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Efficiency of plant extract from *Reynoutria sachalinensis* (Milsana) to control powdery mildew on tomato (*Oidium neolycopersici*)

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Abstract: Powdery mildew caused by *Oidium neolycopersici* is one of the principal diseases on tomato crops grown in greenhouse conditions. Fungicides are widely used to control this disease. Various biological methods have been studied and a plant extract from the giant knotweed *Reynoutria sachalinensis* (Milsana) has shown a high efficiency to control this disease on various crops. Experiments on tomato grown in greenhouse conditions have been conducted during a 3-year period (1999-2001) to test the efficiency of this product for the control of *O. neolycopersici*. Several frequencies of application (7, 14 and 21 days) have been tested. Results obtained were compared with water-treated plants and with a fungicide control (Anvil, 0.6 l/ha). Whatever the frequency of application, plant extract gave a good protection of plants by reducing the number of leaflets attacked and especially by reducing the severity of the disease. Moreover, protection due to the plant extract sprayed every 7 days appeared to be not significantly different from the reference fungicide.

Key words: Tomato, *Oidium neolycopersici*, biological control, plant extract, Milsana

Résumé : L'oïdium (*Oidium neolycopersici*) est l'un des principaux agents de maladies fongiques aériennes sur tomate sous abri. La protection contre cet agent pathogène repose essentiellement sur des interventions chimiques. Différents produits biologiques ont été testés sur les oïdiums et des résultats encourageants ont été obtenus sur différentes cultures avec l'extrait de la plante *Reynoutria sachalinensis* (Milsana). Des essais ont été mis en place sur tomate durant trois années (1999 à 2001) pour étudier l'efficacité de cet extrait de plante. Les modalités étudiées comprennent plusieurs fréquences d'application (7, 14 et 21 jours) en comparaison avec un témoin eau et une référence chimique (Anvil à 0,6 l/ha). A toutes les fréquences d'application, l'extrait de plante a présenté une réduction de l'incidence et de la sévérité d'attaque de l'oïdium. Enfin, une protection du feuillage significative et équivalente au traitement avec le fongicide a été obtenue à la fréquence de 7 jours.

Mots clés: Tomate, *Oidium neolycopersici*, protection biologique, extrait de plante, Milsana

Introduction

Powdery mildew caused by the recently renamed species *Oidium neolycopersici* (Kiss *et al.*, 2001) is one of the principal diseases on tomato crops grown in greenhouse conditions. Fungicides are widely used to control this disease but the low number of fungicides available and the possible emergence of resistant strains of the pathogen complicate this method. Various biological methods, mainly based on the utilisation of antagonistic microorganisms or plant extracts, have been studied to control powdery mildews (Bélanger and Labbé, 2002). A plant extract from the giant knotweed *Reynoutria sachalinensis* (Milsana) has shown a high efficiency to control powdery mildews on various crops including apple, begonia and cucumber (Herger and Klingauf, 1990). The mode of action of this plant extract on cucumber is supposed to be associated with plant induced resistance (Daayf *et al.*, 1997). Experiments on tomato grown in greenhouse conditions have been conducted during a 3-year period (1999-2001) in order to test the efficiency of this product to control *O. neolycopersici*.

Material and methods

The effect of Milsana on the control of powdery mildew was evaluated on plants of tomato cv. *Trust* in 1999 and cv. *Grace* in 2000 and 2001 in a glasshouse at Ctifl. Both cultivars are very susceptible to *O. neolycopersici*. The cultural practices were kept as closely as possible to a commercial situation. Treatments were arranged in a block design with four replicates (60m² compartments) with a plant density of 1.8 plants/m². Eight plants per replicate were evaluated. Two experiments were realised each year

The plant extract was applied at 7, 14 or 21-day intervals starting 7 days before inoculation with *O. neolycopersici*. Powdery mildew was applied as a spore suspension containing 10⁴ spores/ml (except in experiment 2001-1: introduction of contaminated tomato plants). Spores were produced on cotyledons except for experiment 2001-1. Strain Et1 (INRA) was inoculated on plants for each experiment except for experiment 2001-2 where a mix of 3 strains was inoculated. Climatic conditions conducive to the development of powdery mildew were used. Average temperatures were maintained at 19-20° C from February to April and 22-23°C from May to August. Relative humidity (average over a 24 hours period) was comprised between 60% and 75%. The efficiency of Milsana was compared to that of a fungicide (Anvil, 0.6 l/ha) and to that of a control (water-treated plants). Two formulations of the plant extract were provided by Gebrüder Shætte KG., (VP99 in 1999 and 2000 and VP2000 in 2001). Doses of Milsana sprayed on the plants were those recommended by the manufacturer *i.e.* 0.25% for Milsana VP99 and 1.3% for Milsana VP2000.

Leaf infections were monitored weekly after the inoculation of powdery mildew until the end of the experiment. In 1999, disease incidence was recorded on two leaves per plant (with approximately 19 leaflets per leaf) randomly chosen in the treated zone. In 2000 and 2001, disease incidence and severity were recorded on respectively 4 and 3 leaves per plant (on the 3 terminal leaflets) randomly chosen in the treated zone. Severity was estimated based on a visual estimation of the mildewed leaf area infested by powdery mildew (expressed in % of leaf covered with powdery mildew). The efficiency of the control methods

was estimated by the calculation of a protection index computed as the reduction in severity on treated plants relative to severity on the control untreated plants.

Results

Incidence of powdery mildew on tomato

The first symptoms on the control plants appeared on leaves between 1 day (inoculum probably already present) and 20 days after artificial inoculation of *O. neolycopersici*. At the last date of notation, the incidence of powdery mildew on leaflets was high on control plants (up to 75%) and was reduced in all plots treated with Milsana (Table 1).

Severity of powdery mildew on tomato

Kinetics of development of powdery mildew on plants indicated that plants treated with Milsana were well protected and that the product had an effect over a long period, at least up to 30 days after the last application (Figure 1). Whatever intervals of treatments tested, up to 80% protection was provided by Milsana in the 4 tests conducted in 2000 and 2001 at the last date of notation (Table2). Moreover, the protection due to the plant extract sprayed every 7 days, relative to the control plants appeared to be statistically significant in all tests and not significantly different from the reference fungicide.

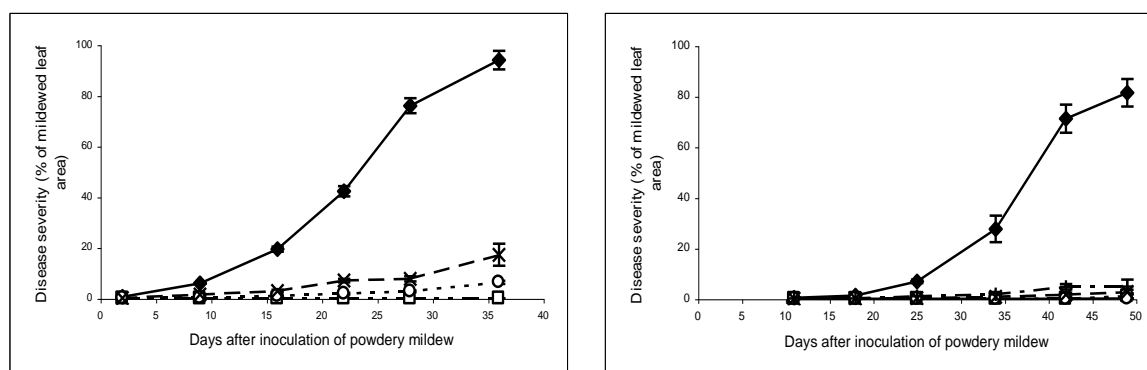
Table 1. Incidence of *Oidium neolycopersici* on leaflets of tomato treated with the plant extract from *Reynoutria sachalinensis* (Milsana VP99).

| Trial | appearance of symptoms (DAI) ^a | last treatment (DAI) | last notation (DAI) | diseased leaflets on the control (%) | diseased leaflets (%) on treated plants | | |
|--------|---|----------------------|---------------------|--------------------------------------|---|-----------------|-----------|
| | | | | | Milsana 7 days | Milsana 14 days | Anvil |
| 1999-1 | 20 | 14 | 50 | 76.4 ± 7.9 ^b | 1.2 ± 0.6 | - | 0.0 ± 0.0 |
| 1999-2 | 1 | 18 | 21 | 90.2 ± 6.9 | 33.4 ± 10.2 | - | 1.6 ± 0.8 |
| 2000-1 | 10 | 14 | 31 | 84.4 ± 9.5 | 15.4 ± 3.6 | 31.8 ± 14.1 | 0.3 ± 0.3 |
| 2000-2 | 3 | 14 | 36 | 99.7 ± 0.3 | 67.9 ± 3.3 | 70.6 ± 7.3 | 0.0 ± 0.0 |

a days after inoculation of powdery mildew on plants

b standard error of the mean

Figure 1: Protection of leaves of tomato against powdery mildew with Milsana in greenhouse 2000-2 (VP99) and 2001-2 (VP2000). Four or five treatments were applied before and after artificial inoculation of *O. neolyopersici*: water (□), Milsana every 7 days (○), Milsana every 14 days, 7 days before inoculation and 7 days after inoculation (◻), Milsana every 21 days, 7



days before inoculation and 14 days after inoculation (+), and the fungicide (◻).

2000-2

2001-2

Table 2. Efficiency of the plant extract from *Reynoutria sachalinensis* (Milsana VP99 and Milsana VP2000) to control *Oidium neolyopersici* on tomato.

| Trial | Mildewed leaf area on control plants (%) | last notation (DAI) | Protection index (%) relative to control plants | | | |
|--------|--|---------------------|---|-----------------|-----------------|------------|
| | | | Milsana 7 days | Milsana 14 days | Milsana 21 days | Anvil |
| 2000-1 | 31.7 ± 15.0 ^a | 31 | 94.8 ± 1.9 | 94.3 ± 2.3 | | 100 |
| 2000-2 | 93.9 ± 3.7 | 36 | 93.3 ± 0.9 | 81.6 ± 5.5 | | 100 |
| 2001-1 | 18.5 ± 9.6 | 62 | 99.9 ± 0.0 | 99.0 ± 0.0 | | 100 |
| 2001-2 | 81.4 ± 5.4 | 49 | 99.8 ± 0.0 | 96.7 ± 1.2 | 93.9 ± 3.6 | 98.8 ± 1.1 |

a standard error of the mean

Inference on the action of Milsana

Protection index could be high with a weak reduction of incidence of powdery mildew (trial 2000-2; Tables 1 and 2). This indicates that Milsana reduced the growth of powdery mildew on the leaves even if the spores were already present. Finally, symptoms of powdery mildew were observed in the non treated zone of the Milsana-treated plants, suggesting an absence of systemic effect of this product (data not shown).

Discussion-conclusion

The plant extract from *R. sachalinensis* significantly reduced the incidence of powdery mildew due to *O. neolycopersici* in the 6 greenhouses trials conducted between 1999 and 2001. Efficiency of this product was consistent whatever the levels of disease pressure and the cultivars of tomato tested. This product has also shown a good efficacy to control the other agent of powdery mildew on tomato, *Leveillula taurica* (Malathrakis *et al.*, 2002). Moreover Nicot *et al.* (this book) have observed that the efficacy of *Microdochium dimerum* strain L13 to control *Botrytis cinerea* on tomato was not altered by the application of Milsana on leaves. It could then contribute to the management of tomato powdery mildews in organic farming. But further work will be needed to assess the compatibility of Milsana with other biocontrol agents or products, in order to include Milsana in an integrated pest management on tomato. This product is currently being used by the agricultural industry in some countries but it is not yet registered in France.

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