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Thermotherapy for *Xanthomonas campestris* pv. *campestris* Control in Kale Organic Seeds

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HIGHLIGHTS

- *Xanthomonas campestris* pv. *campestris* are sensitive to thermotherapy at 50°C.
- Lots of kale seeds with high vigor are recommended for thermotherapy.
- Control of *Xanthomonas campestris* pv. *campestris* in organic kale seeds.

Abstract: Among the vegetables produced in the organic system, there are the brassicas as the largest group in Brazil, and in brassicas, one of the most important pathogens is *Xanthomonas campestris* pv. *campestris* (Xcc), which causes black rot of crucifers. The importance of this bacterium is increased because it is transmitted by seeds. The objective of this research was to evaluate the effect of thermotherapy in kale seeds, produced in the organic system, on the physiological seed quality and on Xcc control. Sixteen treatments were evaluated [factorial scheme: four lots of kale seeds (10714, 10751, 10756 and 10789) and four treatments (control, seeds inoculated with Xcc, seeds inoculated and treated at 50°C for 20 and 40 minutes)], in a completely randomized design, with four replicates. Germination, seed vigor (first count) and Xcc incidence were evaluated. Thermotherapy in kale seeds at 50°C for 20 and 40 minutes of exposure reduces the infection of *Xanthomonas campestris* pv. *campestris* to 10% and 4%, respectively. However, in a seed lot with low initial seed vigor, the heat treatment reduces the germination rate and first count in the germination test. In seed lots with high initial vigor it can be recommended heat treatment at 50°C for up to 40 minutes of exposure for control of Xcc in kale organic seeds.

Keywords: *Brassica oleracea* var. *acephala*; black rot; organic production; heat treatment.

INTRODUCTION

There has been an increase in the demand for vegetables produced in the organic system. Among the vegetables produced in this system, brassicas are the largest group in Brazil and kale is an important crop of this group. In brassicas, one of the most important pathogens is the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc), which causes black rot of crucifers, that is spread worldwide [1]. This pathogen is particularly relevant in kale, as it damages the leaves, making them unfeasible for commercialization. The importance of this bacterium is increased by the fact that it is transmitted by seeds [1,2].

Stated that Xcc can infect plants at any stage of growth [3], and when there are warm and humid periods, the disease severity can increase. In tropical regions, such as Brazil, the control of this disease is difficult, leading to major damage in the field, mainly in organic production system where the use of agrochemicals is not allowed, including in seed treatment. Therefore, it is necessary to study measures of control diseases which are transmitted by seeds in the organic system, in order to avoid pathogens transmission, without affecting the physiological quality of the seed lot [4].

Among the methods allowed in the organic production system there is thermotherapy, a physical treatment method consisting of the exposure of the seeds to the heat action, aiming the control of phytopathogenic organisms [2,5]. For the success of this method, it is necessary to know the appropriate combination of temperature and exposure period, which may vary with the species, cultivar, lot, initial vigor, among other factors [6,7].

Researchers related to the treatment of seeds by thermotherapy still show some divergences in the results, but they show similar temperatures in the control and/or eradication of pathogens in seeds. They described that wet thermotherapy should be applied at a temperature from 45 to 60ºC for a maximum exposure period of 60 minutes, regardless of the species [4-7,8,9].

Although efficient in the control of seed-associated pathogens, thermotherapy, when not properly applied, can cause damage to the seeds, such as the rupture of cell membranes or the denaturation of proteins, severely affecting the physiological quality [5,8]. Thus, it is necessary to verify the effect of this treatment on seed quality, and not only on pathogen control.

Considering that seeds are important agents for the dissemination, survival, and transmission of diseases of economic importance in several plant species [9] and that there is not enough information about the use of seed thermotherapy to control Xcc, this study aimed to evaluate the effect of thermotherapy on kale seeds, produced in the organic system, on the physiological quality of the seeds and on Xcc incidence in seeds inoculated with this bacterium.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Seed Pathology of the School of Agriculture (FCA) of the Sao Paulo State University (UNESP), Botucatu Campus, Brazil.

The research was carried out in two stages. In the first stage the control of *X. campestris* pv. *campestris* (Xcc) in inoculated seeds was evaluated, and in the second the physiological quality of the seeds inoculated and treated by thermotherapy was evaluated.

Sixteen treatments were studied, resulting from the factorial four lots of kale seeds, cultivar Manteiga, and four seed treatments. The seeds lots (10714, 10751, 10756 and 10789) were produced by the company Bionatur in the organic system. The four treatments evaluated were: a) seed inoculated with Xcc and submitted to the heat treatment at 50°C for 20 minutes; b) seeds inoculated with Xcc submitted to the heat treatment at 50°C for 40 minutes; c) seeds inoculated with Xcc and not submitted to heat treatment (“inoculated control”); d) control: seeds that were not inoculated nor treated by thermotherapy. The experimental design was completely randomized, with four replications. These treatments were chosen after preliminary studies, where it was possible to observe that temperatures higher than 50ºC were harmful to the seeds, as well as more than 40 minutes at 50ºC.

Before inoculation, the seeds were previously treated with sodium hypochlorite to eliminate fungi and bacteria on their surface. The immersion method was used for seed inoculation. In this method, approximately 10 g of seeds were immersed in the bacterial cell suspension in the concentration of 10^8 CFU mL^{-1} obtained from pure Xcc cultures, remaining in contact with the inoculum for 48 hours. Then the seeds were dried.

The thermotherapy treatments were performed using a water bath Model 1147 Fanem®, in which two porous nylon bags with five grams of seeds each were allocated, and remained at the temperature of 50 °C for 20 or 40 minutes, depending on the treatment. Posteriorly, the seeds were dried and placed in a dry chamber (20°C and 40% of relative air humidity).

Xcc incidence in the seeds and germination were evaluated. Four replicates of 25 equidistant seeds were distributed over agar nutrient medium in plastic Petri dishes (9.0 cm diameter), in order to evaluate the

Xcc incidence. According to the methodology proposed [10], the seeds were incubated at 26°C for 48h and examined under a stereomicroscope for bacterium detection by the presence of yellowish colonies and mucoid surrounding the seeds in the medium. Results were expressed as percentage of seeds with Xcc incidence.

To evaluate the germination, each plot consisted of 50 seeds distributed on two sheets of germination paper moistened 2.5 times its weight with distilled water, in boxes of transparent plastic (gerbox), covered with transparent plastic bags. The experiment was carried out in a Biochemical Oxygen Demand (B.O.D.) germinator, at 25 ± 1°C and 12 hours of light. The total number of germinated seeds at ten days after sowing (DAS) was considered as the total germination. The first count of the germination test was performed five DAS of the seeds, being considered a vigor test [11].

The results were submitted to analysis of variance, and when significant the means were compared by Tukey test at 5% probability (p<0.05) using the software Sisvar [12].

RESULTS AND DISCUSSION

Control of *Xanthomonas campestris* pv. *campestris*

Both isolated factors (seed lots and seed treatments) were significant by the F test in the evaluation of Xcc incidence in the seeds, as well as the interaction between them.

Even in the absence of inoculation, all lots presented Xcc-infected seeds. The lot 10751 had the highest Xcc incidence (5% of infected seeds), while the lot 10789 presented the lowest incidence (1% of infected seeds) (Table 1). Even after heat treatment at 50ºC for 40 minutes the incidence of Xcc in this lot 10751 was higher than all other lots.

Table 1. Incidence of *Xanthomonas campestris* pv. *campestris* in four kale organic seed lots after inoculation and submitted to the heat treatments. FCA/UNESP, Botucatu/SP.

Seed lot	Control without inoculation	Control inoculated	Thermotherapy (50°C 20 minutes)	Thermotherapy (50°C 40 minutes)
		Incidence (%)		
10714	4.0 AB b[1]	88.0 B a	11.0 AB b	3.3 B b
10751	5.0 A b	97.0 A a	15.0 A b	7.0 A b
10756	3.0 BC b	87.0 B a	9.0 B b	3.0 B b
10789	1.0 C b	87.0 B a	5.5 B b	3.0 B b
CV (%)[2]		14.0		

[1]Means followed by same letter, upper case in colunms and lowercase in lines, do not differ by Tukey`s test at 5% of probability
[2]CV = coefficient of variation

Researchers stated that the pathogen can multiply and develop the disease the sooner it comes into contact with the plant [13] and the use of Xcc-free seeds is indispensable for disease control [14]. In addition, the seed is an efficient initial inoculum due to easy field spread and survival of the pathogen inside and/or on the surface of the seed. The same researches stated that only 0.0003% of infected seeds may be enough to cause black rot epidemics [14]. Therefore, all lots in the present study had high percentages of Xcc presence at levels sufficient to cause damage under field conditions favorable to this bacterium.

The inoculation was efficient, since there was a significant increase in the Xcc incidence for all seed lots, increasing from 1.0-5.0% when there was no inoculation to 87.0-97.0% when the inoculation was performed in the seeds (Table 1). After inoculation, lot 10751 also presented the highest incidence of Xcc, with 97% of infected seeds.

In the heat treatment at 50ºC for 20 minutes, as well as for 40 minutes, there was a significant reduction in Xcc incidence in the seeds of all the lots compared to the inoculated seeds without treatment (Table 1). Despite the decrease in the percentage of contaminated seeds, it was not possible to eradicate the bacterium with the heat treatments used. It is noteworthy that these values were obtained after inoculation in which there was a high incidence of the pathogen (87 to 97%) that is not normally found. Other studies also reported a decrease and not the total eradication of the pathogen. Researchers observed that the heat treatment of cauliflower seeds at 50°C for 15 minutes was not efficient to control this bacterium, obtaining up to 30% of

infected seeds even with this treatment [13]. Others observed a reduction of the Xcc symptoms in brassica seeds after heat treatment at 53°C for 20 minutes, but there was no eradication either [15].

Therefore, the thermotherapy treatments tested proved to be efficient in the reduction of Xcc incidence in kale seeds. However, alone it may not be sufficient for complete eradication of this bacterium. The heat treatment can be an alternative for phytopathogens control in brassica seeds in the organic system [16]. However, it will only be efficient as part of the management strategy, which begins in the field of seed production, with preventive measures against infection by pathogens in the seeds.

Seed germination and vigor after Xcc inoculation and heat treatment

Both isolated factors (seed lots and seed treatments) were significant by the F test in the evaluation of seed germination and vigor, as well as the interaction between them.

The lot 10756 presented the highest germination percentage in all treatments, but differing only from lot 10751, which had the least number of germinated seeds (Table 2). Lot 10751 also had the highest Xcc incidence (Table 1), showing a probable relation between Xcc incidence in seeds lots and total germination of the lot. Also was observed that the germination in some brassica seeds were lower when inoculated with the bacterium Xcc, in relation to the control without inoculation [15]. In organic seed production of Brassica vegetables, infections by pathogens can cause severe losses in seed quality (germination and vigor) [16].

Table 2. Germination and first count in germination test in four kale organic seed lots after inoculation and submitted to the heat treatments. FCA/UNESP, Botucatu/SP.

Seed lot	Control without inoculation	Control inoculated	Thermotherapy (50°C 20 minutes)	Thermotherapy (50°C 40 minutes)
			Germination (%)	
10714	90.0 AB a[1]	85.0 AB a	85.0 A a	79.5 AB a
10751	83.0 B a	77.0 B ab	71.5 B b	71.0 B b
10756	96.5 A a	92.0 A a	92.0 A a	90.5 A a
10789	93.0 AB a	89.0 A a	86.5 A a	86.0 A a
CV (%)			7.6	
			First count (%)	
10714	83.0 A a	76.0 A a	83.0 A a	75.5 A a
10751	69.0 B a	61.0 B ab	60.0 B ab	54.0 B b
10756	89.0 A a	84.0 A a	89.5 A a	86.5 A a
10789	81.5 AB a	78.0 A a	80.5 A a	80.5 A a
CV (%) [2]			9.2	

[1]Means followed by same letter, upper case in colunms and lowercase in lines, do not differ by Tukey`s test at 5% of probability.
[2]CV = coefficient of variation

In spite of the numerical reduction in the germination after seed heat treatments, just for lot 10751 germination was significantly reduced in the treatment at 50°C for 20 and 40 minutes of exposure, being this the lot that always presented the lowest germination in all treatments (Table 2). This result confirms reports that tolerance to thermotherapy without loss of physiological quality may vary according to the lot [6,15] and, if this treatment is tested in only one lot, the results might not be conclusive. Researchers stated less vigorous lots are more likely to be affected by thermotherapy than lots considered more vigorous [14].

Despite the differences, all lots were able to be marketed in the absence of Xcc inoculation. According to the Normative Instruction IN 45, the minimum germination standard is 75% [17]. However, after inoculation and heat treatment at 50ºC for 20 and 40 minutes, the lot 10751 could no longer be marketed because in both treatment (20 and 40 minutes) the values were lower than this minimum. For the lot 10714, the minimum standard for commercialization was not achieved only when the thermotherapy was conducted at 50 °C for 40 minutes of seed exposure.

For the first count in the germination test, lot 10751 presented lower values than the other lots in all treatments, except for the treatment without inoculation of the pathogen when it did not differ from lot 10789. The other lots did not differ significantly among them (Table 2). Despite the numerical reduction in the values, only for lot 10751 germination in the first count was reduced in the treatment at 50°C for 40 minutes of

exposure. Researchers related that treatment with wet thermotherapy at 50ºC for up to 60 minutes did not affect germination, but could reduce the first count in the germination test of kale seeds [4].

Sucessive treatments (desinfestation, inoculation, and thermotherapy) may compromise the germination of seeds, an effect similar to the study of accelerated seed aging, which causes stress through the exposure of seeds immersed in water, and significantly reduces germination and seed vigor [18]. Besides this, the treatment with hot water can cause denaturation of the external tissues and/or rupture of the tegument of the seeds, deteriorating them faster in comparison to the seeds not treated by thermotherapy [8].

The results of this research indicated good prospects for the use of heat treatment for Xcc control in kale organic seeds, almost without harming their physiological quality. However, in seed lots with lower initial vigor this treatment may not be recommended, because it reduces germination at rates lower than those allowed by legislation. In addition, from an environmental point of view, it is a non-polluting method and is allowed in the organic system, so it can be recommended for pathogen control in this production system.

CONCLUSION

Thermotherapy in kale seeds at 50°C for 20 and 40 minutes of exposure reduces the infection of *Xanthomonas campestris* pv. *campestris* to 10% and 4%, respectively. However, seed lot with low initial seed vigor, the heat treatment reduces the germination rate and first count in the germination test.

In seed lots with high initial vigor it can be recommended heat treatment at 50°C for up to 40 minutes of exposure for control of Xcc in kale organic seeds

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Conflicts of Interest: The authors declare no conflict of interest.

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