Application of the Enzyme-Linked Immunosorbent Assay for Detecting Viruses in Soybean Seed and Plants.

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ABSTRACT

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The enzyme-linked immunosorbent assay (ELISA) test procedure easily detected tobacco ringspot virus (TRSV) or soybean mosaic virus (SMV) in individual soybean seed, to 1/2,500 and 1/160 (w/v) dilutions, respectively, of seed extracts made in a phosphate-buffered saline containing polyvinyl pyrrolidone (MW 40,000). Sensitivity for detection of the viruses in leaf extracts relative to seed extracts was similar for TRSV and about four times greater for SMV. The results indicated that both viruses also would be detectable in extracts from seed batches containing low proportions of infected seed: less than 1% for TRSV and about 2-4% for SMV. Germination significantly improved the sensitivity of such tests with SMV. The ELISA tests may be widely applicable in testing seed for viruses, especially with large-seeded species such as legumes and cereals. Testing seedlings instead of seed could further broaden applicability to other systems.

Both of the seed-transmissible viruses, tobacco ringspot (TRSV) and soybean mosaic (SMV), occur in soybean [*Glycine max* (L.) Merrill] in the midwestern USA and elsewhere. Each can be transmitted through a high proportion of seed from infected plants (4, 6), but neither can be tested readily or routinely in seed certification programs because no suitable screening procedure has been developed. Currently, the most useful test for estimating TRSV and SMV in seed is the observation of symptoms in progenies from seed samples germinated and grown to the first or second trifoliate leaf stage in the greenhouse (="sandbench germination test"). This is much too time-consuming and expensive for routine use. An economical, convenient procedure for estimating occurrence of these viruses in soybean seed samples would be useful in seed certification and also in epidemiological studies.

The work reported here shows that the enzyme-linked immunosorbent assay (ELISA) serological test recently applied to plant viruses by Voller et al. (13, 14) and Clark and Adams (5) provides just such a procedure. A short abstract of some of the results was published previously (7).

MATERIALS AND METHODS

Procedures for the ELISA test.—The ELISA test procedures closely followed those developed by Voller et al (13) and Clark and Adams (5). All buffers contained

0.02% sodium azide. The γ-globulins were partially purified by precipitation with ammonium sulfate, equilibration in half-strength PBS, and washing through DEAE cellulose in this buffer (5). Most work was done with γ-globulins at 1 mg protein/ml from an antiserum to TRSV (precipitin titer 1/128) and from an antiserum to SMV (precipitin titer 1/256). These were conjugated with alkaline phosphatase (Type VII, Sigma Chemical Co., St. Louis, MO 63178) at ratios ranging between 2:1 and 8:1 (w/v) globulin:enzyme using 0.06% glutaraldehyde (2, 5), and stored at 4 C with bovine serum albumin after thorough dialysis. Wells in polystyrene hemagglutination plates (Cooke Microtiter Plates, Catalogue No. 1-223-29; Cooke Laboratory Products, Alexandria, VA 22314) were precoated by incubation 2-4 hr at 36 C with 250 μliters/well of nonlabelled γ-globulin diluted 1- to 200-fold in 0.05 M sodium carbonate, pH 9.6. Plates then were rinsed three times with phosphate-buffered saline (PBS) at pH 7.4, containing 0.05% Tween-20, by filling them with a wash bottle and allowing liquid to stand for 2-3 min between rinses.

Crude extracts (see below) or antigen preparations, in PBS containing 0.05% Tween-20 and 2% polyvinyl pyrrolidone (PVP, MW 40,000:Sigma), then were incubated overnight at 4 C in the rinsed wells to react with the bound γ-globulin. After further rinsing, conjugated γ-globulin diluted 1- to 200-fold in a PBS mixture was added to react with bound antigen during 3-5 hr of incubation at 36 C. Besides the Tween-20, PVP and 0.2% ovalbumin recommended by Clark and co-workers (*personal communication*) this buffer mixture also contained extract from healthy control tissue at a ratio of

10-20 times the volume of conjugated γ-globulin used. In my tests this component, which was added first to the mixture, was important in preventing spurious reactions. Its effect may have been twofold: in cross-absorbing antibody to normal host constituents, and in preventing nonspecific binding of free or conjugated enzyme to sites still available in the wells.

Finally, nonreacted conjugate was rinsed away, and specific antibody-antigen reactions were detected by adding p-nitrophenyl phosphate substrate at 0.6-0.75 mg/ml in 10% diethanolamine buffer, pH 9.8. Reactions were arrested after 20-40 min by adding 50 μliters/well of 3 M NaOH. Assay was by visual inspection of the resulting yellow nitrophenol hydrolysis products, or by reading absorbances (A_{405nm}) of the well contents diluted fivefold with water and transferred individually to a cuvette in a Perkin-Elmer spectrophotometer. Automatic apparatus was not available. For simplicity, those reactions giving absorbances equal to or greater than twice the average for healthy control samples in the same experiment were regarded as positive.

The need for most of the specific process times and reagent proportions cited was not established and experience indicated that considerable variation was permissible, according to convenience. However, for uniformity, the protocol given was followed routinely. All data presented are means of duplicate or triplicate tests, and the results obtained were highly reproducible.

Conjugation of enzyme at ratios lower than the recommended 2:1 enzyme:γ-globulin (5) did not appear to improve enzyme economy significantly, as sensitivity was reduced. Also, essentially similar results were obtained using γ-globulins from other antisera to TRSV and SMV with higher titers (up to 1/2,056).

Seed and plant tissue sources and extraction.—Most of the soybean seed extracts examined were from seed of cultivars Midwest and Harosoy which were batches harvested from field-grown infected progeny plants grown from successive generations of seed infected with SMV and TRSV, respectively. Batches of seed of Harosoy and other cultivars from previously healthy plants which had been hand-inoculated with SMV or TRSV well before flowering also were investigated. All seed samples were kindly provided by K. L. Athow and T. S. Abney.

The infected leaf samples examined were from greenhouse-grown plants inoculated with SMV or TRSV derived, respectively, from the Midwest and Harosoy seed infections.

Soybean seeds were soaked overnight in water and seed coats were removed before extraction in buffer. Lots of 1-10 seeds, or small amounts of leaf tissue, were ground with a pestle and mortar with coarse Carborundum powder; larger lots were extracted by thorough blending in a Waring Blendor. For extracts of germinated seeds, entire seedlings were used. These were either germinated in the dark at 25 C in damp, rolled paper towels ("rag dolls"- as used in seed germination tests), or raised in vermiculite or sand in the greenhouse.

Tissues infected with TRSV were extracted with a 1:5 w/v ratio of PBS containing 0.05% Tween-20 and 2% PVP. Tissues infected with SMV were extracted with a 1:5 w/v ratio of 0.2 M phosphate buffer at pH 7.5, and then an equal volume of the PBS extraction mixture was added. This improved extraction efficiency for this virus about twofold over direct extraction in the PBS buffer. Routinely, extracts were not squeezed through cheese-cloth or clarified by centrifugation, because those procedures did not affect the test results.

RESULTS

Detection of purified tobacco ringspot virus by ELISA.—To investigate the conditions required for ELISA testing with the γ-globulins prepared, purified TRSV was titrated when diluted in buffer or plant extracts in tests using coating γ-globulin and enzyme-labeled γ-globulin at various concentrations. The results indicated that, over the range covered, the test was less sensitive to variation in coating γ-globulin concentration than to variation in enzyme-labeled γ-globulin concentration. Closely similar curves for values of absorbance of hydrolyzed substrate with antigen dilution were obtained with coating γ-globulin diluted 1/50 or 1/250, but similar variations in the concentration of enzyme-labeled γ-globulin caused significant differences (Fig. 1).

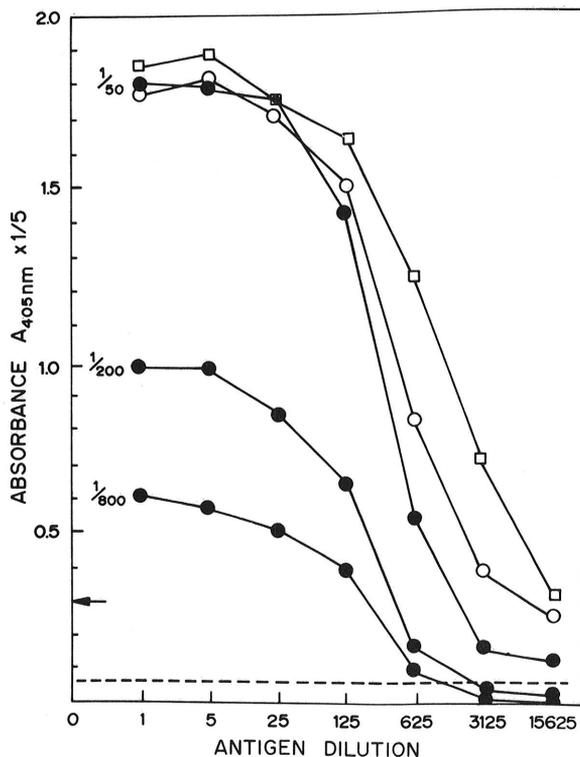With enzyme-labeled γ-globulin at a dilution of 1/50,

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) test absorbances for a preparation of tobacco ringspot virus with initial concentration $A_{260nm} = 0.6$, diluted in phosphate-buffered saline (●————●), or in phosphate-buffered saline extracts of soybean leaf (o————o) or seed (□————□) made at 1:5 w/v. Fractions indicate dilutions of enzyme-labeled γ-globulin that were used. Dotted line indicates absorbance level for non-diluted 1:1 healthy leaf-plus-seed extract. Arrow on absorbance axis indicates minimum level for clearly visualizable reactions.

TRSV diluted in PBS was detectable at concentrations as low as about 50 and 15 ng/ml, when assays were by visual inspection or spectrophotometry, respectively [assuming $A^{0.10\%}_{260\ nm} = 10$ (11)]. Sensitivity was not impaired, but seemingly enhanced, by added extracts from healthy soybean seed or leaf, suggesting that the test would be applicable with both kinds of extracts. It was apparent that valid comparisons of virus concentration could be made between standard virus preparations diluted in buffer and virus in extracts from infected tissue, over a range of concentrations of about 5-500 ng/ml.

Comparisons of ELISA, sandbench, and infectivity tests for estimating percent infection with tobacco ringspot virus in individual seeds.—In preliminary experiments in which extracts from individual soybean seed from infected Harosoy progeny were indexed by ELISA and by infectivity tests with cowpea [*Vigna unguiculata* (L.) Walp.] plants, the results suggested that ELISA testing gave excellent assessments of seed infection (Table 1). Indeed, several extracts that indexed negatively for infectivity gave positive ELISA test results, suggesting that ELISA was more sensitive than infectivity tests. The uniformly negative results in ELISA tests of control extracts suggests that false positives did not occur. Only in one test of the 180 done, did an extract indexing negative by ELISA yields lesions on cowpea. In this case the extract induced only one-to-three lesions per leaf, and TRSV carry over from the previous extract tested, which was highly infectious, seemed likely.

In further experiments (Table 2) estimates of the percentage of infections in batches of seed from TRSV-inoculated plants of several cultivars agreed closely whether assayed by ELISA or by the standard sandbench germination test, which can only estimate the percentage of infection in germinated seeds.

Amount of tobacco ringspot virus in seed extracts.—Figure 2 illustrates typical results for experiments in which the virus content of infected Harosoy soybean seed collected from TRSV-infected progeny plants was estimated in ELISA tests, by comparison with a purified preparation of TRSV. In this case, the absorbance values plotted are means for values for extracts from 10 individual seeds selected from those indexing positive in a parallel test of 50 seeds (Experiment 2, Table 1). Though the extracts selected were representative of the various levels of infectivity obtained (Table 1), variation in the results was small. The comparison indicated that the extracts contained about 50 μg virus/ml, which is similar to the concentration in leaves.

Extension of these assays to seed batches from hand-inoculated field plants of several cultivars showed that typical samples of infected seed from these had similar virus contents. Again, variation in virus content between seeds generally appeared to be low (Table 3). Further individual tests of lots of 50 randomly-selected seed from each of these seed batches and also from the batch from infected Harosoy progeny plants confirmed that, except with the cultivar Cutler-71, the reactions of all infected extracts were quite clearly differentiable from healthy extracts, although some variation in virus content was indicated (Fig. 3). With Cutler-71 seed from inoculated plants there was evidence that some infected seed contained considerably less virus than others; that is, some of the ELISA absorbances rated as positive were relatively low (Fig. 3, middle panel). This was also true of seed from infected Cutler-71 progeny plants. Overall, the results suggested that, except possibly with Cutler-71, germination would not significantly increase virus content or improve differentiation in ELISA test results between healthy and infected seed. The negligible effect of germination on virus content was confirmed in comparative titrations by ELISA of extracts from two 50-seed samples from an 80%-infected batch of Harosoy seed from infected progeny plants (Fig. 4).

TABLE 1. Comparative estimates of tobacco ringspot virus infection in Harosoy seed from infected progeny plants, as determined by enzyme-linked immunosorbent assay (ELISA), by infectivity, and by sandbench tests

Exp. no.	Number of seeds tested[a]		ELISA test[c] (no.)	Seeds indexed as infected by:[b]			
				Infectivity test		Infection	
				Obvious (10-100 lesions) (no.)	Low (1-3 lesions) (no.)	Progeny symptoms[d] (%)	ELISA test (%)
Experiment 1	Infected	30	22	14	3	78	73
	Control	30	0	0	0	0	0
Experiment 2	Infected	50	40	32	9	78	80
	Control	10	0	...	...	...	...
Experiment 3	Infected	50	23	19	0	39 (est.)	46
	Control	10	0	...	...	...	...
Experiment 4	Infected	50	18	15	1	39 (est.)	36
	Control	10	0	...	...	...	...

[a] Infected = seed from infected progeny plants. Control = seed from healthy plants.

[b] These numbers relate to tests of the same individual seed extracts. The symbol ... = not tested.

[c] A positive ELISA test result represents obvious yellow color development.

[d] These percentages based upon a sandbench test for a separate 100-seed sample (See Table 2). For each of experiments 3 and 4, the batches used were sampled from 100:100 seed mixes of the seed from infected progeny plants and seed from healthy plants. The symbol ... = not tested.

Comparative sensitivity of ELISA and gel diffusion tests for detecting tobacco ringspot virus.—The small isometric particles of TRSV readily diffuse into agar and can be detected serologically in double diffusion tests in agar gels (3). However, in comparative tests, the limiting concentration for detection of purified virus by gel diffusion was about 125 $\mu g/ml$, whereas for ELISA tests the limit could be 15 ng/ml (Fig. 1, upper curve). This result conforms well with the findings of Clark and Adams (5) that ELISA tests can be $\times 1,000$ more sensitive than gel diffusion tests.

Also, when extracts from infected seeds were used in gel diffusion tests, the dense background of white opaque material diffusing into the agar completely obscured precipitin lines, unless the plates were repeatedly washed by soaking in PBS during several days. When this was done, virus was detectable in PBS extracts (1:10, w/v) from an 80% infected batch of Harosoy seed, but only to a 1:1 dilution of the extract.

TABLE 2. Comparisons of percent infection of soybean cultivars with tobacco ringspot virus, as determined by enzyme-linked immunosorbent assay (ELISA) and sandbench tests

Cultivars and sample source	Infection		
	ELISA (visual rating) (%)	Progeny symptoms[a] (%)	Germination[a] (%)
Harosoy (from infected progency plants)	83	78	96
Harosoy (from inoculated plants)	68	72	89
Amsoy 71 (from inoculated plants)	86	77	77
Beeson (from inoculated plants)	82	85	84
Culter 71 (from inoculated plants)	80	72	86
Wells (from inoculated plants)	68	80	86
Williams (from inoculated plants)	68	59	91

[a]Progeny symptoms and germination percentages were based on readings for 100-seed lots, and were determined in the same sandbench tests by K. L. Athow. Comparative tests of control seed from healthy plants showed zero infection.

Correlation between ELISA and sandbench tests in estimating percent infection with soybean mosaic in infected seed.—Infectivity assays of seed extracts using the Topcrop (*Phaseolus vulgaris* L.) local lesion assay (8) were unreliable, suggesting low and variable virus content. Therefore estimates of virus infection using ELISA tests were compared only with estimates obtained by the standard sandbench germination test. The results (Table 4) indicated occasional differences between the
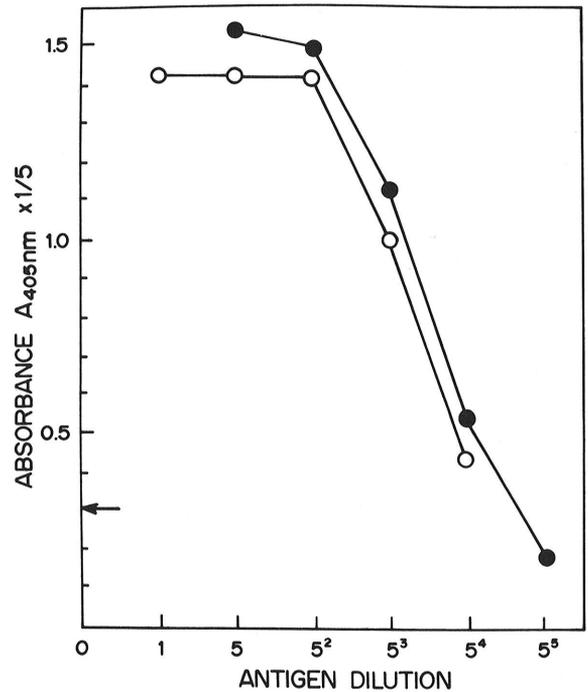

Fig. 2. Comparison of enzyme-linked immunosorbent assay (ELISA) test absorbances for dilutions of extracts of Harosoy soybean seed 100% infected with tobacco ringspot virus (o———o) and a virus preparation with initial $A_{260nm} = 0.6$ (●———●). Extracts from infected seed of Amsoy 71, Beeson, Cutler 71, Wells, and Williams gave similar results. Absorbance levels for undiluted healthy seed extracts were negligible (0.1). Arrow indicates minimum level for clearly visualizable reactions.

TABLE 3. Comparative amounts of tobacco ringspot virus in infected soybean seed extracts as determined by enzyme-linked immunosorbent assay (ELISA)

Dilution of extract[a]	Soybean cultivar[b]						TRSV preparation[c]	Healthy bean extract[a]
	Amsoy 71	Beeson	Cutler 71	Harosoy	Wells	Williams		
5	0.99 (0.14)	1.06 (0.05)	0.99 (0.04)	1.16 (0.14)	1.16 (0.11)	1.05 (0.06)	1.06	0.01
5^2	0.88 (0.11)	0.81 (0.06)	1.00 (0.08)	1.06 (0.06)	0.85 (0.38)	0.96 (0.10)	0.95	0
5^3	0.44 (0.16)	0.27 (0.04)	0.57 (0.13)	0.58 (0.13)	0.50 (0.30)	0.51 (0.10)	0.92	0
5^4	0.11 (0.09)	0.04 (0.02)	0.19 (0.07)	0.16 (0.02)	0.19 (0.18)	0.16 (0.05)	0.78	0
5^5	0.03 (0.02)	0.03 (0.01)	0.06 (0.04)	0.05 (0.01)	0.10 (0.11)	0.06 (0.02)	0.31	0

[a]These dilutions are with respect to original extracts, which were 1:10 (w/v) of bean to extraction buffer.

[b]Means of absorbance (A_{405nm}) readings for fivefold dilutions of reacted substrates, representing in each case duplicate ELISA tests of extracts from three infected soybean seeds (two for Wells). Numbers in parentheses are standard deviations.

[c]Means of absorbance (A_{450nm}) readings for fivefold dilutions of reacted substrates, representing duplicate ELISA tests of dilutions of a preparation of TRSV with an initial concentration of $A_{260nm} = 0.5$.

two kinds of estimate. This is not surprising, because indexing by progeny symptoms is necessarily more subjective than ELISA testing, and unlike ELISA, it can be based only on the seeds that germinate. As expected, the frequency of the seed coat mottling associated with infection of the parent plant bore no relationship to either estimate of infection (4).

Amount of soybean mosaic virus in infected seed.—Based on experiments with batches of seed of the cultivars Midwest and Bansei, each of which were 30% infected with SMV, the relative virus content of non-germinated seed infected with SMV was much lower than that of seed infected with TRSV, but it was significantly increased by germination.

For example, Fig. 5 illustrates a comparison of the

virus content of extracts of germinated and non-germinated Midwest seed, with that of extracts of infected seedling progeny from this seed. The results indicated that germination enhanced SMV concentration, and that the virus content of progeny plants grown for 3 wk in the greenhouse was only about 50% higher than that of seed germinated 1 wk in the dark.

In other comparisons, extracts from 30%-infected seed batches of Bansei and Midwest had similar SMV contents, and both were similarly enhanced by germination–to about 10 μg/ml, assuming $A^{0.10\%}_{260\,nm} = 2.4$, the value for another PVY-type virus, tobacco etch (10) (Fig. 6-A and 6-B). Infected stocks of seed of other soybean cultivars were not available, but extracts from
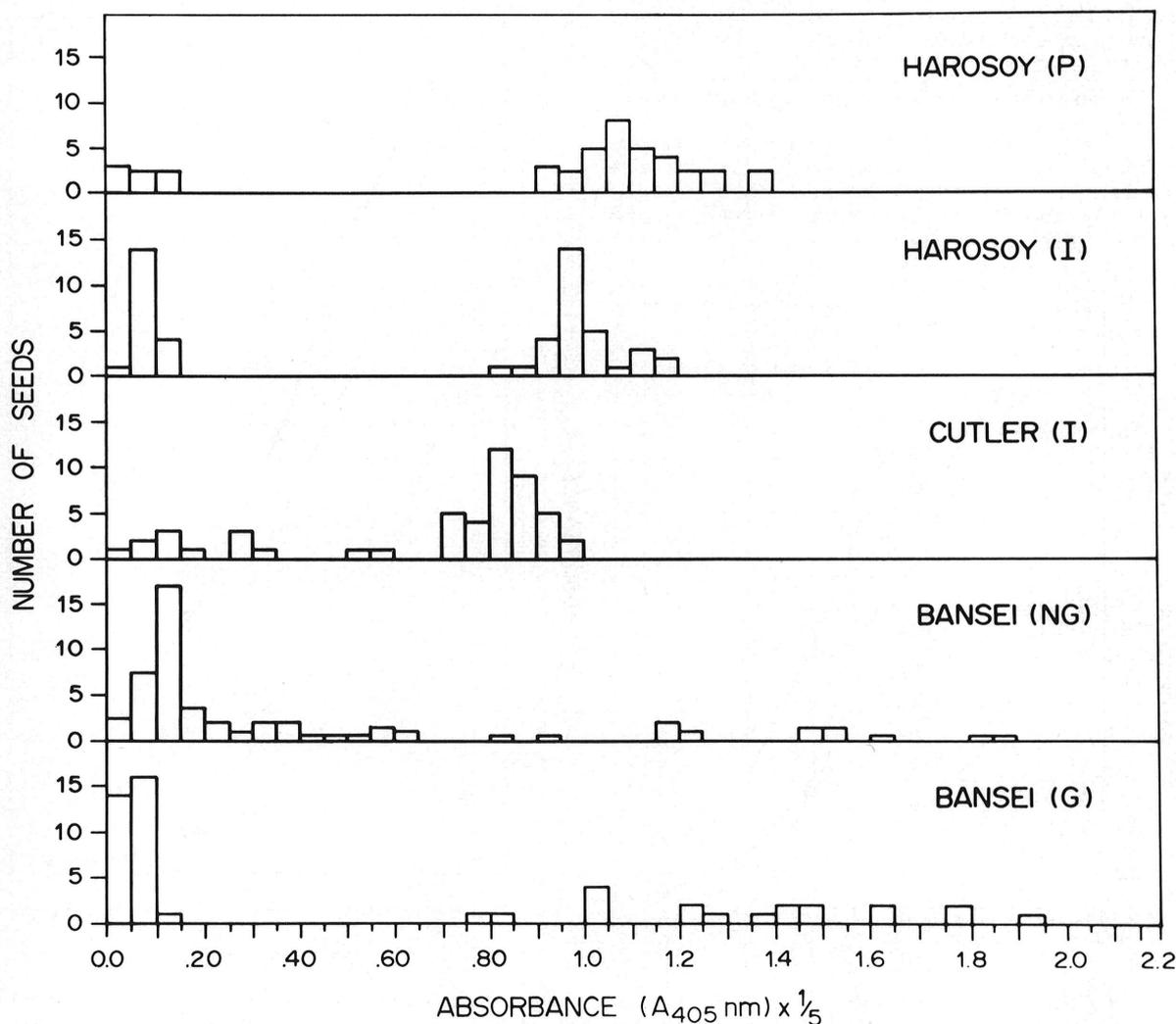

Fig. 3. Frequency distribution plots for enzyme-linked immunosorbent assay (ELISA) test absorbances of extracts of individual soybean seed or seedlings. For Harosoy and Cutler 71, seeds were from: (I), plants inoculated with tobacco ringspot virus, or (P) (Harosoy only), infected progeny plants grown from an infected seed batch. Results obtained with seed from tobacco ringspot virus-inoculated plants of Amsoy 71, Beeson, Wells, and Williams were similar to those of Harosoy. For Bansei, extracts were: (NG) from nongerminated seed from plants inoculated with soybean mosaic virus, or (G), from entire seedlings germinated in the dark for 1 wk. Similar results were obtained with extracts from Midwest seed from soybean mosaic virus-infected progeny plants. In each, the absorbance readings for healthy control extracts averaged 0.05 - 0.07.

systemically infected leaves of greenhouse-inoculated plants of the cultivars Kanrich, Bansei, Wayne, Kent, and Midwest reacted similarly in ELISA tests, suggesting that the concentrations of virus in infected seed of these cultivars might also be similar. In these tests, the virus

TABLE 4. Comparisons of estimates of soybean mosaic virus infection in individual soybean seeds by various methods

Sample	Infection			
	ELISA[a] (visual rating) (%)	Progeny symptoms[b] (%)	Germination[a] (%)	Mottle (%)
Amsoy 71 (1)	7.8 (192)[c]	9.2 (400)	80	69
Amsoy 71 (2)	1.8 (384)	1.1 (800)	82	75
Bansei	36.5 (192)	41.0 (400)	61	48
Midwest (1)	30.0 (100)	33.0 (400)	68	58
Midwest (2)	30.0 (100)	30.0 (200)	46	58
Williams	3.1 (192)	1.8 (400)	71	48

[a]The abbreviation ELISA = enzyme-linked immunosorbent assay.

[b]Percent germination and progeny symptoms were as measured in sandbench tests by T. S. Abney. Percent infections are based on progeny ratings.

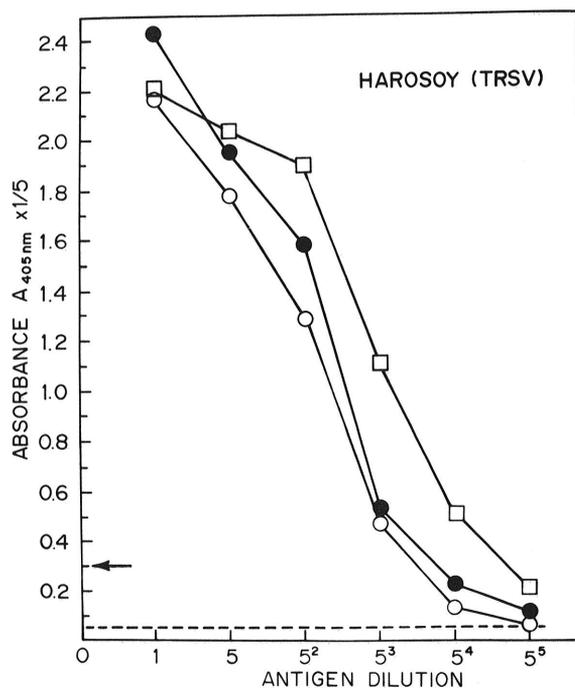[c]Numbers are percentages based on the total numbers of seeds examined, given in parentheses.

concentration indicated for extracts from infected leaves of soybean cultivar Midwest was about four times that for extracts of infected seeds.

Sensitivity of ELISA tests for detecting soybean mosaic virus in seed.—Assuming the relationship shown in Fig. 5 between the virus content of extracts from infected progeny plants grown in the greenhouse and dark-germinated seeds, it was reasonable to estimate the sensitivity of ELISA tests for germinated seeds on the basis of tests of leaf extracts from the greenhouse-grown progeny plants. Accordingly, mixtures containing various ratios of a bulk extract from visibly infected progeny plants with a similar bulk extract from healthy plants were titrated in ELISA tests. Extropolation from the results as plotted graphically (Fig. 7) suggested that

Fig. 4. Enzyme-linked immunosorbent assay (ELISA) test absorbances for dilutions of: a preparation of tobacco ringspot virus with initial A_{260nm} = 0.6 (□——□); extracts from batches of ungerminated seed (80% infected) from tobacco ringspot virus-infected Harosoy progeny plants (o——o); and seed germinated 1 wk in the dark (●——●). Average absorbances for extracts from healthy material were negligible (dotted line). Arrow indicates minimum level for clearly visualizable reactions.
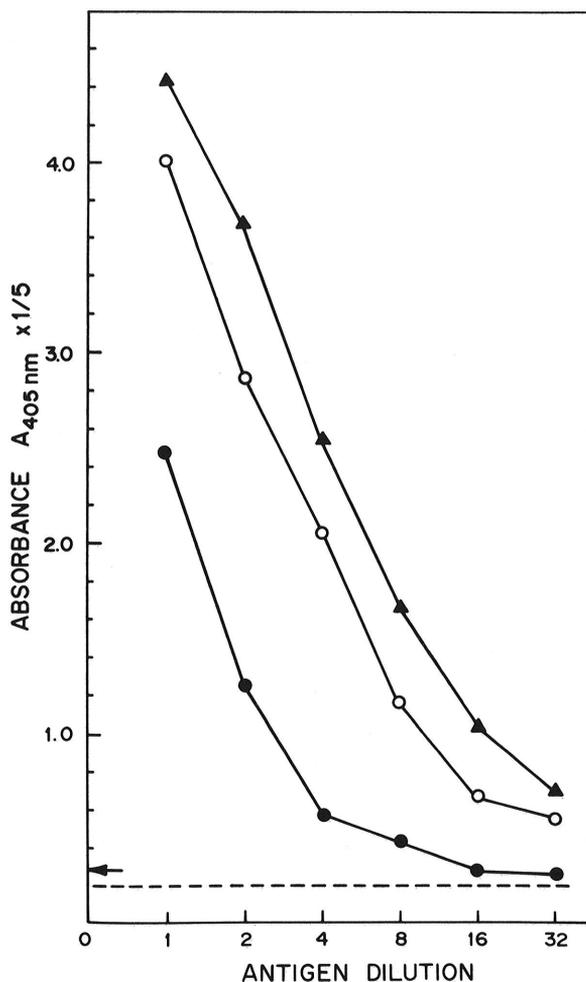

Fig. 5. Mean enzyme-linked immunosorbent assay (ELISA) test absorbances for dilutions of extracts from nonselected triplicate batches containing 50 seeds (●——●), 50 1-wk-old dark-germinated seedlings (o——o), or 50 3-wk-old greenhouse-grown (△——△) Midwest soybeans, from a seed lot 30% infected with soybean mosaic virus. Average absorbances for extracts from healthy materials were negligible (dotted line). Arrow indicates minimum level for clearly visualizable reactions.

undiluted extracts of dark-germinated seed batches containing about 2-4% infected seed would index positive. This is in agreement with the sensitivity obtained in tests of germinated Midwest and Bansei seed (Fig. 6).

In uniformity tests comparing the SMV content of individual seed samples, variation in the results for ungerminated seed of the varieties Midwest and Bansei was much more obvious than that in the experiments with TRSV-infected seed, but differentiation between infected and healthy extracts was improved by germination (Fig. 3). The results were consistent with the occurrence of some virus multiplication during germination, as indicated in Fig. 6.

DISCUSSION

Soybean provided a convenient model system for investigating the applicability of ELISA in testing seed for viruses, and the results confirmed a level of sensitivity far exceeding that of other simple and convenient serological assays. ELISA tests may be widely applicable in testing seed for viruses, especially large-seeded species such as legumes and cereals (9). Testing seedlings instead of seed could further broaden the applicability of ELISA to include other systems.

The results show that ELISA can detect TRSV and SMV infections in individual soybean seeds or seed batches at a level of sensitivity useful for practical tests or for investigating factors affecting seed transmission. Sensitivity was improved for SMV by germinating seed 1 wk in the dark, as is routinely done in tests of germinabil-

ity for seed certification. Probably the level of sensitivity also could be improved in other ways, for example by using specific globulins to improve enzyme-labeling efficiency. Test sensitivity can be predetermined for any particular batch of assays by using standards in preliminary tests of the coating and labeled γ-globulins.

Results from seed testing could be integrated with the results of epidemiological and yield studies, so that appropriate tolerances for infection could be applied in a seed certification program. For example, with TRSV in Indiana, effects on yield were noted only when planting with infected seed resulted in 30% or more of infected plants. The disease tends to be self-eliminating because of the drastic effects of the virus on seed production (1). This suggests that tolerance for TRSV in seed batches for use in Indiana could be set quite high. On the other hand, there is as yet no similar information on the significance of different levels of infection with respect to SMV. In this case, with the possibility of rapid spread under conditions suitable for high aphid populations, tolerances might have to be set lower. Soybean mosaic virus occurs world-wide in soybean growing areas, and the tolerances adopted would depend on the epidemiological conditions found in different soybean growing areas. Tobacco streak virus is another seed-borne virus of soybean (6) for which ELISA testing may be applicable. Clearly, ELISA tests will also have applications in the surveys and field studies required to provide background information on viral epidemiology.

There are further important applications of ELISA to soybean seed testing in relation to breeding programs. World-wide-collected germ plasm is liable to carry seed-
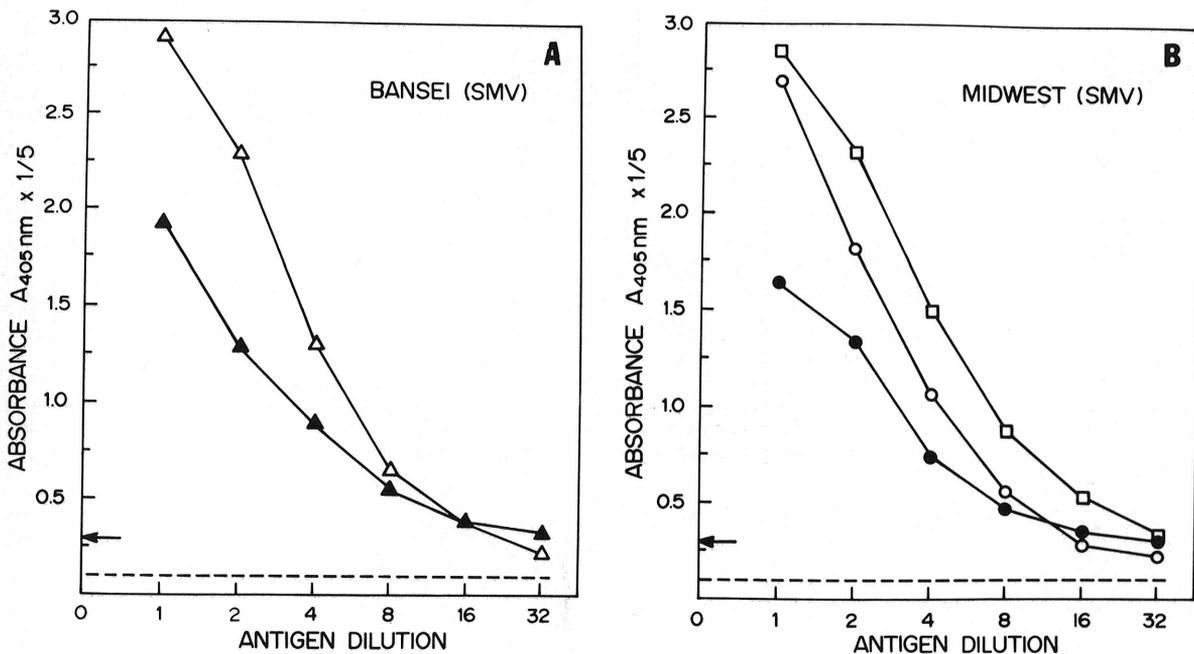

Fig. 6-(A, B). Mean enzyme-linked immunosorbent assay (ELISA) test absorbances for dilutions of extracts from duplicate 60-seed and seedling batches of A) Bansei and B) Midwest soybean, from lots 30% infected with soybean mosaic virus, compared with dilutions of a purified preparation of SMV (□———□) with starting concentration $A_{260nm} = 0.02$. ●———● and ▲———▲ indicate plots for seed extracts: o———o and △———△ indicate plots for seedlings germinated in the dark for 1 wk. Average absorbances for extracts from healthy material were negligible (dotted line). Arrow indicates minimum level for clearly visualizable reactions.

borne viruses, and inspection of the Midwest collections shows this already poses a problem, especially with SMV. ELISA tests should aid in seed selection and elimination of virus infections, thus avoiding loss of important gene sources through virus spread. Where necessary, it would even be possible to test part of individual seeds for viruses, retaining the embryo for germination. The ELISA tests also could aid in selecting virus-free plants in hybrid populations and in roguing for eliminating virus-infected plants in breeder's seed lots.

Perhaps most importantly, in breeding for resistance to virus infection or resistance to the passage of virus through seed to progeny plants, ELISA could aid in rapid, accurate comparisons of the virus content of selected plants and seeds. The precision of the assay might detect degrees of resistance that would pass unnoticed in sandbench or infectivity tests. The results obtained with Cutler-71 seed infected with TRSV, which suggest variation in virus content, may be an instance of this.

The application of ELISA to seed testing is a further example of the power and versatility of the technique in plant virology in its many possible applications (5, 12, 13).
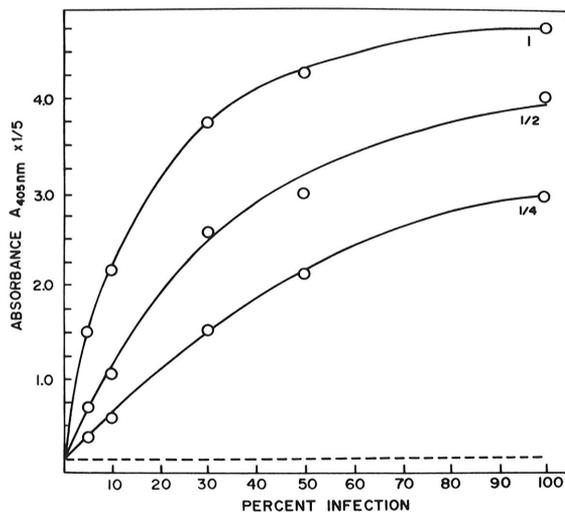

Fig. 7. Plots of enzyme-linked immunosorbent assay (ELISA) test absorbances against "percent infection", derived from plots of variation of absorbances with dilution (using healthy plant extract) for artificial mixtures containing 5, 10, 30, 50, and 100% extracts from 3-wk-old soybean mosaic virus-infected progeny plants mixed with extracts from healthy plants.

Other applications currently under study in this laboratory include detecting tomato ringspot virus in apple leaves and barley yellow dwarf virus in cereal leaves.

LITERATURE CITED

1. ATHOW, K. L., and F. A. LAVIOLETTE. 1961. The relation of seed-transmitted tobacco ringspot virus to soybean yield. Phytopathology 51:341-342.
2. AVRAMEAS, S. 1969. Coupling of enzymes to proteins with glutaraldehyde. Immunochemistry 6:43-52.
3. BALL, E. 1974. Serological tests for the identification of plant viruses. Am. Phytopathol. Soc., St. Paul, Minn. 31 p.
4. BOS, L. 1972. Soybean mosaic virus. No. 93 in Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. (Unpaged).
5. CLARK, M. F., and A. N. ADAMS. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
6. DUNLEAVY, J. M. 1973. Viral diseases. Pages 505-526 in B. E. Caldwell, ed. Soybeans: improvement, production and uses. Am. Soc. Agron., Madison, Wisconsin. 681 p.
7. LISTER, R. M. 1977. Detection of viruses in soybean seed by enzyme-linked immunosorbent assay. Proc. Am. Phytopathol. Soc. 4:132.
8. MILBRATH, G. M., and M. M. SOONG. 1976. A local lesion assay for soybean mosaic virus using Phaseolus vulgaris L. cv. Top Crop. Phytopathol. Z. 87:255-259.
9. SHEPHERD, R. J. 1972. Transmission of viruses through seed and pollen. Pages 267-292 in C. I. Kado and H. O. Agrawal, eds. Principles and techniques in plant virology. Van Nostrand-Reinhold, New York. 688 p.
10. SHEPHERD, R. J., and D. E. PURCIFULL. 1971. Tobacco etch virus. No. 55 in Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. (Unpaged).
11. STACE-SMITH, R. 1970. Tobacco ringspot virus. No. 17 in Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. (Unpaged).
12. THRESH, J. M., A. N. ADAMS, D. J. BARBARA, and M. F. CLARK. 1977. The detection of three viruses of hop (Humulus lupulus) by enzyme-linked immunosorbent assay. Ann. Appl. Biol. 87:57-65.
13. VOLLER, A., A. BARTLETT, D. E. BIDWELL, M. F. CLARK, and A. N. ADAMS. 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). J. Gen. Virol. 33:165-167.
14. VOLLER, A., D. BIDWELL, G. HUIDT, and E. ENGVALL. 1974. A microplate method of enzyme-linked immunosorbent assay and its application to malaria. Bull. W. H. O. 51:209-211.