RESISTANCE OF AGROTRICUMS TO WHEAT STREAK MOSAIC

by

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ACKNOWLEDGMENTS

Antiserum to barley stripe mosaic and wheat streak mosaic virus was kindly provided by M. K. Brakke.

Wheat streak mosaic (WSM) caused by wheat streak mosaic virus (WSMV) continues to be a serious threat to wheat production in the Great Plains. Yield reduction by WSM in Kansas alone was estimated at 20, 40, and 30 million bushels in 1949, 1959, and 1974, respectively (14). The average annual loss in Kansas for the past 5 years has been 13 million bushels (14). There is only a low level of resistance to this disease in present agronomically suitable wheat (Triticum aestivum L. em. Thell).

Several WSM-resistant wheat germplasms containing chromosome substitutions or translocations from Agropyron spp. (Agrotricums) have recently been released. C.I. 15321 and C.I. 15322, disomic substitution and translocation lines from Agropyron elongatum (Host) P.B., respectively, (18) were released by the Agricultural Research Service of the U.S. Department of Agriculture and the Oklahoma Agricultural Experiment Station. These Agrotricums also possess resistance to the wheat curl mite, Aceria tulipae Keifer, the vector of WSMV (13). The South Dakota Agricultural Experiemnt Station released C.I. 15092, a WSM-resistant disomic substitution line, derived from a cross of 'Carsten V' wheat X Agropyron intermedium (L.) S.C. (9). Mites develop readily on C.I. 15092 but resistance to WSM remains (13).

These Agrotricums, especially C.I. 15322, are being widely used by both public and private breeders. This may lead to a widespread introduction of a uniform genotype. Therefore, it is important to determine the nature of this resistance, and to define conditions under which the resistance will be maintained.

MATERIALS AND METHODS

Virus culture and detection. - The culture of WSMV used in these investigations was isolated by C. L. Niblett from wheat collected near Russell, KS in 1970. The virus was maintained on Parker wheat with frequent transfers. An extract from infected plants was prepared by grinding leaves with a mortar and pestle at a 1:5 dilution (w:v) in 0.02 M potassium phosphate buffer, pH 7.0, (KPO_4). Seven days after planting, test plants were dusted with carborundum and the leaves rubbed with the above extract. Plants were maintained in growth chambers at 24 C, and 10,800 lux of fluorescent lighting with a 12-hour photoperiod, unless otherwise indicated.

Inoculum for field experiments was prepared by grinding 14 day infected Parker in a 3.8 l Waring blendor at a 1:20 (w:v) dilution in KPO_4. The extract was squeezed through four layers of cheesecloth and carborundum was added (1.5% by weight). Seedlings were sprayed at the two to three leaf stage from a distance of 2.5 cm and at 655 kPa. The extract was constantly agitated to keep the carborundum in suspension. Four replications were used in a split plot design, with four 3 m rows per plot.

To detect a systemic infection by WSMV, a leaf which developed subsequent to inoculation was ground in 1 ml KPO_4 and rubbed on Parker wheat. If the test plants were systemically infected, mosaic symptoms typical of WSM developed on Parker 6 to 10 days post inoculation (PI).

Experimental growth conditions. - To determine the effects of plant age and environmental factors on WSM resistance, plants were inoculated at different ages and subjected to various light intensities and temperatures after inoculation. To measure plant age effects on resistance, C.I. 15092,

C.I. 15321, C.I. 15322, (hereafter referred to as 092, 321, and 322, respectively) and Parker wheat were maintained in a greenhouse and inoculated 5, 7, 10, 15, 20, 25, 28, 30, and 35 days after seeding. All leaves were inoculated, and the plants were tested for systemic infection 14 days PI.

To measure light intensity effects, 092, 321, 322, and Parker were inoculated and maintained in growth chambers at 27 C with a 12-hour photoperiod. Light intensities of 8600, 21,500, and 37,700 lux were tested, and a set of plants was also maintained under January and February greenhouse conditions. All plants were tested for systemic infection 14 days PI.

To determine the effects of temperature on WSM resistance, 092, 321, 322, and Parker were inoculated and maintained in growth chambers at 18, 27, or 35 C and 10,800 lux with a 12-hour photoperiod. Plants were maintained at 18 C and 27 C for 14 days, but they remained at 35 C for only 3 days and then at 24 C for the remaining 11 days. All plants were tested for systemic infection 14 days PI.

A more intensive temperature study was performed using 092 and Parker. Inoculated plants were placed in a growth chamber at 35 C and 10,800 lux with a 12-hour photoperiod. Heat treatments began the day before and up to 5 days PI. Plants were heated for 24, 48, or 72 hours and then placed in a 24 C growth chamber. All plants were tested for systemic infection 7 days PI.

Metabolic inhibitors. - Inoculated Parker and 092 were treated daily with metabolic inhibitors. Application began 1 day PI and continued for 5 or 6 days. Inhibitors and concentrations used were: tannic acid (17 mg/ml), acridine orange (100 ug/ml), cycloheximide (10 ug/ml), and actinomycin D (100 ug/ml). All were dissolved in a solution of 0.1% aerosol O.T. (a wetting agent) and applied with a cotton swab. Two days

after the final application, all plants were tested for systemic infection.

Additional viruses. - The reaction of 092 to other viruses and the effects of these viruses on WSM resistance were determined. The primary leaf of 092 or Parker was inoculated with barley stripe mosaic virus (BSMV), brome mosaic virus (BMV), or foxtail mosaic virus (FMV). After 7 days, the second leaf was checked for systemic infection. BSMV and BMV were detected using agar double-diffusion serology. FMV was detected by grinding the second leaf and rubbing the extract on Chenopodium amaranticolor Coste and Reyn. Local lesions indicated systemic infection by FMV.

The primary leaf of 092 was inoculated with mixtures of WSMV and BMV, BSMV, or FMV. Systemic infection was determined using serologically specific electron microscopy (7). Grids with parlodion films were carbon coated and floated on the desired antiserum for 30 minutes. The wheat leaf was ground in KPO_4 7 days PI. Antiserum coated grids were floated on the extract for 4 hours, then negatively stained with 2% phosphotungstic acid. A Phillips Model 201 electron microscope operating at 60 kv was used to examine the grids.

Electron microscopy. - Thin sections were cut from primary leaves of healthy and inoculated 092 and Parker. At 5 days PI, all material was fixed and embedded as described by Langenberg and Schroeder (10). Samples were sectioned with a Reichert ultramicrotome, placed on grids, and stained in 5% uranyl acetate in 50% ethanol for 30 minutes followed by a 12-minute treatment with Reynolds lead citrate.

Histochemical detection of callose. - The fluorescent method of Currier and Strugger (6) as modified by Wu, Blakely, and Dimitman (20) was employed for callose detection. Sections were stained for 4 hours,

8 hours, or overnight, and observed with a Leitz fluorescent microscope.

Virus replication and translocation. - Parker and 092 were tested daily from 1 to 10 days PI to determine the extent of viral replication. Leaves were ground (1:8, w:v) in KPO_4 and nine successive two-fold dilutions were inoculated to Parker. The dilution endpoint was reached when less than 75% of the test plants became infected. To determine the direction and extent of WSMV translocation, the upper half or lower half of 092 and Parker primary leaves was inoculated. After 5 days, the leaf was divided and each half was checked for infectivity.

Purification of WSMV. - At 5 days PI, primary leaves of 092 and Parker were harvested. Leaves were blended for 90 seconds in cold 0.06 M dibasic sodium phosphate and 0.01 M sodium diethyldithiocarbamate, pH 9.2, (1) to give a 1:3 (w:v) dilution. The extract was expressed through two layers of cheesecloth. Chloroform was added to 8%, and the mixture was stirred for 2 hours at 4 C, then centrifuged 10 minutes at 13,000g. The pellet was resuspended in 0.1 M tris-0.032 M citrate, pH 6.5, then pelleted through a layer of sucrose (200 mg/ml) for 75 minutes at 192,000g and 8 C. The pellet was resuspended overnight in 2 ml of tris-citrate. The suspension was centrifuged 10 minutes at 1300g and 4 C, and the supernatant was layered on equilibrium gradients (2) of 2 ml each of 400, 500, 600, and 700 mg sucrose/ml. These were centrifuged for 9.5 hours at 205,000g and 8 C. Gradients were scanned at 254 nm with the ISCO UA-2 UV analyzer and Model D density gradient fractionator attached to an external recorder. Fractions were collected and tested for infectivity.

RESULTS

Following inoculation with WSMV, chlorotic lesions developed on inoculated leaves of 092, 321, and 322. These lesions enlarged, coalesced, and eventually the inoculated leaves died. WSMV was readily transmitted from the lesions on all three genotypes and from necrotic leaves for a short time after death, but eventually infectivity was lost. Dark, necrotic lesions first appeared on 092 two days PI and enlarged rapidly along the length and then width of the leaf until the leaf died. Callose was not detected in association with lesions of 092. Lesions first appeared on 321 and 322 4 days PI. The chlorotic spots elongated, coalesced, and eventually the entire leaf became necrotic. Necrosis proceeded much more rapidly in 092 than in 321 and 322. When resistance was expressed, leaves developing subsequent to inoculation were symptomless, and contained no detectable virus.

We observed no symptoms on non-inoculated leaves of 092 under greenhouse conditions, nor could we transmit WSMV from non-inoculated leaves. Due to its spring habit, we did not test 092 in field plots, but it was resistant to WSM in Montana field tests (T. Carroll, personal communication). Under greenhouse and growth chamber conditions, 321 and 322 often developed typical WSM symptoms on non-inoculated leaves of test plants, and WSMV was readily transmitted both from lesions and leaves with systemic symptoms. In field tests with the same seed lot, 321 and 322 were completely resistant. This difference in response between greenhouse/growth chamber and field conditions prompted further investigations using 092.

The resistance of 321 and 322 was not completely broken at any light intensity, plant age, or temperature (Tables 1, 2, 3). 092

remained resistant to systemic infection at all light intensities and plant ages (Tables 1,2). WSMV could not be detected in roots or uninoculated leaves of 092 by infectivity assay. The resistance of 092 was stable at 18 C and 27 C, but was broken at 35 C (Table 3). The percentage of systemically infected plants was determined by the length of heat treatment and the period which elapsed between inoculation and heat treatment (Fig 1).

Various heating patterns were utilized to identify the differences which exist between high temperatures in the field and in growth chambers. The effects of heating were cumulative, that is, a comparable percentage of breakdown was obtained using two half days of heating or 24 hours of continuous heat. Therefore, when heating occurred only during illumination, more successive days of high temperature were required to break resistance. There was a limited period of time after inoculation (about 12-14 days) during which heating broke resistance, and heating before inoculation caused resistance to break in only a small percentage of plants. Resistance was also broken when only the shoot or root portion was heated, and the remaining portion was cooled.

The loss of resistance in 092 due to heat resulted in the appearance of lesions on leaves which developed subsequent to inoculation. Such a response usually resulted in necrosis of all leaves and death of the plant. The few survivors of heating and systemic infections were raised to maturity. Seeds were planted and the progeny were tested for resistance. The progeny were resistant to WSM.

Treatment with metabolic inhibitors was not as effective in breaking resistance as heating. Resistance was broken by daily foliar application of tannic acid, acridine orange, cycloheximide, or actinomycin D

(Table 4). Vacuum infiltration of these inhibitors as well as D-threo-chloramphenicol, erythromycin, rifampicin, 6-methyl-purine, and 6-aza-uracil did not break resistance. Foliar application of D-threo-chlor-amphenicol (1, 3, or 6 mg/ml) and 6-azauracil(2 mg/ml) did not break resistance.

BMV, BSMV, and FMV all caused systemic infection in 092 and Parker. Upon inoculation with each of these viruses plus WSMV, WSMV could not be detected in Parker or inoculated and uninoculated leaves of 092. Extracts were prepared from the uninoculated leaves of 092 and rubbed on 092. Lesions did not develop on 092 plants which were inoculated with this extract. Local lesions were not induced on 092 by BMV, BSMV, FMV, or an extract from healthy Parker plants.

Virus particles and pinwheel inclusion bodies were observed in both inoculated and uninoculated leaves of infected Parker wheat, but were seen only in the inoculated leaves of 092. Virus particles were most readily observed in 092 at the base of lesions fixed 5 days PI. Cells appeared normal at the base of the lesion, but there was increasing cellular disorganization toward the center of the lesion. Generally, the first major indication of lesion formation was disruption of the plasma-lemma, followed by organelle disorganization and rupture of organelle membranes, and finally cell wall collapse. Virus particles were observed in "normal" appearing infected cells, cells with a disrupted plasmalemma, and cells with disrupted organelle membranes.

WSMV replicated in an inoculated leaf of 092 and was translocated basipetally and acropetally within the leaf. Virus titer increased rapidly in 092 until 5 days PI, then declined as necrosis ensued (Fig 2).

WSMV was purified from 092 and Parker at 5 days PI. No differences

in virus concentration could be detected using the described method. This may be due to a lack of difference in virus concentration or to the influence of contaminating host material. The average A_{260}/A_{280} for the purified virus was 1.2 in contrast to an A_{260}/A_{280} of 1.37 obtained by Brakke and Van Pelt (3). An A_{260}/A_{280} of 1.2 was also obtained when the virus was purified as described, but sedimented in a CsCl gradient instead of a sucrose gradient. Virus yield was 1.4 A_{254} units/100 g of tissue in comparison to the yield of 1.2 A_{254} units/100 g of tissue obtained by Brakke and Ball (2).

Half of the plants inoculated at a dilution of 1.2 ug/ml of purified virus (based on A_{260}) developed symptoms. Infectious RNA was extracted from purified virus using the method described by Brakke and Van Pelt (4).

Table 1. Effect of plant age on resistance to wheat streak mosaic under greenhouse conditions

Age (days) at inoculation	Wheat			
	Parker	C.I. 15092	C.I. 15321	C.I. 15322
5	100[a,b]	0	10	61
7	100	0	9	41
10	100	0	24	29
15	100	0	5	58
20	100	0	28	7
25	100	0	10	0
28	100	0	41	9
30	100	0	31	9
35	100	0	39	0

[a]Each test represents 20-40 plants.
[b]Percentage of plants infected.

Table 2. Effect of light intensity on resistance to wheat streak mosaic

	Light intensity (lux)			
Wheat	8600	21,500	37,700	Greenhouse
Parker	100[a,b]	100	100	100
C.I. 15092	0	0	0	0
C.I. 15321	22	29	62	12
C.I. 15322	40	24	62	12

[a]Each test represents 40-60 plants.
[b]Percentage of plants infected.

Table 3. Effect of temperature on resistance to wheat streak mosaic

	Temperature (C)[a]		
Wheat	18	27	35
Parker	100[b,c]	100	100
C.I. 15092	0	0	88
C.I. 15321	5	35	25
C.I. 15322	30	58	43

[a]Plants kept at constant 18 C and 27 C for 14 days. Others kept at 35 C for three days, then at 24 C for 11 days.
[b]Each test represents 20-40 plants.
[c]Percentage of plants infected.

Table 4. Effect of metabolic inhibitors (application beginning 1 day after inoculation) on resistance of C.I. 15092[a] to wheat streak mosaic

Inhibitor	Concentration (mg/ml)	Number of applications	% systemically infected[b]
Tannic acid	17.00	6	86
Actinomycin D	0.10	6	22
Acridine orange	0.10	5	20
Cycloheximide	0.01	5	14
Aerosol O.T.	100.00	6	0

[a]Corresponding treatments with Parker were 100% systemically infected.
[b]Each test represents 10-20 plants.

Fig. 1. Effect of 35 C heating on wheat streak mosaic virus in C.I. 15092 resistance[a]

[a]Unheated C.I. 15092 was completely resistant to wheat streak mosaic. Corresponding Parker treatments were 100% infected.

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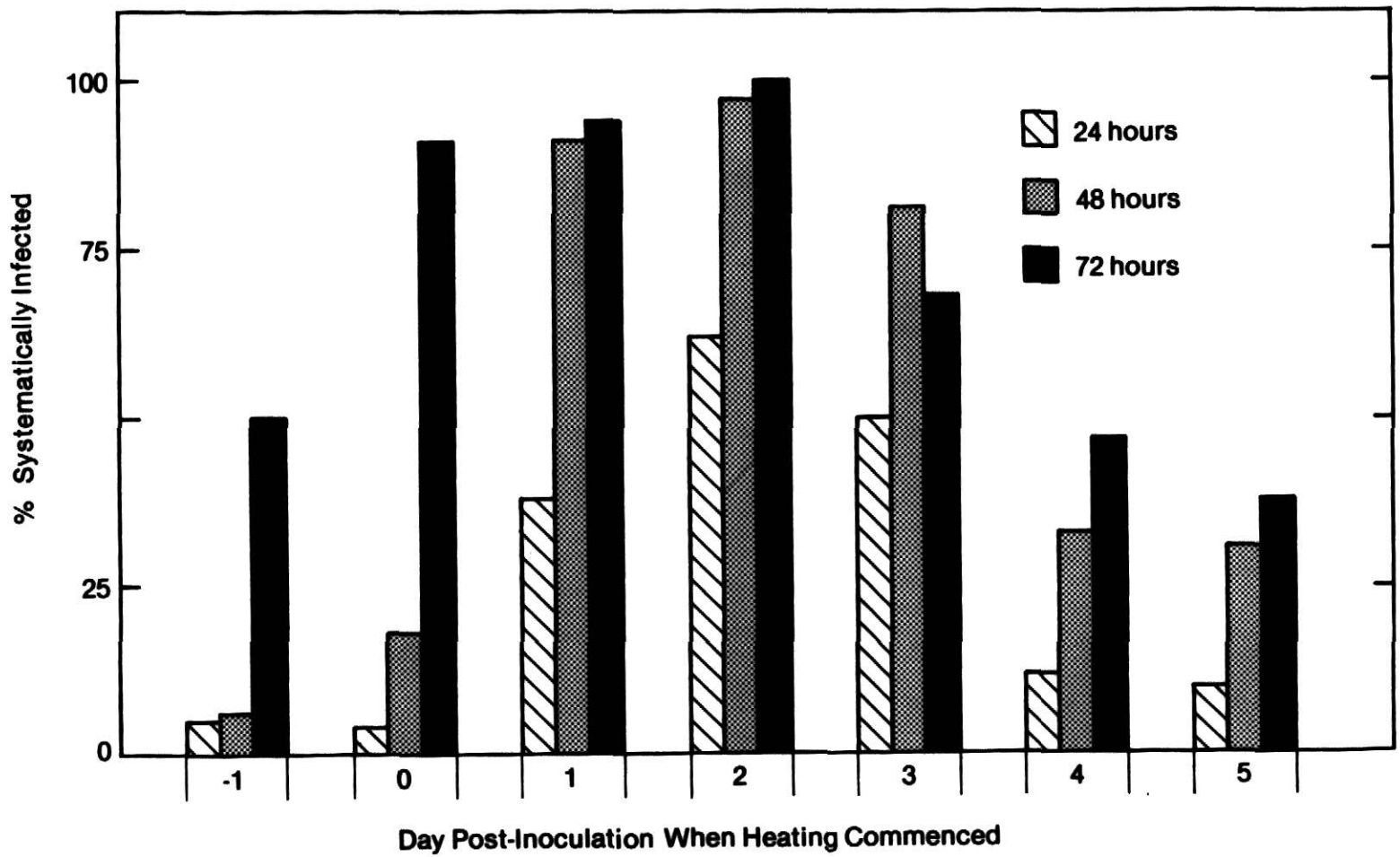
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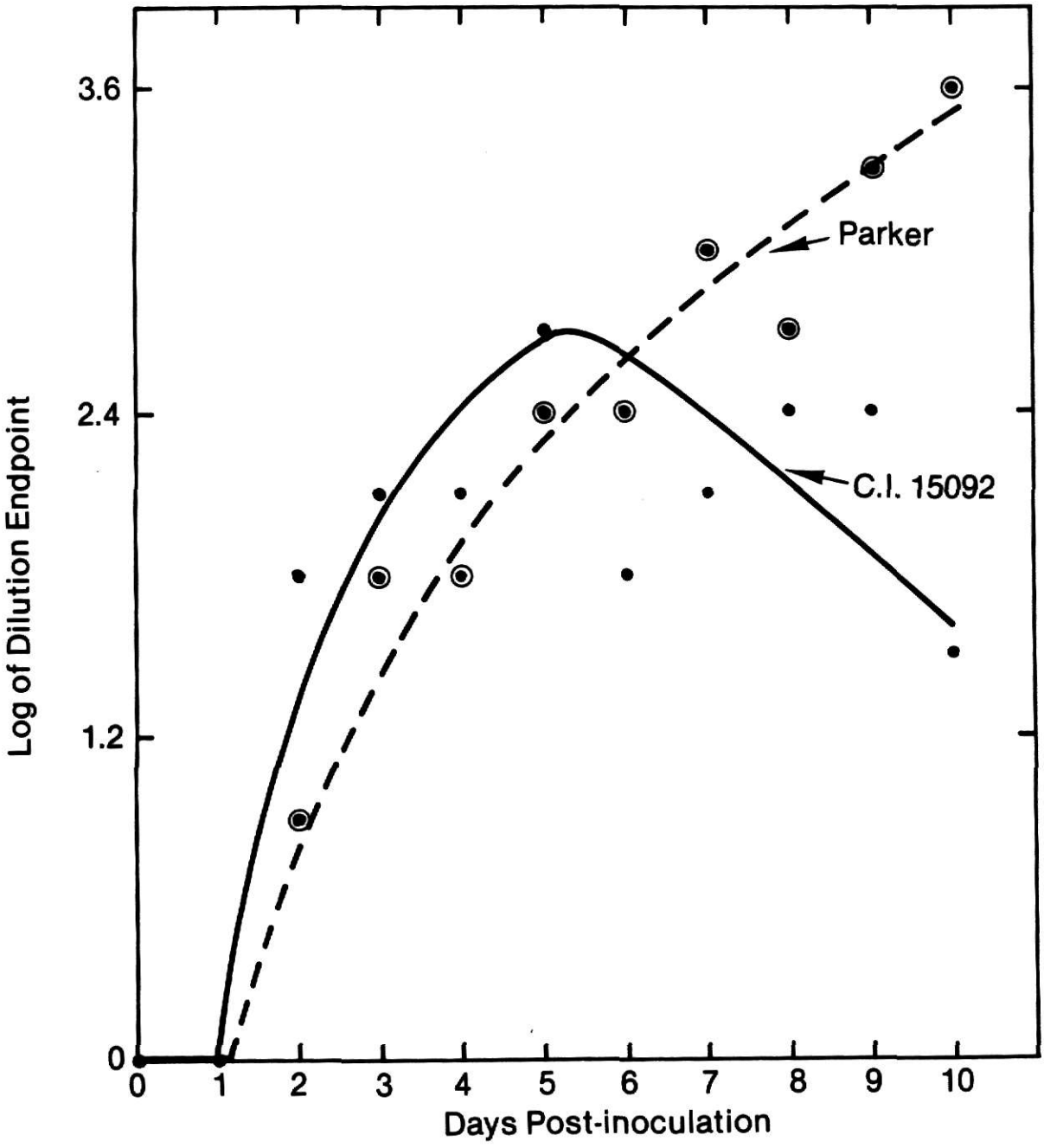
Fig. 2. Replication of wheat streak mosaic in Parker and C.I. 15092.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DISCUSSION

The resistance of 092 to wheat streak mosaic differs from that of 321 and 322 on both a genotypic and phenotypic basis. 092 resulted from a cross of Agropyron intermedium with wheat, its resistance is more temperature sensitive (Table 3), and it is not mite resistant (13). 321 and 322 derive their resistance from Agropyron elongatum and are mite resistant (13). The local lesions also differ markedly. Those of 092 develop rapidly (2 days PI), are necrotic and rapidly expand the length of the leaf. Lesions on 321 and 322 appear later (4 days PI), are distinct chlorotic spots, and only after 12 days expand the length of the leaf. The 092 resistance is stable under a wider variety of conditions, but once resistance has been broken, the plants suffer extreme damage and often death. Resistance in 321 and 322 is more difficult to define, but when resistance breaks, damage is less, with the plants developing a mild systemic infection, while the resistance of 092 is a self-limiting and lethal type of resistance.

Responses of 092 and 321 and 322 to inoculation with WSMV were compared. 092 was always resistant whether tested under field, greenhouse, or growth chamber conditions. 321 and 322 were always resistant under field conditions, but the same seed lot varied from 0 - 100% resistant under greenhouse and growth chamber conditions.

Some plants of 321 and 322 remained resistant at all temperatures, light intensities, and plant ages tested. There was not an obvious relationship between resistance and temperature, light intensity, or plant age. Controlled conditions were not found at which 321 and 322

were totally resistant or susceptible. These genotypes were selected from plants that were resistant under greenhouse conditions in late fall and inoculated at the two to three leaf stage (Sebesta, personal communication). However, we could not identify the environmental factor or factors upon which resistance depends. These factors should be identified, because utilization of a greenhouse screening test for resistance between harvest and planting would insure selection of resistant plants and hasten the development of resistant varieties.

Infectivity be WSMV and the detection of virus particles were associated only with the local lesions on 092. This type of resistance is termed localization (11) since there is viral replication and some translocation, but the virus is restricted to inoculated leaves. The response of 092, therefore, is not immunity as reported by Lay, Wells, and Gardner (9).

The development of local lesions and resistance by 092 was specific for WSMV. No lesions or resistance by 092 were observed after infection by BMV, BSMV, or FMV. This suggests that the resistance of 092 to WSM is an active process requiring a specific recognition between the virus and the host.

The resistance of 092 was broken by high temperatures or metabolic inhibitors. At 35 C, the percentage of plants becoming systemically infected increased as the length of heat treatment increased. Resistance was most susceptible to breakdown when heating was begun 2 days PI. Results suggest that the host process responsible for resistance is initiated about 2 days PI and continues to about 5 days PI (Fig. 1). Heating interferes with this process so that resistance

is not established. Wu et al. demonstrated that the resistance of bean to TMV is reduced upon brief 50 C heating. They suggested this reaction may be due to high temperatures interfering with a host resistance mechanism rather than heat inducing virus replication and translocation.

Temperatures frequently reach 35 C in wheat fields and experimental plots, yet resistance of 092 is maintained. Since resistance was broken when only the shoot or root portion was heated, and the remaining portion was cooled, cooler soil temperatures in the field are not responsible for field resistance. Rather it may result from the short period to which the plants are exposed to maximum temperatures during hours of illumination and long cool nights which break the heating cycle.

Although 100% of the plants did not become susceptible in tests with metabolic inhibitors, the results indicate that resistance is an active phenomenon. Results with the specific metabolic inhibitors (actinomycin D and cycloheximide, respectively) suggest that transcription and translation are necessary for resistance to be expressed. Tannic acid was more effective than the other inhibitors in breaking resistance in 092, but its specific effect on cell metabolism is unknown. A much higher concentration of tannic acid was used in comparison to other inhibitors (Table 4). Even at that concentration, tannic acid was less toxic to the plant. Higher concentrations of the other inhibitors were tested, but they often killed the plants. Another tannin, chlorogenic acid (8), also broke resistance in 092. Cheo and Lindner (5) report that tannic acid complexes with proteins and RNA. In their experiments, the amount of cellular RNA decreased after treatment with tannic acid, but returned to normal after 24 hours. In 092, tannic acid may

complex with proteins and/or RNA synthesized in response to, and necessary for expression of resistance to WSM.

It is essential that the resistance mechanism of 092 and 321 and 322 to wheat streak mosaic be further characterized. They appear to operate by similar but different mechanism, and stem from different origins. Independent utilization of these sources of resistance would avoid presenting a uniform genotype to the pathogen.

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ABSTRACT

The Agrotricums, C.I. 15092, C.I. 15321, and C.I. 15322 possess a hypersensitive type of resistance to wheat streak mosaic. Following infection, local lesions develop on inoculated leaves and increase in size until the leaf is dead. All three genotypes are resistant to wheat streak mosaic in the field, but under greenhouse or growth chamber conditions, only C.I. 15092 remains completely resistant.

Following inoculation with wheat streak mosaic virus (WSMV), C.I. 15092 develops lesions on inoculated leaves. The virus replicates in the leaf, and is translocated basipetally and acropetally. Virus titer increases until 5 days post inoculation, then declines as necrosis ensues. WSMV was purified from inoculated leaves. WSMV was not detected in roots or uninoculated leaves of C.I. 15092 by infectivity assay. Virus particles and pinwheel inclusion bodies are present only in inoculated leaves.

Resistance is unaffected by light intensity or plant age at time of inoculation, but resistance is broken at 35 C. The percentage of plants becoming systemically infected is determined by the length of heat treatment, and the period which elapses between inoculation and heat treatment. Resistance is also broken by daily treatment of inoculated plants with tannic acid, acridine orange, cycloheximide, or actinomycin D. C.I. 15092 is a systemic host of foxtail mosaic virus, brome mosaic virus, and barley stripe mosaic virus. Necrotic local lesions did not follow infection by these viruses.