Detrimental and Protective Effects of Rust in Flax Plants of Varying Age

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The technical assistance of M. Hodgins, R. Klassen, J. Peters, and L. J. Wiebe is acknowledged.

Accepted for publication 13 December 1978.

ABSTRACT

HOES, J. A., and D. G. DORRELL. 1979. Detrimental and protective effects of rust in flax plants of varying age. Phytopathology 69:695-698.

Flax (*Linum usitatissimum*) plants in growth chambers showed differential effects on disease development, agronomic characteristics, and quality of the seed following inoculation one to three times, at different stages of development, with the virulent rust fungus, *Melampsora lini* race 371. Vulnerability increased with age of the plant and reached a maximum at the prebloom stage. Light to moderate infections reduced yield 34–55% and severe infections reduced it 64–79%. Oil and protein contents were reduced when plants were inoculated at prebloom and again at the flowering stage. Iodine number was not affected. Flax inoculated 35–48 days after planting during the vegetative stage, and re-inoculated 11–30 days later at the prebloom stage, showed an induced resistance to rust. Pre-inoculated plants were less severely rusted, and produced more seed capsules and seeds than those inoculated only at the prebloom stage. The induced resistance appeared to be systemic; the effect was apparent in foliage that developed after pre-inoculation. The protective effect lasted at least 30 days in flax cultivar Redwood 65. The significance of the protection phenomenon is discussed.

Additional key words: linoleic acid, linolenic acid, oleic acid, systemic protection.

The effect of the age of the host plant as an epidemiological factor in rust disease (caused by *Melampsora lini* [Pers.] Lév.) of flax (*Linum usitatissimum* L.) is not clearly established. Arif (1) observed flax cultivars that were susceptible prior to bloom and became resistant after bloom. Straib (12), however, noted that some cultivars remained highly susceptible to rust infection after flowering. Yield and seed quality which might have been differentially affected by inoculation with *M. lini* at different stages were not studied by those workers.

In diseases caused by rust fungi, the various pustule types provide a measure of the degrees of resistance or susceptibility of the host and of avirulence or virulence of the parasite (7). Inherent resistance, as determined in race identification, is the typical reaction of 10- to 14-day-old flax seedlings to a single inoculation with an avirulent race of the rust fungus. Resistance also may be induced, and may be localized or systemic. An induced, localized form of resistance to a virulent race of *M. lini* was expressed in flax leaves pre-inoculated a few hours earlier with an avirulent race of the pathogen (9). Pre-inoculation significantly reduced the size, number, and rate of development of uredia produced in the host by the normally pathogenic race. Induced localized resistance also is caused by other avirulent/virulent inoculum combinations of bacterial or fungal pathogens, it generally is nonspecific, generally not translocated, and scarely persistent (10). Systemic and persistent resistance has been induced in plants of various hosts pre-inoculated at an early age with a virulent pathogen and re-inoculated with the same pathogen one to several weeks later (2,3,11).

This paper describes the effects of single and repeated inoculations with *M. lini* on disease development, yield, agronomic characters, fatty acid composition, and oil and protein content of flax plants infected at different stages of development.

MATERIALS AND METHODS

Plants of flax cultivars Noralta, Norland, and Redwood 65, all highly susceptible in the seedling stage to *M. lini* races that attack the N_1 resistance gene (14), were grown in pots 12.5 cm in diameter, each containing four uniform plants. Three experiments were conducted in growth cabinets programmed for 21 C, a photoperiod of 18 hr, and sufficient light to produce vigorous plant growth.

Plants at varying stages of development were inoculated one to three times by dusting with a mixture of *M. lini* uredospores (race 371) and bentonite (1:5, v/v), then misted with tap water, and incubated in the dark for 16–20 hr in closed plastic bags. In experiment 1, plants of cultivar Redwood 65 were inoculated at 48, 59 (prebloom stage [first swelling of flower buds followed by anthesis 2–4 days later]), and 75 days after planting when plants were in full bloom and some seed capsules and seeds had been produced. In experiment 2, plants of cultivar Redwood 65 were inoculated at weekly intervals starting 35 days after planting and ending at the prebloom stage when plants were 63 days old. In experiment 3, plants of cultivars Norland, Noralta, and Redwood 65 were inoculated 41 days after planting and at the prebloom stage, which was 16, 22, and 30 days later, respectively. Schedules of the single and multiple inoculations appear in Tables 1, 2, and 3. Control plants in experiment 1 were vigorous and remained virtually free of rust, those of experiment 2 became heavily rusted, and those of experiment 3 were grown in isolation and developed poorly. Hence, the control plants of experiments 2 and 3 were discarded. However, the plants inoculated earliest that had the least rust symptoms in each of these two experiments were considered a meaningful substitute for the intended control. Contamination among other treatments within the same experiment was negligible. Experiments 1 and 2 were arranged as randomized blocks with 12 and six replications, respectively, and experiment 3 as a split-plot with five replications.

Disease data. *Experiment 1.* Uredial infection was rated when pustule development had ceased and the older rusted leaves had begun to senesce. At this stage, most seed capsules had reached full maturity. Disease classes 'trace', 'light', 'moderate', 'severe', and 'very severe' were established based upon increasing numbers of rusted leaves and increasing pustule density. The total leaf surface covered by rust ranged from less than 1% in plants in the 'trace' class, to 60% or higher in the 'very severe' class. Telial infection was rated at harvest when leaves were absent and the black lesions on stems and peduncles were most obvious. Arbitrary disease classes of 'trace', 'light', 'moderate', and 'severe' were established. In pots rated 'trace', one or two plants showed a few, inconspicuous telia, whereas, in those rated 'severe' the lesions often were several cm long, and their combined lengths were 30% or more of the total length of the stems and peduncles.

Experiment 2. Total plant height (H) and total length of stem with rusted leaves (L) were measured when most seed capsules had matured. The parameter L included all rusted leaves, regardless of inoculation. The proportion (P) of rusted leaves along L was recorded in multiples of 10%. Uredial infections subsequently were expressed as $(L/H) \times P$, and a mean uredial index for each pot was calculated following angular transformation. A telial index for each pot was calculated at harvest by totaling the lengths of all discrete telial lesions on the stems of the four plants in each pot.

Experiment 3. At harvest, the incidence of telia on the peduncles of each plant was scored on an arbitrary scale ranging 1–3. A score of 1 indicated absence of telia; 2, the average length of telia was 3 cm or less; and 3, the length was 4–6 cm. A mean telial index was computed for each pot.

Agronomic and analytical data. Seed yield, number of seed capsules, and number of seeds all were based on the total of four plants in each pot. Oil and protein content and fatty acid composition were determined as described by Dorrell (4). Iodine number, which is a function of the proportions of the unsaturated fatty acids, was calculated from the fatty acid composition. In flax, there is little variation in the number of leaves per centimeter of stem. Thus, height was measured in experiment 2 at each weekly inoculation to obtain an indication of the increase in number of young, potentially susceptible leaves.

RESULTS

Effects of single inoculations. Rust severity, measured by the incidence of uredia and telia, gradually increased and reached a peak at the prebloom stage (Tables 1 and 2). Rust severity decreased when plants were inoculated at the flowering stage (Table 1). In experiment 1, inoculation at 48 days reduced yield by 38%, and at prebloom (59 days) by 77% (Table 1). In experiment 2, plants inoculated 35 to 56 days after planting produced 1.7–2.0 times as much seed as those inoculated at the prebloom (63 days) stage (Table 2). In experiment 3, plants of three different cultivars inoculated at 41 days, yielded 1.3–1.8 times as much as plants inoculated 16–30 days later at prebloom (Table 3). The number of capsules and seeds likewise was depressed most by inoculation at the prebloom stage (Tables 1, 2, and 3).

Seed weight and protein content tended to decrease with delayed inoculation in experiments 1 and 2, whereas oil content was relatively unaffected. For example, protein content declined significantly from 47.4% at the 35-day inoculation to 44.6% at the prebloom inoculation. Seed weight declined significantly from 5.07 to 4.65 g/1,000 seeds during the same period (Table 2).

Fatty acid composition also varied with the plant stages at inoculation. When the inoculation was delayed until 75 days after planting, linoleic acid concentration significantly increased and linolenic acid decreased. Oleic acid varied significantly among treatments, but the changes were not consistent (Table 1). In experiment 2, the concentrations of oleic, linoleic, and linolenic acids were 31.1, 13.3, 47.0% and 28.7, 13.3, 49.2%, for the 35- and 63-day inoculations, respectively. Neither these differences nor those in iodine number were significant.

Telia were produced abundantly on stems and peduncles of the inflorescence in certain treatments of experiments 1 and 2. In experiment 3 however, telia occurred primarily on the peduncles. Telial lesions on stems often covered more than half of the circumference, whereas peduncles were nearly always ringed completely. Discrete lesions in experiment 2 ranged 0.1–22.0 cm in length.

Effects of multiple inoculations. Plants inoculated at least 11

TABLE 1. Effects of single and multiple inoculations with *Melampsora lini* race 371 on rust disease severity[a] and agronomic characters of cultivar Redwood 65 flax (experiment 1)[b]

	Inoculation time effects listed under times (days) from planting to inoculations(s)							
Criteria	Control (not inoculated)	48	59 (prebloom)	75	48&59	48&75	59&75	48,59,&75
Uredial infection[b]	Trace	Light	Very severe	Moderate	Moderate	Moderate	Severe	Severe
Telial infection[b]	Trace	Light	Severe	Moderate	Moderate	Moderate	Severe	Moderate
Seed yield (g)	1.6 a[c]	1.00 b	0.4 e	0.7 cd	1.1 b	0.9 bc	0.3 e	0.6 de
Number of seed capsules	63.00 a	42.1 b	16.7 e	31.8 c	42.9 b	35.5 b	16.0 e	24.5 d
Oil (%)	41.0 a	40.7 a	40.0 a	40.3 a	40.6 a	41.0 a	38.1 b	39.9 a
Protein (%)	45.1 a	43.9 a	43.8 a	42.9 a	43.6 a	44.2 a	39.0 b	42.8 a
Oleic acid (%)	18.1 bc	17.5 c	17.5 c	18.9 b	17.0 c	20.7 a	17.3 c	17.0 c
Linoleic acid (%)	12.9 b	13.4 ab	14.0 a	14.0 a	14.0 a	13.2 b	13.6 ab	13.2 b
Linolenic acid (%)	60.5 a	60.9 a	59.7 ab	58.6 bc	60.4 a	57.6 c	60.1 a	61.1 a

[a]Disease severity rating: trace = less than 1% total leaf surface covered by rust pustules; very severe = 60% or higher covered by rust pustules.
[b]Means of 12 replicates (four plants per replicate).
[c]Means within rows followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 2. Effects of 1 or 2 inoculations with *Melampsora lini* race 371 on rust disease severity and agronomic characters of cultivar Redwood 65 flax (experiment 2)[a]

	Inoculation time effects listed under times (days) from planting to inoculation(s)								
Criteria	35	42	49	56	63 (prebloom)	35&63	42&63	49&63	56&63
Uredial index[b]	22.9 a[c]	24.5 a	29.0 ab	43.7 cd	53.2 ef	35.2 bc	41.7 cd	45.0 de	58.8 f
Telial index[d]	3.3 a	4.8 a	7.8 a	21.3 ab	84.4 d	32.7 bc	20.4 ab	35.9 bc	44.3 c
Yield (g)	3.4 a	3.4 a	3.0 ab	2.9 ab	1.7 c	2.2 bc	3.4 a	1.8 c	1.4 c
Number of capsules	85.5 a	90.2 a	81.3 ab	77.5 ab	49.5 cd	68.8 abc	88.0 a	55.7 bcd	41.3 d
Number of seeds	667.8 a	675.8 a	635.3 a	600.2 a	349.7 b	479.8 ab	667.0 a	396.5 b	315.7 b
1,000-seed wt. (g)	5.1 ab	5.0 ab	4.7 abcd	4.8 abc	4.7 bcd	4.3 cd	5.1 a	4.4 cd	4.3 d
Protein (%)	47.4 a	45.9 bc	46.8 ab	44.7 cd	44.6 cd	44.4 d	45.7 bcd	44.9 cd	45.6 bcd

[a]Means of six replicates (four plants per replicate).
[b]Angular-transformed data of $(L/H) \times P$, where L is total stem length along which rusted leaves occurred, H is plant height, and P is percentage rusted leaves along L.
[c]Figures within rows followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.
[d]Length (cm) of discrete telial lesions.

days before prebloom and again at prebloom were less severely rusted than those inoculated only at the prebloom stage (Tables 1, 2, and 3).

Data in Table 1 indicate that multiple inoculation (at 48 and 59 days) reduced development of uredia and telia to a level intermediate between those of the two individual inoculations. The effect of the pre-inoculation was more pronounced on seed yield and number of seed capsules. For example, those for the 48- and 59-day inoculations were not significantly lower than for the 48-day treatment, and were significantly higher than for the 59-day treatment alone. The rust severity rating and seed yield of the 48 and 75-day treatment were not significantly different from the 75-day treatment alone. However, plants in the multiple inoculation group produced significantly more seed capsules. Inoculation at 59 days was more detrimental than at 75 days, therefore an additive effect of the 59 and 75-day treatment could be expected. However, the effects of this multiple treatment on rust severity, yield, and capsule production were not significantly different from those of the 59-day treatment alone (Table 1). The effect of the 48-day pre-inoculation was noted in the 48, 59, and 75-day treatment. This multiple inoculation produced significantly more seed capsules and had a lower telial severity rating than did the 59 and 75-day treatment.

The effects of pre-inoculation on fatty acid composition were less pronounced than those on rust severity, yield, and seed capsule production. Nevertheless, levels of linoleic acid for plants that received the 48 and 75-day, and 48, 59, and 75-day treatments were significantly lower than that for the 75-day treatment alone (Table 1). Compared to other treatments, the 48 and 75-day treatment had an unusually high level of oleic acid and a correspondingly low level of linolenic acid. Iodine number was not affected. Oil and protein content were reduced significantly in the 59- and 75- day treatments, but this effect was offset with the addition of the 48-day inoculation.

The detrimental effects of a single inoculation at prebloom in experiment 2 also were reduced by a preceding inoculation. An initial inoculation at 42 days was the most effective; it significantly reduced uredial and telial severity, and increased seed yield, number of seed capsules and seeds, and 1,000-seed weight. Inoculations at 35, 49, or 56 days significantly reduced uredial and telial severity, but did not affect other parameters. These pre-inoculations did not offset the reduction in protein content observed with the prebloom treatment (Table 2). Levels of oil, fatty acids, and iodine number of plants inoculated twice did not differ significantly from those inoculated once.

In experiment 3, plants of three cultivars inoculated at 41 days, and again at prebloom, showed a reduction in telial severity on peduncles, and increases in yield and number of seed capsules and seeds compared to plants inoculated at prebloom (Table 3).

Increases in stem height. Mean stem heights in experiment 2 at 35, 42, 49, 56, and 63 (prebloom) days after planting were 17.4, 20.8, 21.2, 28.0, and 41.2 cm, respectively. Plant height increased by 3.8 cm in the 35- to 49-day period, by 6.8 cm in the 50- to 56-day period, and by 13.2 cm during the week preceding prebloom. Increases in stem height indicated corresponding increases in number of young leaves.

DISCUSSION

Infection by *M. lini* reduced yield and number of seed capsules and seeds. Light infections reduced yield by 38%, moderate infections by 34–55%, and severe to very severe infections by 64–79%. Thus, yields corresponded closely to the relative amounts of rust as noted earlier by Flor (6). Rust reduced oil and protein content when plants were inoculated at both prebloom and flowering stages. Single inoculations affected concentrations of fatty acids, but the changes were inconsistent. As expected, however, when oleic and linoleic acids increased, the concentration of linolenic acid decreased, and vice versa. Rust infections, even when severe, did not affect the iodine number.

Vulnerability to rust, as indicated by results of single inoculations, increased toward the end of the vegetative growth period and reached a maximum at the prebloom stage. Uredial and telial infections were more severe, more leaves were rusted, and number of seed capsules and seed production were lower on plants inoculated at the prebloom stage than on those inoculated earlier. Plant growth rates increased more rapidly at the 56- and 63-day stages, and increased numbers of young and susceptible leaves were produced. Logically, the prebloom stage, which follows a period of maximum growth, is highly vulnerable. Plants became less vulnerable during flowering, and yield reduction was smaller than that resulting from prebloom inoculation. This was not unexpected because seed-bearing capsules were present by this time. Complete resistance to rust during the flowering period, as found in field studies by Arif (1), was not observed in this study.

Pre-inoculation induced resistance and reduced the detrimental effects of a later inoculation. Plants of cultivar Redwood 65 inoculated 41–48 days after planting and again 11–30 days later at the prebloom stage were less severely rusted and produced more seed capsules and seeds than those inoculated only at the prebloom stage. In one experiment, pre-inoculation offset the reduction in seed weight experienced with inoculation at the prebloom stage. In another experiment, pre-inoculation offset the effects on linoleic acid, and on oil and protein content associated with rust inoculation at the flowering stage. The extent of protection against uredial development is indicated by the indices in Table 2. The initial inoculation in the 42- and 63-day treatment reduced the potential uredial index of the 63-day inoculation by 11.5 units. It appears that about 11% of all leaves present at the prebloom inoculation were protected by pre-inoculation. Considering the 100% increase in number of leaves during the period between the two inoculations, it appears that 22% of the new leaves were protected. The effect of pre-inoculation on telial infection was even stronger (Table 2).

Littlefield (9) indicated that pre-inoculation of flax with an avirulent race of *M. lini*, or with the alien rust fungi *Puccinia graminis tritici* and *P. recondita tritici*, induced a localized, nonspecific resistance that lasted for 7 days. This induced resistance was localized because lesion production was reduced only on the apical or basal leaf half that had been pre-inoculated,

TABLE 3. Effects of one or two inoculations of plants of three flax cultivars with *Melampsora lini* race 371 on rust disease severity and agronomic characters (experiment 3)

	Inoculation time effects listed under cultivar and times (days) from planting to inoculation(s)											
	Norland			Noralta			Redwood 65			Mean		
Criteria	41	57 (PB)[a]	41&57	41	63 (PB)[a]	41&63	41	71 (PB)[a]	41&71	41	PB[a]	41&PB
Telial index[b]	1.0	1.5	1.3	1.0	2.4	2.2	1.0	2.0	1.5	1.0 c[c]	2.0 a	1.5 b
Yield (g)	2.9	2.2	2.8	1.8	1.3	2.0	1.3	0.7	1.4	2.0 a	1.4 b	2.1 a
Number of seed capsules[d]	83.6	71.2	77.6	115.8	82.2	109.4	44.0	23.8	59.6	81.1 a	59.1 b	82.2 a
Number of seeds[d]	488.4	385.6	445.0	444.6	334.0	520.6	325.8	174.2	359.6	419.6 a	297.3 b	441.7 a

[a]Abbreviation PB = prebloom stage.
[b]Mean total length of telial lesions on peduncles of 20 plants. Rating scale: 1 = no telia; 2 = up to 3 cm of lesions; and 3 = 4–6 cm of lesions.
[c]Means within rows followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.
[d]Means of five replicates (four plants per replicate).

and not on the untreated leaf half. Instances are reported in which protection extended beyond the site of induction. Yarwood (13) found that established infections of bean rust reduced subsequent infections by the same rust on the same leaf up to 50 mm beyond the first infection. Elliston et al (5) reported that etiolated bean hypocotyls pre-inoculated with a nonpathogenic race of *Colletrotrichum lindemuthianum* or a nonpathogenic *Colletotrichum* sp. were protected against anthracnose caused by a pathogenic race of *C. lindemuthianum* at distances of up to 5 mm. Protection in bean against the rust and anthracnose pathogens extended only a short distance on the same leaf or hypocotyl.

The systemic protection induced by virulent pathogens in other systems is expressed in uninoculated, younger leaves, and over a much more extensive area. Muskmelon and watermelon pre-inoculated on the first leaf with virulent *Colletotrichum lagenarium* appeared to be systemically protected when re-inoculated 4 wk later with the same pathogen (2). At that time the plants possessed 10–14 true leaves, and protection was noted as a reduction in the number and size of lesions. Similar results were noted on cucumber (3). In the case of tobacco necrosis virus which incites local lesions on tobacco, lesion production on the upper leaves of conditioned plants was reduced when re-inoculation took place 7 or 14 days later (11). The induced resistance of flax to rust in this study was conditioned by a virulent race and was systemic because it was expressed in plant parts that had developed after the initial inoculation. As many as 22% of new leaves did not become rusted as a result of the pre-inoculation. The resistance was induced in several cultivars and persisted for 30 days in Redwood 65. This is the first instance of systemic protection in a host-rust system.

Induced systemic resistance that is extensive has been found in some highly diverse host-pathogen systems, and may be more widespread than is indicated by this and previous studies (2,3,11). Kuć et al (8) believe that under certain conditions the protection phenomenon may actually protect crops from severe damage. Further studies on the transmissibility of protection to rust in flax are underway.

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