高温调控对黄瓜霜霉病菌侵染的影响

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摘要:采取温湿度相结合的方法来研究高温处理对黄瓜霜霉病菌(*Pseudoperonospora cubensis* Rostov)侵染的影响.比较研究了35~50℃4个温度梯度,50%~90%5个湿度梯度组合处理对病菌致病性的影响。同一相对湿度条件下,随着温度的上升病菌致病性降低;40~50℃的高温范围内、在同一温度下随着湿度的不断升高,受处理病菌的致病能力逐渐下降。在RH80%以上、温度40℃以上时,病菌的致病力随着处理时间的延长而变弱;45℃以上的高温高湿处理病菌超过1h,病菌基本上没有致病性。

通过高温控制苗期黄瓜霜霉病的研究,确定高温高湿防治苗期黄瓜霜霉病的最佳温湿度为45℃1h(RH80%),通过高温致死菌诱导植株抗性的研究,初步明确高温处理致死的病菌可以短期诱导植株的抗性。

关键词:黄瓜霜霉病;高温;湿度;诱导抗性

The effect of high temperature regulation on the infection of *Pseudoperonospora cubensis*

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Abstract: Environmental condition is a key factor affecting the outbreak of cucumber downy mildew. To develop effective bionomic control strategies, it is important to decipher the essential condition and restrictive factors influencing the occurrence of this disease. "High-temperature sealing greenhouse", which was used to control cucumber downy mildews, is a representative of the ecological control technique of vegetable disease. This method has been developed for over 20 years, but it was limited because the detailed studies on the correlated environmental factors has been not yet carried out. This study were to understand the effects of high temperature regulation on the infection of *Pseudoperonospora cubensis* by using the method of combination of temperature and humidity, and find the optimum temperature and humidity to control the cucumber downy mildew. Thus this information will probably provide new theoretical foundation for ecological control.

Diseased leaves of cucumber downy mildew with mould produced by placing in moist chamber were incubated in a growth chamber set to 50, 45, 40, 35℃ and relative humidity (RH) 50%, 60%, 70%, 80%, 90%, respectively, for 2 hours. Plants were inoculated by placing droplets of sporangial suspension on the surfaces of cotyledon. The disease index was investigated 4 days after inoculation. The results indicated that at the same humidity, the pathogenicity of the pathogen was decreased with the temperature increasing when *Pseudoperonospora cubensis* was treated in a growth chamber at high temperature from 35 to 50℃ and RH from 50% to 90% for 2 hours. At the same temperature, the pathogenicity was significantly reduced with the humidity increasing when the temperature ranged 40 to 50℃, which showed the above

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40℃ high temperature and high humidity apparently has significant lethal action to *Pseudoperonospora cubensis*. Whereas the pathogenicity of the pathogen was not obviously reduced when treatment temperature was below 40℃.

And *Pseudoperonospora cubensis* with pathogenicity were treated in a growth chamber at 50, 45, 40 and 35℃ and RH 60%, 80% for 30, 60, 90 and 120 minutes, respectively, then sporangial suspension(4×10^3 sporangia/ml) was prepared by washing sporangia from the treated leaves. The disease index was investigated 4 days after inoculation. The results showed that the pathogenicity of the pathogen was reduced with lengthening the treatment time when the RH was adjusted to 80% and the temperature ranged from 40℃ to 50℃. The pathogenicity became weak when the pathogen was treated for over 30 minutes at the temperature of 50℃ or 45℃, RH 80%, respectively. Remarkably, it was observed that the pathogenicity was significantly decreased when the pathogen was pathogenicity after treated for over 120 minutes at 40℃, RH 80%.

These results showed that the effect of killing pathogen became more obvious with lengthening the time of the treatment when the temperature and RH was set to above 45℃ and 60 % respectively.

By the above result and the experiment which controlled cucumber downy mildew by the high temperature and high relative humidity . The research results indicated the seedling treated by 50℃ was seriously harmed , even killed by burning, the treatment of 45℃ for 2 hours also had slight harm to seedling. But the seedlings were not damaged when they were treated by 45℃ for 1 hour or 40℃ for 2 hours . These showed the ability of tolerance to heat of the host had limit. However, further field experiment still need to perform.

Therefore, at the same temperature, the control effects were different as the different periods when the treatment of high temperature and high relative humidity were carried out after inoculation, the control effect was more efficient, the time of infection was more short. The effect was best when the treatment was carried out 2 hours after inoculation. At that time, *Pseudoperonospora cubensis* on the leaves was treated by high temperature and high relative humidity; However, the effects of treatment that were carried out 1 and 3 day after inoculation was lower than that of 2 hours after inoculation. This showed that the control effect would reduce if the seedlings were treated by high temperature after out－breaks of disease. At high humidity, disease was inhibited significantly if cucumber seedlings were treated for 1 hour at 45℃, whereas the seedlings were not harmed. Thus, "High-temperature sealing greenhouse" was to treat the seedlings by 45℃ and RH 80% for 1 hour, the control effect when the plants were treated at the early stage of infection was more efficient than that at the late stage.

Furthermore, on the basis of the research to high temperature, the lethal pathogen by high temperature could induce cucumber producing resistance. Diseased leaves of cucumber downy mildew with mould produced by placing in a moist chamber were incubated in a growth chamber at 45℃ and 80% RH for 2 hours. Sporangia was brushed to prepare sporangial suspension (2×10^5 sporangia /ml). The seedlings were inoculated by spraying the sporangial suspension on the cotyledons. Then the cotyledons were inoculated with virulent pathogen (4×10^3 sporangia /ml) 1, 3 and 5 days after induction inoculation with lethal pathogen caused by high temperature, respectively. The plants that were not inoculated with virulent pathogen after induction inoculation were used as control. Each induction time had a control and designed as CK_{1d}, CK_{2d} and CK_{5d}, respectively. The disease index was surveyed 4 days after inoculation. The results indicated that the resistance of leaves could be induced to enhance through inoculating the seedlings with the lethal pathogen. The disease index of each treatment that the seedlings were inoculated by virulent pathogen 1, 3 and 5 days after the lethal pathogen inoculation was lower than that of the correspond

ing control. The control effect was the best when challenge inoculation was carried out at the interval of 1 day after induction inoculation, the control effect became lower as the interval was lengthened. This result indicated that the plants do not enable to retain induced resistance for a longer time. The further study on systemic induced resistance of plant itself is in progress.

At present, many researchers have interested in the plant' reaction to heat shock. The changes of resistance can be induced when the plants are treated by high temperature, similarly, a lot of data demonstrated that weak-virulent pathogen or nonpathogenic pathogen can induce resistance of plants. The pathogenicity is reduced or pathogen is killed after the fungus is treated by high temperature in greenhouse. Our hypothesis is that high temperature can make virulence of pathogen become weak or result in pathogen dead, and then the weak-virulent or lethal pathogen can induce host to produce immunity reaction, i.e. "High-temperature sealing greenhouse" may result in twice induction of resistance to the host. However, there are not reports that whether either the weak-virulent strains caused by high temperature or elicitor can induce plants to produce "the second resistance induction". Our studies provided some evidence for this hypothesis. We think it appears likely that "twice resistance induction" has wide prospective for disease control, particularly for the vegetable diseases in greenhouse.

Key words: high temperature regulation; infection; *Pseudoperonospora cubensis*

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"高温闷棚"防治黄瓜霜霉病是蔬菜病害生态防治中有代表性的方法[illegible]。但在该技术提出后的20a内,尚未对其相关的环境因子进行详细的研究,影响了该技术的推广。在棚室可控高温条件下,湿度对病原菌致死的影响国内外还未见系统的报道。本文采取温湿度相结合的方法来研究高温处理对黄瓜霜霉病菌侵染的影响,以期找到防治黄瓜霜霉病的最适温湿度条件,从而为该生态防治方法在田间应用提供新的理论依据。

1 材料与方法

1.1 供试材料

1.1.1 植物材料 供试植株为黄瓜感霜霉病品种——"长春密刺",在装有蛭石和草炭(1∶1)的营养钵中播种,20~35℃温室内培养,当长出1片真叶时待用。

1.1.2 供试菌种 试验所用霜霉病菌菌株是从北京市海淀区东升乡科技园区大棚中的黄瓜霜霉病叶片上获得,并鉴定具有较强的致病性。

1.1.3 接种体的制备 将采回的霜霉病病叶用清水洗净,放入培养皿中,下面垫上湿润的纱布置于18~20℃的温度条件下保湿培养1 d后,用毛笔将叶片背面新产生的霉层刷入烧杯中,用无离子水配制成一定浓度的孢子囊悬浮液,供接种使用。

1.2 试验方法

1.2.1 高温高湿对病菌致病力的影响 (1)不同高温高湿处理对黄瓜霜霉病菌致病性的影响 将保湿后产生大量霉层的霜霉病病叶置于温度分别为50℃、45℃、40℃、35℃,相对湿度分别为50%、60%、70%、80%、90%的人工气候箱中(哈尔滨东联电子技术开发有限公司制造)处理2h,然后用毛笔刷下霉层,用无离子水配制成浓度为4×10^4个孢子囊/ml的悬浮液,用点滴子叶法接种于黄瓜子叶上,以未处理霜霉病菌孢子囊,相同浓度点滴接种子叶设为对照CK,每个处理20株苗,3次重复。接种后第4天调查发病情况。(2)高温高湿处理不同时间对黄瓜霜霉病菌的致病性的影响 在温度为50℃、45℃、40℃、35℃,相对湿度(RH)为60%、80%的人工气候箱中分别处理有致病性的黄瓜霜霉病菌30、60、90、120min,再用无离子水配制成浓度为4×10^4个孢子囊/ml的悬浮液,用点滴子叶法接种于黄瓜子叶上,未处理霜霉病菌设为对照CK,每个处理20株苗,3次重复。接种后第4天调查发病情况。

1.2.2 高温高湿控制黄瓜霜霉病的研究 将培养好的霜霉病菌配制成浓度为2×10^4个孢子囊/ml的悬

浮液，用喷雾法接种第 1 真叶黄瓜苗，在接种后 2 h、1 d、3 d 将这批黄瓜苗分别放在温度为 50℃、45℃、40℃、35℃，相对湿度为 80%的人工气候箱中处理 1 h、2 h（高温处理前将育苗钵浇足水），以接种但不进行高温处理的黄瓜苗为对照 CK，共 25 个处理，每个处理 20 株苗，3 次重复，接种后第 4 天调查发病情况。

1.2.3　高温致死病菌诱导植株抗性的研究　将保湿后产生大量霉层的霜霉病病叶置于温度为 45℃，相对湿度为 80%的人工气候箱中处理 2 h，然后用毛笔刷下霉层，用无离子水配制成浓度为 $2\times10^{?}$ 个孢子囊/ml 的悬浮液，用喷雾法接种于黄瓜苗子叶上，诱导后分别间隔 1 d、3 d、5 d 再接种浓度为 $4\times10^{?}$ 个孢子囊/ml 的有活力的霜霉病致病菌，诱导后未接种有活力的致病菌设为对照 CK_0，每一诱导时间分别设一对照，即 CK_{1d}、CK_{3d}、CK_{5d}。每个处理 20 株苗，3 次重复，在相应处理后第 4 天调查发病情况。

1.3　分级标准

1.3.1　子叶病情分级标准　0 级：无病症；1 级：接种点有轻微小斑点，直径小于 0.5 cm；2 级：病斑明显，直径为 0.5～1.0 cm；3 级：病斑直径 1 cm 至叶面积的 1/3；4 级：病斑面积占子叶面积的 1/3～2/3；5 级：病斑面积占子叶面积的 2/3 以上。

1.3.2　真叶病情分级标准　0 级：无病症；1 级：病斑面积不超过叶面积 1/10；3 级：病斑面积占叶面积的 1/10～1/4；5 级：病斑面积占叶面积的 1/4～1/2；7 级：病斑面积占叶面积的 1/2～3/4；9 级：病斑面积占叶面积的 3/4 以上。

2　结果与分析

2.1　高温高湿对病菌致病力的影响

2.1.1　不同高温高湿处理对黄瓜霜霉病菌致病力的作用　黄瓜霜霉病菌在高温 35～50℃、相对湿度 50%～90%的人工气候箱中处理 2 h，其致病性见表 1。可以看出，在同一湿度条件下，随着温度的升高，处理后病菌的致病性降低；40～50℃温度范围内，在同一高温条件下，随着湿度的升高，被处理病菌的致病性明显减弱，说明 40℃以上的高温高湿对黄瓜霜霉病菌的致死作用明显；而经 35℃处理的病菌，随着湿度的上升其致病性没有规律性，说明 35℃的高温对霜霉病菌没有杀伤效果。

表 1　高温高湿处理对霜霉病菌致病性的影响

Table 1　The effect of high temperature and high humidity treatment to the zoosporange on the pathogenicity

处理温度 Temperature(℃)	处理相对湿度 The treatment of relative humidity(%) 50		60		70		80		90		CK*
	病情指数 Infective index	病害降低(%) Disease Reduction	病情指数 Infective index	病害降低(%) Disease Reduction	病情指数 Infective index	病害降低(%) Disease Reduction	病情指数 Infective index	病害降低(%) Disease Reduction	病情指数 Infective index	病害降低(%) Disease Reduction	病情指数 Infective index
50	7.45	88.77	4.18	93.70	0.00	100	0.00	100	0.00	100	66.35
45	10.13	84.96	8.53	87.33	3.66	94.56	0.00	100	0.00	100	67.31
40	14.72	10.84	40.67	18.92	38.42	23.40	35.66	28.91	32.57	35.07	50.16
35	51.50	4.18	50.13	6.73	61.59	−14.58	58.65	−9.12	49.35	8.18	53.75

CK：病菌不经过高温处理 No high temperature treatment

通过高温条件下不同湿度对病菌致病性的影响可以看出，45℃以上的高温 RH 80%处理 2 h 足以能够致死霜霉病菌。

2.1.2　高温高湿处理不同时间对黄瓜霜霉病菌致病性的影响　从表 2 中可以看出，在高湿 RH 为 80%时，40～50℃处理的病菌都随着处理时间的延长其致病力依次减弱。而相对湿度 RH 60%的规律性不强。50℃、45℃RH 为 80%处理 30 min 以上病菌致病力明显变弱，而在 40℃RH80%处理 120 min 以上时病菌致弱效果明显。

由此可见，45℃以上的高温 RH 为 60%时，随着处理时间的延长杀菌效果明显，处理 2 h，基本上已能完全杀死病菌，可以控制该病害的发生。

2.2　高温控制黄瓜霜霉病的研究

在高湿的情况下，高温处理接种后的黄瓜苗，随着处理温度的升高，病情指数越来越低，相应的防效也

越来越高,这与前面所处理病菌的结果是一致的(表 3)。但经 50℃高温处理的黄瓜幼苗,伤害严重,以至全部灼烧而死,45℃处理 2 h 的也有少量灼伤,45℃处理 1 h、40℃处理 2 h 及其以下的温度处理对黄瓜苗无伤害。

表 2 高温高湿处理不同时间对病菌致病性的影响

Table 2 The effect of different time treatment by high temperature and high humidity on pathogenicity

温度(℃) Temperature	病情指数 Infective index									
	80% RH					60% RH				
	30	60	90	120	CK*	30	60	90	120	CK*
50	4.26	2.66	0.00	0.00	58.75	6.17	4.49	3.92	1.18	58.75
45	5.11	4.50	3.65	0.00	73.29	33.67	31.25	8.52	3.33	73.29
40	45.34	44.21	40.04	25.66	50.61	57.13	59.37	16.75	39.67	50.61
35	52.41	57.72	50.09	57.65	55.45	62.84	67.73	66.49	50.13	55.45

CK:病菌不经过高温处理 No high temperature treatment

表 3 接种后不同时间高温高湿处理防治效果

Table 3 The protection of high temperature and high humidity after different period time of inoculation

82 处理温度 (℃) Temperature	处理时间 (h) Time treatment	接种后 2 h 处理 2 h after inoculation		接种后 1 d 处理 1 d after inoculation		接种后 3 d 处理 3 d after inoculation	
		病情指数 Infective index	病害降低(%) Disease reduction	病情指数 Infective index	病害降低(%) Disease reduction	病情指数 Infective index	病害降低(%) Disease reduction
35	1	31.29	9.75	33.38	3.72	34.15	1.50
	2	29.65	14.48	32.72	5.62	34.98	-0.89
40	1	16.14	53.45	34.53	0.40	36.92	-6.19
	2	12.70	63.37	35.14	-1.36	37.72	8.80
45	1	0.00	100.00	1.32	96.19	10.98	68.33
	2	0.00	100.00	0	100.00	8.81	60.17
50	1	*	—	*	—	*	
	2	*		*		*	
CK**		34.67	-	34.67	—	34.67	-

* 黄瓜苗灼伤;CK** 为接种后幼苗不经过高温处理。

表 4 高温致死菌诱导植株抗性的效果

Table 4 The effect of induced resistance to cucumber using lethalic pathogen caused by high temperature

处理时间 Time treatment	第 1 次调查		第 2 次调查	
	病情指数 Infective index	病害降低(%) Disease reduction	病情指数 Infective index	病害降低(%) Disease reduction
诱导 1 d 后接种	8.93	63.02	34.69	46.65
CK_{1d}	24.15	—	75.28	-
诱导 3 d 后接种	15.42	32.16	58.15	17.43
CK_{3d}	22.73	—	70.43	—
诱导 5 d 后接种	16.34	19.98	63.57	7.12
CK_{5d}	20.42	—	68.41	—
CK_0	0.00	—	0.00	

CK:黄瓜苗不经过致死菌诱导。

在同一温度情况下,接种后不同时间高温高湿处理黄瓜苗,可以看出,病菌侵染的时间越短,防治效果越好。防效最好的是在接种后 2 h 处理,此时相当于高温高湿处理叶面的霜霉病菌;而在此后 1 d、3 d 处理的防效均低于 2 h 后处理的防效,可以看出,发病后再进行高温处理,防效有所下降。在高湿情况下,45℃高温处理 1 h 对病害有很好的抑制作用,此时幼苗尚未受到损害,是高温高湿控制霜霉病的理想温度。

2.3 高温致死菌诱导植株抗性的研究

将高温致死菌接种到植株叶片后,可以诱导叶片抗病性增强。从表 4 可以看出,黄瓜苗接种致死菌后

分别间隔 1 d、3 d、5 d 再接种有活力的致病菌，其病情指数都低于各自的对照，其中间隔 1 d 接种的防效最好，随着间隔时间的延长，其防效越来越低，说明这种诱导抗性不能长期地保持下去。

3　结论与讨论

3.1　通过不同高温高湿处理黄瓜霜霉病菌对其致病力影响的研究表明，在同一湿度下，随着处理温度的升高，相应的病菌致病性降低；40℃以上的高温条件下，随着湿度的升高及时间的延长，受处理的病菌致病能力逐渐下降；45℃以上的高温高湿处理病菌超过 1 h，霜霉病菌基本上没有致病性；40℃以下的高温处理的病菌则没有明显的致病力变弱现象。

3.2　通过高温控制苗期黄瓜霜霉病的研究，初步确定"高温闷棚"防治苗期霜霉病的最佳温湿度为 RH80%、45℃ 1 h。在黄瓜感病初期进行高温防治效果高于发病后防治。

3.3　通过对黄瓜幼苗在接种正常霜霉病菌后进行不同高温不同时间处理发现，虽然在高湿条件下温度越高控制霜霉病的效果越好，但寄主对高温高湿的承受力是有限的。50℃高温处理的黄瓜苗全部灼烧而死，45℃高温处理 2 h 也出现轻微灼烧。这与前人所确定的"高温闷棚"温度范围(40～47℃处理 2 h)略有不同。在高湿的情况下高温闷棚防治黄瓜霜霉病，不宜超过 45℃、2 h，其田间植株试验尚待进一步研究。

3.4　通过高温致死菌诱导植株抗性的研究，可以发现通过高温处理而致死的霜霉病菌可以诱导黄瓜植株产生抗病性，诱导后 1 d 再挑战接种的植株抗病性表达效果最明显。但是这种抗性并不能长期地保持下去其植株本身系统性的诱导抗病性还有待进一步研究。

3.5　目前，植物对高温热击的反应已引起人们的广泛关注。当一定的高温作用于植株后，可诱导植株的抗病性发生改变，同时诸多研究已证明弱毒或灭活的病菌具有诱导抗病性的作用[5~7]。在棚室的环境下，当一定的高温作用于病菌时，可以使病菌致死或致病力变弱，该致弱或致死菌可能诱导寄主产生免疫反应——即对寄主抗病性产生第二次诱导。但是在设施蔬菜栽培条件下，国内外尚无高温所致弱毒株或诱抗菌激发了对寄主是否存在"两次抗病性诱导"的报道，本研究为该推测提供了例证，同时看到了在病害防治，尤其在棚室蔬菜防治中应用的可喜前景。

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study，we focused on the interaction of tree species in same community，and just used the basic equations of tree growth part. The model parameters were estimated by deviation from physics-math equation and experimentally statistical analysis fitting methods. Data that was used to estimated parameters and tested the model come from management practices and field surveys.