The Relationship Between Glycoalkaloids and Disease Resistance in Potatoes

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ABSTRACT

Ten potato cultivars were grown in replicated field-disease test plots in 1972 and 1973 at Presque Isle, Maine. The four diseases tested included early and late blight, common scab, and Verticillium wilt. During the infection period in each disease test, the part of the plant that was invaded by the individual pathogen was analyzed for total glycoalkaloid (TGA) content. The data obtained in 1972 and 1973 indicated a lack of correlation between disease resistance in potato plants and TGA content.

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Glycoalkaloids have been implicated as factors in resistance of plants towards fungal pathogens (1, 2, 3, 7, 10, 18) and insects (5, 12, 16, 19). Glycoalkaloid content in potato plants has been shown to be under genetic control (15). In potato plants these compounds (primarily solanine and chaconine) are naturally present and their concentration may increase when plants are under stress (8, 9). This biological stress reaction was used to implicate glycoalkaloids as possible resistance factors in potatoes toward *Phytophthora infestans* (Mont.) de Bary (2, 9). However, Deahl, Young, and Sinden (6) reported no apparent association between glycoalkaloid content and multigenic resistance to *P. infestans*.

Potato glycoalkaloids have also been reported to cause human disorders, including gastrointestinal disturbances, nervous depression, and death (20). Recently, a hypothesis suggesting glycoalkaloids as possible teratogens was proposed by Renwick (14). He also suggested a relationship between late blight resistance and glycoalkaloids. Results from feeding studies using marmosets added further to the public concern about the possible consumption of these blighted tubers (13).

This study was conducted to determine whether the disease resistance in potatoes is related to total glycoalkaloid content (TGA) during the infection period.

MATERIALS AND METHODS.—The U. S. Department of Agriculture (USDA) national potato breeding program conducts annual disease evaluations on all advanced breeding lines. Tests are conducted in Presque Isle, Maine, in individually isolated field plots. Fungicide and insecticide sprays are applied as necessary to insure a valid test of clonal reaction to the individual pathogen in each plot. The four disease tests incorporated into this research were late blight, early blight, Verticillium wilt, and common scab. The USDA breeding program also analyzes the advanced breeding lines for total glycoalkaloid (TGA) content as a routine procedure.

As a preliminary to this research, data from USDA potato clones that were tested for resistance to common scab in 1970 to 1971 were statistically analyzed for correlation between resistance to common scab and TGA levels. The scab test plot in Presque Isle is used yearly without a rotation program, and treated with ground limestone to maintain a soil pH of about 6.5 to 7.5. The scab organism, *Streptomyces scabies* (Thaxt.) Waks. and Henrici, was allowed to develop symptomatic lesions on the tubers. At harvest, to evaluate each clone, the area of the tubers covered with lesions was observed, and lesion size was rated. Six tubers of each clone were analyzed for total tuber TGA. These samples were analyzed by the short method described by Bretzloff (4).

To assess further the relationship between disease resistance and TGA levels, 10 potato cultivars were chosen for replicated field tests in the USDA disease plots. Each test included one known resistant cultivar and one cultivar susceptible to the respective pathogen. The cultivar Wauseon, which is generally low in TGA, and the clone B 5141-6, which is generally high, were included in all test plots. The remaining cultivars in each test were selected because of their wide range in TGA values, as indicated from previous testing. In 1973, four of these cultivars were removed, and cultivars which showed a wider range of disease reactions were substituted. Kennebec was selected as the resistant cultivar for the early and late blight tests. It is resistant to *Phytophthora infestans*, race O, which was used in the late blight trial, and it also has some degree of resistance to *Alternaria solani* (Ell. and G. Martin) Sor., the early blight pathogen. Cultivar Abnaki was selected as the cultivar resistant to *Verticillium albo-atrum* Reinke & Berth, and Cherokee as resistant to the common scab organism, *Streptomyces scabies*.

These resistant cultivars and the high- and low-TGA cultivars were grown in both 1972 and 1973. The following clones were also tested in both years: B-6955-4, B-6990-88, and B-7159-25. In 1972, the clones B-6980-38, B-6985-36, B-7164-2, and B-7164-18 were included, but clones B-6998-15, B-7572-3, B-6983-11, and B-7582-5 were substituted for them in 1973. The test plots were generally planted in the latter part of May, with the scab plot being planted first to ensure adequate tuber infection for evaluation. The tests consisted of four hills planted in each of four replications.

The inoculation procedure for the Verticillium wilt plot involved cutting the seed in the field before planting, dipping the seed pieces into a spore suspension of *V. albo-*

atrum (8×10^4 spores per milliliter), planting, and covering immediately. Symptoms usually developed within 6-8 weeks after inoculation. At this time, two plants were dug from each replication, and their root systems and lower stems were removed for TGA analysis. The remaining plants were evaluated weekly until harvest for their disease reaction on a 1 to 5 scale, with 1 indicating no disease and 5 signifying plant death. This rating scale was also used for plants inoculated with the other two fungi.

The late blight plot was inoculated in mid-July, when temperatures and relative humidity were optimum for infection. The border rows and every third row in the plot were planted with the blight-susceptible cultivar, Green Mountain, to serve as pathogen spore spreader rows. Zoospore suspensions of *P. infestans*, race 0, were sprayed on the spreader rows at dusk every third night, until the disease was established. Once lesions were noticed on the test plants, all leaves were removed to be analyzed for TGA levels. The remaining plants were evaluated weekly for disease development.

The early blight plot was planted so that the border rows and every third row consisted of the susceptible clone B-5281-1. Spore suspensions of *A. solani* were sprayed on the spreader rows in mid-July during a period of high relative humidity, to establish the pathogen in the plot. Once lesions were noted on the test plants, all leaves were removed for TGA analysis. The remaining plants were evaluated weekly until harvest, for their disease reactions.

The scab plot depended on natural infection, and every third row in the plot was planted with the susceptible cultivar Green Mountain to determine when infection occurred. The soil was removed weekly from around the base of the Green Mountain plants once tuberization had begun, and scab development was observed. Once lesions were observed, two of the test plants in each replication were dug, and tubers were removed for TGA analysis. During harvest in early September, the remaining plants were dug, and the tubers were evaluated for their disease reactions. The ratings evaluated the area of tuber infected and also the lesion type. Both area and type ratings were made on a 0 to 5 scale, with 0 indicating no disease. The area ratings were finally multiplied by type ratings, to provide a single figure for computation of correlation coefficients.

All field plots were fertilized with 15-15-15 at the rate of 21.6 kg N per hectare (ha) and treated as necessary with endosulfan [1.17 liter per hectare (ha)] and methyl demeton (1.75 liter per ha). The scab and Verticillium wilt plots were sprayed weekly from July until harvest with zinc ion maneb (2.25 kg per ha) to control late blight and early blight. An isolated control plot was fertilized and given the same pesticide treatments as mentioned above. This plot was planted in 10-hill replications, so that adequate plant material was available for controls for all four disease tests.

In all disease tests, when tubers, leaves, or roots were removed from the field for TGA analysis, the plant parts were immediately placed in brown paper bags, to prevent light from affecting TGA levels. The majority of the foliage in the inoculated blight plots had small, pinpoint lesions visible on susceptible cultivars. All samples from individual plants of each cultivar were pooled, weighed, and a 250-g sample was selected for TGA analysis. The samples were cut into small pieces and homogenized in a blender with 95% EtOH containing 2.5% acetic acid. This slurry was processed for total glycoalkaloids by the method of Sanford and Sinden (15). This same procedure was repeated in 1973. The correlation coefficients between disease readings and TGA levels were computed by the values determined from within the same test plot.

RESULTS AND DISCUSSION.—The potato clones tested in the 1970 and 1971 USDA common scab field

TABLE 1. Late blight ratings and total glycoalkaloid (TGA) content of leaves for potato cultivars and clones evaluated in 1972 and 1973 field trials

	1972			1973		
	Inoculated		Control	Inoculated		Control
Clone or cultivar	Disease rating	TGA value	TGA value	Disease rating	TGA value	TGA value
B 5141-6	5.0[a]	78.0[b]	53.1	3.7	166.6	143.4
B 6955-4	3.5	70.4	71.0	3.9	113.2	70.4
B 6990-88	3.0	104.9	135.0	3.7	114.0	127.6
B 7159-25	5.0	60.0	51.5	2.6	51.6	68.4
Wauseon	4.0	53.0	50.0	3.4	76.6	58.7
Kennebec	3.5	49.0	46.6	2.2	115.8	122.2
B 6980-38	4.0	134.1	154.0	. . .	. . .	. . .
B 6985-36	5.0	28.0	28.0	. . .	. . .	. . .
B 7164-2	5.0	94.0	94.3	. . .	. . .	. . .
B 7164-18	4.5	92.0	58.9	. . .	. . .	. . .
B 6998-15	. . .	. . .	. . .	3.5	81.6	57.2
B 7572-3	. . .	. . .	. . .	3.9	81.7	86.7
B 6983-11	. . .	. . .	. . .	3.1	83.9	50.5
B 7582-5	. . .	. . .	. . .	2.8	134.2	174.9
LSD($P = 0.05$)	0.67			0.54		
		r = 0.1			r = 0.17	

[a]Disease rating is based on a 1 to 5 scale, with 1 indicating no disease and 5 indicating plant death.
[b]TGA value is presented as mg TGA in 100 g plant tissue.

trials had no significant correlation between their scab resistance and TGA values. The 98 clones evaluated in 1970 had a correlation coefficient of 0.0 (r = 0.0). Their value for 78 clones in 1971 was 0.03. This data is extremely meaningful, because the disease ratings were made on the tubers, which have generally been used for TGA analysis. Some TGA levels and resistance have been compared while tuber TGA levels and foliar disease resistance were being compared. Late-blight resistance in potato foliage does not necessarily signify tuber resistance to the same pathogen.

The data obtained for the individual disease test in 1972 and 1973 are presented in Tables 1 to 4, with each table comprising a specific disease test. The correlation coefficient between resistance and TGA level is lower than 0.35 in all tests, except the 1973 common scab test, in which r = 0.71. The TGA levels for tubers from this plot indicate a significant correlation ($P = 0.05$) between TGA and disease reaction. However, the correlation indicates that tubers high in TGA tend to be more susceptible to scab than tubers having low TGA levels. The overall disease reactions for the early blight, late blight, and

TABLE 2. Early blight disease ratings and total glycoalkaloid (TGA) content of leaves for potato cultivars and clones evaluated in 1972 and 1973 field trials

	1972			1973		
	Inoculated		Control	Inoculated		Control
Clone or cultivar	Disease rating	TGA value	TGA value	Disease rating	TGA value	TGA value
B 5141.6	4.0[a]	93.1[b]	53.0	3.4	100.8	143.4
B 6955-4	4.5	64.0	71.0	2.9	104.8	70.4
B 6990-88	3.5	92.0	135.0	1.5	106.9	127.6
B 7159-25	4.0	75.4	51.2	4.7	30.1	68.4
Wauseon	3.5	44.3	50.1	3.1	60.3	58.7
Kennebec	3.0	31.0	46.0	2.5	53.0	122.2
B 6980-38	4.5	139.6	154.0	. . .	. . .	. . .
B 6985-36	5.0	39.0	46.7	. . .	. . .	. . .
B 7164-2	5.0	100.2	94.0	. . .	. . .	. . .
B 7164-18	5.0	72.7	58.9	. . .	. . .	. . .
B 6998-15	. . .	. . .	. . .	4.2	58.4	57.2
B 7572-3	. . .	. . .	. . .	4.7	80.9	86.7
B 6983-11	. . .	. . .	. . .	4.0	101.2	50.5
B 7582-5	. . .	. . .	. . .	3.9	106.4	174.9
LSD($P = 0.05$)	0.79			0.60		
		r = 0.30			r = −0.35	

[a]Disease rating is based on a 1 to 5 scale, with 1 indicating no disease and 5 indicating plant death.
[b]TGA value is presented as mg TGA in 100 g plant tissue.

TABLE 3. Verticillium wilt disease ratings and total glycoalkaloid (TGA) content of roots and stems for potato cultivars and clones evaluated in 1972 and 1973 field trials

	1972			1973		
	Inoculated		Control	Inoculated		Control
Clone or cultivar	Disease rating	TGA value	TGA value	Disease rating	TGA value	TGA value
B 5141-6	4.0[a]	34.0[b]	30.1	3.0	82.4	118.6
B 6955-4	5.0	26.5	23.2	4.7	53.2	85.0
B 6990-88	4.0	24.2	31.0	3.6	58.4	93.4
B 7159-25	2.0	24.0	28.1	1.1	74.7	79.0
Wauseon	3.5	22.7	21.0	2.4	40.2	84.8
Abnaki	1.5	22.3	25.0	1.0	67.2	73.2
B 6980-38	5.0	25.0	25.3	. . .	. . .	. . .
B 6985-36	5.0	28.1	28.0	. . .	. . .	. . .
B 7164-2	5.0	40.6	35.1	. . .	. . .	. . .
B 7164-18	3.5	27.1	33.0	. . .	. . .	. . .
B 6998-15	. . .	. . .	. . .	2.4	66.4	76.0
B 7572-3	. . .	. . .	. . .	3.9	85.4	107.4
B 6983-11	. . .	. . .	. . .	3.5	73.9	79.4
B 7582-5	. . .	. . .	. . .	2.6	64.1	81.8
LSD ($P = 0.05$)	0.94			1.02		
		r = −0.30			r = −0.05	

[a]Disease rating is based on a 1 to 5 scale, with 1 indicating no disease and 5 indicating plant death.
[b]TGA value is presented as mg TGA in 100 g plant tissue.

TABLE 4. Common scab disease ratings and total glycoalkaloid (TGA) content of tubers for potato cultivars and clones evaluated in 1972 and 1973 field trials

	1972			1973		
	Inoculated		Control	Inoculated		Control
Clone or cultivar	Disease rating	TGA value	TGA value	Disease rating	TGA value	TGA value
B 5141-6	1.3[a]	31.8[b]	32.2	9.6	87.8	99.0
B 6955-4	0.6	2.2	1.9	3.2	10.8	22.9
B 6990-88	3.6	19.5	19.2	3.6	25.7	18.6
B 7159-25	0.0	3.6	29.7	0.3	21.9	86.4
Wauseon	0.1	2.6	0.6	0.6	11.2	32.2
Cherokee	0.4	1.2	0.9	0.7	6.4	11.1
B 6980-38	0.1	20.8	1.1	...	...	...
B 6985-36	7.2	22.1	24.1	...	...	...
B 7164-2	2.8	31.4	42.6	...	...	...
B 7164-18	12.5	20.8	40.8	...	...	...
B 6998-15	...	...	...	4.8	29.8	54.2
B 7572-3	...	...	...	3.1	23.9	30.3
B 6983-11	...	...	...	4.9	49.7	60.0
B 7582-5	...	...	...	3.1	84.2	76.0
LSD($P = 0.05$)	2.20			3.49		
		r = 0.1			r = 0.71	

[a]Disease rating is based on area of tuber covered (1 to 5 scale, with 1 indicating 20% coverage, 5 indicating 100%) multiplied by the lesion type (1 to 5 scale, with 1 indicating small lesions and 5 indicating large, sunken lesions).

[b]TGA value is presented as mg TGA in 100 g plant tissue.

Verticillium wilt tests were generally more severe in 1972 than in 1973 (Tables 1 to 3), whereas the scab reactions were more severe in 1973 (Table 4). One of the reasons for the differences in disease reactions was the substitution of the four new clones in all 1973 tests. The environmental variables favored scab in 1973, whereas they favored the other three diseases in 1972.

Another general observation was that TGA levels increased in 1973 over those of 1972. Because the growing season in 1973 was shorter than that in 1972 due to late planting, the level of plant maturity at the infection period was lower in 1973. The relationship between maturity of potato foliage and TGA levels has been discussed by Sinden et al. (18).

The purpose of this study was to determine whether or not TGA levels are related to disease resistance. The data in Tables 1 to 4 indicate no relationship between TGA levels and disease resistance. For example, the cultivar Wauseon was resistant to scab in both 1972 and 1973, and it had low TGA levels. The clone B-5141-6 was more susceptible than Wauseon in both years, and its TGA levels were much higher. If glycoalkaloids were part of the resistance reaction and act like phytoalexins, their concentrations should increase immediately after infection. If the glycoalkaloids acted like naturally occurring toxins, one would expect the resistant cultivars to have higher TGA levels even before infection. Neither of these two situations existed in the data for this paper.

An interesting development in relation to potato disease resistance was the suggestion that TGA accumulation was suppressed and TGA levels were actually decreased during infection (11, 17). The indication in both papers was that the potato phytoalexins increased via the acetate-mevalonate pathway at the expense of steroid glycoalkaloids. These data indicated that the phytoalexins are the responsible components for disease resistance. This research was carried out on tubers removed from the field and on some cut tuber slices. In our data, TGA levels show no pattern for decrease or increase immediately after infection with any pathogen. In general, the conclusion is that TGA has no direct relationship to resistance against the four diseases tested, and that TGA should be eliminated or severely reduced in all breeding stocks. That way, the detrimental effects of these compounds upon consumers can be avoided.

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