EVALUATION OF ALTERNATIVE PRODUCTS TO REDUCE THE ASCOSPORE POTENTIAL OF *Venturia inaequalis*

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ABSTRACT

A low level of inoculum is a prerequisite for planning an effective protection strategy against apple scab. Currently, inoculum reduction approaches proven to be effective include treatments with urea, dolomitic lime, and the removal of leaf litter from the orchard. An experiment was conducted to evaluate the effectiveness of alternative product applications in reducing the apple scab inoculum on artificial leaf litter. Remedier®, *Trichoderma asperellum* ICC012, and *T. gamsii* ICC080-based products, urea as an inorganic fertilizer, and whey as an organic amendment were compared in a study spanning three overwintering periods. A partial reduction in ascospore production compared to untreated leaf litter was achieved through autumn application of urea, spring application of Remedier, and both autumn and spring applications of whey. Over the two-year assessment period, spore reduction was approximately 60% for urea, 50% for Remedier, and 40% for whey. The Trichoderma-based product could be an interesting option for reducing apple scab inoculum, particularly in organic farming where other means, such as costly mechanical operations and the use of dolomitic lime, are not available.

Keywords: apple scab, inoculum, *Trichoderma* spp., urea, whey

1 INTRODUCTION

Apple scab, caused by the ascomycete *Venturia inaequalis* (Cooke) G. Wint. (anamorph *Spilocaea pomi* Fr.), represents the most significant disease globally, especially in areas characterized by cool and humid springs. Annually, the fungus reproduces by forming pseudothecia, which overwinter in fallen leaf debris, protecting the ascospores formed inside. During spring, as novel vegetation tissues are produced by the host, ascospores are released into the air, initiating the primary infection phase. After infection symptoms appear on the vegetation, newly formed conidia initiate the secondary infection phase (Vaillancourt and Hartman, 2000). Protection against apple scab relies on fungicides, resistant varieties, and sanitation practices. Sanitation aims to reduce ascospore production or pseudothecia formation by destroying or removing fallen leaves, thereby enhancing disease management during the apple vegetative

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season, particularly effective in susceptible cultivars and areas with a high disease burden from the previous year.
Urea and dolomitic lime have proven to be effective interventions (Spotts et al., 1997). Urea, an inorganic fertilizer, inhibits *V. inaequalis* by promoting competition from microorganisms on leaf surfaces and providing nitrogen as a nutritional source for soil microorganisms but not for the pathogen (Burchill, 1968; Burchill et al., 1965; Ross and Burchill, 1968).
Organic matter theoretically stimulates the growth of antagonistic microorganisms and impedes soil-borne pathogens. While the precise aspects of its use in biological control require definition, successful cases provide a promising starting point for further investigation (Fry, 1982). Whey, investigated as a plant nutrient and soil amendment, reduces ascospore levels by providing nutrients to microorganisms naturally present on leaves or soil surfaces (Sharratt et al., 1959).
Trichoderma, a group of fungi abundant in ecosystems, has been extensively studied and used as a biocontrol agent against various crop diseases. However, its practical application in field crop protection faces challenges due to variable, sometimes contrary, efficacy influenced by factors such as weather conditions, isolate type, host, inoculum density, and application methods (Zaidi and Singh, 2017, Chammen et al., 2022). Recent studies have demonstrated Trichoderma's efficacy in reducing inoculum sources of *Stemphylium vesicarium*, the causal agent of pear brown spot (Rossi and Pattori, 2009). Remedier®, containing *Trichoderma asperellum* ICC012 and *T. gamsii* ICC080, is registered by Gowan Italia for biological control of pear brown spot, showing promise in reducing apple scab primary inoculum.

2 MATERIAL AND METHODS

The experiment was conducted over three overwintering periods, spanning from 2020 to 2022, at Fondazione Edmund Mach in San Michele all'Adige (TN). Artificial leaf litter sheets were set up to compare different treatments. Leaves were collected from untreated plots in a local apple orchard naturally infected by Venturia. The leaf litter consisted of two 1 m^2 metallic wire sheets with a mesh size of 20 x 20 mm, placed over an Agro-nonwoven white sheet. These leaf litters were anchored to the soil in the orchard, spaced 1.5 m apart along a row of trees, and maintained from November to May.
The experimental design was a complete randomized block with four different treatments, including an untreated control, each replicated three times (table 1).

Table 1: Principal characteristics of the tested products.

Products	Active ingredients	Form.	Conc. a.i.	Field rate g/hL
Remedier®	*Trichoderma asperellum* ICC012; *Trichoderma gamsii* ICC080	WP	2%; 2%	250
Biuron®	N	WG	46%	2000
Whey	Milk whey	WP	-	6000

In 2020, both leaf litter treatments with urea (20 kg/ha) and Remedier (2.5 kg/ha) were applied in late autumn. An additional treatment with Remedier (2.5 kg/ha) was applied the following spring.
In autumn 2021, three different treatments were applied: urea (20 kg/ha), Remedier (2.5 kg/ha), and whey (60 kg/ha). Treatments with Remedier and whey were repeated the following spring.
In 2022, treatments with whey (60 kg/ha) were applied in late autumn and early spring according to the study protocol. Treatments with Remedier (2.5 kg/ha) and a mixture of Remedier with urea (2.5+20 kg/ha) were applied only in spring. Due to heavy rainfall events, all spring applications were repeated.
All treatments were applied using a knapsack electric sprayer at a water volume of 1000 L/ha until complete wetness of the leaf litters was achieved. Remedier was suspended in a small volume of water approximately twenty-four hours before use. Meteorological data were recorded by the nearby weather station. During the ascospore release period, particularly in April and May, leaf samples were collected weekly from each litter to assess ascospore quantity, estimated by forcibly discharging them in water per gram of leaf litter (Hutton and Burchill, 1965). The efficacy of treatments was calculated using Abbott's formula (Abbott, 1925), and spore counts were conducted using a microscope with a hemocytometer. All data collected were analyzed using ANOVA, followed by Tukey's test to determine mean differences between the different treatments under study.

3 RESULTS

3.1 Experimental trial in 2020-2021

The meteorological data registered during the first year of experiment are shown in fig. 1.

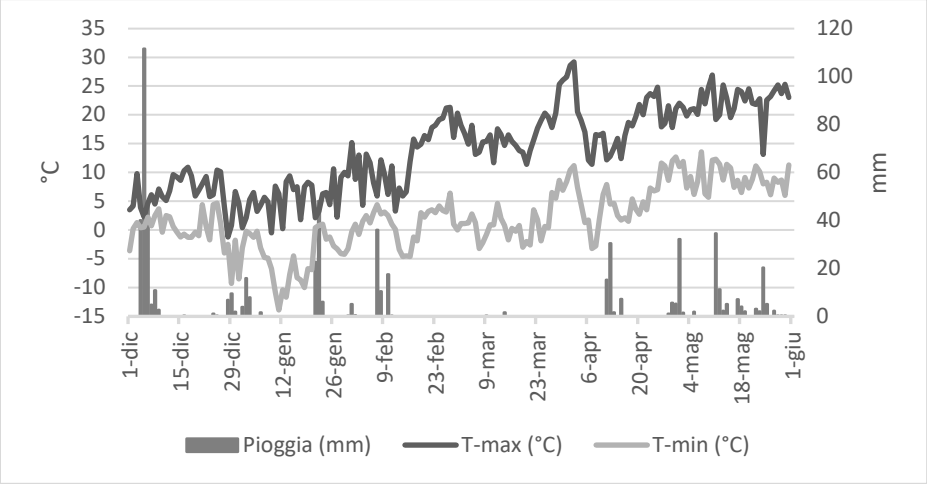

Figure 1: Meteorogical data recorded in the study site of S.Michele a/A – 2020/21.

The potential ascospore production was assessed weekly for 11 weeks from March to May. Compared to the untreated control, Remedier, when applied in either autumn or spring, reduced the production of ascospores per gram of leaf litter by 26% and 59%, respectively. However, the urea treatment demonstrated the most substantial reduction with a significant decrease of 71% (table 2).

Table 2: Experimental trial 2020/21: mean of ascospores released from leaf litters.

Treatment/Product	Application timing	n. ascospores/g leaf litter	Efficacy % (Abbott)
Untreated	-	151963,7 a***	-
Remedier	autumn*	113257,4 ab	25,5
Remedier	spring**	62121,9 ab	59,1
Biuron	autumn*	44769,9 b	70,5

*16/12/20: **30/03/21: ***Values followed by different letters were significantly different (Tukey's HSD, p<0,05)

3.2 Experimental trial in 2021-2022

The meteorological data recorded during the second year of experiment are shown in fig. 2.

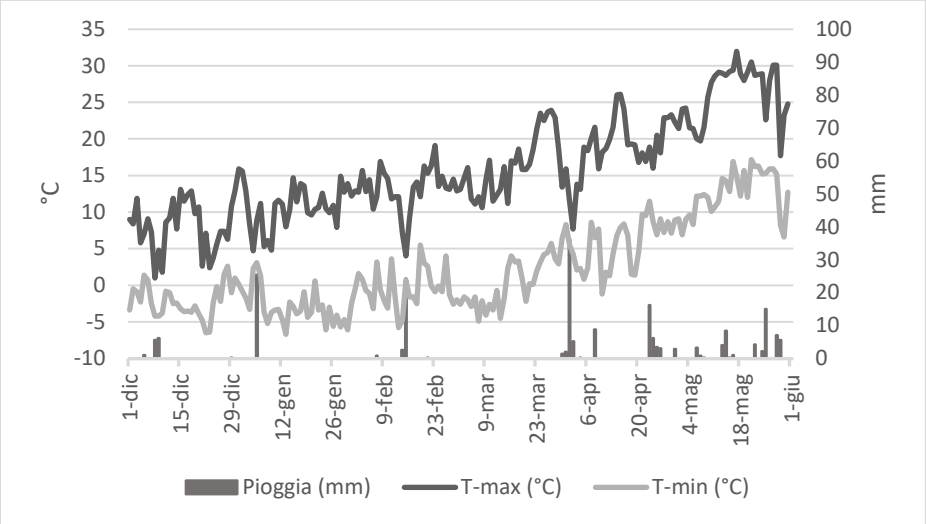

Figure 2: Meteoreogical data recorded in the study site of S.Michele a/A – 2021/22.

At beginning of January a snow cover was present on all artificial leaf litters for a period of two weeks. The potential ascospore discharge was assessed weekly for 6 weeks from April to May. There was no statistical significant difference between treatments, including the untreated control. However, the application of Remedier in both autumn and spring, appeared resulting in a 19% increase in ascospore production compared to the untreated control. Conversely, whey application during the same periods resulted in a 24% reduction. The distribution of urea in autumn exhibited the highest efficacy, with a 44% reduction in ascospore production (Table 3).

Table 3: Experimental trial 2021/22: mean of ascospores released from leaf litters.

Treatment/Product	Application timing	n. ascospores/g leaf litter	Efficacy % (Abbott)
Untreated	-	48332,2	-
Remedier	autumn* + spring**	57260,7	-18,5
Whey	autumn* + spring**	36901,0	23,7
Biuron	autumn*	27117,5	43,9

*22/11/21: **15/03/22

3.3 Experimental trial in 2022-2023

The meteorological data recorded during the last year of experiment are shown in fig. 3.

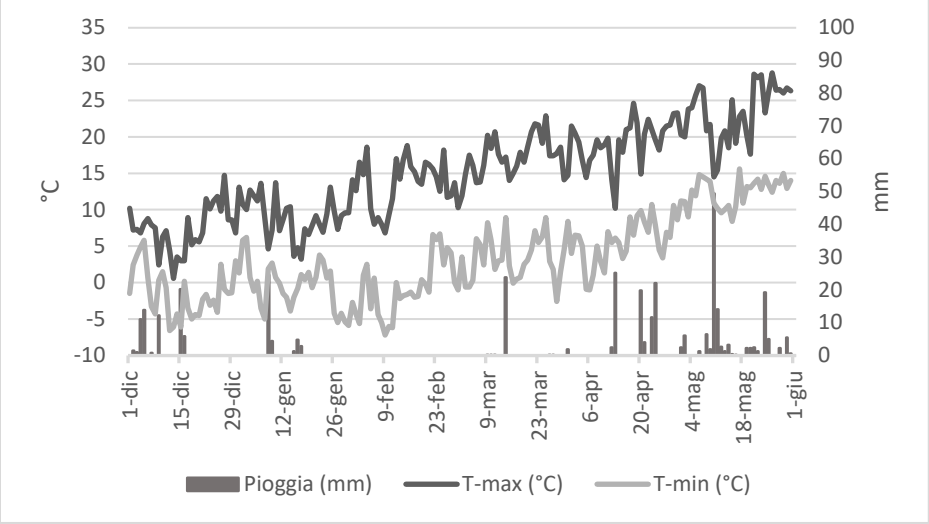

Figure 3: Meteoreogical data recorded in the study site of S.Michele a/A – 2022/23.

The potential ascospore discharge was registered weekly for consecutive 6 weeks period from April to May. In comparison to the untreated control, the spring application of Remedier resulted in 48% reduction in the amount of ascospores per gram of leaf litter. When Remedier was used in combination with urea, the reduction level increased to 59% which was statistically similar to the 67% reduction achieved by whey in dual treatment (table 4).

Table 4. Experimental trial 2022/23: mean of ascospores released from leaf litters.

Treatment/Product	Application timing	n. ascospores/g leaf litter	Efficacy % (Abbott)
Untreated	-	97799,6 a***	-
Remedier	spring**	50472,3 ab	48,4
Remedier + Biuron	spring**	40427,3 b	58,7
Whey	autumn* + spring**	31847,8 b	67,4

*06/12/22; **24/03/23; ***Values followed by different letters were significantly different (Tukey's HSD, $p<0,05$)

The reduction in ascospore production, averaged over two years and compared to untreated leaf litters, was approximately 60% for urea applied in autumn, 50% for Remedier applied in spring, and 40% for whey applied in both seasons (see Figure 4).

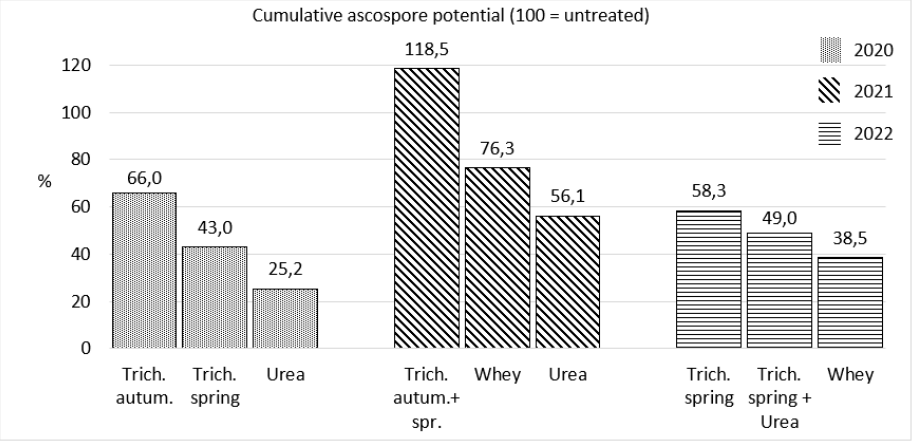

Figure 4: Efficacy of the a.i. to reduce the primary ascospore inoculum of *Venturia inaequalis*.

4 DISCUSSION AND CONCLUSION

The conclusions are referred as two years average results of the treatments compared during the study. The autumnal application of urea scored the most significant results

in terms of reducing apple scab inoculum during the study, which encompassed various weather conditions and disease pressures. Remedier, *Trichoderma asperellum* ICC012 and *T. gamsii* ICC080, when applied once in spring, reduced the disease inoculum potential by approximately 50% compared to untreated conditions. Combining Remedier with inorganic fertilizers such as urea may enhance its efficacy, although further experimentation is necessary to validate this hypothesis. At contrary it seemed not profitable if not negative, to distribute the product both in autumn and spring. Whey, used as a soil amendment, demonstrated a clear antagonistic effect against the pathogen during the experiment. However, before its practical implementation in the field, it requires thorough evaluation in terms of economic and environmental sustainability. The primary goal of the study was to reduce the potential Venturia inoculum and subsequently alleviate disease pressure. Nevertheless, it remains imperative to continue fungicide applications throughout the season based on plant phenological phases and disease forecast models. Biological control agents (BCAs) and amendment products could serve as valuable supplements to fungicides in managing apple scab and other pathogens overwintering in leaf litter (e.g., Alternaria, Colletotrichum, Glomerella, *Marssonina* spp.), providing beneficial effects to both soil and plants even in the absence of disease.

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