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Control of Root-knot Nematodes on Tomato by Lectins

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Abstract: Significant control of tomato root knot was achieved by applications of the lectins Concanavalin A (Con A) and *Limax flavus* agglutinin in greenhouse, growth chamber, and microplot trials. Four consecutive weekly applications at lower concentrations of Con A yielded better control than single applications at a higher total concentration. The present state of knowledge on binding of Con A to soil nematodes and the in vitro effect of this lectin in chemotactic behavior are discussed. The mode of action of Con A on root-knot control is unknown.

Key words: Concanavalin A, *Limax flavus* agglutinin, *Lycopersicon esculentum, Meloidogyne incognita,* root knot, tomato.

The possibility that lectins can block recognition of chemotactic factors by nematodes and thereby modify responses to chemoattractants was proposed by Zuckerman (9) and Zuckerman and Jansson (10). This hypothesis was tested in vitro to evaluate the degree of chemoattraction of the bacteriophagous nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* to sterile filtrates from cultures of *Escherichia coli* (5,6). The results showed a temporary inhibition of attraction of the nematode to the filtrates following exposure to Concanavalin A (Con A) and limulin (LPA) and two enzymes, mannosidase and neuraminidase. The lectins or enzymes did not affect nematode development or locomotion at the concentrations applied, thereby supporting the premise that the effects were due to binding to, or to conformational changes of, nematode chemoreceptors which mediate the recognition function. In a similar approach, Bone and Bottjer (2) found a significant inhibition of the feeding stimulus of the animal-parasitic nematode *Trichostrongylus colubriformis* following exposure to the glucose-mannose-specific lectin *Lens culinaris* agglutinin and concluded that this effect was due to lectin binding to nematode receptors.

The objective of this research was to examine the efficacy of two lectins against root-knot nematode on tomato.

MATERIALS AND METHODS

Cultures of *Meloidogyne incognita* (Kofoid and White) Chitwood for the Massachusetts trials were from Dr. M. McClure, University of Arizona, Tucson, and Mexican populations maintained in the greenhouse at the Colegio de Postgraduados, Montecillos.

Lycopersicon esculentum Mill. cv. 857 Floramerica was used in all trials except Experiments 4 and 7 in which cv. Rutgers was used.

Experimental treatments consisted of a range of concentration levels of the lectins Concanavalin A (Sigma Chemical Company, St. Louis, Missouri) and *Limax flavus* agglutinin (LFA) (E-Y Laboratories, San Mateo, California). The several variations of the treatments with these lectins are specified under the description of each experiment.

Each experiment included a lectin treatment, an aldicarb (Temik 15 G) application, and untreated control plants inoculated with *M. incognita.* The procedure was to apply lectins to 2–3-week-old tomato seedlings grown in steam-sterilized soil. Lectins were dissolved in 10 mM Tris buff-

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FIG. 1. Microplots at Tecamachalco, Mexico, on day of harvest showing the experimental design and type of container used.

er (pH 7.2) and applied at the desired concentration to the soil around the plant roots. The soil was irrigated to incorporate the lectins as specified in the description of each experiment. At Amherst the same amount of 10 mM Tris buffer (pH 7.2) was applied to the untreated controls and to the lectin treatments. Aldicarb treatments were concurrent with those of the lectins. Hatched second-stage juveniles in tap water were added to the soil 24 hours after application of lectins or aldicarb.

The trials were terminated 42–60 days after nematode inoculation. Data were taken on the total number of root galls, fresh root weights, and plant tops.

Single application of lectins: Experiment 1 consisted of a single application of Con A or LFA at concentrations of 3, 30, 90, or 180 μg/pot containing 250 cm^3 steam-sterilized potting soil. Lectins were applied to 20-day-old tomato seedlings as a soil drench in 20 ml of 10 mM tris buffer (pH 7.2) 5 days after transplanting. Aldicarb at 4.5 mg/pot and untreated control plants were included. One day following treatments, 300 freshly hatched second-stage *M. incognita* were added to each pot. Each treatment contained eight replicates, giving a total of 96 pots for the experiment. The experiment, performed in growth chambers at Chapingo, was terminated 42 days after nematode inoculation.

Experiment 2 was similar to Experiment 1, but contained 10 replicates for each treatment, for a total of 120 pots, and 700 second-stage juveniles were added to each pot. Experiment 3 also was similar to Experiment 1, but was performed in the greenhouse at Amherst. Each variant was replicated 10 times, and 500 second-stage juveniles were added to each pot. The experiment terminated 45 days after nematode inoculation.

Single and combination lectin applications: In Experiments 4 and 5, single applications of lectins and combinations of LFA and Con A were tested for possible synergistic effects. Treatments were LFA at 3 μg and 30 μg/pot, Con A at 30 μg/pot, LFA 3 μg + Con A 15 μg/pot, LFA 15 μg + Con A 15 μg/pot, aldicarb 4.5 mg/pot, and controls inoculated with *M. incognita.* Each treatment was replicated six times for a total of 42 pots in each experiment, and 500 freshly hatched second-stage *M. incognita* were added to each pot. These trials were performed in the greenhouse at Amherst and terminated 42 days after nematode inoculation.

Multiple applications of lectin: Multiple applications of LFA and Con A were evaluated in a growth chamber at Chapingo. Treatments were Con A or LFA at 3 μg applied 1, 2, 3, or 4 times, and 350 *M. incognita* were added to each pot. Nematicide and untreated controls were as in other experiments. Each treatment was replicated six times. The plants were harvested 42 days after inoculation.

Microplot test: A microplot series (Experiment 7) was installed at Tecamachalco, Mexico. Each microplot consisted of a can containing 20,000 cm^3 steam sterilized soil (Fig. 1). Treatments were Con A at 30 μg, Con A 3 μg, LFA 30 μg, aldicarb 4.5 mg/plot, and controls to which *M. incognita* juveniles were added with no other treatment. Lectins were applied in Tris buffer (pH 7.2), then irrigated with 200 ml water to incorporate. Each treatment was replicated 10 times, and the duration was 60 days. Five hundred second-stage juveniles were added to each microplot.

The data were analyzed by a one-way classification analysis of variance. Dunnett's procedure (3) was used to test for the

TABLE 1. Tomato root galling by *Meloidogyne incognita* with and without Concanavalin A (Con A), *Limax flavus* agglutinin (LFA), or aldicarb.

Treatment	Concentration†	No. galls per root system		
		Exp. 1	Exp. 2	Exp. 3
Con A	180	29*	29*	5*
	90	29*	32*	10*
	30	27*	22*	6*
	3	39*	27*	8*
LFA	180	26	45	3*
	90	20*	35*	3*
	30	31	38*	8*
	3	33	40*	3*
Aldicarb				
Con A series	4.5 mg	1*	1*	2*
LFA series		1*	1*	2*
Untreated				
Con A series		47	62	37
LFA series		35	62	23

Experiment 1 had eight replicates of each treatment; Experiments 2 and 3 each had 10 replicates. The data are means of the total number of galls per plant.

* Significantly different ($P = 0.05$) from the untreated.

† Values are micrograms per pot unless otherwise indicated.

significance of differences of treated groups from the control groups.

RESULTS

Single applications of lectins: Con A generally significantly reduced root galling at concentrations as low as 3 μg Con A (Table 1). Significant control was achieved also at dosages of 30, 90, and 180 μg, but the consistent performance at lower concentrations led to the selection of 3 μg and 30 μg for further testing. Generally LFA gave poorer control than did Con A, but in several combinations significant reductions in galling occurred (Table 1).

Single and combination lectin applications: Both combinations of LFA and Con A evaluated resulted in an insignificant reduction of root galling. There was no evidence of synergistic activity from these combined lectin treatments (Table 2). The results of these two experiments support the findings that 30 μg Con A gave more effective control than LFA at a comparable concentration, but as before, significant reductions in root galling were achieved with both lectins.

Multiple applications of lectins: Con A at 3 μg increasingly reduced galling as the number of weekly applications increased (Fig. 2). The four application series resulted in 75% reduction in root galling, the highest level of control achieved in the experiments reported here. LFA again gave lower and inconsistent levels of control.

Microplot test: In the microplot series, Con A at 30 μg resulted in a 55% reduction in root galling (Fig. 3). Con A at 3 μg and LFA at 30 μg each gave a lower level of control of root knot.

The wet weights of roots and plant tops were not significantly different between tomatoes treated with 30 μg Con A and untreated plants in most experiments, but the microplot series (Experiment 7) and Experiment 5, in which both roots and tops of plants treated at 30 μg Con A were significantly larger than those of untreated plants ($P = 0.5$), were exceptions. The relatively short duration of the experiments (other than the microplots) may explain the lack of significant growth differences. For this reason, the experimental data for plant growth are not presented in this paper. Significant reductions in root or top

TABLE 2. Galling of tomato by *Meloidogyne incognita* as affected by single or combination treatments of Concanavalin A (Con A), *Limax flavus* agglutinin (LFA), or aldicarb compared to an untreated check.

	Lectin (μg/pot)						
	Con A 30	LFA 3	LFA 30	LFA 3 + Con A 15	LFA 15 + Con A 15	Aldicarb 4.5 mg	Untreated
Exp. 4	24*	39	27*	56	40	1*	60
Exp. 5	31*	59	35*	67	48	1*	75

Six replicates of each treatment. The data are means of the total number of galls per plant.

* Significantly different ($P = 0.05$) from the untreated.

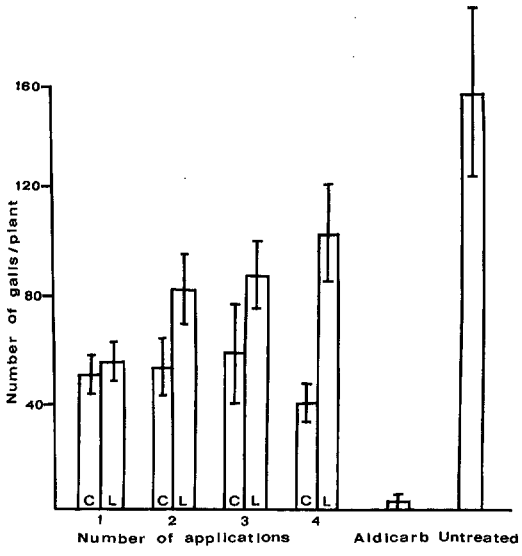

FIG. 2. Data from Experiment 6 showing significant reduction ($P = 0.05$) in root galling of *M. incognita* on tomato by Con A following multiple applications of LFA (L) or Con A (C).

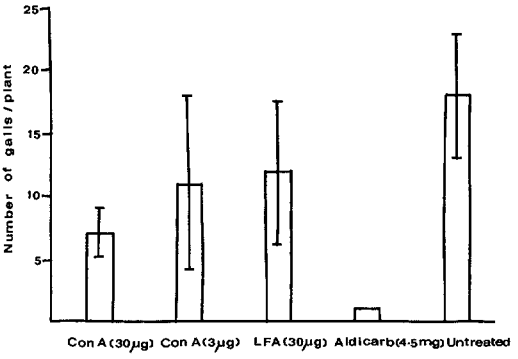

FIG. 3. Data from Experiment 7 showing significant ($P = 0.05$) reduction in root galling of *M. incognita* on tomato by single applications of 30 μg Con A and aldicarb.

growth associated with lectin treatments did not occur.

DISCUSSION

The lowest consistently effective single dosage application of Con A which reduced root galling was 30 μg; LFA was much less effective than Con A. Combinations of Con A and LFA did not enhance root-knot control, suggesting that no synergistic action occurs between the two lectins. Up to four applications of Con A at weekly intervals significantly increased root-knot control at lower total dosages. For example, 12 μg Con A applied over a period of 4 weeks gave 75% control.

The experimental observations of Con A binding specifically to the head of *M. incognita* (7) and to four populations of *Globodera rostochiensis* and *G. pallida* (4), as well as specific localization of sialic acid residues on the head region of *Xiphinema index* (8), suggest a logical line of speculation as to the mode of action of Con A on root knot of tomatoes. Binding of the ligand presumably could result in blocking or altering the conformation of the nematode chemoreceptors. The resumption of normal chemotactic behavior by *C. elegans* 8 hours after treatment with Con A suggests that receptor function is initially destroyed, and that receptor renewal is initiated almost immediately. The general observation that plasma membrane receptors have a half life of about 24 hours supports this suggested mechanism of action (1). In the experiments reported here, the nematodes would be exposed to continued contact with lectin, so that blocking of chemoreception would be a recurring process. One may also speculate that the lectin, a protein, would be degraded in the soil in a relatively short time. Multiple applications therefore would be required for more effective control. Another possibility that should be considered is that the lectin directly affected the plant by inducing resistance to root knot. However, we emphasize that at present there is no direct evidence on the mode of action of Con A on root-knot nematodes.

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