inhibiting metabolites.

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