THE RESPONSE OF OILSEED RAPE (*BRASSICA NAPUS* L.) SEEDLINGS TO *PERONOSPORA PARASITICA* AND *ALTERNARIA BRASSICAE* FOLLOWING TREATMENT WITH SALICYLIC ACID OR METHYL JASMONATE

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ABSTRACT

In preliminary experiments on the effect of treatment with abiotic elicitors on disease reaction in oilseed rape seedlings, salicylic acid reduced the severity of infection by two fungal pathogens, *Peronospora parasitica* and *Alternaria brassicae*, but methyl jasmonate affected only the reaction to *P.parasitica*. Treatments also led to the selective accumulation of different types of glucosinolates.

INTRODUCTION

Salicylic acid (SA) and methyl jasmonate (MJ) are implicated in the signalling processes that occur when plants become infected by micro-organisms (Bennett and Wallsgrove, 1994). In earlier work, we have shown that glucosinolates, compounds that accumulate in infected oilseed rape (*Brassica napus* L.) leaves (Doughty *et al.*, 1991), possibly contributing to disease resistance, can also be made to accumulate artificially by treating with SA and MJ (Kiddle *et al.*, 1994; Doughty *et al.*, 1995). We now report preliminary experiments investigating the response of seedlings treated with SA and MJ to the downy mildew (*Peronospora parasitica*) and dark leaf spot (*Alternaria brassicae*) pathogens, in relation to the accumulation of glucosinolates following treatment with these elicitors.

EXPERIMENTAL

Seeds of oilseed rape cv. Bienvenu were sown in Prescription compost in plastic propagator trays. After 7 days, seedlings were treated with one of the two elicitors as follows: SA was applied directly to the compost in 0.5mM, 2mM, or 5mM aqueous solutions; MJ was sprayed onto seedlings electrostatically (Doughty *et al.*, 1995) in 0.5mM, 5mM or 50mM ethanolic solutions. Controls were distilled water (for SA and MJ) and ethanol alone (for MJ only). Treated seedlings were incubated in illuminated controlled-environment cabinets at 18/15°C, 16/8h day/night.

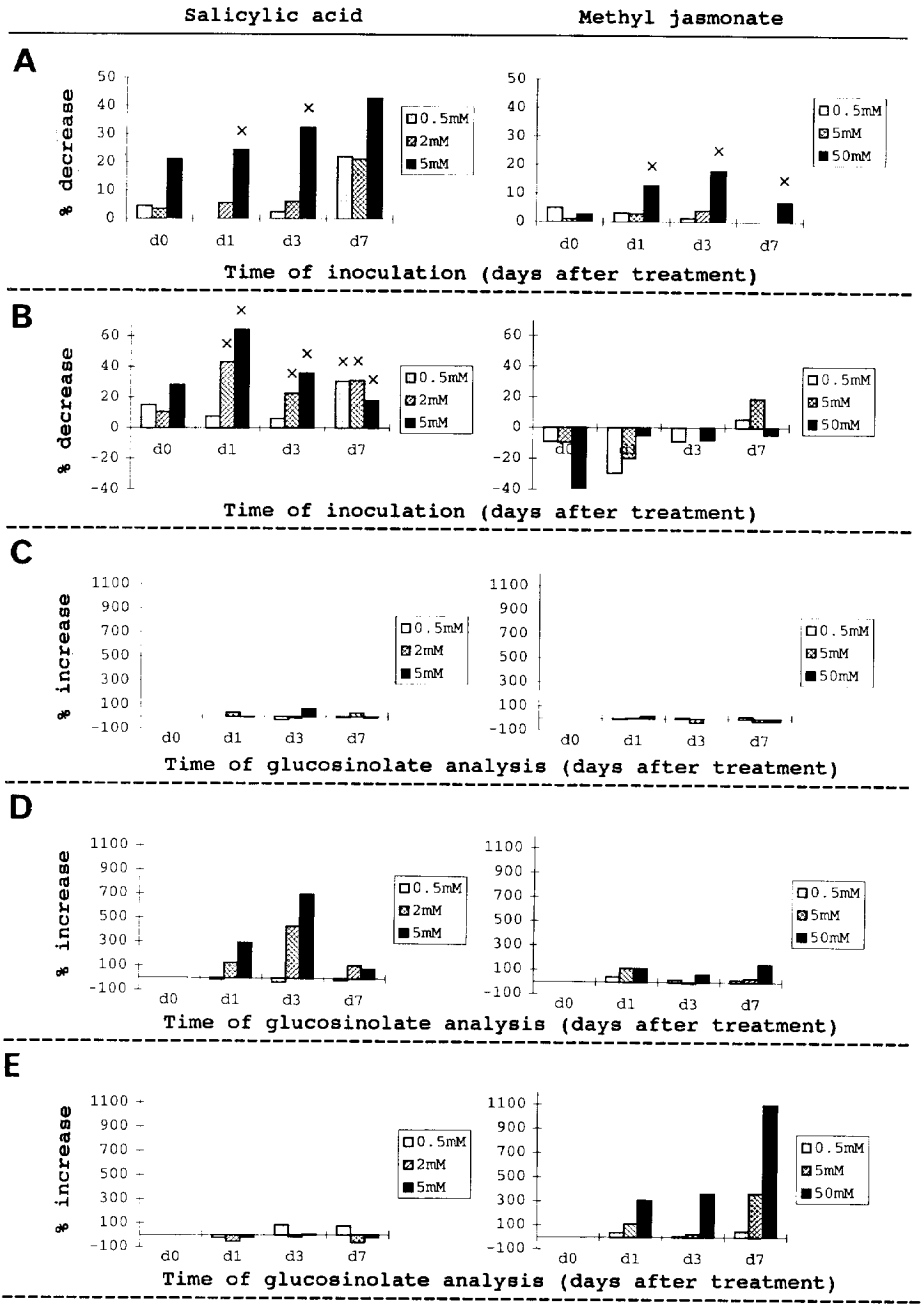
In one set of experiments, separate batches of treated and untreated seedlings were then inoculated with conidial suspensions of a *P.parasitica* isolate (R1) (10μL on each cotyledon, $2X10^5$ spores/mL) or an *A.brassicae* isolate (25μL on each cotyledon, $2x10^3$ spores/mL), either at the time of treatment with SA or MJ, or 1, 3 or 7 days after treatment. Both isolates were known to be virulent on cv. Bienvenu. Immediately after inoculation, propagators were sealed, in order to maintain high humidity for infection. Disease reaction was estimated seven days after inoculation, as an "interaction phenotype" for *P.parasitica* (after Nashaat and Rawlinson [1994]) or an "infection score" for *A.brassicae* (the number of lesions per seedling multiplied by the approximate size [mm^2] of each lesion).

In a second set of experiments, cotyledon samples were taken for the estimation of glucosinolate concentrations in cotyledons at the time of treatment with SA or MJ, or 1, 3 or 7 days after treatment: analyses were done by HPLC, using a slight modification of the method of Heaney *et al.* (1986).

RESULTS

Figure 1 shows the changes (percent decrease vs control) that occurred in interaction phenotype (*P.parasitica*) or infection score (*A.brassicae*) in relation to treatment with elicitors, and compares them to the corresponding state, at the time of inoculation, of the concentrations (percent increase vs control) of the three classes of glucosinolate: aliphatic, aromatic and indolyl. Treatment with SA caused a decrease in the severity of symptoms when cotyledons were subsequently inoculated with *P.parasitica* or *A.brassicae* (Figure 1 A,B). In contrast,

Figure 1. Comparison of changes in disease reaction to *Peronospora parasitica* (A) and *Alternaria brassicae* (B), and changes in aliphatic (C), aromatic (D) and indolyl (E) glucosinolate concentrations in cotyledons of oilseed rape seedlings treated with SA and MJ



Notes: Changes in disease reaction and glucosinolate concentrations are expressed relative to respective controls (for MJ, the ethanol control is used). Changes in disease reaction that are significant at $P<0.05$ are denoted by a ×. Disease reaction data were taken from at least 100 seedlings; glucosinolate data are means for 2 samples.

treatment with MJ only caused a decrease in the subsequent severity of symptoms of *P.parasitica* (Figure 1, A).

Neither elicitor caused an accumulation of aliphatic glucosinolates (Fig. 1, C). But treatment with SA caused the selective accumulation of the aromatic compound, 2-phenylethylglucosinolate (Fig. 1, D). Treatment with MJ caused a small accumulation of this compound, but its main effect was to cause an accumulation of the two indolyl compounds, 3-indolylmethyl- and 1-methoxy-3-indolylmethylglucosinolates (Fig. 1, E).

DISCUSSION

These results show that it is possible to induce changes in disease reaction in oilseed rape seedlings by treating with SA or MJ. The pattern of accumulation of glucosinolates in seedlings was consistent with what occurs in true leaves treated with these elicitors (Kiddle *et al.*, 1994; Doughty *et al.*, 1995). Induced changes in disease reaction to either pathogen were not associated with an accumulation of aliphatic glucosinolates at the time of inoculation, whichever elicitor was used. Conversely, the accumulation of indolyl glucosinolates that occurred in MJ-treated seedlings did not affect the subsequent development of *A.brassicae*. But the induced reduction in the severity of symptoms of this pathogen, and of *P.parasitica*, in SA-treated seedlings was associated with an accumulation of 2-phenylethyl glucosinolate. The contribution of this compound to disease resistance in *Brassica* is as yet unclear: other work (Blight *et al.*, 1995) found no evidence that it is degraded to its isothiocyanate (a highly fungitoxic derivate) during infection of *Brassica rapa* seedlings by *A.brassicae*. Furthermore, the accumulation of glucosinolates occurred against a background of other changes. For example, among the various enzyme activities that were also monitored following treatment with SA or MJ, increased activity of peroxidases was also induced (data not shown).

Further work will use SA, MJ and other elicitors of glucosinolates in oilseed rape to generate a wider range of elicitor treatment/disease response interactions. This approach will be used to investigate the relative contribution of induced glucosinolates to disease resistance, compared to that of other inducible systems.

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