Detection of *Phytophthora capsici* in Pepper and Cucurbit Crops in Ohio with Two Commercial Immunoassay Kits

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ABSTRACT

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Phytophthora capsici was shown to be the principal species associated with Phytophthora blight of peppers and cucurbits in Ohio during 1992 and 1993. Both A^1 (31.5%) and A^2 (64.2%) compatibility types were found in peppers, and only A^2 isolates were obtained from cucurbits. *Phytophthora cactorum* was recovered from two cucurbit samples. The Alert Phytophthora "flow-through" immunoassay and the Agri-Screen multiwell ELISA kit E for *Phytophthora* were compared for their efficacy. The former detected *P. capsici* in pepper and cucurbit crops, and had the advantage of being rapid (10 min) and easy to perform. The latter was effective in detecting *P. capsici* in pepper tissue, but absorbance values for healthy cucurbit tissues were relatively high. Agreement between ELISA and isolation of *P. capsici* or *P. cactorum* on semiselective medium was excellent for both kits. *Phytophthora* antigen was detected in three of seven field soils using the ELISA kit E combined with a soil organic matter extraction concentration protocol.

Additional keywords: enzyme-linked immunosorbent assay

Phytophthora capsici Leonian is a highly destructive pathogen of pepper (*Capsicum annuum* L.) and cucurbit crops. Phytophthora blight occurs sporadically in Ohio, primarily when rainfall is excessive during the growing season. *P. capsici* is heterothallic (10) and is thought to survive through the winter as oospores in soil and plant debris (3). Both the A^1 and A^2 compatibility types of this pathogen have been found in the same field in New Jersey (6) and North Carolina (8), but the occurrence of both types in Ohio vegetable production fields has not been established. Management of this disease in both peppers and cucurbits is best accomplished through a combination of practices, including crop rotation, water management, and fungicide use (11). The ability to detect overwintering propagules of *P. capsici* would be advantageous in field site selection and/or planning disease management programs.

Commercial kits for *Phytophthora* detection (originally developed by Agri-Diagnostics Assoc., Cinnaminson, NJ 08019, now a product of Neogen Corporation, Lansing, MI 48912) based on the enzyme-linked immunosorbent assay (ELISA) have been available for several years and have been shown to be effective in diagnosing diseases caused by numerous *Phytophthora* species (2,4,5,7). They have also been used to detect *Phytophthora* spp. in irrigation water (1) and soil (13). The Agri-Screen Phytophthora kit (multiwell kit E) contains both polyclonal and *Phytophthora* genus-specific monoclonal antibodies in a conventional double antibody multiwell plate format. The Alert Phytophthora tests contain the same antibodies in a rapid "flow-through" format (flow-through kit). In this study, we evaluated both kits for efficacy in diagnosing Phytophthora blight in peppers and cucurbits, including pumpkin and summer (yellow) squash (*Cucurbita pepo* L.) and cantaloupe (*Cucumus melo* L.). The multiwell ELISA kit was also tested for efficacy in detecting *P. capsici* propagules in field soil. We also identified compatibility types of *P. capsici* in Ohio pepper and cucurbit fields.

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MATERIALS AND METHODS

Tissue assays. All symptomatic and healthy pepper and cucurbit tissues were obtained from commercial production fields or OARDC greenhouses in Ohio. They were dipped in 70% ethyl alcohol for 5 sec, rinsed with sterile distilled water, and blotted on sterile paper towels. Small pieces of tissue from the margins of lesions or from randomly chosen asymptomatic areas were excised aseptically and plated on PBNIC medium (9) semiselective for *Phytophthora* spp.

Flow-through kit. Tissue pieces from the same areas selected for plating on PBNIC were ground on abrasive pads (supplied with the kits) until the surface of the pads was completely covered. The abrasive pads were added to kit extraction buffer, and the tests were run according to supplied instructions. The intensity of color development in test wells of the rapid assay was measured by using a hand-held reflectance device (Agri-Meter II, Neogen Corp., Lansing, MI); measurements were based on a scale of 0 (white) to 100 (dark purple).

Multiwell kit. Pepper samples were obtained from plants used in a bioassay of natural field soils (see below); stem and root samples were taken from different bioassay experiments. Stem samples for pathogen isolation and for ELISA were prepared as described above. Roots were washed to remove soil particles, cut into 1- to 2-cm pieces, and mixed, and subsamples were extracted for ELISA or plated as described above. For the cucurbit samples, the same filtered extracts prepared for the Phytophthora rapid assay were used. Assays were run according to kit instructions in duplicate wells. Absorbance values (405 nm) were determined using a Microplate EL 309 automated reader (BIO-TEK Instruments, Inc., Winooski, VT 05404-0998). The positive-negative threshold value for each set of tests was determined as the mean of the healthy plant control samples plus three standard deviations (12).

Soil assays. Soil samples were collected from five fields (designated Mitchell, Whitten, Pugh, Oakie, and Belpre) in southern Ohio and from two different areas of one field (Celeryville) in northwestern Ohio. The former samples were collected in April 1992 from fields in which a *P. capsici*-susceptible crop had not been grown for at least 2 yr. The Celeryville samples were collected in October 1993 from a field in which *P. capsici* had been isolated in 1992 and 1993. One set of six samples (Celeryville A) was collected in the vicinity of pepper plants with symptoms of Phytophthora blight, while the other six samples (Celeryville B) were collected near apparently healthy plants in a different part of the field. Each sample consisted of two 1.5-L volumes of soil collected using a golf course cup cutter (LESCO, Sebring, FL 33870), which were thoroughly mixed. One aliquot of approximately 500 g of soil from each sample was air-dried and finely ground using a Burr mill. A portion of the remaining soil was used to fill one 15-cm-diameter pot per sample for a pepper seedling bioassay.

Bioassay. A pepper seedling bioassay (3) was used to determine the presence or absence of *P. capsici* in soil samples. Pots containing soil samples collected in autumn or winter were covered with plastic bags and allowed to stand 2 wk at room temperature (19–21 C), while those collected in the presence of a pepper crop were tested immediately. Three 4- to 6-wk-old pepper seedlings (cv. Marengo) were planted in each pot and maintained in a greenhouse with average day/night temperatures of 27 C/21 C. Two, four, and six weeks after planting, pots were flooded with tap water for 6–8 hr and allowed to drain. Symptoms of Phytophthora blight on roots and/or stems of assay plants were recorded 2 wk after the final flooding.

ELISA. Organic debris was concentrated, collected, and extracted from soil samples as described previously (13), with several modifications. For each sample, 50 g of dried soil was placed in a 125-ml plastic bottle, 75 ml of distilled water was added, and the bottle was shaken vigorously for 1 min. Water was then added until a positive meniscus formed at the top of the bottle, and a 3.6-cm^2 plastic square (capture slip), approximately 1 mm thick, was placed on the surface. The suspension was allowed to stand for 30 min, then organic debris was collected by quickly picking the capture slip straight up and off the water. Debris that remained attached to the capture slip were placed in a plastic dentist's amalgam-grinding capsule containing a ball bearing. The capture slip was rinsed with 50 μl of water from the soil suspension into the grinding capsule. The contents of the capsule were then disrupted in a dentist's amalgam shaker.

One hundred microliters of 0.1 M $CaCl_2$ was added to the capsule along with enough distilled water to fill the capsule (bottom section only). The capsule (both top and bottom, open end down) and contents were then added to a 16 × 100 mm test tube containing 1.5 ml 5× extraction buffer concentrate (from kit), vortexed, and centrifuged at 3,000 rpm for 5 min. The sample was filtered through a serum separator-type filter pushed down into the test tube. Final extracts (100 μl per well) were tested in duplicate in the Phytophthora multiwell kit E as described above. *Phytophthora* antigen units were calculated for each sample based on a standard curve developed from dilutions of *Phytophthora sojae* antigen supplied with the kit. According to the manufacturer, 1 Phytophthora unit is approximately equivalent to one *P. sojae* oospore. Three or four replicates were tested for each soil sample.

Determination of *P. capsici* compatibility types. *P. capsici* was obtained from peppers or cucurbits from eight fields in Ohio, either by direct isolation from naturally infected plants or from bioassay plants grown in field soil. Some of the isolates (Celeryville, Okie, Belpre) were from the same soils for which ELISA and bioassays for *Phytophthora* were carried out. Other isolates were obtained from plant and soil samples collected during a routine survey of Phytophthora diseases of vegetables in 1992. Isolations were carried out as described above. Compatibility type was determined by pairing isolates with A^1 tester isolates (PCW 3A, T 586, and W 4a) and A^2 tester isolates (SCITal, P891, and W5a) originating from North Carolina, California, and Florida, respectively.

RESULTS

Tissue assays. *Flow-through kits.* Detection of *P. capsici* in symptomatic pepper stems, roots, and fruit was nearly identical using ELISA compared to isolation on PBNIC (Table 1). Reflectance values for two negative samples were less than 8, while the mean reflectance value for 12 positive stem samples was 59.9, with a range of 21–80. Discolored roots and fruit lesions from which *P. capsici* was isolated also yielded high values in ELISA (Table 1). The positive–negative threshold for these samples was 13.1 reflectance units. *Phytophthora* spp. were also readily detected in fruit and vegetative parts of minipumpkin, squash, and cantaloupe (Table 2). *P. cactorum* was isolated with *P. capsici* from one minipumpkin fruit, but all other isolates were *P. capsici.* Reflectance readings for healthy tissue were <10, while values for samples from which *P. capsici* was isolated were generally >20. The positive–negative threshold calculated from values for healthy tissues was 13.3 reflectance units. One yellow squash sample from which *P. capsici* was isolated yielded a test reading of 11 units, below the positive–negative threshold. However, nonspecific background color development in the negative control well was very high, and greater color development in the sample well could easily be distinguished by eye. For both pepper and cucurbit samples, flow-through tests were completed in approximately 10 min each.

Multiwell kits. For pepper stem samples, results of the immunoassay and isolation on PBNIC medium were in agreement for 92 of 94 stem and root samples (Table 3). Positive–negative A_{405} threshold values for stem and root

Table 1. Comparative detection of *Phytophthora capsici* in peppers using Alert Phytophthora flow-through ELISA or isolation on selective medium

Tissue source	No. of samples	ELISA reflectance[a] Mean	ELISA reflectance[a] Range	Recovery of *P. capsici*[b]
Stem lesion	10	59.9	21–80	10/10
Roots, discolored	3	43.3	35–48	3/3
Fruit lesion	2	48.5	41–56	2/2
Stem lesion	1	6		0/1[c]
Stem, healthy	3	2	0–6	0/2
Roots, healthy	2	0		0/2
Fruit, healthy	1	7		0/1

[a] Reflectance based on scale of 0–100.
[b] Successful isolations/total plated on PBNIC medium.
[c] *Erwinia carotovora* subsp. *carotovora* isolated.

Table 2. Detection of *Phytophthora* spp. in cucurbits by Alert Phytophthora flow-through ELISA

Host	Tissue source	No. of samples	ELISA reflectance[a] Mean	ELISA reflectance[a] Range	*P. capsici* isolated[b] (+/−)
Minipumpkin	Brown fruit lesion	1	78		+
	Fruit lesion	1	53		+[c]
	Fruit lesion	1	72		+
	Fruit lesion	3	12.6	2–22	−
	Healthy fruit	2	1.5	0–3	−
Yellow squash	Fruit lesion	5	43.6	11[d]–79	+
	Collapsed vine	2	40.0	33–47	+
	Discolored root	1	21		+
	Discolored vine	1	6		−
	Healthy fruit	2	0		−
	Healthy vine	2	3.0	0–6	−
Cantaloupe	Fruit lesion	3	47.3	26–58	+
	Collapsed vine	1	64		+
	Healthy fruit	1	5		−
	Healthy vine	2	4.5	0–9	−

[a] Reflectance based on scale of 0–100.
[b] Isolation on PBNIC semi-selective medium.
[c] *P. cactorum* also isolated.
[d] High background in negative well.

samples were 0.12 and 0.24, respectively. For extracts of the cucurbit samples, A_{405} values for healthy control samples ranged from 0.13 to 0.75 (Fig. 1), considerably higher than observed for pepper and other tissues. Samples from which *P. capsici* was isolated resulted in high A_{405} values, ranging from 1.48 to >3.0. Multiwell kit E tests required 45–60 min to complete.

Soil assays. *ELISA.* Five of six subsamples collected in or near the root zone of *P. capsici*-infected peppers (Celeryville A) contained detectable amounts of *Phytophthora* antigen (the positive–negative threshold determined by the manufacturer is 10–12 units) (Table 4). None of the subsamples collected in or near the root zones of healthy plants (Celeryville B) were positive for *Phytophthora*. Of the five southern Ohio field soils collected prior to planting, *Phytophthora* antigen was detected in two. Three of six subsamples were positive in the Whitten soil, and one of six was positive in the Oakie soil.

Bioassay. Bioassay results were negative for all soil samples, except one symptomatic plant in one subsample (Belpre soil).

***P. capsici* compatibility types.** A total of 163 *P. capsici* and two *P. cactorum* cultures was isolated from fields throughout Ohio in 1992 and 1993 (Table 5). Of the *P. capsici* isolates, 31.5% were compatibility type A^1, while 64.2% were compatibility type A^2. Both A^1 and A^2 compatibility types were obtained from the three soils from which most of the isolates originated (Worthington, Bench, and Celeryville) (Table 5). Only A^2 isolates were obtained from squash, cantaloupe, and pumpkin from Celeryville, whereas both A^1 and A^2 types were obtained from pepper. From five locations (Okie, Wagner, Sparling, Belpre, and Bratton) with limited sampling, A^1 was obtained from two, A^2 was obtained from two, and both A^1 and A^2 were obtained from one. *P. cactorum* and three *Phytophthora* cultures that did not form oospores were isolated from minipumpkins (Two-Bit Farm).

Table 3. Comparative detection of *Phytophthora capsici* in pepper stem and root samples using the Agri-Screen Phytophthora multiwell ELISA kit E or isolation on semiselective medium

ELISA[a]	Semi-selective medium[a]	No. of samples[b]	ELISA absorbance (405 nm)	
			Mean	Range
+	+	37[c]	2.70	1.42–3.0+
+	−	2[d]	2.47	1.94–3.0+
−	+	0		
−	−	4[c]	0.10	0.10–0.11
−	−	51[d]	0.17	0.15–0.22

[a]ELISA = samples positive (+) or negative (−) in the multiwell kit E; medium = *P. capsici* isolated (+) or not (−) on PBNIC medium.
[b]Number of samples in each category.
[c]Stem samples were used for ELISA and isolation.
[d]Root samples were used for ELISA and isolation.

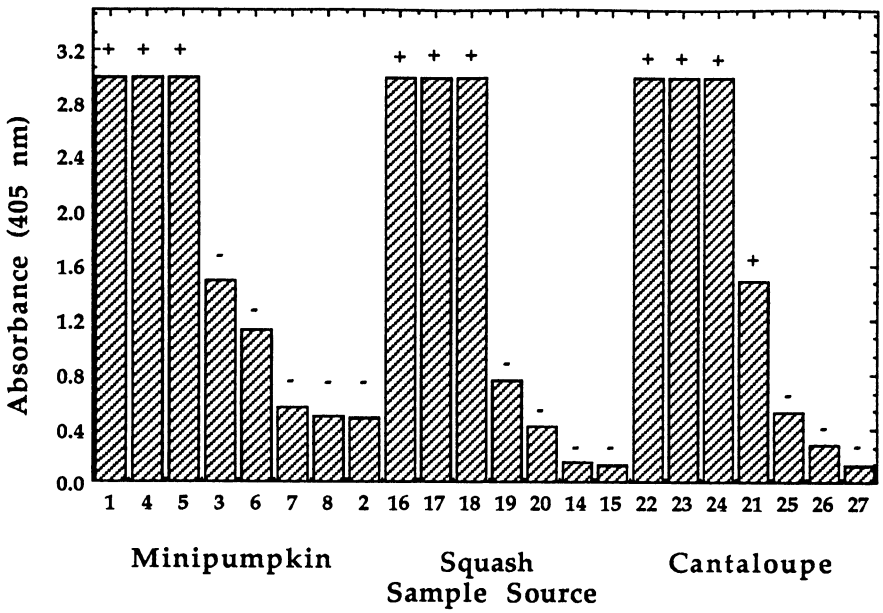

Fig. 1. Comparative detection of *Phytophthora capsici* in cucurbit crops by microtiter-plate ELISA and isolation on PBNIC semiselective medium (+ = *P. capsici* isolated; − = *P. capsici* not isolated).

DISCUSSION

P. capsici has been shown to be the more prevalent species associated with Phytophthora blight of peppers and cucurbits in Ohio. The role of *P. cactorum* in causing fruit rot of minipumpkins appears to be minor, since it was isolated from lesions on one minipumpkin fruit, together with *P. capsici.* Both A^1 and A^2 compatibility types were found in Ohio, although only A^2 isolates were obtained from cucurbits. Both compatibility types were found in four of the 10 fields studied; the isolation of only one compatibility type in the remaining fields may be due to limited sampling in these fields. It is surprising that only A^2 isolates were obtained from cucurbits, since the cucurbit field sampled most extensively was adjacent

Table 4. Detection of *Phytophthora capsici* in field soils by the Agri-Screen Phytophthora multiwell kit E

Field[a]	*Phytophthora* units[b] Sample no.[c]						Average[d]
	1	2	3	4	5	6	
Celeryville A	80.3 ± 12.0	53.5 ± 1.8	31.0 ± 4.3	122.3 ± 9.9	6.0 ± 2.9	35.8 ± 3.56	54.8 ± 8.2
Celeryville B	0.0 ± 0.0	2.8 ± 2.1	1.3 ± 1.1	7.3 ± 6.3	1.3 ± 1.1	1.8 ± 1.2	2.4 ± 1.3
Mitchell	5.7 ± 3.5	6.7 ± 4.3	4.3 ± 3.5	3.1 ± 2.4	3.3 ± 2.7	6.0 ± 3.8	4.8 ± 1.4
Whitten	4.3 ± 2.8	8.0 ± 3.4	5.0 ± 3.7	14.7 ± 6.0	30.7 ± 23.9	17.0 ± 7.8	13.3 ± 4.9
Pugh	3.0 ± 2.1	4.7 ± 3.8	5.7 ± 2.8	3.7 ± 1.9	6.5 ± 3.8	7.4 ± 4.3	5.9 ± 1.5
Oakie	0.0 ± 0.0	3.6 ± 2.1	27.1 ± 15.7	3.7 ± 2.1	6.6 ± 3.8	4.8 ± 2.8	9.3 ± 4.1
Belpre	0.7 ± 0.5	10.3 ± 6.2	4.3 ± 2.4	0.2 ± 0.2	3.7 ± 3.0		3.9 ± 1.7

[a]Celeryville A soil collected near peppers with Phytophthora blight symptoms; Celeryville B soil collected near asymptomatic plants; all other soil samples collected prior to planting.
[b]Units based on standard curve in ELISA of *P. sojae* antigen.
[c]Mean ± standard error of four (Celeryville) or three (all others) replicates/soil sample.
[d]Mean ± standard error of five (Belpre) or six (all others) soil samples from each location.

to a pepper field from which both types were obtained. Papavizas et al (6) isolated only the A^1 or A^2 compatibility type, but not both, from each of three squash fields in New Jersey. Similar results were obtained in North Carolina (8), where seven cucurbit fields were sampled, except that both compatibility types were found in one field, in a single plant. In the Ohio fields in which both the A^1 and A^2 compatibility types were found, oospores may be the overwintering structures for *P. capsici.*

The Alert Phytophthora flow-through assay was effective in detecting *P. capsici* in pepper and cucurbit crops, and had the advantage of being rapid (10 min) and easy to perform. In all but one case, color development in the negative control for each test device was very limited. This allowed clear differentiation of positive and negative results both visually and by using a simple reflectometer. For both pepper and cucurbit tissues, results of flow-through ELISA and isolation on semiselective medium were the same in these experiments. A high degree of correlation between the results of ELISA and culture plate methods for *Phytophthora* spp. has been observed for other crops as well (2,4,5). While Phytophthora blight symptoms on pepper stems and crowns are distinctive and usually diagnostic, similar lesions caused by other pathogens, e.g., *Erwinia carotovora* subsp. *carotovora,* can be confused with Phytophthora blight. Root symptoms of the disease, as well as vine, root, and fruit symptoms on cucurbit crops, are generally not distinctive or easily diagnosed. Thus, the Alert Phytophthora assays may be useful in providing primary or confirming field diagnoses of Phytophthora blight in peppers and cucurbits.

The multiwell ELISA, designed to be carried out in a laboratory, was very sensitive and effective in detecting *P. capsici* in pepper tissue. In two pepper samples that were negative by isolation but positive with ELISA, isolation results were questionable due to rapid growth of non-*Phytophthora* spp. on the isolation medium. It is possible that *P. capsici* was present in these samples, but was obscured by other fungi. The multiwell ELISA is also relatively rapid and can be completed in a minimum of about 45 min. For cucurbit crops, relatively high absorbance values for apparently healthy tissues from which *Phytophthora* was not isolated made the assay results difficult to interpret. However, precipitating debris from the tissue extract by a short centrifugation, or freezing and thawing the extract prior to testing in ELISA, may help to reduce nonspecific background reactions from healthy tissue.

Preliminary results have indicated that the *Phytophthora* microtiter-plate ELISA has promise for detection of *P. capsici* in soil. Positive results were obtained for five of six soil samples collected near pepper plants showing symptoms of Phytophthora blight, and all six subsamples collected near healthy plants were negative in the assay. In general, variation between replicate ELISAs for individual soil subsamples was low, as indicated by low standard error values. Soil samples collected in southern Ohio were mostly negative for *Phytophthora* in ELISA, with the exception of three of six subsamples in the Whitten field and one of six in the Oakie field. None of these positive ELISA samples was positive in the pepper seedling bioassay, while one positive bioassay sample (Belpre) was negative in ELISA. These discrepancies could be the result of uneven distribution of propagules of *P. capsici* in field soil, propagule dormancy, or other factors such as lack of specificity in the immunoassay or detection on nonviable propagules. These results, while preliminary, indicate that multiple sampling from a field will be necessary to accurately detect *P. capsici* infestation. Time of sampling may also be a factor, as populations of *P. capsici* are known to decline significantly in soil at low temperatures (3). Although ELISA is a quantitative assay for *Phytophthora* antigen, it is not known if ELISA units obtained in the assay accurately reflect the number of *P. capsici* propagules present in a soil sample. All sexual and asexual structures of *Phytophthora* can be detected with this ELISA kit (Agri-Diagnostics Assoc., *unpublished*), which could result in an overestimation of *P. capsici* populations. However, populations of *P. parasitica* and *P. citrophthora* in citrus rhizosphere soils in California determined by soil dilution plating were shown to be positively correlated with Phytophthora ELISA values (13). Finally, since the Phytophthora assay is genus- but not species-specific, it is possible that other *Phytophthora* spp. that do not cause diseases in peppers and cucurbits may be detected in these soils. However, with the exception of one isolate of *P. cactorum,* we did not detect other *Phytophthora* species in the fields sampled. Therefore, it is likely that in areas of intensive pepper and cucurbit production in Ohio, *P. capsici* may be the predominant species present in the soil, and ELISA could be used to determine the presence or absence of the pathogen.

Table 5. *Phytophthora capsici* compatibility types in Ohio vegetable fields

Field/location[a]	Source[b]	Compatibility type: No. of isolates	A^1	A^2	Others
Worthington/SE	Field pepper	15	12	3	
Worthington/SE	Bioassay	25	2	22	1 no oospores
Okie/SE	Bioassay	5	5	0	
Wagner/SE	Bioassay	2	1	1	
Sparling/SE	Bioassay	7	0	7	
Belpre/SE	Bioassay	3	0	3	
Bench/NW	Field pepper	12	7	5	
Bench/NW	Bioassay	14	0	14	
Celeryville/NC	Field pepper	26	14	11	1 no oospores
Celeryville/NC	Bioassay	15	7	8	
Celeryville/NC	Field squash	8	0	8	
Celeryville/NC	Field melon	13	0	13	
Celeryville/NC	Field pumpkin	11	0	11	
Bratton/NW	Field pepper	4	4	0	
Two-Bit/NC	Field minipumpkin	4	0	0	2 *P. cactorum* 2 no oospores
Gale/NC	Field minipumpkin	1	0	0	1 no oospores

[a]Southeast (SE), northwest (NW), or northcentral (NC) region of Ohio.
[b]Isolates recovered from peppers or cucurbits either growing in the field or as bait plants growing in field soil brought into the greenhouse.

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