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[See table of contents](#)

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Article abstract

Homodestruxin B, a compound produced by *Alternaria brassicae*, the causal organism of the blackspot disease of rapeseed, is known to be phytotoxic to the leaves of *Brassica napus*. In this study we found that its phytotoxicity was comparable to that of destruxin B, another toxin produced by the pathogen when tested on *B. napus*. Homodestruxin B caused symptoms of different severities in leaves of various non-host plants. The results suggest that homodestruxin B is a non-host-specific toxin.

A note on phytotoxicity of homodestruxin B - a compound produced by *Alternaria brassicae*

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Homodestruxin B, a compound produced by *Alternaria brassicae*, the causal organism of the blackspot disease of rapeseed, is known to be phytotoxic to the leaves of *Brassica napus*. In this study we found that its phytotoxicity was comparable to that of destruxin B, another toxin produced by the pathogen when tested on *B. napus*. Homodestruxin B caused symptoms of different severities in leaves of various non-host plants. The results suggest that homodestruxin B is a non-host-specific toxin.

Bains, P.S., J.P. Tewari et W.A. Ayer. 1993. Une note sur la phytotoxicité de l'homodestruxine B - un composé produit par l'*Alternaria brassicae*. PHYTO-PROTECTION 74: 157-160.

L'homodestruxine B, un composé produit par l'*Alternaria brassicae*, agent pathogène de la tache noire du colza, est reconnue pour sa phytotoxicité envers les feuilles du *Brassica napus*. Nous avons trouvé que sa phytotoxicité était comparable à celle de la destruxine B, une autre toxine produite par l'agent pathogène lorsque vérifié sur le *B. napus*. L'homodestruxine B a causé des symptômes de diverses gravités sur les feuilles de plusieurs plantes non-hôtes. Ceci suggère que l'homodestruxine B est une toxine non-spécifique à une plante-hôte.

Alternaria brassicae (Berk.) Sacc. causes the blackspot disease of rapeseed (*Brassica rapa* L. and *Brassica napus* L.) and many other crucifers (Ellis 1971; Kolte 1985). It appears that several host and pathogen factors are important in the initiation and severity of this disease (Tewari 1991). Toxins produced by the pathogen are one such factor. To date, *A. brassicae* is known to produce four destruxins namely, destruxin B, homodestruxin B, destruxin B₂, and desmethyl-

destruxin B (Ayer and Peña-Rodriguez 1987; Bains and Tewari 1987; Buchwaldt and Jensen 1991). Of these, destruxin B (Ayer and Peña-Rodriguez 1987; Bains and Tewari 1987; Buchwaldt and Green 1992), destruxin B₂ (Buchwaldt and Green 1992) and homodestruxin B (Buchwaldt and Green 1992; Tewari 1991) are reported to have phytotoxic activity.

Among the phytotoxins, only destruxin B has been studied for its host-specificity and its role in blackspot pathogenesis (Bains 1989; Bains and Tewari 1987; Buchwaldt and Green 1992). No such detailed information is available for homodestruxin B and destruxin B₂ except that these were found to be toxic to the leaves of *B. napus* at 20.1×10^{-5} mol L⁻¹, and could have phytotoxicities similar to that of destruxin B (Buchwaldt and Green 1992). This study was undertaken to examine the host-specificity of homodestruxin B.

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Homodestruxin B and destruxin B were isolated from V8 juice culture broth of *A. brassicae* according to the method given by Ayer and Peña-Rodriguez (1987). ^1H NMR and IR spectra and HPLC profiles confirmed the identity and purity of the compounds.

The phytotoxicity of destruxin B and homodestruxin B were compared in a detached leaf bioassay using the third and fourth leaves of greenhouse-grown *B. napus* cv. Westar [growth stages 3.3 to 4.2 (Harper and Berkenkamp 1975)]. The surface of each half leaf was gently scratched with the tip of a pasteur pipette at two sites (each approximately 3 mm^2) and $20\ \mu\text{L}$ of test solution was placed on the scratched area. One leaf was treated with $4.2 \times 10^{-5}\text{ mol L}^{-1}$ destruxin B on one half leaf and with $4.2 \times 10^{-5}\text{ mol L}^{-1}$ homodestruxin B on the opposite half leaf. A second leaf was treated in the same manner with $8.4 \times 10^{-5}\text{ mol L}^{-1}$ of the two toxins. A third leaf was inoculated with a spore suspension of *A. brassicae* (10^5 spores mL^{-1}) on one half and with distilled water on the opposite half, which served as positive and negative controls, respectively. There were three replicates of each treatment and the experiment was repeated twice. All leaves were incubated on moist filter paper in petri dishes under continuous fluorescent light at room temperature (approximate-

ly 22°C). Reactions of leaves were noted after about 66 h of incubation.

Sixteen non-host plants of *A. brassicae* belonging to eight families were tested for their reaction to inoculation with *A. brassicae* and treatment with 5.0 , 25.3 , and $50.6 \times 10^{-5}\text{ mol L}^{-1}$ homodestruxin B. The tests were conducted using a detached leaf bioassay as described earlier except that smaller leaves and leaves with parallel venation were tested by applying only one treatment per leaf. There were three replicates of each concentration on each plant species and the experiment was repeated twice.

Homodestruxin B caused chlorosis and necrosis in 'Westar' leaves similar to that caused by *A. brassicae* (Figure 1). Identical concentrations (4.2 and $8.4 \times 10^{-5}\text{ mol L}^{-1}$) of homodestruxin B and destruxin B tested on opposite half-leaves of cv. Westar produced symptoms of similar severity, suggesting that the toxins have similar activity (Figure 1). This is not surprising because the only difference between homodestruxin B and destruxin B is an additional methylene group in homodestruxin B (Ayer and Peña-Rodriguez 1987). The results confirmed the suggestion made earlier that the phytotoxicities of these toxins are similar (Buchwaldt and Green 1992).

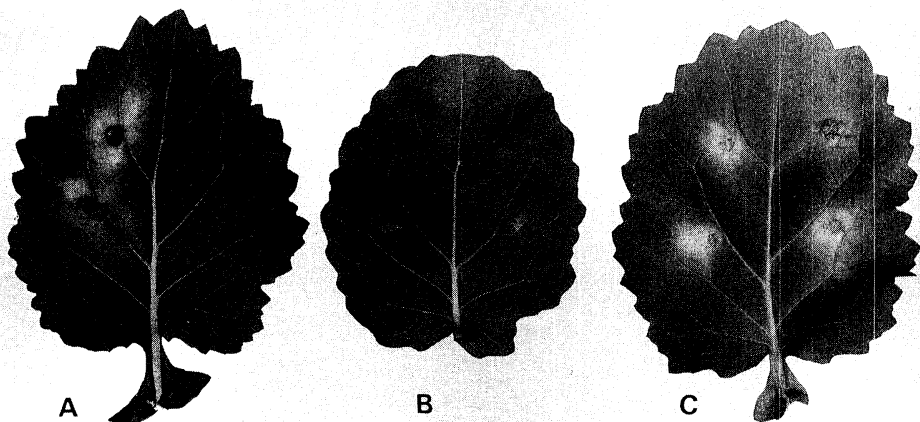


Figure 1. Response of detached leaves of *Brassica napus* cv. Westar to inoculation with *Alternaria brassicae* and treatment with destruxin B and homodestruxin B. The sequence of treatments for each half leaf is from left to right: A) *A. brassicae* (10^5 spores mL^{-1}), distilled water; B) $4.2 \times 10^{-5}\text{ mol L}^{-1}$ destruxin B, $4.2 \times 10^{-5}\text{ mol L}^{-1}$ homodestruxin B; C) $8.4 \times 10^{-5}\text{ mol L}^{-1}$ destruxin B, and $8.4 \times 10^{-5}\text{ mol L}^{-1}$ homodestruxin B.

Table 1. Symptom intensities caused by *Alternaria brassicae* and homodestruxin B on detached leaves of various plant species

| Species | Symptom intensity ^a | |
|--|--------------------------------|-----------------|
| | <i>A. brassicae</i> | Homodestruxin B |
| <i>Avena sativa</i> (oat) | — | — |
| <i>Euphorbia pulcherrima</i> (poinsettia) | — | — |
| <i>Hordeum vulgare</i> (barley) | — | — |
| <i>Triticum aestivum</i> (wheat) | — | — |
| <i>Zea mays</i> (corn) | — | — |
| <i>Chenopodium amaranticolor</i> (amaranticolor) | — | +++ |
| <i>Chenopodium quinoa</i> (quinoa) | — | + |
| <i>Callistephus chinensis</i> (china-aster) | — | + |
| <i>Cucumis sativus</i> (cucumber) | — | +++ |
| <i>Helianthus annuus</i> (common sunflower) | — | +++ |
| <i>Lycopersicon esculentum</i> (tomato) | — | + |
| <i>Nicotiana glutinosa</i> (tobacco) | — | +++ |
| <i>Petunia hybrida</i> (garden petunia) | — | + |
| <i>Phaseolus vulgaris</i> (red kidney bean) | — | +++ |
| <i>Solanum tuberosum</i> (potato) | — | +++ |
| <i>Vigna unguiculata</i> (cowpea) | — | +++ |
| <i>Brassica napus</i> cv. Westar (canola) | + | +++ |

^a — = no visible chlorosis or necrosis produced after inoculation with *A. brassicae* or treatment with 50.6×10^{-5} mol L⁻¹ homodestruxin B; + = visible chlorosis and necrosis produced after inoculation with *A. brassicae* or treatment with 25.3×10^{-5} mol L⁻¹ homodestruxin B; +++ = visible chlorosis and necrosis produced after treatment with 5.0×10^{-5} mol L⁻¹ homodestruxin B.

Homodestruxin B may play a minor role in blackspot pathogenesis compared with that of destruxin B, because it is produced in much smaller quantities (Ayer and Peña-Rodríguez 1987; Buchwaldt and Jensen 1991). Production of more than one homolog of a toxin is also known from other pathogens including *Alternaria alternata* apple pathotype (Kohmoto *et al.* 1976), *A. alternata* Japanese pear pathotype (Otani *et al.* 1985), and *A. alternata* strawberry pathotype (Maekawa *et al.* 1984). None of the four graminaceous plants developed any visible chlorosis or necrosis with homodestruxin B. However, the dicotyledonous non-host plants reacted variably to treatment with homodestruxin B. The range of symptoms developed in the leaves of non-host plants varied from no visible chlorosis or necrosis with 50.6×10^{-5} mol L⁻¹ homodestruxin B to visible chlorosis and necrosis with 5.0×10^{-5}

mol L⁻¹ of the toxin (Table 1). The results suggested that homodestruxin B is a non-host-specific toxin because such toxins are toxic to many plants, whether they are hosts or non-hosts of the producing pathogens (Mitchell 1984).

At this point a comparison of the host-specificity of homodestruxin B and destruxin B would be relevant, however, a discrepancy exists regarding host-specificity of destruxin B. Bains and Tewari (1987) described it as a host-specific toxin, whereas, Buchwaldt and Green (1992) described it as a host-selective toxin and suggested that the discrepancy could be due to different toxin treatment methods used for host and non-host plants by Bains and Tewari (1987). The discrepancy could also be due to the methods used to monitor the symptoms caused by the toxin. Bains and Tewari (1987) monitored the symptoms visually, whereas, Buchwaldt and Green (1992)

used a more sensitive technique and observed symptoms by light microscopy. The technique enabled them to observe symptoms that might not be visible to the naked eye. However, these observations need to be reassessed as most of the symptoms in non-host plants were visible only under the microscope and the control treatment examined similarly, would likely have similar symptoms. Damage to cells from scratching of the leaves prior to the placement of test solution may also have confounded the effect of control and low concentration toxin treatments. Further investigations are required to settle this discrepancy, and comparison of host-specificity of homodestruxin B and destruxin B might have to wait for the results of such investigations.

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