Sensitization of Leaves and Pathogens to Cold

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ABSTRACT

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When healthy or diseased leaves were exposed to 0 C for 1 sec after a sublethal dosage of heat, the injury or therapy was usually greater than when the tissue was not chilled. Cold alone was not injurious or therapeutic at the dosages used. The sensitization of bean, corn, cowpea, cucumber, and thistle to cold was greater if the plants were held in darkness or grown at high temperature before the heat and cold treatment. The greatest sensitization to cold was with infections of *Puccinia pelargoni-zonale* in *Pelargonium* × *hortorum* for which the dosage of heat for therapy was 12 times as great for heat alone (72 sec at 45 C) as for heat followed by cold (6 sec at 45 C). Sensitization to cold also was demonstrated for *Puccinia antirrhini* in snapdragon, *Uromyces fabae* in broad bean, *Uromyces phaseoli* in French bean, *Pseudoperonospora cubensis* in cucumber, and tobacco necrosis virus infection in cucumber and cowpea. No sensitization to cold was detected with two viruses, three powdery mildews, two aphids, or one mite. With *Thielaviopsis basicola* in carrot slices, chilling of tissues after heating reduced the therapeutic effect.

Additional key words: *Puccinia pelargonia-zonale*, heat injury, heat therapy, cold therapy.

Sensitization to cold refers to the greater injury or therapy which results when plants and pathogens are exposed to cold following a sublethal dosage of heat, than when exposed to the same or greater dosages of cold alone or heat alone. Precht et al. (3) cited several references to studies of the effects of heat and cold shock (mostly with bacteria or animals) but none of the type reported here. This is an extension of my previous studies of cold therapy of pathogens (4, 5), but covering more organisms and conditions of treatment. Previous trials of sensitization of leaves to cold under ordinary daylight indicated that any sensitization was slight. Discovery of the importance of darkening the plants before treatment shows that sensitization of leaves to cold can be great.

METHODS

Methods were mostly as previously described (4, 5). Trials were usually by twin- or half-leaf methods with plants grown in the greenhouse at about 21 C in 8-cm diameter pots of a sand:peat:fertilizer mixture and used at about 9 days after seeding. The standard treatment for beans, cowpeas, and cucumbers was to heat both attached primary leaves simultaneously at 55 C in a waterbath at a range of dosages and then one twin leaf was immediately immersed for 5 sec in ice water. Injury results usually were recorded at about 3 days after heating. For rusts, powdery mildews, and downy mildews, leaves at 1-6 days after inoculation were heated and the ED_{50} (mean effective dose) was determined from the number of infections which resulted. For inactivation of viruses, infected leaf pieces were heated, the tissue was ground and used as inoculum, and the ED_{50} determined from the number of assay lesions.

For virus infections (distinct from inactivation of viruses in infected tissues) the leaves were inoculated and immediately heated and chilled, and the ED_{50} was determined from the number of local lesions on the leaves. For *Thielaviopsis basicola*, infections on carrot slices in petri dishes were heated at about 12 hr after inoculation, and the ED_{50} values were determined from the number of colonies counted after 4 days. For aphids and mites, detached leaf pieces on which the animals were feeding were heated and chilled, and the number of living and dead animals determined about 2 days later. Each trial consisted of a series of heat dosages (e.g., 3, 6, 9, 12, 15, and 18 sec at 55 C) and the ED_{50} (dosage in seconds for 50% injury, 50% thereapy, or 50% kill) was determined by observational or graphical interpolation. The cold sensitization index is the ratio of the ED_{50} after heating with chilling, to the ED_{50} without chilling. Previous trials (4, 5) have shown that the duration of treatment in ice water up to 24 hr was not important, but a very short interval from heating to chilling was important, and these aspects were usually kept constant, but not further investigated.

RESULTS

Most results are given in Table 1. Only cold-sensitization values of less than 0.85 are regarded as highly significant. Sensitization to cold was demonstrated in eight of nine species of higher plants and was clearest in bean, where it was most studied. Placing plants in the dark before heat treatment increased sensitization in four of the five hosts studied. This effect was greatest with bean, in which the ED_{50} of heat if followed by cold was only 0.28 of the dosage necessary if the plants were not

TABLE 1. Sensitization of leaves and pathogens to cold

Indicator and sensitization temperature	Variation from standard treatment[a]	No. of trials	ED_{50} (seconds for injury at sensitization temperature)		Cold sensitization index[b]
			Nonchilled	Chilled	
Leaves of higher plants:					
Chenopodium quinoa, 55 C	None	2	26	26	1.0
Cirsium lanceolatum, 55 C	None	9	14	11	0.79
Cucumis sativus, 55 C	None	6	27	26	0.96
Cucumis sativus, 55 C	0-20 hr dark before heat	6	28	19	0.68
Nicotiana tabacum, 55 C	None	2	12	12	1.0
Phaseolus vulgaris, 55 C	None, or miscellaneous	82	14.2	12.0	0.84
Phaseolus vulgaris, 55 C	0-20 hr dark before heat	30	14.3	6.4	0.45
Phaseolus vulgaris, 55 C	20-80 hr dark before heat	55	14.4	3.3	0.23
Phaseolus vulgaris, 55 C	Grown at 32C, 10-30 hr dark before heat	31	19.5	2.1	0.11
Phaseolus vulgaris, 55 C	10-30 hr dark before heat, chilled at 10 C	2	14.0	8.5	0.59
Phaseolus vulgaris, 55 C	10-30 hr dark before heat, chilled at 20 C	4	14	13	0.93
Salix sp., 55 C	None	2	19	12	0.64
Salix sp., 55 C	0-20 hr dark before heat	2	10	7	0.7
Stachys rigida, 55 C	None	1	22	17	0.77
Vigna sinensis, 55 C	None	3	14	13	0.93
Vigna sinensis, 55 C	10-30 hr dark before heat	3	10.6	7.8	0.73
Zea mays, 55 C	None	3	28	23	0.82
Zea mays, 55 C	24 hr dark before heat	1	28	17	0.61
Rust infections caused by:					
Puccinia antirrhini, 45 C	None	3	38	21	0.55
P. pelargoni-zonale, 45 C	None	3	73	5.7	0.078
P. pelargoni-zonale, 50 C	None	3	10	2.0	0.55
Uromyces fabae, 45 C	None	2	37	17	0.46
Uromyces phaseoli, 40 C	None	23	350	78	0.22
Uromyces phaseoli, 45 C	None	51	38	14	0.37
Uromyces phaseoli, 50 C	None	20	3.7	2.0	0.54
Uromyces phaseoli, 45 C	1- to 4-day infection before heat	19	28	12	0.43
Uromyces phaseoli, 50 C	6- to 12-day infection before heat	14	47	10	0.21
Powdery mildew infections caused by:					
Erysiphe graminis, 45 C	None	6	21	21	1.0
Erysiphe graminis, 50 C	None	3	2.0	2.0	1.0
Erysiphe polygoni, 45 C	None	8	62	62	1.0
Erysiphe polygoni, 50 C	None	7	7.3	7.3	1.0
Sphaerotheca fuliginea, 45 C	None	2	100	100	1.0
Sphaerotheca fuliginea, 50 C	None	7	15	15	1.0

TABLE 1. (continued)

Indicator and sensitization temperature	Variation from standard treatment[a]	No. of trials	ED_{50} (seconds for injury at sensitization temperature)		Cold sensitization index[b]
			Nonchilled	Chilled	
Viruses:					
Cucumber mosaic virus, 55 C	None	2	20	20	1.0
Tobacco mosaic virus, 90 C	None	2	125	125	1.0
Tobacco necrosis virus, 75 C	None	2	50	50	1.0
Tobacco necrosis virus, 55 C	Infections in cucumber	11	6.4	4.9	0.77
Tobacco necrosis virus, 55 C	Infections in cowpea	3	3.9	2.2	0.57
Miscellaneous:					
Pseudoperonospora cubensis, 45 C	Infections in cucumber	7	18	6.9	0.38
Thielaviopsis basicola, 50 C	Infections in carrot	8	32	34	1.1
Thielaviopsis basicola, 50 C	Infections in carrot	10	12	20	1.7
Acyrthosiphon pisum, 45 C	None	3	30	30	1.0
Acyrthosiphon pisum, 50 C	None	1	6	6	1.0
Brevicoryne brassicae, 45 C	None	3	66	65	1.0
Brevicoryne brassicae, 50 C	None	2	8	8	1.0
Tetranychus urticae, 50 C	None	4	49	45	0.92
Tetranychus urticae, 55 C	None	1	13	13	1.0

[a]Standard treatment was to take healthy or diseased leaves on plants growing in natural daylight in a greenhouse environment, heat them for a range of times at the sensitization temperature indicated, then immerse them in ice water (0 C) for 5 sec and then return them to the greenhouse bench.

[b]The cold-sensitization index is the ED_{50} for chilled leaves divided by the ED_{50} for unchilled leaves.

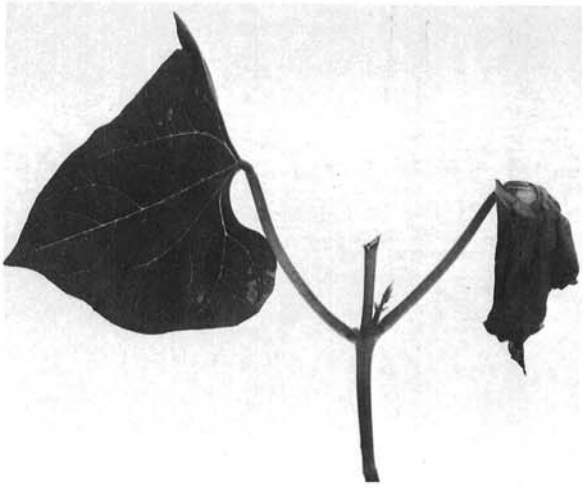

Fig. 1. Sensitization of bean to cold by heat treatment. Seeded 13 November, placed in dark at 0600 hours 30 November, both leaves heated 4 sec at 55 C at 1500 hours on 1 December, and the right leaf dipped in ice water immediately after heating. Photographed 4 December. The right leaf shows cold injury.

given the dark treatment. Growing beans at 32 C rather than 21 C, plus a dark treatment before heating, gave the highest degree of cold sensitization (index of 0.11) observed for any higher plant (Fig. 1).

The apparently insignificant sensitization to cold of bean leaves grown under standard conditions may be misleading in that injury increased with time after treatment, but increased more rapidly for nonchilled leaves than for chilled leaves. In several trials, injury to the same leaves was recorded at intervals ranging from 1 hr to 180 hr. That is, the cold sensitization was clear at 1 hr (sensitization index of 0.62) but doubtful at 180 hr (sensitization index of 0.95).

Increased therapy as a result of cold treatment after heat was demonstrated in all four rusts that were tested. The greatest cold sensitization with any living system tested was with *Puccinia pelargoni-zonale* for which the ED_{50} of heat followed by cold (55 C for 5.7 sec) was only 0.078 of that required for ED_{50} if the heat was not followed by cold.

No effect of cold on heat inactivation was demonstrated with any of three viruses that were tested, but infection with TNV was decreased as a result of chilling the freshly inoculated leaves immediately after heating. In this case the dosage of heat necessary to inactivate the virus was much greater than the dosage necessary to prevent the infection process. It seems likely that the effect of heat on the infection process is primarily an effect on the host rather than on the virus. *Pseudoperonospora cubensis* infection of cucumber leaves was the only downy mildew tested and it clearly was vulnerable to cold therapy.

Thielaviopsis basicola was the only facultative parasite tested; infection of carrot slices by *T. basicola* was the only case observed in which more heat was necessary for therapy if the tissues were chilled after heating than if they were not. This may be associated with the much greater thickness of carrot slices than of any leaves tested. No sensitization to cold was demonstrated with two aphids and one mite.

Chilling at other than 0 C (ice water) after heat treatment was tested only with beans. When chilling was at 10 C, cold injury was clearly demonstrated, but no cold injury was detected at 20 C (Table 1).

Of secondary interest is the high tolerance to heat alone, or no change in tolerance to heat, of leaves that had been kept in the dark. It is commonly believed, based on logic and the data of Heyne and Laude (1) that holding plants in the dark increases their sensitivity to heat. However, Illert (2) found that darkness could increase heat tolerance in oxalis. The data for beans in Table 1 agree somewhat with those of Illert in that there was no clear increased sensitivity to heat as a result of holding plants in the dark; however, I do not question that, in general, holding plants in the dark increases their sensitivity to heat.

DISCUSSION

It seems likely that sensitization to cold is a widespread phenomenon, perhaps a universal phenomenon applying to all living things. The failure to detect it in many trials in this study may be due to inappropriate conditions. This is supported by the results with bean; initial results with ordinary greenhouse plants yielded inconclusive results until the practice of darkening the plants before heat treatment and growing plants at 32 C was tested. There are many other obvious treatments such as nutrition, water availability, etc., that have not been tested which might yield results as dramatic as those of the dark treatments.

LITERATURE CITED

1. HEYNE, E. G., and H. H. LAUDE. 1940. Resistance of corn seedlings to high temperature in laboratory tests. J. Am. Soc. Agron. 32:116-126.
2. ILLERT, H. 1924. Botansiche Untersuchungen über Hitzetod und Stoffwechselgift. Bot. Arch. 1:133-141.
3. PRECHT, H., J. CHRISTOPHERSEN, H. HENSEL, and W. LARCHER. (eds.). 1973. Temperature and life. Springer-Verlag, New York. 279 p.
4. YARWOOD, C. E. 1974. Cold therapy of bean rust. Phytopathology 64:1044-1046.
5. YARWOOD, C. E. 1975. Cold therapy of one downy mildew and three rusts. Reports VIII. Pages 284-291 *in* Proc. Int. Congr. Plant Protection Sec. VI.