Comparative Purification of Two Luteoviruses

Cleora J. D'Arcy, A. D. Hewings, P. A. Burnett, and H. Jedlinski

Assistant professor, graduate student, pathologist/breeder, and research plant pathologist and associate professor, respectively. First and second authors, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801; third author, CIMMYT, Londres 40-101, Apdo. 6-641, Mexico; fourth author, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

The authors wish to thank W. F. Rochow for performing ELISA tests and George Gregerson for technical assistance.

Mention of a trademark, proprietary product, or specific equipment does not constitute a guarantee or warranty of the product by the USDA, or imply approval to the exclusion of other products that also may be suitable.

Accepted for publication 18 November 1982.

ABSTRACT

D'Arcy, C. J., Hewings, A. D., Burnett, P. A., and Jedlinski, H. 1983. Comparative purification of two luteoviruses. Phytopathology 73:755-759.

An Illinois vector-nonspecific isolate of barley yellow dwarf virus (BYDV) was purified from oats (*Avena byzantina* 'Coast Black') and a California isolate of beet western yellows virus (BWYV) was purified from shepherd's purse (*Capsella bursa-pastoris*). Root tissue yielded four times as much BYDV as shoots when ground to a very fine powder in liquid nitrogen with a mortar and pestle and homogenized in a blender prior to clarification. BYDV yields ($E_{260\,nm}^{0.1\%} = 8$) from roots harvested in March through October averaged 0.7 mg/kg, while in November through February yields averaged 4.4 mg/kg. Average yields of 1.1 mg/kg ($E_{260\,nm}^{0.1\%} = 8$) of BWYV were obtained when shoots were frozen in liquid nitrogen, powdered in a blender, and stirred for 24 hr at room temperature in 0.1 M phosphate buffer (pH 6.0) with 0.5% sodium azide and 1.5% Rohament P, a macerating enzyme. The titer of BYDV peaked sharply in roots 10–14 days after inoculation, whereas BWYV yields were consistently high from shoots harvested from 10 to more than 20 days after inoculation. Purified BYDV and BWYV had $A_{260/280}$ ratios of 1.89 and 1.65, respectively.

Barley yellow dwarf virus (BYDV) and beet western yellows virus (BWYV) are members of the luteovirus group of plant viruses (16). Luteoviruses are difficult to purify, probably because they are restricted to phloem tissue. Of the luteoviruses, purification methodology has been studied for BYDV (3,5,18,21,22), BWYV (4,9), carrot red leaf virus (CRLV) (26), pea leafroll virus (PeLRV) (2), potato leafroll virus (PLRV) (10–12,14,15,17,23–25), soybean dwarf virus (SDV) (13), and tobacco necrotic dwarf virus (TNDV) (25). Yields of purified virus are typically less than 1.0 mg/kg of infected plant tissue, with the exception of enzyme-assisted purification of PLRV and TNDV (25), and extraction of an isolate of BYDV specific for *Sitobion avenae* from oats with a Wiley mill (18). Average yield of the vector-specific BYDV was 1.36 mg/kg. In contrast, a vector-nonspecific isolate of BYDV yielded only 0.52 mg/kg (18).

Vector-nonspecific strains of BYDV have been reported to be predominant in nature in the U.S. and Canada (6–8,19,20). The importance of improving methods for handling these typically severe isolates is thus increased. BWYV causes economically important diseases of several dicotyledonous species worldwide, but the virus itself has been little studied. We have examined several aspects of the purification of an Illinois vector-nonspecific isolate of BYDV, and a California legume isolate of BWYV, and here report improved purification methods.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Virus propagation. An Illinois isolate of BYDV previously shown to be transmitted by both *Sitobion* (=*Macrosiphum*) *avenae* Fabricius and *Rhopalosiphum padi* L. was used in all experiments. The isolate was tested in an enzyme-linked immunosorbent assay system and reacted like the New York vector-nonspecific (PAV) isolate (W. F. Rochow, *personal communication*). BYDV was maintained in barley (*Hordeum vulgare* L. 'Hudson') by transferring 20–40 *R. padi* from a viruliferous colony to new plants 3 wk after sowing (two-leaf stage). The plants with aphids were caged and grown at 24 C in an ISCO model E3 growth chamber under 15 hr fluorescent and incandescent illumination (9,000 lux). New viruliferous colonies were set up every 2 wk.

A California isolate of BWYV obtained by J. E. Duffus from field-infected broadbean and transmitted by *Myzus persicae* Sulz. was used. The isolate was maintained in radish (*Raphanus sativus* L.) by transferring 20–40 *M. persicae* from a viruliferous colony to 4-wk-old plants. The plants and aphids were caged and grown in a model M-31 growth chamber (Environmental Growth Chambers, Inc., Chagrin Falls, OH 44022) (EGC) at 20 C under 12 hr of fluorescent and incandescent illumination (8,000 lux). New colonies were established every 3 wk or as needed.

Tissue for virus purification. Plants for BYDV purification were grown in a mixture of steamed soil and vermiculite (2:1, v/v) in 50 × 35 × 9-cm flats in an insect-free greenhouse at temperatures between 23–30 C. Sixteen hours of supplemental fluorescent and incandescent light at 4,000 lux were provided in winter. Oats (*Avena byzantina* C. Koch 'Coast Black' and *Avena sativum* L. 'Clintland') were inoculated 3 wk after planting; Hudson barley was

inoculated 2 or 3 wk after planting. BYDV inoculation was by viruliferous *R. padi* (10–20 aphids per plant) allowed to feed for 2–3 days in the EGC chamber at 23 C under 14 hr fluorescent and incandescent illumination (8,000 lux). Aphids were killed by fumigation with Vapona, and plants were returned to the greenhouse as above until harvest. After shoots were cut, roots and stem bases were washed from the potting mixture. Most tissue was processed immediately; where indicated, tissue was frozen at −80 C.

Several cultivated and weed species were tested as propagation hosts for BWYV: *Beta vulgaris* L., *Capsella bursa-pastoris* L., *Crambe abyssinica* Hochst., *Lactuca sativa* L., *Pisum sativum* L., *Thlaspi arvense* L., and *Vicia faba* L. Plants were inoculated with BWYV and grown in a greenhouse at temperatures ranging 16–30 C with 14 hr of supplemental fluorescent and incandescent illumination (2,500 lux). A California biotype of shepherd's purse (*C. bursa-pastoris* L.) obtained from J. E. Duffus was selected as the BWYV propagation host. Seedlings were grown in a steam-pasteurized mixture of soil, peat, and sand (2:1:1, v/v) in 35 × 20 × 9-cm flats. Seedbeds were prepared by layering a single sheet of Kleenex tissue over firmly packed soil mixture and soaking with 500 ml of 0.2% KNO_3 per flat. Seeds were sown directly onto the soaked tissue. A sheet of Saran wrap was stretched over the uncovered seeds until the cotyledons were 0.3–0.5 cm long. Seedlings were maintained in the EGC chamber at 20 C with a 12-hr photoperiod (8,000 lux). Plants were inoculated with BWYV 3 wk after seeding by allowing viruliferous *M. persicae* (5–10 per plant) to feed for 3–4 days after which the plants were sprayed with Pirimor to kill the aphids and returned to the growth chamber as above. Leaf tissue was harvested 10–22 days after inoculation and used fresh or frozen at −80 C until processed.

Virus purification. Two purification schemes were developed and tested on BYDV-infected root and shoot tissue and BWYV-infected shoot tissue. All procedures, except where noted, were at 4 C. Sodium phosphate buffer, 0.1 M at pH 7.0, was used throughout, except during enzyme incubation.

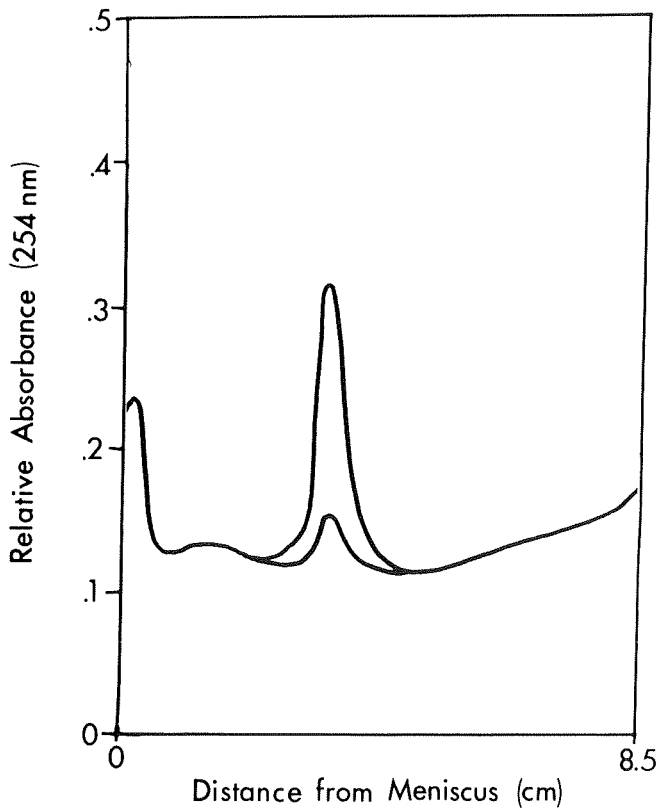

Fig. 1. Ultraviolet absorption profiles of BYDV preparations purified from 100 g of cultivar Coast Black oat roots (top) and shoots (bottom) and separated on linear 10–40% sucrose gradients in 0.1 M sodium phosphate, pH 7.0, centrifuged at 25,000 rpm for 5 hr in a Beckman SW27 rotor.

In scheme one, a combination of methods for BYDV was utilized (3,18). Tissue was ground in liquid nitrogen in a large mortar with a pestle, then homogenized in a Waring Blendor with two to three volumes of buffer for 1 min. The homogenate was strained through four layers of cheesecloth or a single layer of 155-μm (92-mesh) polyester material, and the residue reground in liquid nitrogen to a fine powder. This powder was mixed with the liquid from the first homogenization, homogenized again for 1 min, and refiltered.

In scheme two, tissue was frozen in liquid nitrogen, transferred dry to a Waring Blendor, and ground to a fine powder. Sodium phosphate buffer (0.1 M, pH 6.0), 2–3 ml/g of tissue, was added and the mixture was blended for 1 min. The homogenate was stirred with 0.5% sodium azide and 1.5% Rohament P (Fermco Biochemics Inc., Elk Grove Village, IL 60007) for 24 hr at room temperature. The temperature of the mixture was approximately 30 C after 24 hr.

In some early experiments, use of 6% polyethylene glycol 6000 (PEG) and 0.3 M NaCl for clarification and concentration was investigated in both schemes. PEG was added to the crude sap, stirred for 2 hr, and centrifuged for 20 min at 8,700 rpm in a Sorvall GSA rotor. The pellets were resuspended and recentrifuged two times and the supernatants were combined. In later experiments, instead of PEG, Triton X-100 and chloroform were used for clarification. One percent Triton X-100 was added to the crude sap and stirred at room temperature for 10 min in the first scheme and 30 min in the second scheme. In both schemes, 33% chloroform was added and the emulsion stirred for 15 min at room temperature. The emulsion was broken by centrifugation for 5 min at 5,000 rpm in a Sorvall GSA rotor. The aqueous phase was centrifuged for 4 hr at 28,000 rpm in a Beckman 30 rotor and the resulting pellets were covered with buffer (0.2–0.5 ml per pellet) and left overnight to resuspend. The partially resuspended pellets were homogenized in a ground glass homogenizer and centrifuged for 10 min at 10,000 rpm in a Sorvall SS-34 rotor.

The supernatant from either PEG or chloroform-Triton clarification was layered onto 3 ml of 30% sucrose in buffer, and centrifuged 2.5 hr at 38,000 rpm in a Beckman 40 rotor. The resulting pellets were covered with 1 ml of buffer and left overnight. After homogenization and, if necessary, centrifugation at 10,000 rpm for 10 min in a SS-34 rotor, the opalescent samples were layered onto linear 10–40% sucrose density gradients and centrifuged for 5 hr at 25,000 rpm in a Beckman SW-27 rotor. Gradients were scanned with an ISCO fractionator and absorbance monitor. The virus peak or zone approximately 4 cm below the meniscus was collected and pelleted in a Beckman 40 rotor at 38,000 rpm for 2.5 hr. The pellet was covered with 0.5–1.0 ml buffer overnight. If a zone had been collected, the material was separated through a second density gradient to obtain a virus peak. Ultraviolet absorption scans (220–320 nm) of virus preparations were made in a Beckman model 34 spectrophotometer.

Infectivity assay. To assay for infectivity, preparations of purified virus were made 20% sucrose (w/v) and fed to nonviruliferous aphids (*R. padi* for BYDV, *M. persicae* for BWYV) through stretched Parafilm membranes for 3 days. The aphids were transferred to healthy test seedlings (three to eight aphids per plant), which were fumigated or sprayed 3 days later and observed for symptom development.

To confirm that the purified BYDV was vector-nonspecific, both *R. padi* and *S. avenae* were fed for 2 days on leaf sections from plants that had been infected by membrane-fed *R. padi*. These aphids were then allowed inoculation accesses of 3 days on Coast Black oat seedlings. The plants were fumigated and observed for symptom development.

RESULTS

Symptoms. BYDV-inoculated Coast Black oats developed symptoms of progressive water-soaking, chlorosis, and necrosis 1–2 wk after inoculation. Clintland oats reacted similarly, but Hudson barley required 2–3 wk for initial symptom appearance, a chlorosis and curling of leaf tips. The disease was very severe on the

two oat cultivars, but remained mild on the barley for up to 12 wk after inoculation.

A variety of symptoms were observed on plants inoculated with BWYV: downward spiralling of leaves (radish), interveinal chlorosis (*Capsella bursa-pastoris* and *Thlaspi arvense*), reddening of leaves (*C. bursa-pastoris* and *Crambe abyssinica*), and leathery leaves with an atypical upright growth habit but no chlorosis (sugar beet and lettuce). Most hosts showed some degree of stunting, except sugar beet. Broadbean and pea showed no symptoms.

Tissue for purification. No BYDV peaks were obtained upon UV scanning (OD scale = 0–0.5) of density gradient preparations from barley shoots or roots harvested 3, 4, 5, or 6 wk after inoculation. More than twice as much BYDV was obtained from Coast Black than from Clintland oats. In two experiments, an equal weight of Coast Black oat roots yielded more than four times as much virus as shoots (Fig. 1). Root yields ranged from 0.6–3.2 mg BYDV/kg. To determine the optimum yield of BYDV, roots were harvested in time-course experiments. In the first trial, fresh roots from each of four flats of Coast Black oats of the same age were harvested at 6, 8, 10, and 12 days after inoculation. Symptoms first appeared 9 days after inoculation, and necrosis was obvious on day 10. Virus yield was highest in the 10-day postinoculation purification (Fig. 2). In the second experiment, symptoms appeared on day 10, but necrosis was not distinct until day 14. Roots were harvested every other day from 6 to 16 days after inoculation, and virus yield was greatest on day 14.

Yields of 0.6–0.9 mg BWYV/kg tissue were obtained from *C. bursa-pastoris* and *T. arvense* harvested 10–22 days after inoculation (Fig. 3). Less than 10% as much virus was obtained from *C. abyssinica* when shoots were harvested 10–16 days after inoculation. *C. bursa-pastoris* was selected for further purification studies because of ease of propagation.

Purification. Both purification schemes were tested for each virus. The enzyme extraction procedure resulted in less than 10% of the amount of BYDV obtained from the liquid nitrogen grinding procedure. For BWYV, however, the situation was reversed. Enzyme extraction yielded approximately two times as much virus as did grinding the tissue in liquid nitrogen (Fig. 4).

Virus peaks were obtained upon UV scanning of sucrose density gradients when PEG was used as a clarification and concentration agent for either virus. However, as much virus was left in the PEG pellet as was released from it. Two washes of the PEG pellet failed to remove all trapped virus. The yields of BYDV or BWYV from the PEG clarifications were consistently less than half those from the chloroform-Triton procedure.

In BYDV purifications from greenhouse-grown tissue during March through October 1980, a virus peak was never obtained in the first density gradient. Two methods to obtain a virus peak were investigated: two successive density gradients (14), or dialysis of the material immediately prior to a single density gradient run. Dialysis was for 16 hr against two 6-L changes of phosphate buffer. In some instances, dialysis was a better procedure than two density gradient runs. Virus yields of more than 1.5 mg/kg were obtained, compared to an average yield of 0.7 mg/kg when two density gradients were used. However, dialysis often failed to result in a virus peak, and the double density gradient procedure was adopted. In purifications from November 1980 through February 1981 the first density gradient yielded a virus peak. Also during this time we discovered that by using an excess of liquid nitrogen (5–10 times more than was required to freeze the tissue) during the two extractions yields could be doubled. BYDV yields from six experiments ranged from 3.2–6.1 mg virus per kilogram of root tissue ($E^{0.1\%}_{260\ nm} = 8$), with an average yield of 4.4 mg/kg. In March 1981 purifications, two density gradients were again required to obtain a BYDV peak. Use of a growth chamber during warm weather allowed production of tissue, which gave a BYDV peak in the first density gradient.

A similar loss of purity of BWYV preparations made from tissue grown in warm weather (June) in the greenhouse was not noted. BWYV yields from nine experiments ranged from 0.9–1.2 mg of virus per kilogram of shoot tissue ($E^{0.1\%}_{260\ nm} = 8$), with an average

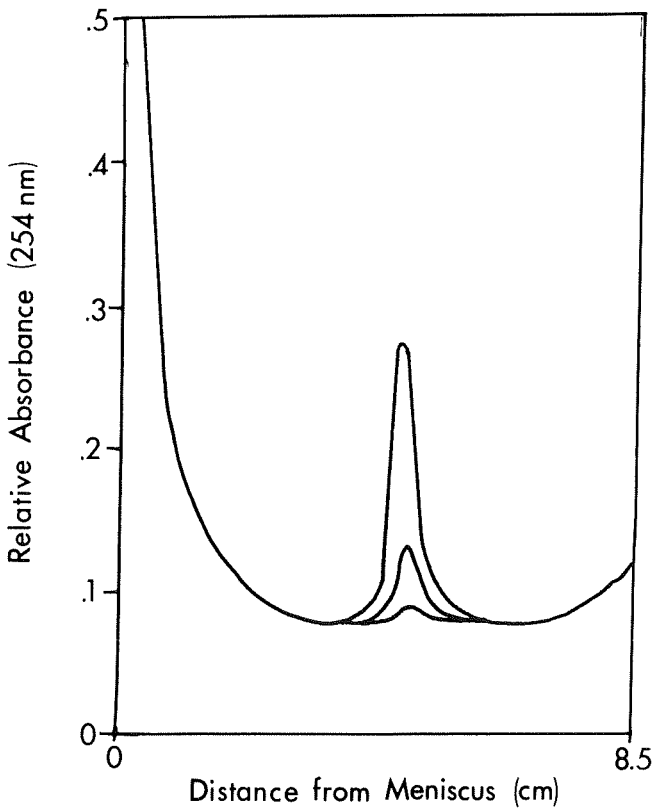

Fig. 2. Ultraviolet absorption profiles of BYDV preparations purified from 43 g of cultivar Coast Black oat roots 10 days (top), 8 or 12 days (middle), or 6 days (bottom) after inoculation. Gradient composition and centrifugation as described in Fig. 1.

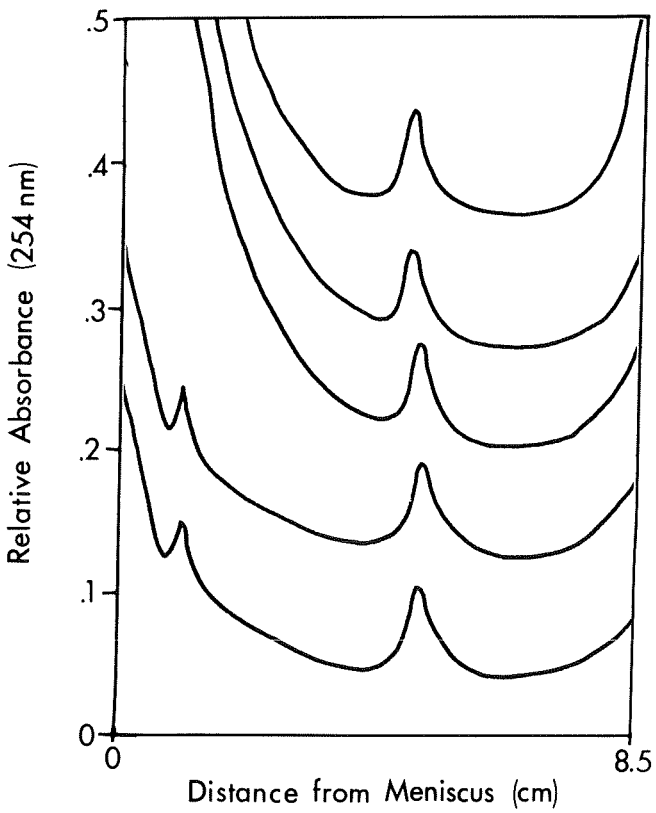

Fig. 3. Ultraviolet absorption profiles of BWYV preparations purified from 40 g of shepherd's purse shoots 14, 16, 18, 20, and 22 days (bottom to top) after inoculation. Gradient composition and centrifugation as described in Fig. 1.

yield of 1.1 mg/kg.

Coast Black oat root tissue harvested November–February and frozen at −80 C for 4 wk yielded as much BYDV as fresh tissue. However, tissue harvested and frozen in warm weather when the greenhouse temperature often rose above 27 C (March–October), yielded less BYDV than fresh. Fresh and frozen BWYV-infected sheperd's purse tissue always gave equivalent virus yields.

Electron microscopic examination of purified preparations of both viruses revealed small, spherical particles 25–30 nm in diameter. Purified BYDV had an $A_{260/280}$ of 1.89; the ratio for BWYV was 1.65.

Infectivity. Membrane feeding experiments showed that infectious BYDV or BWYV could be obtained by both the liquid nitrogen and enzyme extraction methods, and by both the chloroform-Triton and PEG clarification methods. All plants infested with aphids fed on virus preparations developed typical symptoms in 10–20 days. Control plants infested with aphids with no exposure to virus preparations remained symptomless. BYDV was subsequently transmitted to oat seedlings by both *S. avenae* and *R. padi*, confirming its vector-nonspecificity.

DISCUSSION

Extraction from phloem tissue is the most important step in luteovirus purification. The most efficient extraction of the BYDV and BWYV isolates used in this study was obtained by two different methods. This difference is possibly due to the different host species and tissues used for purification, rather than to differences between the two viruses. In the one previous comparative study of luteovirus purification, the same host species was used for two viruses (25).

Enzyme extractions of luteoviruses have employed cellulase and Driselase, a commercial enzyme preparation from the wood-rotting fungus *Irpex lacteus*, which contains amylase, cellulase, laminarinase, pectinase, and xylanase (18,25,26). Rohament P, a pectin glycosidase from an *Aspergillus* species, is both cheaper and more readily available. Despite the fact that there may be more BYDV in roots than shoots (1), the oat root tissue did not release much virus during pectinase extraction, whereas shepherd's purse shoot tissue released much BWYV. The success of this substitution indicates that different macerating enzyme preparations may be useful for luteovirus purification.

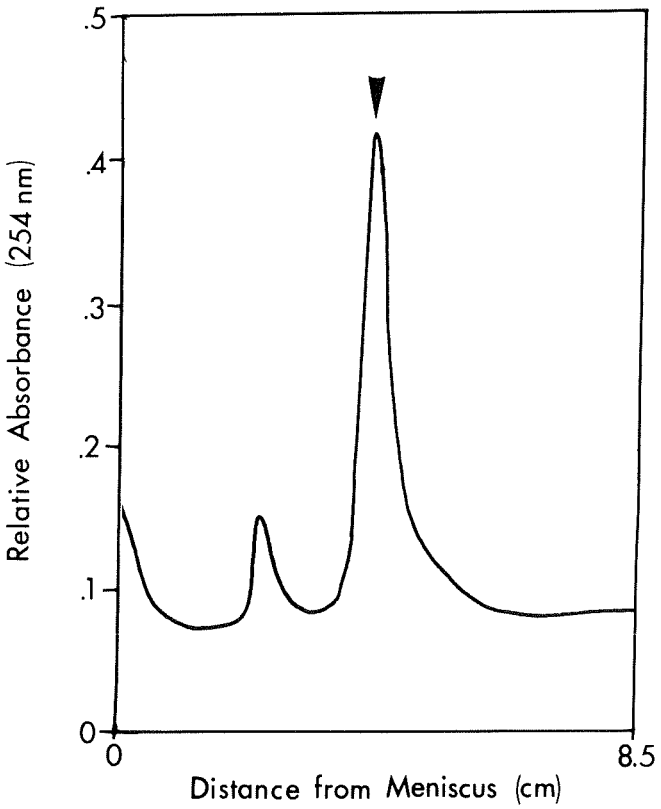

Fig. 4. Ultraviolet absorption profile of a BWYV preparation purified from 100 g of shepherd's purse shoots. Arrow indicates the virus band; the nature of the upper band will be the subject of a further report. Gradient composition and centrifugation as described in Fig. 1.

Liquid nitrogen extraction of our BYDV isolate from roots released more virus than did pulverization of freeze-dried tissue in a Wiley mill (18). The use of excess liquid nitrogen made grinding easier and pulverized the tissue much more quickly than when only enough nitrogen to freeze the tissue was used.

Vector-nonspecific isolates of BYDV are typically severe, causing extensive leaf necrosis and often plant death (21). The Illinois isolate used in this study follows this pattern on the oat hosts tested; however, on the barley cultivar Hudson it was quite mild, even when inoculated at a very early stage of plant growth. The oats were found to be better sources of purified virus, as others have reported (21). However, with a strain as severe as this, purification must be done very soon after inoculation. We began virus purification between 8 and 14 days after the beginning of the aphid inoculation period. The optimal time varied, influenced by environmental conditions, both daily and seasonal. For this isolate the best method to determine when to harvest tissue for purification was to follow symptom development, rather than to count a specific number of days after inoculation. Highest yields of BYDV were obtained from tissue harvested on the first day that leaf necrosis became distinct. With a system where there is a sharp peak of virus titer in the plants, time of tissue harvest is critical to obtaining good virus yields. Our average yield during winter months of 4.4 mg/kg is the highest reported for a vector-nonspecific strain of BYDV.

For BWYV, in contrast, time of harvest was not a critical factor in determining virus yield. Shepherd's purse harvested at any time over a 2-wk period gave similar virus yields. No previously published yield figures for BWYV are available for comparison with our average yield of 1.1 mg/kg. However, this is a high yield for a luteovirus. The difference in importance of time of harvest for an acute (BYDV) and a chronic (BWYV) luteovirus infection is crucial for optimization of virus purification.

Several of our findings are similar to those reported for other luteoviruses. BYDV purifications from tissue grown during cool weather yielded more virus than those from tissue grown during warm weather (21), although this difference was not noted for BWYV. Oat roots yielded more BYDV than shoots, as Ashby found for PeLRV purified from peas (2). In the spring of 1980, when little liquid nitrogen was used, we found, as has been reported for PLRV (15,23) and PeLRV (2), that higher BYDV yields were obtained from fresh than from frozen tissue. However, in the winter of 1980, when excess liquid nitrogen was used, frozen tissue yielded as much BYDV as fresh. This may have been due to the state of the tissue, the procedure, or a combination of both. BWYV yields were equivalent from fresh and frozen tissue. The problem of loss of virus in a PEG pellet has also been noted in purifications of BYDV (21) and PLRV (24).

LITERATURE CITED

1. Amici, A., Faoro, F., and Tornaghi, R. 1979. Intracellular crystallization in roots of *Leersia oryzoides* of a strain of barley yellow dwarf virus. Micron 10:5-12.
2. Ashby, J. W., and Huttinga, H. 1979. Purification and some properties of pea leafroll virus. Neth. J. Plant Pathol. 85:113-123.
3. Brakke, M. K., and Rochow, W. F. 1974. Ribonucleic acid of barley yellow dwarf virus. Virology 61:240-248.
4. Duffus, J. E., and Gold, A. H. 1965. Transmission of beet western yellows virus by aphids feeding through a membrane. Virology 27:388-390.
5. Foxe, M. J., and Rochow, W. F. 1975. Importance of virus source leaves in vector specificity of barley yellow dwarf virus. Phytopathology 65:1124-1129.
6. Gildow, F. E., and Rochow, W. F. 1982. Identification of vector-specific barley yellow dwarf virus (BYDV) isolates and their aphid vectors in California. Phytopathology 72:963.

7. Gill, C. C. 1975. An epidemic of barley yellow dwarf in Manitoba and Saskatchewan in 1974. Plant Dis. Rep. 59:814-818.
8. Gill, C. C. 1980. Assessment of losses on spring wheat naturally infected with barley yellow dwarf virus. Plant Dis. 64:197-203.
9. Gold, A. H., and Duffus, J. E. 1967. Infectivity neutralization—a serological method as applied to persistent viruses of beets. Virology 31:308-313.
10. Gugleri, P. 1979. Potato virus A and potato leafroll virus: purification, antiserum production and serological detection in potato and test plants by enzyme-linked immunosorbent assay. Phytopathol. Z. 96:97-107.
11. Hepp, R. F., and deZoeten, G. A. 1978. Purification of potato leaf roll virus and an evaluation of methods for its diagnosis. Am. Potato J. 55:125-139.
12. Kojima, M., and Murayama, D. 1972. The application of polyethylene glycol precipitation for purification of potato leaf-roll virus. Ann. Phytopathol. Soc. Jpn. 38:431-433.
13. Kojima, M., and Tamada, T. 1976. Purification and serology of soybean dwarf virus. Phytopathol. Z. 85:237-250.
14. Kojima, M., Shikata, E., Sugawara, M., and Murayama, D. 1969. Purification and electron microscopy of potato leafroll virus. Virology 39:162-174.
15. Maat, D. Z., and deBokx, J. A. 1978. Potato leafroll virus: antiserum preparation and detection in leaves and sprouts with the enzyme-linked immunosorbent assay (ELISA). Neth. J. Plant Pathol. 84:149-156.
16. Matthews, R. E. F. 1979. Classification and nomenclature of viruses. Intervirology 12:132-160.
17. Murayama, D., and Kojima, M. 1974. Antigenicity of potato leaf-roll virus. Proc. Japan Acad., Series B, Phys. Biol. Sci. 50:322-327.
18. Paliwal, Y. C. 1978. Purification and some properties of barley yellow dwarf virus. Phytopathol. Z. 92:240-246.
19. Paliwal, Y. C. 1982. Identification and annual variation of variants of barley yellow dwarf virus in Ontario and Quebec. Can. J. Plant Pathol. 4:59-64.
20. Rochow, W. F. 1979. Field variants of barley yellow dwarf virus: detection and fluctuation during twenty years. Phytopathology 69:655-660.
21. Rochow, W. F., Aapola, A. I. E., Brakke, M. K., and Carmichael, L. E. 1971. Purification and antigenicity of three isolates of barley yellow dwarf virus. Virology 46:117-126.
22. Rochow, W. F., and Brakke, M. K. 1964. Purification of barley yellow dwarf virus. Virology 24:310-322.
23. Rowhani, A., and Stace-Smith, R. 1979. Purification and characterization of potato leafroll virus. Virology 98:45-54.
24. Sarkar, S. 1976. Potato leafroll virus contains a double-stranded DNA. Virology 70:265-273.
25. Takanami, Y., and Kubo, S. 1979. Enzyme-assisted purification of two phloem-limited plant viruses: tobacco necrotic dwarf and potato leafroll. J. Gen. Virol. 44:153-159.
26. Waterhouse, P. M., and Murant, A. F. 1981. Purification of carrot red leaf virus and evidence from four serological tests for its relationship to luteoviruses. Ann. Appl. Biol. 97:191-204.