Effects of Host Variety, Photoperiod and Chemical Treatments on Hatching of Globodera rostochiensis[1]

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Abstract: Golden nematode cysts were collected after they had completed one generation on an early, a mid-season, or a late-maturing potato variety ('Superior,' 'Katahdin,' and 'Sebago,' respectively). The plants were grown in 8.5- or 16-h photoperiods and treated with gibberellic acid (GA) or xylocaine or both. When treated subsequently with potato root-diffusate few (13.3%) juveniles hatched from cysts collected from Sebago, more (22.3%) from cysts collected from Katahdin, and most (36.0%) from cysts collected from Superior. Hatching was greater from cysts collected from plants treated with GA than from untreated plants. Short photoperiods decreased hatching from cysts collected from Katahdin but increased hatching from cysts collected from Superior and Sebago. Gibberellic acid had no detectable effect on hatching, but EDTA had some. *Key words:* potato cyst-nematode, golden nematode.

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Winter dormancy allows nematode life cycles to be synchronized with the life cycles of their hosts and prevents excessive mortality by ensuring that nematodes are active only when suitable hosts are available. Shepherd and Cox (12) showed that laboratory hatching from cysts of *Globodera rostochiensis* (Woll.) was reduced during autumn and winter, but that this effect was lessened if the cysts were removed from the field before August. Mägi (8) observed a possible diapause that started in July and affected cysts formed in the previous year, but Rode (11) found no evidence of diapause. If dormancy occurs in *G. rostochiensis,* it would be interesting to know what controls its induction. Generally *G. rostochiensis* produces only one complete generation per year (7). A partial second generation can often be found, but the induction of dormancy in the newly formed eggs seems to prevent most of them from hatching. The induction of dormancy may be beneficial to the nematode: although healthy host roots are present when the first generation completes its development (usually July in northern temperate latitudes), it is unlikely that a second generation could complete its development before the crop is harvested. McKenna and Winslow (9) were able to produce six generations of *G. rostochiensis* in one year in a glasshouse experiment, but the conditions were very unlike those of plants growing in the field. Major physiological changes occur in field-grown potato plants at about the time that dormancy is reported to begin in the nematode: tubers are initiated (and become a primary sink for nutrients) and flowering occurs. To help assess the roles of these changes a multi-factorial experiment was designed to observe the effects of delaying host maturation on the nematode.

MATERIALS AND METHODS

Three potato (*Solanum tuberosum*) varieties ('Superior' which tuberises early, 'Katahdin' which is a mid-season variety, and 'Sebago' which tuberises late) were used. Pieces of tubers with single sprouts were planted in 10-cm plastic pots containing sterilized organic loam soil and grown in a greenhouse with supplemental heating. Photoperiod was either 16 h (natural day-length extended by fluorescent lighting) or 8.5 h (achieved by covering the plants). Twelve days after planting, 5,000 juveniles of *G. rostochiensis* (pathotype Ro1, European notation, from the township of Prattsburg, Steuben County N.Y., U.S.A.) were added to each pot. Fifteen days later, and at weekly intervals until six applications had been made, plants were sprayed with gibberellic acid (GA) or xylocaine (lidocaine hydrochloride, a lipid antioxidant which delays aging [10] supplied by Astra Pharmaceutical Products Inc., Framingham, Massachusetts) or both of these chemicals. Gibberellic acid (50 ppm) and xylocaine

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(200 ppm) aqueous solutions were applied with a mist sprayer to run-off; distilled water was applied similarly to the control plants. There were five plants in each treatment combination.

Two months after inoculation three batches of 100 mature brown cysts were picked off the roots of plants in each treatment, and 2 wk later tubers were harvested, counted, and weighed. Each batch of cysts was placed in a compartment of a 25-compartment petri dish, immersed in distilled water for 1 wk and then potato root diffusate for 5 wk, and kept in the dark at 20 C. The potato root diffusate was collected by leaching pots of sterilized soil in which Katahdin potato plants were growing with distilled water. Emerged juveniles were counted and hatching solutions changed weekly. At the end of the test, the numbers of unhatched juveniles remaining in the cysts were estimated.

To determine if GA induces hatching, or at least preconditions juveniles in cysts to hatch readily, the acid was supplied to mature cysts in the presence and absence of hatching agents. Since calcium uptake by eggs just prior to hatching has been reported to be an important feature in the hatching process (1), cysts were also treated with EDTA (ethylenediaminetetraacetic acid) to bind the calcium and perhaps reduce hatching. Three replicate batches of 100 cysts were soaked for 1 wk in water or aqueous solutions of GA (50 or 500 ppm) or EDTA (50 or 250 ppm). The solutions were then replaced by 1:1 mixtures of the pretreatment solution with water, potato root-diffusate, or 0.3 m*M* picrolonic acid (an artificial hatching agent [5]). Hatched juveniles were counted and solutions changed at weekly intervals for 5 wk, after which time unhatched eggs remaining in cysts were counted.

RESULTS

Growth of the plants: All plants grown in 16-h photoperiods were taller and stronger than those grown in 8.5-h photoperiods (Fig. 1); the latter had larger, paler leaves and weaker stems. Plants that had been treated with GA were taller than others, but those treated with xylocaine were taller than untreated plants. Katahdin and Sebago plants grown in 16-h photoperiods had many stolons growing out of the pots by 4 wk after inoculation. None of the plants grown in 8.5-h photoperiods flowered, but flowering began 3–4 wk after inoculation on plants grown in 16-h photoperiods and continued until the plants were harvested (10 wk after inoculation). Gibberellic acid and xylocaine delayed flowering of Superior by about 1 wk but did not delay flowering of the other two varieties.

Chemical treatments had little effect on tuber number and yield from plants grown in 8.5-h photoperiods, nor did they affect yield of the variety Superior in 16-h photoperiods, although Superior potatoes treated with GA formed more tubers in 16-h photoperiods (Table 1). Yields of Katahdin and Sebago were very small when treated with GA, greater when treated with xylocaine, and greatest in untreated plants.

Hatching tests: Hatch was greatest from cysts taken from Superior and least from those taken from Sebago; Katahdin was midway between the two (Table 2). Significantly greater hatch was observed from cysts taken from GA treated plants than from untreated plants, but treating plants with xylocaine did not have this effect. More juveniles hatched from cysts collected from Superior and Sebago grown in short photoperiods than long, but the reverse was true for Katahdin.

Pretreatment of cysts with GA, EDTA, or water did not affect subsequent hatching in root diffusate or picrolonic acid, but more juveniles always hatched with picrolonic acid (mean 75.6%) than with root diffusate (mean 62.1%) or water (mean 5.8%) (Table 3). Gibberellic acid alone or in combination with a hatching stimulant did not affect hatching. However, EDTA at 250 ppm for 1 wk followed by EDTA at 125 ppm (i.e., a 1:1 mixture with distilled water) caused significantly more juveniles to hatch than were hatched in water alone.

DISCUSSION

All of the treatments (host variety, photoperiod, and chemical treatments) influenced hatching significantly. It seems unlikely that the treatments affected the rates

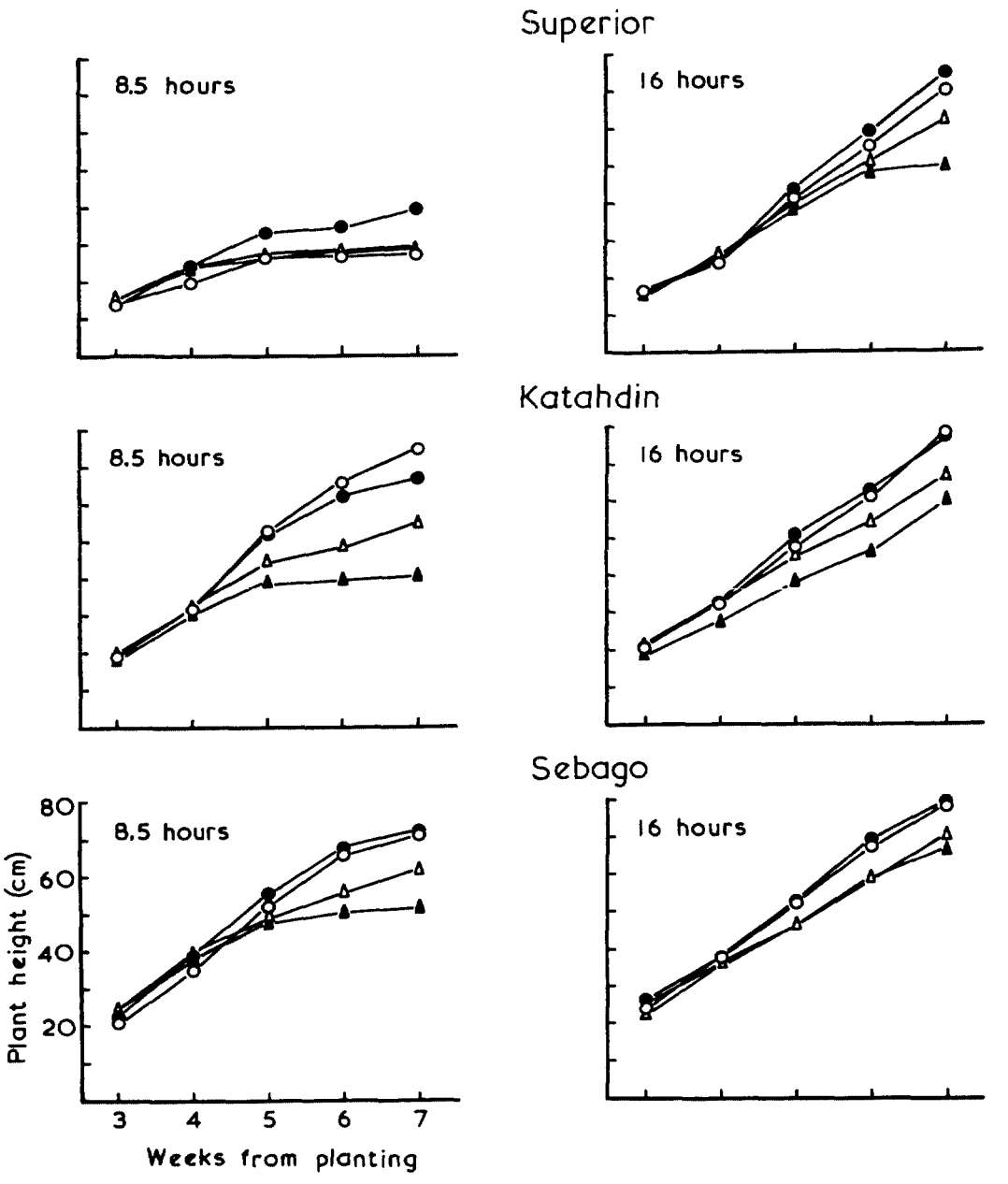

Fig. 1. Plant height measurements from 'Superior,' 'Katahdin,' and 'Sebago' potato plants grown in 8.5- or 16-h photoperiods, with foliar applications, to run-off, of gibberellic acid and/or xylocaine, with distilled water checks (controls).

- ○ Gibberellic acid at 50 ppm
- ● Gibberellic acid at 50 ppm plus xylocaine at 200 ppm
- △ Xylocaine at 200 ppm
- ▲ Distilled water

of development of the nematode, since all eggs appeared to contain fully formed second-stage juveniles and average temperature (24.1 C) over the growing period was high enough for complete development of the nematodes (3). Therefore it is likely that the nematodes responded by becoming more dormant after some treatments than after others.

If first generation juveniles hatch readily

Table 1. Numbers and weights (g) of tubers from 'Superior,' 'Katahdin,' and 'Sebago' potato plants grown in 8.5- or 16-h photoperiod, with gibberellic acid (GA) or xylocaine (X) treatment (totals from five plants).

Variety	Treatment							
	GA (50 ppm)*		GA + X		X (200 ppm)*		No treatment	
	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.
				8.5-h photoperiod				
Superior	15	194	17	166	13	150	8	160
Katahdin	7	131	7	88	12	155	12	174
Sebago	14	147	17	177	15	172	15	163
				16-h photoperiod				
Superior	19	168	17	189	8	201	7	204
Katahdin	6	24	7	19	8	67	6	141
Sebago	5	8	3	6	7	76	6	99

*Aqueous solutions applied to run off on six weekly occasions.

only when the hosts are still physiologically young, there would be sufficient time for them to complete a second generation. On this basis it was expected that hatching would be greatest from cysts collected from Sebago (the latest maturing variety), from plants grown in 16-h photoperiods (because long days delay tuber formation [6]) and from plants treated with GA, which also delays host maturation. However, although hatching was increased by GA treatment, it was usually decreased by 16-h photoperiods and was much less from cysts reared on Sebago than on the other two varieties. Banyer and Fisher (2) report similar results for *H. avenae:* hatching was faster from cysts reared on an early maturing barley variety than from cysts reared on a later maturing variety. Perhaps such results are obtained because cysts from the early ma-

Table 2. Percentage hatch from cysts removed from the roots of 'Superior,' 'Katahdin,' and 'Sebago' potato plants grown in 8.5- or 16-h photoperiod, with gibberellic acid (GA) or xylocaine (X) treatment.

Variety	Photoperiod (h)	Chemical treatment				Mean	Mean for varieties
		GA (50 ppm)*	GA + X	X (200 ppm)*	No treatment		
Superior	8.5	57.0	43.4	32.0	28.1	40.1	
	16	36.9	40.2	23.1	26.9	31.8	36.0
Mean		47.0	41.8	27.6	27.5		
Katahdin	8.5	27.1	19.8	16.9	15.4	19.8	22.3
	16	24.6	28.5	18.7	27.0	24.7	
Mean		25.9	24.2	17.8	21.2		
Sebago	8.5	23.3	17.3	11.5	10.5	15.7	13.3
	16	13.3	9.2	8.5	12.6	10.9	
Mean		18.3	13.3	10.0	11.6		
Mean for chemical treatments		30.4	26.4	18.5	20.1		23.9

Least significant differences:

Effect	$P = 0.01$	$P = 0.001$
Variety	3.9	5.2
Chemical	4.7	6.4
Variety × day	6.1	8.7
Variety × chemical	11.2	19.1
Day × chemical	7.6	11.4

*Aqueous solutions applied to run off on six weekly occasions.

Table 3. Mean percentage and standard error for juveniles hatching from cysts pretreated with gibberellic acid (GA), ethylenediaminetetraacetic acid (EDTA), or water, followed by exposure to water, potato root diffusate, or picrolonic acid.*

Pretreatment		Hatching agents		
		Distilled water	Root diffusate	Picrolonic acid†
GA_3	50 ppm	2.4 ± 0.39	62.2 ± 1.60	75.1 ± 1.18
GA_3	500 ppm	3.3 ± 0.92	61.0 ± 1.92	68.6 ± 1.76
EDTA	50 ppm	3.9 ± 0.29	65.0 ± 1.86	89.5 ± 1.22
EDTA	250 ppm	17.2 ± 1.80	56.2 ± 3.24	67.1 ± 4.48
Distilled water		2.1 ± 0.59	66.2 ± 2.47	77.7 ± 5.13
	Means	5.8 ± 1.58	62.1 ± 1.28	75.6 ± 2.48

*Means and standard errors of three replicate batches of 100 cysts; hatching solutions were 1:1 mixtures of pretreatment solutions and hatching agents.

†0.3m*M* (79 ppm) aqueous solution.

turing varieties are in some way more mature.

It is possible that the EDTA stimulated hatching by interfering with calcium cross-linkages in the eggshell or its lining membrane so bringing about a conformational and permeability change which encouraged hatching in the manner suggested by Clarke and Perry (4). Picrolonic acid also has a great affinity for calcium and may be a successful artificial hatching agent for similar reasons. The changes in calcium concentrations noted by Atkinson et al. (1) may have been the result of eggshell permeability changes rather than a more direct part of the hatching process.

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