Effects of homeopathic and mineral treatments on dark leaf spot caused by *Alternaria brassicicola* on cauliflower

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Abstract

This research aimed at verifying the efficacy of some homeopathic and mineral treatments on Alternaria brassicicola/cauliflower interaction. Growth chamber experiments and a field trial were performed, using Brassica plants artificially inoculated with the fungus. In growth chamber experiments, infection was significantly reduced by arsenic trioxide 35 decimal potency (As_2O_3 35 d) and in field trial by both As_2O_3 35 d and bentonite treatments.

Introduction

The aim of this work is to give a contribution on the effects of homeopathic treatments on dark leaf spot caused by *Alternaria brassicicola* (Schw.) Wiltshire on cauliflower. This disease, very common in *Brassica* crops (Humpherson-Jones, 1983), appears as small dark spots at all growth stages of the plant. In organic agriculture, the control of dark leaf spot, as well as of most fungal diseases, is based on the use of mineral products such as copper, that has a high efficacy and a long-lasting action. Unfortunately, copper use presents some disadvantages: it can be phytotoxic, and it can accumulate in the ground with negative consequences on soil microflora and microfauna. For these reasons, European Union delivered a directive (Commission Regulation EC no. 473/2002) that mandates a reduction in copper use in organic agriculture. In this context, homeopathic preparations, due to their extreme dilutions, could represent suitable treatments, complementary to copper, in organic agricultural protocols. Homeopathic treatments are prepared starting from a mother tincture of different substances, according to a standardized protocol which consists in serial aqueous dilutions (decimal or centesimal, d and c, respectively) coupled with dynamization phases (mechanical agitation of the dilution). An hypothesis of the action mechanism of homeopathic remedies is the following: the manufacturing process employed for the preparation of homeopathic remedies would induce a dynamic 'ordering' of water's constantly switching network of intermolecular hydrogen bonds (Chaplin 2007). This could lead to a long-range molecular 'coherence' between trillions of mobile water molecules (Elia et al, 2004; Milgron 2006). The literature on the effects of homeopathy on plants provides several papers on germination and growth tests on different species, some on phytopathological models, whereas very few descriptions concerning field trials are available (Betti *et al*., 2007).

Materials and methods

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Plants of *Brassica oleracea* L. cultivar clx 33247 were used for both growth chamber and field experiments. Plants, at the stage of three true leaves, were artificially inoculated by spraying a fungal suspension ($1x10^7$ conidia ml^{-1}) on the leaves. In the first experiment, arsenic trioxide, As_2O_3 35 d (As) and a bentonite treatment (bent., provided by the company Cosmoonda s.n.c.) at 10 g/l were compared with copper oxiclorure (Cu) at 0.3, 1, and 3 g/l, the control being water. In the second experiment, the treatments with As, bent. and Cu 3 g/l (as positive control) were repeated and compared with As diluted 1:5000 (As dil.) and ß-aminobutyric acid (BABA, 5 mM). As_2O_3 was chosen according to the homeopathic law of similarity (Bellavite *et al.*, 1997): in ponderal concentration it induced on leaves necrotic spots similar to those provoked by *A. brassicicola* infection. Bentonite was chosen because of its inhibiting effect on *in vitro* spore germination and BABA because it is a well-known resistance inducer (Cohen 2002). In the field trial, the same treatments of the first growth chamber experiment were tested. The field was divided in plots consisting of 6 plants/treatment (separated each other by two not-treated healthy plants), each treatment being replicated four times in a randomized complete block design. Treatments were sprayed weekly on the leaves 3 times before and 4 times after artificial fungal inoculation. The evaluation of infection level on leaves (growth chamber experiments) or head (field trial) was carried out blind by two different operators (in order to exclude unconscious influences). A visual assessment of the necrotic area on each plant was performed on the basis of an infection scale, previously defined and then reported in percentage, referred to control. Data were subjected to analysis of variance (ANOVA), followed by Dunnett post-hoc test.

Results

In the preliminary screening of homeopathic treatments, the best disease control was obtained by As, which induced a reduced infection of about 20% (data not shown).

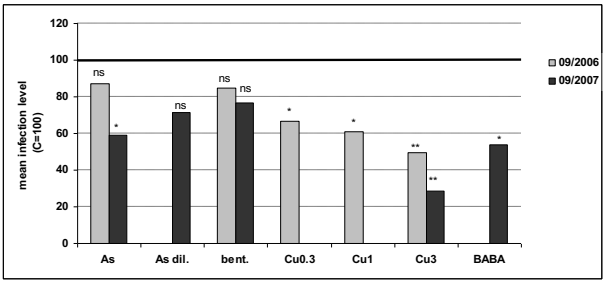

Figure 1: Different treatment effects on mean infection level in growth chamber experiments. Bold line represents control equal to 100.
n = 12 and 18 plants/treatment in 2006 and 2007 experiments, respectively
*** significant for p<0.05; ** significant for p<0.01**

Growth chamber experiment results, shown in Figure 1, confirmed the significant effect in disease control of As in the second experiment (infection level reduction vs. control of about 40%). A reduction of 15-25%, but not significant, was obtained with bent; Cu at all concentrations and BABA significantly reduced disease severity. In the field trial, disease assessments on cauliflower heads, performed in 3 successive times (Figure 2), showed in the last measurement a similar and significant reduction of

disease symptoms for As, bent. and Cu 3 g/l, with a relative efficacy vs. control of 46%, 42%, 45%, respectively.

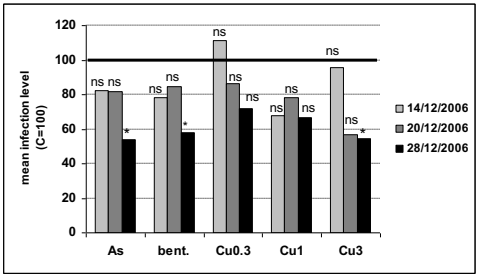

Figure 2: Different treatment effects on mean infection level in field trial. Bold line represents control equal to 100.
n = 4 replicates/treatment ; * significant for p<0.05

Discussion

In literature there are some evidences on the efficacy of homeopathic arsenic in the control of plant diseases (Scofield, 1984) and a resistance increase in tobacco plants against tobacco mosaic virus following treatments with As_2O_3 45 d has been already reported (Betti *et al*., 2003). The growth chamber experiment showed that As_2O_3 35 d significantly controlled dark leaf spot disease only in one case, even if a trend towards a symptom reduction can be observed. It is noteworthy that in different plant/pathogen interactions different homeopathic dilutions of the same treatment can have different efficacy. Moreover, since As_2O_3 35 d is diluted above Avogadro's number, there are no arsenic molecules in the treatment and thus it can be used in agricultural practice. Cu treatments confirmed the well known antifungal activity, particularly at 3g/l, and BABA its characteristics of resistance inducer. In particular, BABA was chosen because in a recent work a protection of *Brassica* plants against *Aternaria brassicae* following BABA treatment has been reported (Kamble and Bhargava, 2007). In the field trial, significant positive effects in the last assessment of infection level on corymb have been observed following arsenic, bentonite and copper oxiclorure at 3g/l. Since fungal inoculation was performed on the leaves before flowering, we can hypothesize that arsenic homeopathic treatment and bentonite induced a plant resistance increase to fungal infection. The symptom reduction due to copper oxiclorure, similar in our experimental trial to that induced by arsenic and bentonite, confirms the well known inhibiting effect of Cu^{2+} ions on fungal spore germination (Borkow *et al*., 2005).

Conclusions

The obtained results need further investigations to indicate a real measurable effect of homeopathic treatments, and rather the existence of a significant effect by chance. Our experimentation is still in progress with another field trial. The aim is to check the effects of the above mentioned treatments against a natural infection of *A. brassicicola*. Besides phytopathological analyses, an evaluation of organolectic characteristics and nutraceutical properties of differently treated plants will be performed. In particular, glucosinolates, a class of plant secondary metabolites typical of *Brassicacae*, will be analysed: these organic compounds seem to participate in the plant resistance mechanisms (Ménard *et al*., 1999) and present a potential activity as

"plant food protection agents" (Talalay *et al*., 2001). If homeopathic treatments will induce significant effects, an agricultural application of homeopathy ("agrohomeopathy") could be possible, at least as integrative to conventional agricultural practices. The privileged target of agrohomeopathy could be small farms (and in particular, those of nutraceutical and herbalist sectors) practicing organic farming that strive to be environmentally responsible, economically viable, and socially just.

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