Neth. J. Pl. Path. 83 (1977) 161–163 SHORT COMMUNICATION

Induced pterocarpan formation in two Melilotus species

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Accepted 16 February 1977

After a period of night frosts in autumn, stems of *Melilotus albus* plants, infected with *Botrytis cinerea*, showed a strong hypersensitive reaction. As hypersensitive reactions in leguminous hosts are reported to be often associated with phytoalexin formation (Bailey and Burden, 1973), extracts of these plants were screened for the presence of fungitoxic compounds by means of a bioassay on thin layer chromatograms (TLC). The appearance of an inhibition zone indicated the presence of a fungitoxic compound not present in healthy tissue.

Further investigations on the induction of this phytoalexin were performed, using the conventional methods of a) treatment with $HgCl_2$ 10^{-4} M or $CuCl_2$ 3×10^{-3} M, or b) challenge with various fungi.

a. Young shoots (just at the onset of bloom) of *Melilotus albus* Medicus (plants with high coumarin content, grown outdoors from seed collected near Gerolstein, F. R. G.) and *M. altissimus* Thuill. (plants collected in the vicinity of Wageningen) were treated by dipping into a 10^{-4} M $HgCl_2$ solution with a detergent. These shoots were then incubated during two days in glass trays at 23 °C, subsequently extracted with ethanol, the extract purified in various steps, finally by preparative TLC and the fungitoxic compound isolated. The yield of the phytoalexin was very low, only about 3 mg/kg fresh weight. The isolated compound from both *Melilotus* species was characterized by ultra-violet, proton magnetic resonance and mass spectroscopy as 3-hydroxy-9-methoxypterocarpan (medicarpin, Fig. 1).

b. Challenge of stems and leaves with several fungi was not very successful. Conidial suspensions of *Penicillium corymbiferum*, *P. brevicompactum*, *P. expansum*, *Aspergillus nidulans*, *Cladosporium cucumerinum*, *Colletotrichum acutatum*, *Ascochyta pisi*, *Fusarium graminearum* and mycelial mats of *Helminthosporium turcicum*, *Phytophthora cryptogea*, *Fusarium oxysporum* f. *pisi*, both at 15 °C and 20 °C did not induce a trace of medicarpin nor elicit a hypersensitive reaction.

Unlike the situation in *Pisum sativum*, where powdery mildew infection is reported to cause pisatin production in large amounts (Oku et al., 1975), *Melilotus albus* leaves infected by *Erysiphe martii* never contained a trace of medicarpin, either at 20 °C or after heating to 30 °C or 35 °C for 24 hours, which might shift the host-pathogen relationship in a direction unfavourable for the pathogen, giving rise to phytoalexin

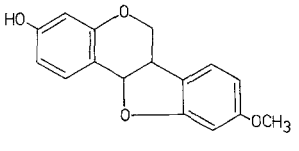

Fig. 1. Medicarpin.

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formation (Rahe, 1973). Similar observations were made on leaves infected by *Peronospora meliloti* and treated in the same way.

However, phytoalexin formation could be induced after establishing an infection by *Botrytis cinerea* at 15 °C, during which period no phytoalexin could be demonstrated, and subsequent maintenance at 30 °C for 24 hours, resulting in a hypersensitive reaction.

In healthy untreated plants coumarin is present as a glucoside in an amount of about 1 % of the dry weight. In most treatments coumarin was present, whether medicarpin was formed or not. On the other hand, medicarpin never occurred without coumarin. In those rare cases, where coumarin was accompanied by medicarpin, the inhibition zone on thin layer chromatograms, with *Penicillium expansum* and *P. corymbiferum* as test organisms, due to coumarin was always much larger than that due to medicarpin. So medicarpin may be regarded as playing only a secondary role in general disease resistance in *Melilotus* species, similar to pterocarpan formation in *Trifolium pratense* infected by *Kabatiella caulivora* (Sakuma et al., 1974).

The high amount of coumarin formed may also explain the low yield of phytoalexin. It has been reported, that pterocarpan formation is very low in the presence of other fungitoxic compounds, as is the case with *Vicia faba* where wyerone and wyeronic acid are predominant (Hargreaves et al., 1976), or even absent as in *Lupinus* species (Harborne et al., 1976).

Samenvatting

Fytoalexinevorming in twee Melilotus soorten

De vorming van een pterocarpan-fytoalexine werd waargenomen in twee *Melilotus* spp. Het fytoalexine werd geïdentificeerd als 3-hydroxy-9 methoxypterocarpan (medicarpine, Fig. 1). De inductie vond slechts plaats onder invloed van zware metalen of bij een overgevoeligheidsreactie en ook dan nog in lage hoeveelheden.

Aangezien de fungitoxiciteit van het aanwezige cumarine (in een biotoets op TLC) altijd veel groter was dan van het fytoalexine, werd geconcludeerd, dat medicarpine in dit geval geen grote rol kan spelen in het resistentiemechanisme van de onderzochte *Melilotus* soorten.

Acknowledgments

My thanks are due to Dr J. C. Overeem and Dr J. W. Vonk of the Organic Chemical Institute-TNO at Utrecht for help with the PMR and MS spectra.

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