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Field study on the efficacy of plant activators against *Plasmopara viticola*

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Abstract: Benzothiadiazole (BTH) and chitosan (CHT) belong to a class of agrochemicals activating the plant defense mechanisms against various pathogens. In this work, their efficacy against downy mildew, one of the most widespread and destructive diseases of the grapevine in the world, was evaluated. Open-field treatments were carried out in a vineyard of Groppello, a native cultivar of northern Italy, for two consecutive phytoiatric campaigns. In both years, BTH and CHT proved to be active, resulting in significantly lower percentages of disease incidence and disease severity than those recorded in the control plots. In particular, the CHT-copper combination was able to decrease the infection symptoms with values ranging from 60% to over 90% depending on the environmental conditions and the disease pressure. Our results suggested that plant defense inducers could be successfully used in crop protection as part of integrated pest management due to their effectiveness, as well as reduced environmental impact and low fitness costs for the plant.

Key Words: *benzothiadiazole, chitosan, fungal diseases, grapes, plant defence inducers, downy mildew*

INTRODUCTION

More than 10.000 species of Oomycetes and fungi can be pathogenic and cause diseases in plants worldwide [Bonaurio et al., 2009]. Downy mildew represents a major disease able to threaten seriously grapevine (*Vitis vinifera* L.) cultivation. The causal agent is the Oomycete *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni that infects all the plant green parts characterized by the presence of stomata, generally causing yellow discoloration, necrosis and distortion (Fig. 1). The bunch infections are responsible for the yield losses, while leaf damage decreases the carbon fixation, which negatively affects the grape quality, the accumulation of carbohydrates and the plant vigor in the following season [Jermini et al., 2010; Gessler et al., 2011].

Different strategies can be used to control fungal diseases and prevent associated yield losses. The fungicide application is the most effective and widely used method in case of high pathogen pressure, but there is an urgent need for alternative approaches to reduce the risks and impacts on human health and the environment (Directive 2009/128/EC). In this view, the stimulation of the plant's systemic acquired resistance (SAR) offers the perspective of long-lasting, broad-spectrum disease control through activation of the plant own defense machinery. Plant activators are products employed in crop protection able to elicit SAR and trigger a number of defense responses against pathogen attacks, including the biosynthesis of phytoalexins, plant secondary metabolites with a broad spectrum biological activity [Iriti et al., 2011]. Two SAR inducers, chitosan (CHT) and benzothiadiazole (BTH) deserve particular attention, because of their efficacy and low toxicity. In particular, CHT (Fig. 2) is a natural, biodegradable and low-cost polymer obtained from the deacetylation of crustacean chitin. BTH – benzo-(1,2,3)-thiadiazole-7- carbothioic acid S-methyl ester (Fig. 3) is a synthetic, photostable molecule and a functional analogue of salycilic acid, a plant hormone-like compound deeply involved in resistance against pathogens. In order to improve the knowledge on the potential of SAR inducers in crop protection, the aim of this work was to evaluate the efficacy of both elicitors in controlling downy mildew in a native grapevine cultivar of northern Italy growing in an area predisposed to fungal epidemics.

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Figure 1. Downy mildew symptoms in leaves (upper and lower surface) and bunches.

MATERIAL AND METHODS

Plant material and treatments. Two phytoiatric campaigns were planned, in 2015 and 2016, on Groppello, a red grapevine (*Vitis vinifera* L.) variety autochthonous of Lombardy, cultivated in an experimental vineyard located in Raffa di Puegnago (Brescia, Italy) (Fig. 4). Open field treatments were: i) 0.03% (w/v) CHT (76 kDa molecular weight and 85% deacetylation degree), ii) 0.03% CHT in combination with 150 g hL^{-1} copper hydroxide (CHT/Cu) and iii) 0.3 mM BTH. Untreated vines were used as negative controls. In both field surveys, the trial was set up as a complete randomized block design in 4 replications, with 10 vines (a plot) per treatment in each block. Plants were sprayed approximately every 10 days, according to the meteorological conditions, from the beginning of grapevine susceptibility to fungal diseases until the complete veraison (i.e., approximately from the middle of April to the end of July). To avoid spray drift to neighbouring plot, treatments were carried out with a spray lance powered by a walking-type motor pump, distributing a volume equivalent to 800-1000 L ha^{-1}.

Disease symptom assessment. Symptoms were assessed

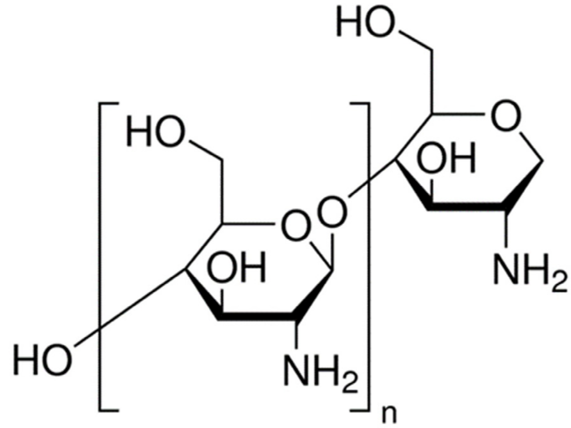

Figure 2. Chitosan (CHT) chemical structure.

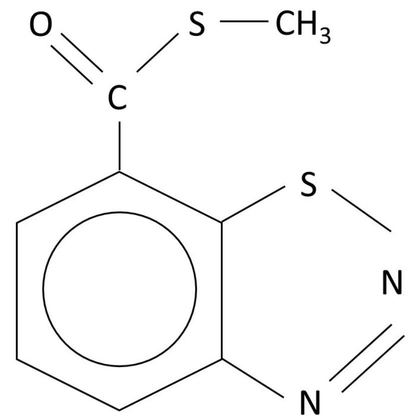

Figure 3. Benzothiadiazole (BTH) chemical structure.

weekly on bunches, by visual inspections, and the following two parameters were recorded: (i) disease incidence, as the percentage of infected bunches; (ii) disease severity, evaluated on a scale of 0–5, where 0 = no symptoms; 1 = 1– 10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; and 5 = 75–100% of infected berries per bunch [Zahavi et al., 2001]. Data regarding disease severity were processed according to Townsend–Heuberger formula (Eq. 1), in order to calculate the percentage of infection (I%):

$$I\% = [\sum(n \cdot v)/z \cdot N] \cdot 100$$

where n = number of bunches in each class; v = class value; z = highest class value; and N = total amount of assessed bunches.

Statistical analysis. For each time-step and treatment, the mean and standard deviation values of disease incidence and disease severity were computed, and the one-way ANOVA analysis and Turkey's test for post hoc analysis were carried out on the collected

Figure 4. Vines treated with plant activators in the study area.

data, determining the significant differences among the treatments CHT/Cu, CHT and BTH, compared to the control, at *p*-value < 0.001***.

RESULTS AND DISCUSSION

The results of the three most representative disease evaluations, performed on 1, 10 and 25 July in 2015 and 2016, are shown in Tables 1 and 2. In general, in both years, the treatments were effective, with disease incidence and severity values significantly different from those of the controls (*p*-value = 0.000). Particularly, as a result of the 2015 phytoiatric campaign, the data obtained showed that CHT/Cu was able to delay the pathogen infection (until early July) and reduce it by about 60%, both in terms of disease incidence and severity after the last treatment (at the end of July) (Tab. 1). Otherwise, the CHT and BTH efficacy against downy mildew was lower. Both inducers provided an intermediate level of protection against disease decreasing its incidence and severity by 34% and 46%-51%, respectively, compared to untreated grapevines (Tab. 1).

Despite the lower pathogen pressure in the study area during 2016, the plant activator efficacy was confirmed in the second phytoiatric campaign (Tab. 2). Indeed, disease incidence and severity were both significantly affected by CHT/Cu (-92% and -95%, respectively) followed by BTH (-60% and -74%) and CHT (-64% and -60%) at last assessment.

The obtained results showed a high performance of BTH and CHT in open fields, activating the plant resistance mechanisms and reducing downy mildew symptoms. In addition, Groppello variety could be a responsive cultivar able to maximize the efficacy of the SAR inducers [Banani et al., 2014]. Overall, our data confirmed the results of previous studies showing the BTH and CHT ability to provide protection against grape downy mildew, even when carried out under different experimental conditions and on different cultivars [Harm et al., 2011; Farouk et al., 2017]. A number of defense mechanisms could be elicited by CHT and BTH, including stomatal closure, oxidative burst, hypersensitive response, biosynthesis of phytoalexins and pathogenesis-related proteins.

The high destructive potential of downy mildew has required the development of strategies that minimize yield losses. The use of plant protection products with anti-peronosporic activity has been necessary due to the ineffectiveness of alternative control methods and the high susceptibility of the vine to the pathogen, especially in areas characterized by weather conditions favorable

Table 1. Disease incidence (expressed as % of infected bunches, I%) and severity (expressed as % of infection according to Townsend–Heuberger formula, S%) in the untreated parcels (CTRL) of Groppello grapevines or in plots treated with chitosan (CHT), chitosan in combination with copper hydroxide (CHT/Cu), or benzothiadiazole (BTH) during 2015.

Treatments	1st July		10th July		25th July	
	% Incidence	% Severity	% Incidence	% Severity	% Incidence	% Severity
CTRL	13.4 ± 1.3 a	5.0 ± 0.8 a	17.8 ± 1.6 a	11.8 ± 1.3 a	57.0 ± 3.4 a	30.5 ± 3.8 a
BTH	3.3 ± 0.5 b	1.4 ± 0.4 b	3.4 ± 0.7 c	4.8 ± 0.4 b	37.8 ± 3.0 b	14.9 ± 1.2 bc
CHT	3.0 ± 0.4 b	2.2 ± 0.3 b	6.0 ± 0.3 b	4.3 ± 0.4 b	36.8 ± 3.3 b	16.4 ± 1.7 b
CHT/Cu	0.0 ± 0.0 c	0.0 ± 0.0 c	5.1 ± 0.7 b	4.9 ± 0.6 b	22.8 ± 1.6 c	11.5 ± 1.6 c
F	272	77	192	84	94	53
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. Disease incidence (expressed as % of infected bunches) and severity (expressed as % of infection according to Townsend–Heuberger formula) in the untreated parcels (CTRL) of Groppello grapevines or in plots treated with chitosan (CHT), chitosan in combination with copper hydroxide (CHT/Cu), or benzothiadiazole (BTH) during 2016.

Treatments	1st July		10th July		25th July	
	% Incidence	% Severity	% Incidence	% Severity	% Incidence	% Severity
CTRL	16.0 ± 0.8 a	6.1 ± 0.6 a	22.5 ± 0.9 a	8.3 ± 0.6 a	36.8 ± 1.4 a	18.6 ± 2.3 a
BTH	7.2 ± 0.0 c	2.4 ± 0.0 c	11.1 ± 0.7 b	4.5 ± 0.0 c	14.8 ± 0.5 b	4.8 ± 0.0 c
CHT	8.7 ± 0.6 b	3.0 ± 0.3 b	10.5 ± 1.2 b	5.8 ± 0.3 b	13.3 ± 0.5 b	7.5 ± 0.4 b
CHT/Cu	0.9 ± 0.2 d	0.3 ± 0.2 b	1.2 ± 0.3 c	0.5 ± 0.3 d	2.8 ± 0.3 c	1.0 ± 0.2 d
F	572	202	429	349	1236	165
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Note: Values are mean ± standard deviation. Different letters indicate statistically significant differences at p value < 0.001 among treatments.

(warm and wet climates) to the occurrence of epidemics. The active substance traditionally as anti-peronosporic agent is copper. However, it can be phytotoxic and the current provisions regarding its use in conventional and organic viticulture place considerable limitations in order to avoid accumulation in the soil [EC, 2018]. In this context, the plant defense inducers could be a promising alternative because of their efficacy and low health and environmental impact. Moreover, both BTH and CHT provide advantages in terms of low fitness costs to the host plant and avoid resistance phenomena by pathogens [Iriti et al., 2010; Dufour et al., 2016]. In conclusion, our results suggested that the chitosan-(low dose) copper combination is promising against *P. viticola* in grapevine, as part of an integrated pest management. This approach could further contribute to reduce the copper input in crop protection.

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Bitki aktivatorlarının *Plasmopara viticola* əleyhinə effektivliyi ilə bağlı çöl tədqiqatları

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Benzotiadiazol (BTH) və xitosan (CHT) bitkilərin müxtəlif növ patogenlərə qarşı müdafiə mexanizmlərini aktivləşdirən aqrokimyəvi maddələr sinfinə aiddir. Məqalədə onların dünyada ən geniş yayılmış və üzümlükər üçün təhlükəli olan yalançı unlu şeh xəstəliyinə qarşı effektivliyi qiymətləndirilmişdir. Şimali İtaliyanın yerli sortu olan Groppello üzümlüyündə açıq sahədə iki ardıcıl fitoiatrik tədbir keçirilmişdir. Hər iki ildə BTH və CHT-nin aktiv olması müəyyən edilmiş və nəzarət sahələrində qeydə alınanlarla müqayisədə əhəmiyyətli dərəcədə aşağı xəstəlik faizi və xəstəlik dərəcəsi ilə nəticələnmişdir. Xüsusilə, CHT-mis birləşməsi ətraf mühit şəraitindən və xəstəlik təzyiqindən asılı olaraq infeksiya simptomlarını 60%-dan 90%-dək azalda bilib. Nəticələrimiz göstərdi ki, effektivliyinə, ətraf mühitə təsirlərinin az olmasına və az məsrəf tələb etdiyinə görə müdafiə induktorları bitkilərin mühafizəsində zərərvericilərin inteqrasiya olunmuş idarəetməsinin tərkib hissəsi kimi uğurla tətbiq edilə bilər.

Açar sözlər: *benzothiadiazole, xitosan, göbələk xəstəlikləri, üzümlüklər, bitki müdafiə induktorları, yalançı unlu şeh*

Полевое исследование эффективности растительных активаторов против *Plasmopara viticola*

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Бензотиадиазол (BTH) и хитозан (CHT) относятся к классу агрохимикатов, активирующих механизмы защиты растений от различных патогенов. В данной работе оценивалась их эффективность против ложной мучнистой росы, одной из самых распространенных и разрушительных болезней виноградной лозы в мире. Обработки в открытом грунте проводились на винограднике Гроппелло, местного сорта северной Италии, в течение двух последовательных фитоиатрических кампаний. В оба года BTH и CHT оказались активными, что привело к значительно более низким процентным показателям заболеваемости и тяжести заболевания, чем те, которые были зарегистрированы на контрольных участках. В частности, комбинация CHT-медь смогла уменьшить симптомы инфекции со значениями в диапазоне от 60% до более 90% в зависимости от условий окружающей среды и тяжести заболевания. Наши результаты показали, что индукторы защиты растений могут быть успешно использованы в защите растений в рамках комплексной борьбы с вредителями благодаря их эффективности, а также снижению воздействия на окружающую среду и низкой стоимости пригодных растений.

Ключевые слова: *бензотиадиазол, хитозан, грибные болезни, винограды, индукторы защиты растений, ложная мучнистая роса*