Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 259-260, 14 May, 2012
Available online http:// academicjournals.org/JAERD
DOI: 10.5897/JAERD12.064
ISSN 2141-2154 ©2012 Academic Journals

Extended Abstract

Evaluating methyl jasmonate for induction of resistance against *Fusarium oxysporum*, *Fusarium circinatum* and *Ophiostoma novo-ulmi*

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Accepted 30 November, 2011

Worldwide, damping off is probably the most severe disease affecting seedlings in forests nurseries. In south-western Europe, the pitch canker and the Dutch elm disease cause relevant economic looses in forests, mostly in adult trees. Plants protect themselves against a diversity of attackers through constitutive and inducible defense strategies. Exogenous applications of Methyl Jasmonate (MeJA) have been successfully used to artificially induce chemical defense responses and resistance of forest trees against several insects and pathogens (Huber et al., 2005; Zeneli et al., 2006; Moreira et al., 2009). MeJA is usually mixed with the surfactant Tween 20 (0.1% v/v) and applied by spraying or by brushing the plant stem. Conscious that induced resistance occurs in pines and elms (Huber et al., 2005; Martín et al., 2010); we conducted three different experiments to evaluate if MeJA induces resistance in *Pinus pinaster* against *Fusarium oxysporum* and *Fusarium circinatum*, and in *Ulmus minor* against *Ophiostoma novo-ulmi*.

First, different concentrations of an aqueous solution of MeJA were applied to *P. pinaster* seeds and seedlings. Seeds were treated by (i) spray with 5 mM of MeJA solution, (ii) immersion 10-min in 5 mM of MeJA solution, or (iii) spray with water. During sowing, seeds were inoculated with *F. oxysporum* (5 mL; 10^5 and 10^7 spores mL^{-1}) on the ground. In non-inoculated seeds, MeJA treatments did not significantly affect seed germination but originated 44% of seedling mortality 1 month after treatments. In inoculated seeds, MeJA treatments caused a significantly higher mortality by *F. oxysporum* in relation to the control treatment (P=0.008). Seedlings were treated with MeJA solutions (0, 0.1, 0.5, 1, 5 and 10 mM) by spraying the stems. Time-periods between treatments and challenge inoculations (10^5 and 10^7 spores mL^{-1}) were 1 or 7 days. Seedlings treated at doses above 1 mM MeJA showed symptoms of toxicity. Seedlings treated at doses below 1 mM showed higher mortality rates than untreated seedlings. Final mortality of seedlings depended on the concentration of MeJA and the dose of *F. oxysporum* used, but the challenging treatments did not significantly protect the seedlings against the pathogen.

In the second experiment, 6-months-old *P. pinaster* seedlings were sprayed with 0 and 25 mM of MeJA. One month after treatments, half of the seedlings were inoculated by contact mycelium of *F. circinatum* with a wound onto the stem. The exogenous application of MeJA in non-inoculated seedlings significantly reduced above and belowground plant growth (P<0.05), increased the average number of resin ducts per transverse section (P<0.01) and marginally increased the relative conductive area of resin ducts (P=0.056) in relation to the control plants. Mortality of seedlings inoculated with *F. circinatum* was 58%, and mortality of seedlings treated with MeJA and subsequently inoculated with *F. circinatum* was 60%, thus the challenging treatment did not show any positive effect against the pathogen tested.

Finally, 4-year-old *U. minor* trees were sprayed with 0, 50 and 100 mM of MeJA. Half month after treatments, half of trees were inoculated with *O. novo-ulmi*. 100 mM of MeJA was slightly toxic to the trees, causing leave spots and some wilting. However, time to bud burst and tree growth was not altered by MeJA treatments. Dieback symptoms, evaluated 120 days and one year after inoculations, revealed that MeJA did not protect the plants against *O. novo-ulmi*. In fact, one year after inoculation the trees treated with MeJA showed higher dieback symptoms than the control trees (P=0.03).

MeJA did not protect *P. pinaster* seeds and seedlings against *F. oxysporum*, probably because plants were too young for the physiological mechanisms responsible for resistance to be induced. Based on the morphological changes observed in the treated 6-months-old *P. pinaster* seedlings (reduction of growth, increased resin duct density), there is evidence that MeJA could have activated the mechanisms of resistance. However, 25 mM MeJA did not reduce plant

mortality, probably because the spread of the virulent *F. circinatum* strain within the tree tissues was faster than the formation of effective defense responses. Based on the lack of phenological changes observed in the treated elms, there is no evidence that MeJA would cause induction of resistance. This is the first work reporting the effect of MeJA on *U. minor* and *P. pinaster* seeds, and the first approach to test MeJA against three pathogens previously not used for this purpose. Based on our results, the use of MeJA to prevent *F. oxysporum* and *F. circinatum* in *P. pinaster* seedlings in nurseries and *O. novo-ulmi* in *U. minor* trees should be discarded.

Key words: *Pinus pinaster, Ulmus minor*, Induced resistance.

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