

The Use of *Ascochyta caulina* Phytotoxins for the Control of Common Ragweed

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Abstract

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage. Previous research had led to the identification of a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., having high toxicity against both host and non-host plant leaves, with lack of antibiotic and zootoxic activities. Recently, a two-year research project named ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop the technologies to obtain a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the possibility of its practical use. Among the selected target weeds, *A. artemisiifolia* showed an evident response to the toxin application, probably due to the presence of trichomes on the leaves that allow a quick absorption of the metabolites, with fast appearance of leaf necrosis and plant desiccation. Greenhouse bioassays as well as open field experiments clearly indicate the lethal effects on the young ragweed plants.

Introduction

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage.

Previous research had led to identify a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., showing a wide range of phytotoxicity against different weed species.

Recently, a two-year research project named the ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop technologies for obtaining a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the potential of its practical use.

During 2010 and 2011, in the framework of the ECOVIA research project, among the 16 weed species we tested, *A. artemisiifolia* was evaluated both in laboratory and semi-field conditions.

Materials and Methods

Toxin production

The fungus, *A. caulina*, a biological control agent of the weed *C. album*, was grown on a liquid defined mineral medium (named M1-D) for 4 weeks in static conditions as previously described (Evidente et al., 1998). Toxins were purified as described (Evidente et al., 1998; 2000) and obtained as a pure mixture containing three main phytotoxic compounds (i.e., ascaulitoxin, its aglycone, and trans-4-aminoproline; Fig. 1).

Laboratory and screen house bioassays

Young potted ragweed plantlets were sprayed uniformly with an 8 mg/ml water solution of the purified fungal toxins by uniform nebulisation of leaf surface. A relative humidity of 100% was maintained for 24 hours after each application. The experiment was successively repeated with a lower concentration of toxins solution (2 mg/ml) on cut leaves in Petri dishes and on potted plantlets.

In order to enhance the efficacy of the toxins, further bioassays were performed by adding a wetting agent to the toxin solution. The following four commercial products, which appear to best fit our needs, were used at the labelled concentration: Biopower®, Adigor®, Etravon® and Codacide®.

Results

A. artemisiifolia showed an evident response to the toxin application. In particular, the 8 mg/ml solution showed a high herbicide effect on young plantlets with 100% leaf damage within 3-5 days after the application (Fig. 2). The sensitivity of the target

weed is likely due to the presence of high numbers of trichomes on the leaves (Grangeot et al. 2006) that allow a quick absorption of the metabolites, with the fast appearance of leaf necrosis and plant desiccation. We obtained a significant damage with cut leaves treated with the 2 mg/ml solution in Petri dishes (Fig. 3). The same solution (2 mg/ml) caused a leaf damage of less than 50% when sprayed on the plantlets, but its phytotoxicity has been significantly improved using the toxin in combination with the Codacide®.

Results presented here show promise that these toxins can be effective in controlling common ragweed. Bioassays are in progress to further develop methods of application of the toxin mixture in order to achieve higher efficacy as a natural herbicide for the control of common ragweed.

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Figure 3. Effects of a 2 mg/ml toxin solution on ragweed cut leaves (control - left, treated - right).