MINISTERE DE L'AGRICULTURE ET DE LA PECHE
DIRECTION REGIONALE DE L'AGRICULTURE ET DE LA FORET D'AUVERGNE
SERVICE REGIONAL DE LA PROTECTION DES VEGETAUX
BP 45 - Marmilhat - 63370 LEMPDES

VISITE DE LA STATION DE QUARANTAINE DES VEGETAUX LIGNEUX DU CANADA

Compte-rendu de la mission effectuée par
J-F CHAUVEAU
du 18 au 25 septembre 1994

cofinancée par :

FONDATION NATIONALE ENTREPRISE ET PERFORMANCE

FEDERATION REGIONALE AUVERGNE DE DEFENSE CONTRE LES ENNEMIS DES CULTURES

Cette mission n'aurait pu avoir lieu sans l'intervention de nombreuses personnes que je tiens à remercier ici.

Ma reconnaissance s'adresse tout d'abord à la **Fondation Nationale Entreprise et Performance** qui en acceptant de financer le déplacement m'a offert la possibilité de réaliser le projet de visite. La **Fédération Régionale Auvergne de Défense contre les Ennemis des Cultures**, en apportant le complément financier nécessaire, a permis de mener à bien cette opération. **Je tiens à leur témoigner ma profonde gratitude.**

Mme ROUX (Fondation Nationale Entreprise et Performance) a organisé le déplacement avec compétence et dévouement. La réussite de la mission lui doit beaucoup. Qu'elle trouve ici l'expression de ma reconnaissance.

Mes remerciements s'adressent aussi à tous les **collègues du Service de la Protection des Végétaux** qui m'ont aidé à préparer cette visite.

Parmi eux, il convient de mentionner particulièrement **M LELION et Mme PETTER** (DGAL-SDPV-RPRI) et **MM JARDILLET et PRADIER** (Station de Quarantaine des Végétaux Ligneux à Clermont-Ferrand) dont les conseils et les encouragements ont été très profitables.

La qualité et la quantité des informations recueillies au Canada sont la conséquence la qualité de l'accueil et du dialogue de mes hôtes canadiens. Je tiens à leur témoigner toute ma reconnaissance.

L'accueil chaleureux et la bienveillance de **MM LANTERMAN, LAM, SINGH, MONETTE, JAMES et MACKENZIE** ont rendu la visite fructueuse. Qu'ils en soient remerciés. Le dévouement et la compétence, l'amabilité de **Ray JOHNSON**, ont éclairé le séjour à Sidney. Je tiens à lui exprimer ma profonde gratitude et à l'assurer de mon amitié.

Monsieur l'Ambassadeur de France et son collaborateur, **M CHAUVIN**, par l'intérêt porté à cette mission, m'ont témoigné leur soutien. **Ma gratitude s'adresse** aussi **à eux.**

A tous ceux que j'ai omis ici, mais qui ont néanmoins, à un moment ou un autre, aidé à la réalisation de ce projet, j'adresse également mes remerciements.

Depuis plusieurs années le Service de la Protection des Végétaux étudie la possibilité de doter la Station de Quarantaine des Végétaux Ligneux de Clermont-Ferrand des serres et laboratoires indispensables au fonctionnement d'une station de quarantaine moderne.

Parvenu dans sa phase de réalisation, ce projet devait être comparé aux structures existantes ailleurs. Cela a été fait en Europe tout d'abord, avec les stations orientées vers les pommes de terre et les plantes herbacées. Il convenait, en complément, de visiter la station la plus proche spécialisée sur les plantes ligneuses. Elle se trouve au Canada.

Une demande de prise en charge a donc été présentée à la Fondation Nationale Entreprise et Performance. La proposition a été acceptée et le projet mis à exécution. Le rapport suivant résume le déroulement de la visite.

1. PREPARATION DE LA MISSION

Outre les aspects matériels qui ont été réglés directement avec la Fondation Nationale Entreprise et Performance (FNEP) et les hôtes canadiens, le Service de la Protection des Plantes (Agriculture Canada) et la Station de Quarantaine de Saanichton notamment, les questions techniques ont été traitées avec le Ministère de l'Agriculture et de la Pêche (DGAL), et l'Ambassade de France à Ottawa.

1.1 Aspects matériels

Des documents joints en annexe 1 précisent l'organisation de la mission.

1.2 Aspects techniques

L'attaché agricole à l'Ambassade de France a souhaité qu'une information me soit transmise sur la question des exportations de plants de vigne vers le Canada. Un dossier complet, accompagné d'explications orales a été fourni par M LELION (DGAL-SDPV-RPRI).

La DGAL-SDPV et les responsables de secteurs du SPV ont été interrogés sur les questions relevant de leur domaine d'intervention qu'ils souhaitaient voir abordées au cours de la mission. Les réponses peuvent être résumées ainsi :

- le dossier export plants de vigne, et notamment la multiplication des plants français par la Station de Quarantaine en vue de leur fourniture à des pépiniéristes canadiens,
- l'organisation générale des services phytosanitaires canadiens,
- l'organisation des laboratoires du Service de la Protection des Plantes,
- les méthodologies employées à la Station de Quarantaine,
- le point des connaissances sur le Peach Rosette Mosaïc Virus et son vecteur *Longidorus diadecturus*.

Une autorisation d'importation à titre scientifique à été demandée et accordée par la DGAL-SDPV en vue de rapporter des greffons infectés par divers virus exotiques et de conserver ces témoins à la Station de Quarantaine de Clermont-Ferrand (annexe 1)

1.3 Questions administratives

Un ordre de mission a été signé par le Ministère de l'Agriculture (annexe 1). La Direction de la Production et des Echanges a été tenue informée du dossier de préparation de la mission.

Un rendez-vous a été pris avec l'attaché agricole à l'Ambassade de France à Ottawa.

2. RAPPEL DES BUTS DE LA MISSION

2.1 Entretiens avec Agriculture Canada

Objet de cette rencontre :

- présentation de la mission,
- présentation du projet français de Station de Quarantaine des Végétaux Ligneux à Clermont-Ferrand,
- recherche d'informations sur l'organisation générale des Services de la Protection des Plantes canadiens.

2.2 Visite de la Station de Quarantaine de Saanichton

Objet de la visite :

- discussions sur les contraintes techniques de construction d'une station de quarantaine,
- présentation du projet français,
- information sur les procédures utilisées à la Station de Saanichton : procédure générale et méthodes spécifiques,
- informations sur les procédures de quarantaine pour la vigne,
- informations scientifiques sur les parasites de quarantaine de la vigne et des arbres fruitiers,
- informations sur la maladie du Little Cherry,
- informations sur le Peach Rosette Mosaïc Virus et son vecteur,
- collecte d'échantillons infectés par divers virus.

3. RAPPORT FINANCIER

Les frais de mission ont été pris en charge par la FNEP pour un montant de 12000 F. Le complément a été financé par la Fédération Régionale Auvergne de Défense contre les Ennemis des Cultures (FRADEC), dans le cadre des subventions qu'elle reçoit du Ministère de l'Agriculture et de la Pêche pour l'aide aux analyses de diagnostic et de détection réalisées par la Station de Quarantaine des Végétaux Ligneux de Clermont-Ferrand.

Le montant total des frais occasionnés par cette mission s'élève à 15277,97 F (annexe 2).

4. DEROULEMENT DE LA MISSION

La journée du 19 septembre a été consacrée aux rencontres avec les autorités fédérales canadiennes et l'Ambassade de France.

J'ai été reçu au Service de la Protection des Plantes par :

- M LANTERMAN, Directeur de la Station de Quarantaine de Saanichton, assurant l'intérim de M BRADNOCK absent,
- M LAM, responsable des questions réglementaires,
- et M SINGH, responsable des opérations.

J'ai également pris contact avec M SHEPPARD, responsable du laboratoire des semences que je connaissais du fait de mes activités antérieures.

M l'Ambassadeur de France m'a accordé une audience, et M CHAUVIN, attaché agricole m'a reçu.

Du 20 au 23 septembre M Ray JOHNSON, responsable de la section vigne à la Station de Quarantaine de Saanichton, a assuré avec beaucoup de dévouement et de gentillesse l'encadrement de la visite. J'ai rencontré également :

- M MACKENZIE, responsable du laboratoire de biotechnologie,
- M JAMES, responsable du laboratoire de recherches sur la virologie des arbres fruitiers,
- et M MONETTE, responsable du laboratoire de recherches sur la virologie de la vigne.

Au total, la mission a duré 8 jours dont 5 ouvrables. Les divers trajets ont duré deux jours et demi. Les entretiens et visites se sont étalés sur quatre jours et demi. J'ai bénéficié d'une journée de repos (samedi 24 septembre).

5. BILAN DES ENTRETIENS ET DES VISITES

5.1 Organisation générale du Service de la Protection des Plantes

Le Service de la Protection des Plantes est une division de la Direction Générale de la Santé des Animaux et des Plantes. Il est dirigé par M BRADNOCK et divisé en quatre sections :

- les politiques réglementaires (responsable Andrew LAM) qui traite des questions réglementaires et des contacts bilatéraux,
- les opérations (responsable Yudi SINGH) qui gère l'application des politiques sur le terrain et les problèmes quotidiens,
- la coordination (responsable I. MACDONNELL) qui s'occupe des coordinations internationales (NAPPO ...etc.),
- le bureau national de la pomme de terre (responsable Reinouw BAST-TJEERDE) qui gère la certification génétique et la quarantaine propres à cette production.

Les documents 1 à 6 en précisent l'organisation.

Au total, la division compte 38 personnes. L'inspectorat emploie en outre l'équivalent de 330 personnes à plein temps pour les contrôles sur le terrain, avec deux pôles d'action principaux : la Colombie Britannique et la Côte Atlantique.

Les laboratoires qui apportent leur appui à la division de la Protection des Plantes, n'appartiennent pas à cette dernière mais à une autre division qui comprend également les laboratoires vétérinaires. Ces laboratoires sont au nombre de trois :

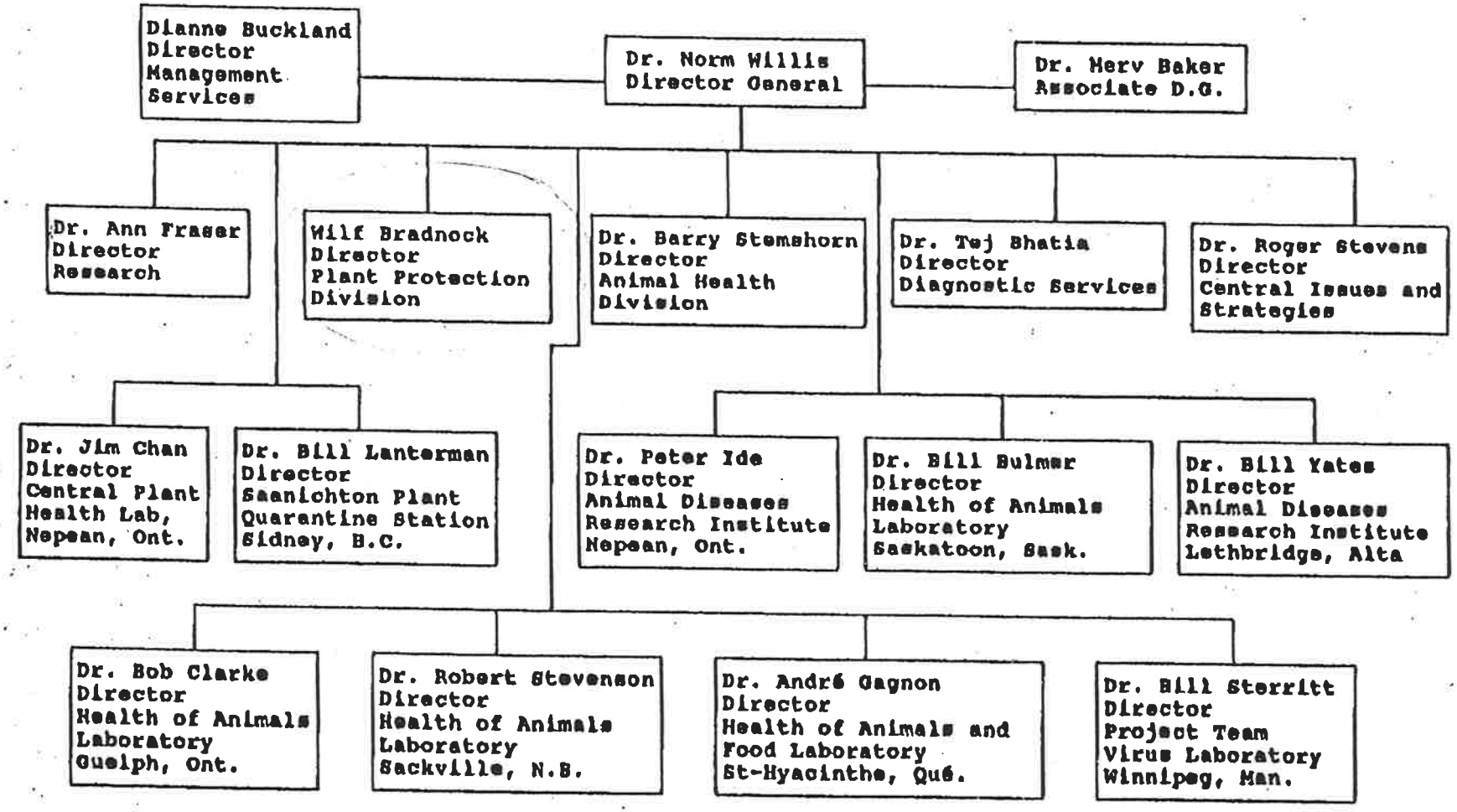
- la Station de Quarantaine de Saanichton (Center for Plant Health) s'occupe essentiellement des maladies à virus et à mycoplasmes des arbres fruitiers et de la vigne,
- le Laboratoire Central (Central Plant Health Laboratory) situé à Ottawa prend en charge les identifications, les tests et l'analyse des risques liés aux organismes pathogènes ("pest risk analysis") : nématodes, insectes, champignons, bactéries,
- une unité située à Charlottetown à l'est du Canada est orientée vers la pomme de terre, bien qu'actuellement elle diversifie ses activités (bactéries et champignons).

Au contraire des laboratoires vétérinaires, les laboratoires spécialisés en maladies des plantes ne font pas de recherche à proprement parler. Celle-ci est prise en charge par la branche Recherche, équivalent de l'INRA, qui compte 3000 personnes. Agriculture Canada a un arrangement formel avec cette branche, accord par lequel c'est Agriculture Canada qui définit les priorités de recherche pour les programmes concernés. Il existe aussi le Canadian Agricultural Service Coordinating Committee qui coordonne les programmes de recherches avec tous les intervenants et notamment les Universités.

Animal and Plant Health Directorate

Deputy Ministry: Mr. Rob Wright

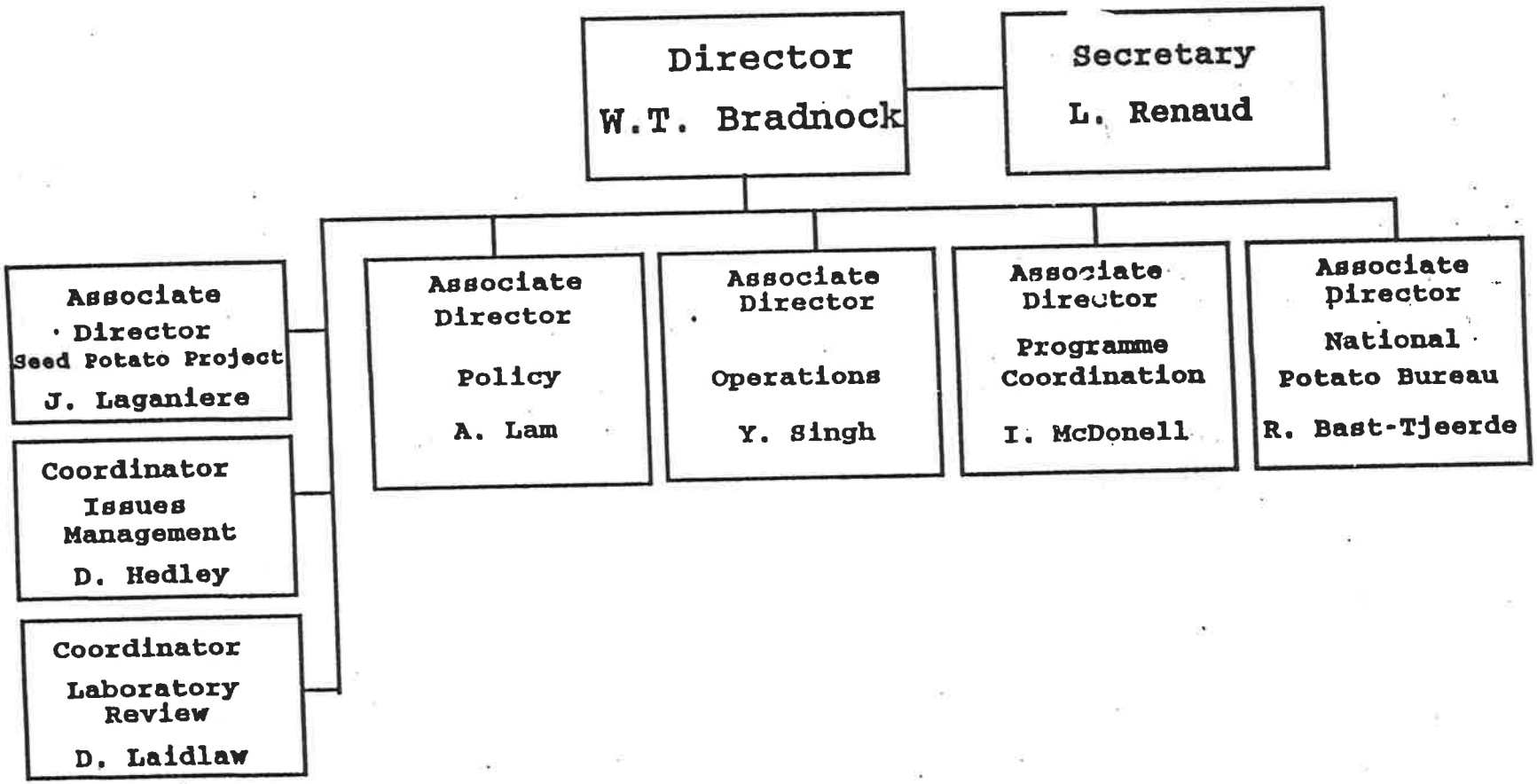
Assistant Deputy Minister: Dr. A.O. Olson



DOCUMENT 1

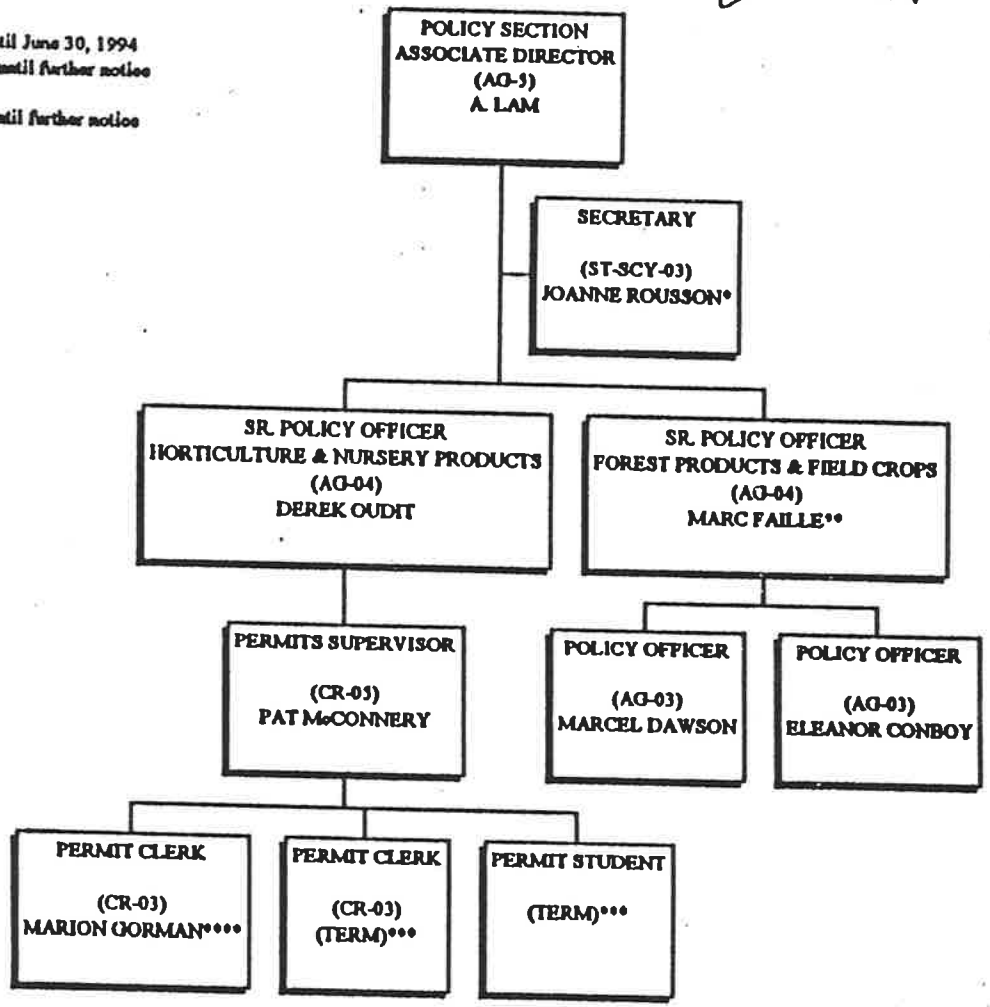
DOCUMENT 2

Plant Protection Division



ORGANIZATION OF POLICY SECTION
PLANT PROTECTION DIVISION

* On leave, replaced by Joanne Paradis until June 30, 1994
** Seconded to "Beneficiary Pays" project until further notice
*** Temporary until no longer required
**** On leave, replaced by Christine Lailey until further notice



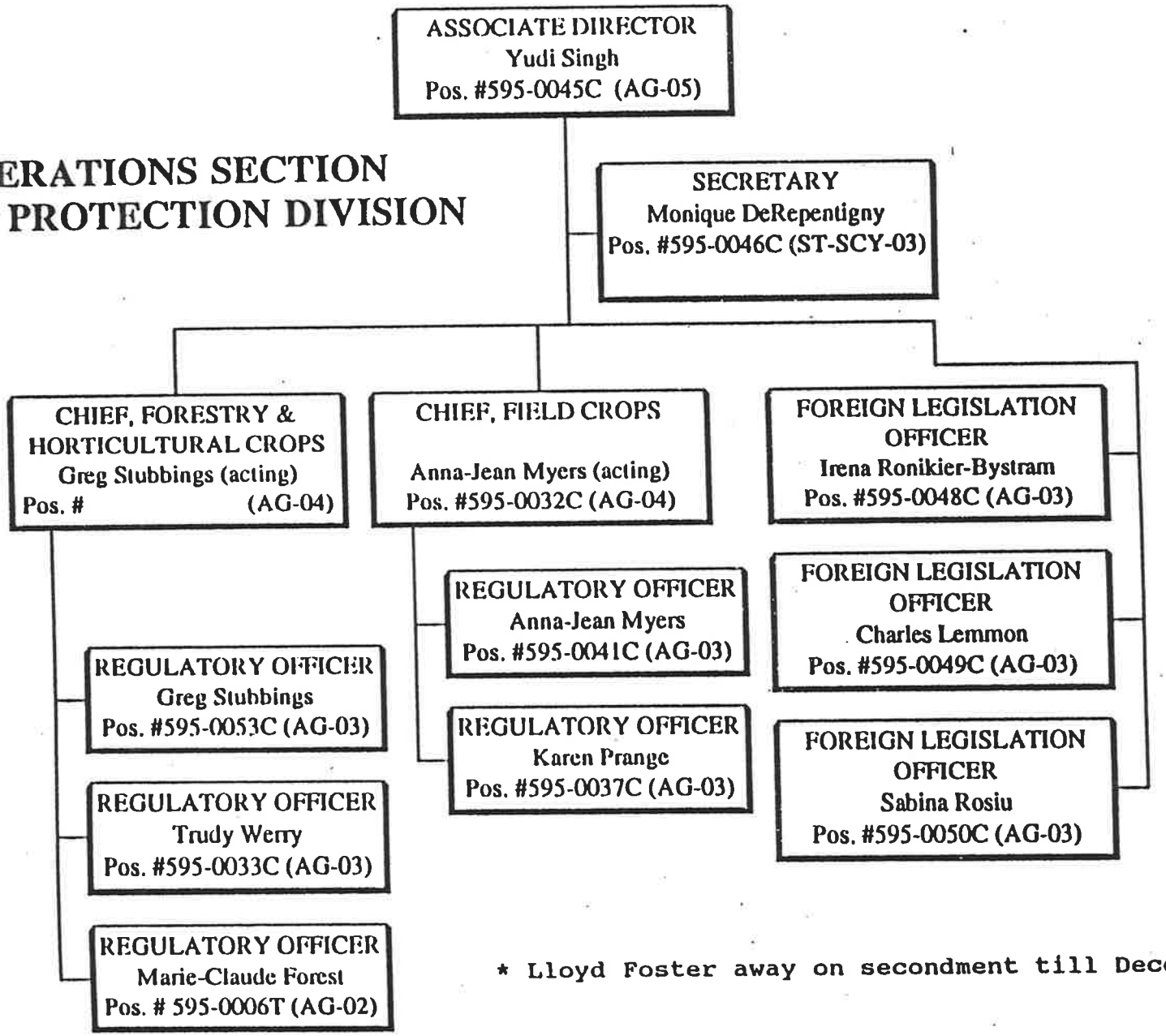
A. LAM, ASSOCIATE DIRECTOR
POLICY SECTION

DATE SIGNED

DOCUMENT 3

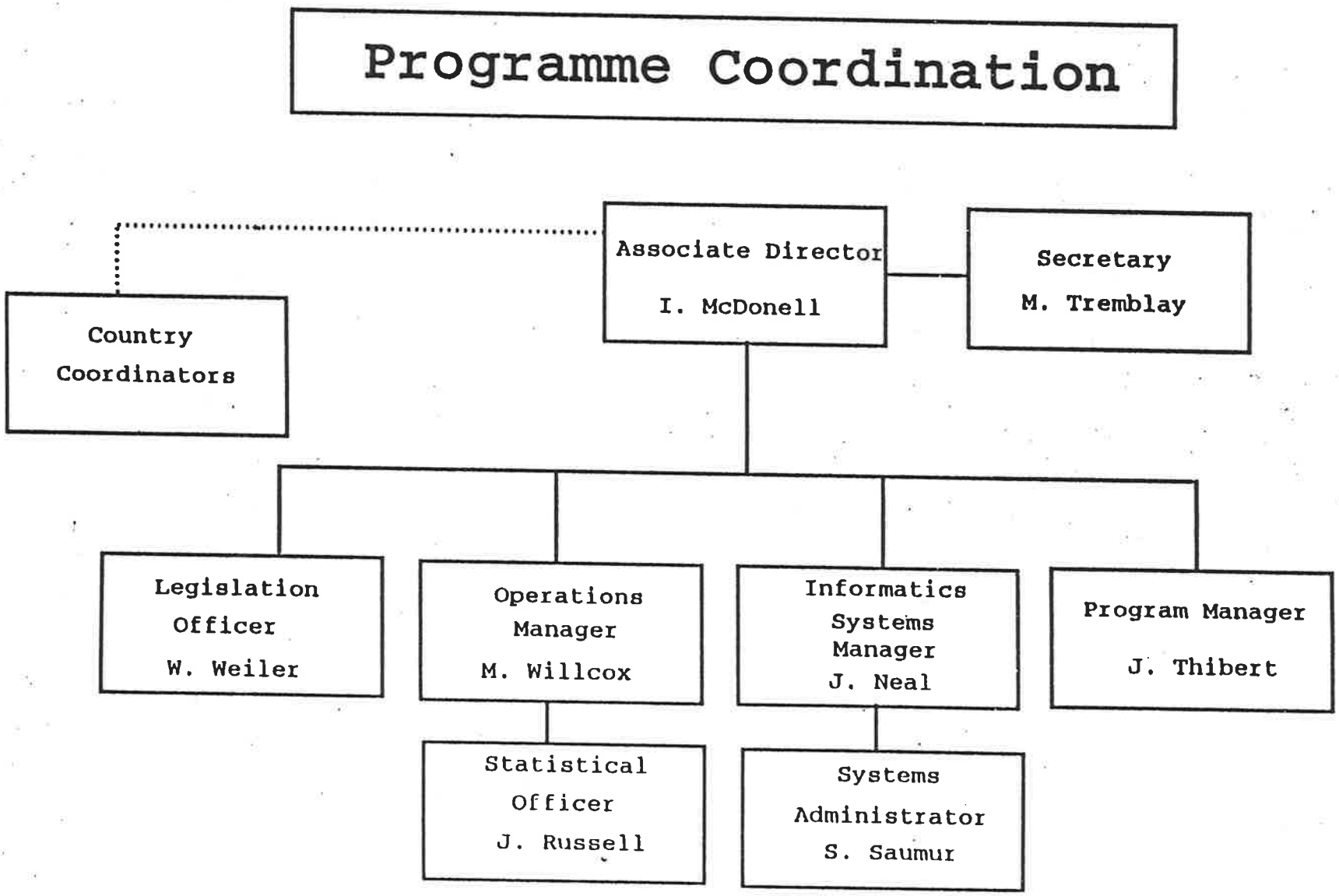
DOCUMENT 4

OPERATIONS SECTION
PLANT PROTECTION DIVISION

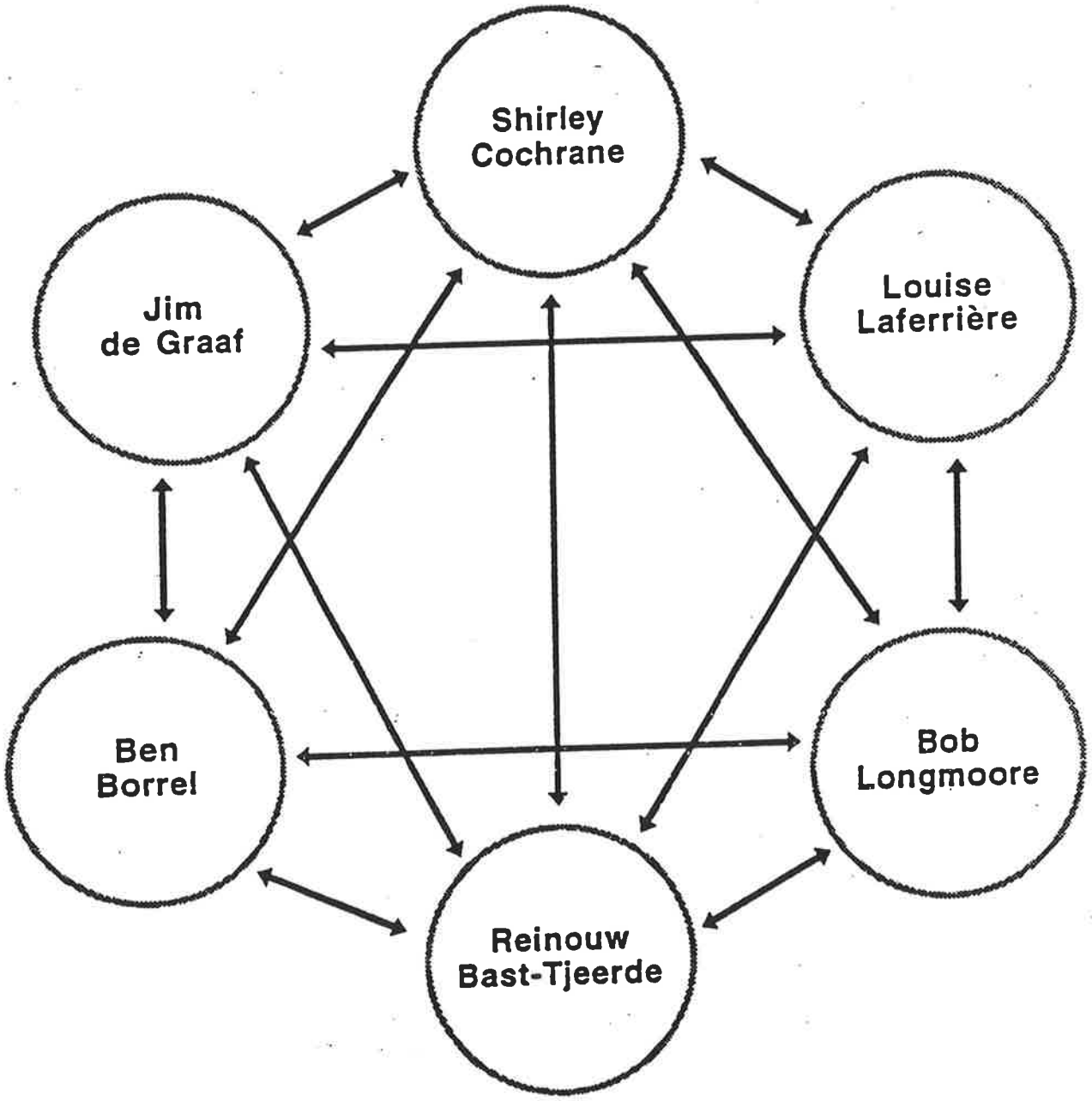


* Lloyd Foster away on secondment till December 1994. *

JANUARY 1994


Programme Coordination
Associate Director
I. McDonell
Secretary
M. Tremblay
Country
Coordinators
Legislation
Officer
W. Weiler
Operations
Manager
M. Willcox
Informatics
Systems
Manager
J. Neal
Program Manager
J. Thibert
Statistical
Officer
J. Russell
Systems
Administrator
S. Saumur

National Potato Bureau
Bureau national des pommes de terre



La réglementation canadienne interdit les introductions de végétaux sans autorisation préalable. Les lots expérimentaux sont dirigés vers la station de Saanichton.
Les demandes d'importation sont tout d'abord analysées par les scientifiques selon le protocole d'analyse des risques élaboré en commun par l'O.E.P.P. et la N.A.P.P.O. Un avis est transmis à la division de la Protection des Plantes. Celle-ci prend la décision administrative définitive. Les récents accords du GATT imposent des changements de conception des règlements phytosanitaires. C'est désormais l'importateur qui doit apporter la preuve de la réalité du risque. Pour cette raison les protocoles d'analyse des risques sont de plus en plus largement utilisés. Ils sont complétés par des enquêtes nationales exhaustives tendant à prouver l'absence de tel ou tel agent pathogène sur le territoire national.

Les efforts de mise au point sont actuellement orientés essentiellement vers le développement des méthodes issues de la biologie moléculaire (amplification génique etc.). Ces techniques seront utilisée parallèlement au procédures traditionnelles pendant quelques années. Selon Jim SHEPPARD, tous calculs faits, l'amplification génique est plus onéreuse que les techniques sérologiques telles qu'ELISA, qui sont elles-mêmes plus chères que les méthodes traditionnelles (du moins en ce qui concerne la pathologie des semences). Par contre ces techniques sont plus rapides.

Nota : Au cours de nos conversations, J. SHEPPARD m'a montré le logiciel TenCore qui permet d'intégrer texte et images. Avec cet outil, il a réalisé une base documentaire sur les procédures de contrôle des semences. Ce logiciel paraît très intéressant pour décrire les procédures utilisées par le Service de la Protection des Végétaux.

5.2 La Station de Quarantaine de Saanichton

Elle est située à Sidney sur l'île de Vancouver. La figure 1 en montre une vue générale. On distingue les bâtiments abritant les laboratoires et les bureaux, les serres et tunnels et l'un des vergers d'indexage. La figure 2 donne un exemple des serres conservatoires. La surface totale occupée par la station est de 48 ha. Elle a été implantée sur ce site en 1965. Le choix du lieu est un compromis entre les diverses contraintes liées au concept de la quarantaine : isolement, qualité du sol, climat favorable aux cultures fruitières et viticoles...etc. Le document joint en annexe 3 décrit succinctement la station.

Elle est dirigée par M LANTERMAN et est divisée en 7 unités (document 7) :

- une unité administrative (responsable Mme CAMPBELL),
- une unité chargée des opérations de maintenance (bâtiments et matériels) et des travaux dans les serres et les vergers (responsable M ALSBURY),
- une unité chargée des contrôles sur la vigne et les plantes ornementales (responsable M JOHNSON),
- une unité chargée des contrôles sur les arbres fruitiers (responsable M THOMPSON),
- deux unités de recherche chargées de développer de nouvelles techniques de diagnostics, l'une sur les maladies de la vigne (responsable M MONETTE), l'autre sur les maladies des arbres fruitiers (responsable M JAMES),
- une unité de biotechnologie (responsable M MACKENZIE) chargée de fournir un appui aux autres unités en matière de cultures in vitro, de microscopie électronique et de biologie moléculaire.

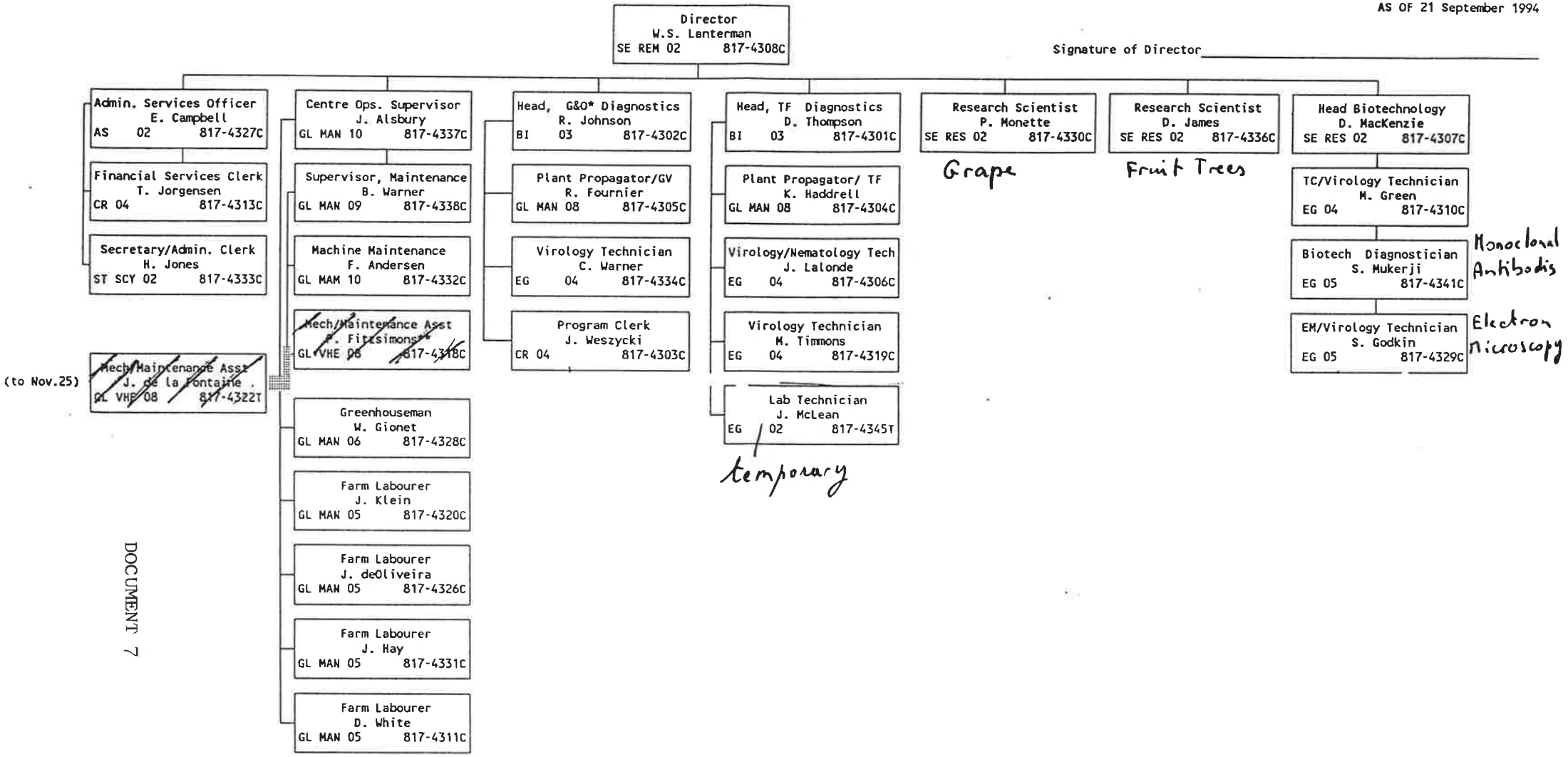
Ce sont au total 26 personnes qui travaillent à la station. Il convient de noter que pour chaque type de culture (arbres fruitiers ou vigne) la station dispose d'un spécialiste pour la propagation (greffage) et d'un scientifique pour la mise au point de méthodes. La tenue des fichiers informatisés est prise en compte par un seul agent pour toutes les cultures. On verra plus loin qu'un système autorisant une traçabilité totale a été instauré.

Figure 1 : Vue générale de la station de quarantaine de Saanichton

Figure 2 : Vue générale des tunnels conservatoires

CENTRE FOR PLANT HEALTH - SAANICHTON
AS OF 21 September 1994

Signature of Director _____



DOCUMENT 7

* G&O - Grapevine & Ornamental Diagnostics
TF - Tree Fruit TC - Tissue Culture GV - Grapevine

** Indeterminate employee (Fitzsimons) on D.I.

Le document joint en annexe 4 précise les missions et les activités de la station. On peut les résumer ainsi :

- contrôle des végétaux importés (arbres fruitiers et d'ornements, vigne),
- contrôle des nouveaux clones et variétés sélectionnés par les pépiniéristes canadiens (équivalent au système français de certification),
- mise en oeuvre des techniques de thermothérapie pour éliminer les virus et autres agents infectieux présents sur des végétaux particulièrement intéressants,
- contrôle de la fiabilité des systèmes de certification étrangers par sondages lors des introductions sur le territoire canadien,
- maintien en bon état végétatif et sanitaire de variétés d'arbres fruitiers (1062) et de vigne (451) en vue de la fourniture de greffons aux professionnels canadiens,
- développement de techniques de détection rapides et sensibles,
- contrôle du nématode doré pour la région ouest du Canada.

Comparativement à l'organisation mise en place en France, la station assure les missions de la Station de Quarantaine des Végétaux Ligneux de Clermont-Ferrand, les missions de certification gérées par le CTIFL et l'ANTAV, et des missions relevant des Services Régionaux de la Protection des Végétaux.

Les végétaux contrôlés peuvent provenir d'importations non certifiées, de la production nationale pour exportation ou pour les besoins internes (certification ou autre), d'importations certifiées dans le cadre de l'audit des certifications étrangères.

Les principales plantes testées autres que les arbres fruitiers et la vigne sont le saule, l'orme et le chrysanthème.

La thermothérapie est réservée à l'assainissement de variétés présentant un grand intérêt. Elle permet notamment l'introduction et l'utilisation de plants contaminés.

Le conservatoire de plants sains permet de fournir des végétaux aux importateurs, aux producteurs de plants certifiés et aux échanges internationaux de matériel génétique. Ce rôle serait actuellement remis en question.

Enfin, la station maintient des plantes infectées par diverses maladies américaines et européennes. Ces témoins malades sont destinés à une utilisation interne.

5.3 Les procédures utilisées à la station de Saanichton

5.3.1 Descritpion générale

- Les envois d'échantillons de végétaux sont ouverts au laboratoire sans précautions particulières. Ils sont soumis à un examen visuel. Dans tous les cas ils ont, au préalable été inspecté au point d'entrée sur le territoire canadien. Les responsables de la station estiment que des mesures devraient être prises lors de l'ouverture des colis afin d'éviter la dissémination éventuelle d'organismes pathogènes portés par les végétaux introduits. C'est dans ce sens que le projet de modernisation de la station a été conçu (cf. paragraphe 5.4.2).
- Les échantillons pour lesquels l'examen visuel n'a pas révélé la présence de parasites de quarantaine (dans le cas inverse les échantillons sont détruits) sont numérotés et enregistrés.
- Si nécessaire, il est procédé à une fumigation. Ce traitement est particulièrement important pour la vigne.

- A partir des échantillons introduits 4 pieds-mères sont produits.
  - Trois d'entre eux sont placés en réserve.
  - Le dernier est indexé.
    - Si aucune maladie n'est détectée, ce plant sert à produire deux pieds-mères définitifs et les plantes en réserve sont détruites.
    - Si la présence de pathogènes est mise en évidence, l'échantillon peut être soumis à une thermothérapie ou bien les pieds en réserve peuvent être utilisés pour l'indexage. Ceux-ci sont également utiles dans le cas de non reprise du plant destiné à l'indexage ou de mort de celui-ci en cours d'analyse (l'indexage sur plantes ligneuses tel qu'il est pratiqué à la station requiert de deux à trois années).

Les procédures de réception et de traitement des échantillons sont précisées dans les documents ci-après. Le document 8 décrit la phase de réception et de décision des opérations à effectuer. Le document 9 est le formulaire complété pour chaque échantillon. Il précise les tests à effectuer et sert de support d'information entre le responsable et les membres de son équipe. Les suivants détaillent les analyses pratiquées pour la recherche des différents organismes pathogènes et les indicateurs utilisés dans chaque cas. Le document 10 concerne les arbres fruitiers, le 11 la vigne. Dans ce cas, tous les indicateurs ne donnant pas les mêmes résultats suivant les conditions locales, deux indicateurs différents sont utilisés pour la détection de chaque parasite.

Les opération réalisées à la station sont tarifées. L'indexage des plants fruitiers coûte 800$, celui de la vigne 500$, et la thermothérapie 2000$. L'objectif fixé par le Ministère de l'Agriculture est de parvenir à une facturation représentant 50% du coût réel des analyses.

5.3.2 Responsabilités

Le responsable de chaque unité :
- procède à l'ouverture des colis,
- décide les tests à réaliser,
- effectue les lectures d'indexage
- et valide l'ensemble des résultats obtenus.

Il dirige l'ensemble des opérations de contrôle qui sont menées dans son secteur. Autrement dit, il valide l'ensemble des résultats obtenus par quelque méthode que ce soit. Mais, il est le seul habilité à effectuer les lectures d'indexage car celles-ci exigent une très grande expérience.

5.3.3 Traçabilité

La traçabilité est assurée par l'enregistrement de tous les actes pratiqués sur l'échantillon et l'archivage de tous les documents le concernant. Cet enregistrement informatisé depuis de nombreuses années comporte les informations suivantes :
- l'identité et l'origine de l'échantillon,
- la filiation complète de l'échantillon dans la station et sa situation physique,
- les tests réalisés et les résultats de ces tests,
- les décisions prises,
- les personnes physiques ou morales auxquelles l'échantillon a été distribué.

Le formulaire 12 montre un exemple fictif d'enregistrement d'un échantillon en cours de testage à la station.

RECEIVING AND PROCESSING OF NEW SAMPLES

1. Complete and verify VIRUS INDEXING APPLICATION form.

2. Make and affix Q NUMBER label to sample.

3. Complete 'NEW SHIPMENT INFORMATION AND FOLLOWUP FORM '. For grapes use the GRAPEVINES version. Make copies for the propagator and the technician.

4. Give the SAMPLE, a copy of the INDEXING APPLICATION FORM, and a copy of the NEW SHIPMENT INFORMATION AND FOLLOWUP FORM - GRAPEVINES to the grape propagator.

5. Give a copy of the NEW SHIPMENT INFORMATION AND FOLLOWUP FORM - GRAPEVINES to the technician.

6. Give the other INDEXING APPLICATION FORM copies and the original NEW SHIPMENT INFORMATION FORM AND FOLLOWUP FORM to the clerk.

7. Verify that information entered into the computer is complete and factual. This could save us a lot of grief in the future by ensuring that we do not have to hunt for missing information in case of a problem.

NEW SHIPMENT INFORMATION AND FOLLOWUP SHEET - GRAPEVINES

SHIPMENT:	ARRIVAL DATE:

		DATE DONE	INITIALS
APPLICATION FORM COMPLETED:			
ENTERED INTO COMPUTER:			
SEND APPLICATION COPIES TO:	NOBODY		
	CONSIGNEE		
	PORT OFFICE		
	OTHER		
CLONE REPORT MAILED:			
CLONE REPORT RETURNED:			
CLONE INFORMATION UPDATED IN COMPUTER:			

PROPAGATION REQUIREMENTS:	FUMIGATION REQUIRED: YES NO

INDEXING REQUIREMENTS: (NOTE that letter 'G' means to be indexed by greenhouse indexing as well as field indicators.)

INOCULUM	110R	5BB	BACO	LN33	PN	RIPA	SG		DAPI	EL	HE	ISEM

GIVEN TO: BY: DATE:

INDEXING TECHNIQUES AND INDICATOR RANGES USED AT THE SAANICHTON PLANT QUARANTINE STATION

Number of replicates (rep.), temperature conditions (C) for greenhouse indexing, duration of test (days = d, weeks = w, months = m, years = y, crops = c).

VIRUS AND VIRUS-LIKE DISEASES OF APPLE

Serological Tests

- Apple chlorotic leafspot (CLSV)
- Apple stem grooving (ASGV)
- Apple union necrosis = Tomato ringspot (TomRSV)

Herbaceous Indexing		
Chenopodium quinoa	4 rep, 20 C, 20 d	CLSV, ASGV, Nepovirses
Nicotiana occidentalis 37B	2 rep, 20 C, 20 d	CLSV, ASGV, Stem pitting
Greenhouse Indexing		
R 12740 7A	2 rep, 18 C, 2 m	CLSV
Spy 227	2 rep, 22 C, 3 m	CLSV, Stem pitting
Virginia Crab	3 rep, 22 C, 5 m	ASGV, Stem pitting
Field Indexing		
Cox's Orange Pippin (Europe)	2 rep, 2 C	Russet wart, Star crack, Ringspot, Green crinkle
Golden Delicious	2 rep (5 rep), 2 c	Apple mosaic, Star crack, Proliferation (5 rep), Rough skin, Green crinkle, Russet ring, Ring spot, Leaf pucker
Granny Smith (S. Hemisphere)	2 rep, 2 c	Apple ringspot, Green crinkle
Gravenstein	2 rep, 3 y	Flat limb
Lord Lambourne	2 rep, 3 y, 2 c	Rubbery wood, Chat fruit, Apple mosaic, Dapple apple
Robusta #5 (British Columbia)	2 rep, 2 y	Robusta decline
Spartan	2 rep, 2 c	Leaf pucker, Russet ring, Dapple apple (Scar skin)
Virginia Crab	2 rep, 3 y	ASGV, Stem pitting, Dapple apple

VIRUS AND VIRUS-LIKE DISEASES OF PEAR AND QUINCE

Serological Tests

- Apple chlorotic leafspot (CLSV)
- Apple stem grooving (ASGV)

Herbaceous Indexing		
Chenopodium quinoa	4 rep, 20 C, 20 d	CLSV, ASGV
Nicotiana occidentalis 37B	2 rep, 20 C, 20 d	CLSV, ASGV, Stem pitting (Vein yellows)
Greenhouse Indexing		
Jules D'Airolles	2 rep, 22 C, 2 m	Vein yellows
Pyronia veitchii	3 rep, 22 C, 5 m	CLSV, ASGV, Vein yellows (Quince sooty ringspot), Quince stunt
Field Indexing		
Bartlett (oriental rootstock)	2 rep, 3 y	Bark split, Rough bark, Blister canker, Pear decline
Bosc	2 rep, 2 c	Stony pit, Freckle pit
Lord Lambourne	2 rep, 2 y	Rubbery wood, Dapple apple

VIRUS AND VIRUS-LIKE DISEASES OF PRUNUS

Serological Testing

- Prunus necrotic ringspot (NRSV)
- Prune dwarf (PDV)
- Apple chlorotic leafspot (CLSV)
- Plum pox (PPV)
- Tomato ringspot (TomRSV)

Herbaceous Indexing		
Chenopodium quinoa	4 rep, 20 C, 20 d	CLSV, NRSV, Nepoviruses, Tomato bushy stunt
Chenopodium foetidium	2 rep, 20 C, 20 d	PPV
Cucumis sativus	5 rep, 20 C, 20 d	Ilarviruses, Tomato bushy stunt, some Nepoviruses

Greenhouse Indexing		
Apricot seedling (European)	2 rep, 22 C, 2 m	PPV
Kwanzan	2 rep, 18 C, 2 m	Green ring mottle
Peach seedling (European)	2 rep, 22 C, 2 m	PPV, CLSV
Shiro plum	2 rep, 18 C, 2 m	Plum line pattern, Peach wart
Field Indexing		
Bing (on F 12/1)	2 rep, 2 y	Cherry albino, Rusty mottles, Mottle leaf, Cherry leaf roll, Twisted leaf, Detrimental canker, Short stem, Spur cherry, Raspleaf, X-disease, Peach asteriod spot, Black canker, Strawberry latent ringspot, Arabis mosaic
Elberta (North American)	2 rep, 2 y	NRSV, PDV, CLSV, PPV, TomRSV, Peach asteroid spot, X-disease, Peach wart, Peach blotch, Peach calico, Peach mosaic, Peach rosette, Peach rosette mosaic, Phony peach, Peach yellows, Peach latent mosaic, Willow leaf rosette, Almond bud failure
Lambert (Western N.A.)	2 rep, 2 y	Rusty mottles, Cherry albino, X-disease, Short Stem
Luizet (European)	2 rep, 2 y	Apricot chlorotic leafroll
Sam	2 rep, 2 y	Little cherry, Rusty mottles
Shirofugen	4 buds, 8 w	NRSV, PDV
Tilton (North American)	2 rep, 2 y	Apricot ring pox, Apricot leaf pucker, PPV

GRAPEVINE VIRUS INDEXING PROCEDURES

Saanichton Plant Quarantine Station
September 16, 1991

All plant samples received at the Plant Quarantine Station are verified for the presence of quarantinable diseases by serology, inoculation onto herbaceous indicators and inoculation onto woody indicators.

SEROLOGY

The standard double antibody sandwich ELISA or indirect ELISA is used to detect the following viruses: grapevine fanleaf virus, arabis mosaic virus, tomato ringspot virus, and tomato black ring virus. Serological detection is carried out at least once/year for 2 subsequent years. Foliar tissue taken from grapevines is ground in standard ELISA grinding buffer containing 2.5% nicotine.

HERBACEOUS INDEXING

Herbaceous indicators are used to detect nepoviruses known to infect grapevine around the world. Routine indicators used are: *Chenopodium quinoa*, New York source, *C. quinoa*, Summerland source, and *C. amaranticolor*. Inoculations are carried out using foliar tissue at least once/year for 2 subsequent years. Additional indicators are used on an irregular basis when needed to confirm the detection of various viruses.

WOODY INDEXING

All plant samples are chip-bud inoculated onto woody indicators. Four replicates of each indicator are made, each with 2 chips or buds. Symptoms are observed on the foliage and shoots initially in the greenhouse and then in the field for three growing seasons. The bark is removed and pitting or grooving symptoms are observed on the *Vitis* hybrid LN-33 and *Vitis rupestris* St. George indicators after the third growing season.

Woody indicators

Indicator	Disease Detected
V. rupestris St. George	nepoviruses grapevine fleck disease asteroid mosaic virus rupestris stem pitting disease
V. hybrid LN-33	grapevine corky bark disease grapevine enations disease LN-33 stem grooving disease grapevine leafroll disease
V. vinifera Pinot Noir	grapevine leafroll disease
V. vinifera Baco 22A	grapevine leafroll disease Flavescence Dorée

Additional indicators will be used on an audit basis in 1992 to detect other diseases. These are:

V. hybrid Kober 5BB	Kober stem grooving disease
V. hybrid 110 Richter	grapevine vein necrosis disease

Ray Johnson

DISEASE DIAGNOSTIC MATRIX FOR NAPPO QUARANTINE PESTS

PEST CATEGORY: VIRUSES, VIROIDS AND VIRUS-LIKE DISEASES GENUS: VITIS COUNTRY: Sample only of MATRIX format

A1/A2 PEST	QUARANTINE PEST	HOST CROP	INDEXING PROCEDURE						
			VISUAL INSPECTION	SEROLOGY	HERBACEOUS INDEXING	WOODY INDEXING GREENHOUSE (G) AND FIELD (F)	LABORATORY HYBRIDIZATION PROBES	LAB CULTURE TECHNIQUES	OTHER
NEPOVIRUSES									
A1	arabis mosaic virus	grapevine	not reliable, requires bioassay and/or serology	1) ELISA 2) ISEM	Chenopodium quinoa, C. amaranticolor - 3 reps repeated twice for two years.	(F): Vitis rupestris St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	artichoke Italian latent virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	grapevine Bulgarian latent virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	grapevine chrome mosaic virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George, V. vinifera Pinot noir - 2 chips budded onto 4 reps - observed for 3 seasons			
A2	grapevine fanleaf virus	grapevine	not reliable, requires bioassay and/or serology	1) ELISA 2) ISEM	C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George, V. vinifera Baco 22A, V. hybrid LN 33 - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	peach rosette virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			

A1/A2 PEST	QUARANTINE PEST	HOST CROP	INDEXING PROCEDURE						
			VISUAL INSPECTION	SEROLOGY	HERBACEOUS INDEXING	WOODY INDEXING GREENHOUSE (G) AND FIELD (F)	LABORATORY HYBRIDIZATION PROBES	LAB CULTURE TECHNIQUES	OTHER
A1	raspberry ringspot virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	strawberry latent ringspot virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	tobacco ringspot virus	grapevine	not reliable, requires bioassay and/or serology		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A2	tomato black ring virus	grapevine	not reliable, requires bioassay and/or serology	1) ELISA 2) ISEM	C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A2	tomato ringspot virus	grapevine	not reliable, requires bioassay and/or serology	1) ELISA 2) ISEM	C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years	(F): St. george - 2 chips budded onto 4 reps - observed for 3 seasons			
NON-TRANSMISSABLE VIRUSES									
1. Rugose wood complex									
A1	grapevine corky bark disease	grapevine	not reliable, requires bioassay			(F): V. hybrid LN 33 and St. George - 2 chips budded onto 4 reps, observed for 3 seasons			
	rupestris stem pitting disease	grapevine	not reliable, requires bioassay			(F): St. George - 2 chips budded onto 4 reps - observed for 3 seasons			

A1/A2 PEST	QUARANTINE PEST	HOST CROP	INDEXING PROCEDURE						
			VISUAL INSPECTION	SEROLOGY	HERBACEOUS INDEXING	WOODY INDEXING GREENHOUSE (G) AND FIELD (F)	LABORATORY HYBRIDIZATION PROBES	LAB CULTURE TECHNIQUES	OTHER
A1	Kober stem grooving disease	grapevine	not reliable			(F): V. berlandieri x V. riparia Kober 5BB - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	LN33 stem grooving disease	grapevine	not reliable			(F): LN 33 - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	grapevine legno riccio disease	grapevine	not reliable			(F): LN, St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
2. Other diseases									
A1	grapevine asteroid mosaic virus	grapevine	possible but not definitive			St. George - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	grapevine bratislava mosaic virus	grapevine	possible but not reliable		C. quinoa, C. amaranticolor - 3 reps repeated twice for 2 years				
A1	grapevine Chasselas latent disease	grapevine	not reliable						
A1	grapevine enation disease	grapevine	yes, but not consistent			(F): LN 33 - 2 chips budded onto 4 reps - observed for 3 seasons			
A1	grapevine leafroll disease	grapevine	possible, but requires bioassay and/or serology	ELISA, ISEM and Western blot for closteroviruses for which antisera are available. Bioassay required.		(F): LN 33 and V. vinifera cv Pinot noir - 2 chips budded onto 4 reps - observed for 3 seasons			

A1/A2 PEST	QUARANTINE PEST	HOST CROP	INDEXING PROCEDURE						
			VISUAL INSPECTION	SEROLOGY	HERBACEOUS INDEXING	WOODY INDEXING GREENHOUSE (G) AND FIELD (F)	LABORATORY HYBRIDIZATION PROBES	LAB CULTURE TECHNIQUES	OTHER
A1	grapevine little leaf disease	grapevine	yes						
A1	grapevine vein mosaic disease	grapevine	not reliable, requires bioassay			(F): LN 33 - 2 chips budded onto 4 reps and observed for 3 seasons			
A1	grapevine vein necrosis disease	grapevine	not reliable, requires bioassay			(F): V. rupestris x V. berlandierii 110 Richter - 2 chips budded onto 4 reps and observed for 3 seasons			
A1	tobacco necrosis virus	grapevine							
A1	tomato bushy stunt virus	grapevine							

DISEASE DIAGNOSTIC MATRIX FOR NAPPO QUARANTINE PESTS

PEST CATEGORY: MYCOPLASMATA GENUS: VITIS COUNTRY: CANADA

A1/A2 PEST	QUARANTINE PEST	HOST CROP	INDEXING PROCEDURE						
			VISUAL INSPECTION	SEROLOGY	HERBACEOUS INDEXING	WOODY INDEXING GREENHOUSE (G) AND FIELD (F)	LABORATORY HYBRIDIZATION PROBES	LAB CULTURE TECHNIQUES	OTHER
A1	Flavescence Dorée	grapevine	yes, but requires confirmation by other means.			(F): V. hybrid 8aco 22A and V. vinifera cv Chardonnay. Indicator grafted onto rooted plant. Symptoms appear at least 2-3 months after inoculation			DAPI technique.
A1	blackwood (bois noir)	grapevine	yes, but requires confirmationed by other means			(F): V. vinifera cvs Chardonnay, 8aco 22A - 2 chips budded onto 4 reps - observed for 3 seasons in field			

VIRUS INDEXING APPLICATION - DEMANDE D'INDEXAGE DE VIRUS

Centre for Plant Health
8801 East Saanich Road
Sidney, B.C. V8L 1H3
Tel: (604) 363-6650

Centre de la Defence des Vegetaux
8801 Rue Saanich Est
Sidney, B.C. V8L 1H3
Fax: (604) 363-6661

Canada
Agriculture
Food Production and Inspection Branch

Q 1831

Instructions:

1. Complete form as thoroughly as possible
2. Do not complete shaded areas
3. Send original and one copy with material
4. If test fees are applicable, an invoice will be sent to the consignee

1. Completez le formule le plus completement possible
2. Ne pas remplir les sections ombragees
3. Envoyer l'originale et une copie avec les materiaux
4. Une facture sera envoyee au destinataire s'il y a des couts associes au type d'essai

SAMPLING - DATE - D'ECHANTILLONNAGE D-J M Y-A	ARRIVAL - DATE - D'ARRIVEE D-J M Y-A	COUNTRY OF ORIGIN - PAYS D'ORIGINE	STATE OF ORIGIN - ETAT D'ORIGINE
	21 - September - 1994	U.S.A.	OR

REPORT - RAPPORT (A,C,R)	INDEX (A,U,R)	SOURCE (A,Q,U,I)	FUMIGATION (Y,N)	PORT OFFICE - BUREAU	PERMIT NO. DE PERMIS
R	R	U		VANCOUVER	123456

CONSIGNEE - DESTINATAIRE	CONSIGNOR - EXPEDITEUR
CANADA PLANTS	BAILEY NURSERIES INC.
ADDRESS - ADRESSE RR 2	ADDRESS - ADRESSE 18616 N.W. REEDER RD
ADDRESS - ADRESSE	ADDRESS - ADRESSE

CITY - VILLE	PROV/STATE - PROVINCE/ETAT	POSTAL CODE - CODE POSTAL	CITY - VILLE	PROV/STATE - PROVINCE/ETAT	POSTAL CODE - CODE POSTAL
OLIVER	BC	V0H 1T0	PORTLAND	OR	
COUNTRY CANADA			COUNTRY U.S.A.		

SHIPMENT REMARKS - REMARQUES
TEST SAMPLE AS INPUT EXAMPLE

TEST TYPE - TYPE D'ESSAI:
01 VIRUS INDEXING - INDEXAGE DE VIRUS
02 VIRUS ELIMINATION - ELIMINATION DE VIRUS
03 VIRUS INDEXING AND ELIMINATION - INDEXAGE ET ELIMINATION

FORM - FORME: S - SCION; C - CUTTING - BOUTURE; D - SEED - SEMENCE; O - ROOTED PLANT - PLANT ENRACINE; G - GRAFTED PLANT - PLANT GREFFE; T - TISSUE CULTURE - CULTURE IN VITRO; X - OTHER - AUTRES

V	TEST TYPE D'ESSAI	COST - COUT	VARIETY - VARIETE	ROOTSTOCK - PORTE GREFFE
1			HARVEST QUEEN	1 MAZZARD

COMMON NAME - NOM COMMUN	GENUS - GENRE	SPECIES - ESPECE	TYPE	GROWER NO. DU PRODUCTEUR	FORM - FORME
PEAR	PYRUS	COMMUNIS	ROOTSTOCK		G

QUANT	DECISION	DECISION DATE	VIRUS	OWNER (Y/N)	HT	HT PRIOR (1,2,3)
10	INCOMPLETE	SEP/21/1994		Y	Y	1

VARIETY REMARKS - REMARQUES SUR LA VARIETE
TEST SAMPLE

On peut ainsi à tout moment connaître, pour chaque échantillon introduit depuis 1965, son origine, les contrôles effectués et les endroits où il a été distribué. Par mesure de sécurité, les enregistrements informatiques sont sauvegardés sur un support informatique. De plus, deux duplications imprimées de ces mêmes enregistrements sont conservées, l'une d'entre elles se trouvant protégée contre l'incendie, dans un endroit différent de la station.

5.3.4 Méthodes d'analyse

Les procédures particulières pour la mise en évidence des organismes pathogènes sont en cours de rédaction. Dans ce domaine, la pathologie végétale est en retard sur la pathologie animale. Elle utilise donc les descriptions réalisées pour les analyses vétérinaires comme modèles pour l'élaboration de ses propres procédures. On trouvera en annexe 5 un exemple concernant la détection du virus de la diarrhée virale bovine.
Les éléments suivants doivent figurer obligatoirement dans les descriptions :

- généralités sur la maladie et sur le test,
- équipement nécessaire,
- réactifs utilisés,
- étapes de préparation,
- test,
- interprétation des résultats.

Les points importants et les points critiques sont mentionnés.

5.3.5 Propriété des échantillons

La liste des variétés d'arbres fruitiers et de vigne conservées à la station figure en annexe 6. A la question sur la propriété des végétaux, la réponse suivante a été apportée. Pendant la phase d'indexage, le produit végétal reste propriété de l'importateur. Ensuite, après rétrocession d'une partie des pieds-mères, si l'importateur n'a pas spécifié de restrictions particulières, le produit est fourni à qui le demande. Dans le cas inverse, les conditions particulières sont respectées. C'est l'explication apportée à la diffusion de variétés de vigne françaises introduites puis bloquées pour cause de présence de virus.

5.4 Les équipements de la station et le projet d'extension

5.4.1 Les équipements existants

Comme précisé plus haut, la station est équipée de laboratoires, de serres, de tunnels et de terrains expérimentaux.

Les serres ne sont pas étanches et elles n'ont pas de doubles parois (figures 3 et 4). Elles ne sont pas pourvues de sas d'entrée. La climatisation est assurée par un système de cooling et des ouvrants équipés de filets "insect-proof". Le cooling manque d'efficacité car il est installé dans la longueur des compartiments de serre. Le climat extérieur est moins continental qu'à Clermont-Ferrand : 0 à 4°C l'hiver (-10°C au maximum) et 25°C l'été, ce qui autorise malgré tout un contrôle satisfaisant des conditions de culture.

Lors d'indexages sur plantes herbacées, les échantillons sont préparés et inoculés sur place, sur une paillasse placée dans la chapelle concernée.

Dans les tunnels où des plantes indexées sont conservées longtemps, le sol est recouvert d'une bâche sur laquelle une épaisse couche de copeaux de bois est déposée. Les pots sont enfoncés dans ces copeaux, ce qui évite les problèmes de gel des racines des plantes l'hiver (figure 5).

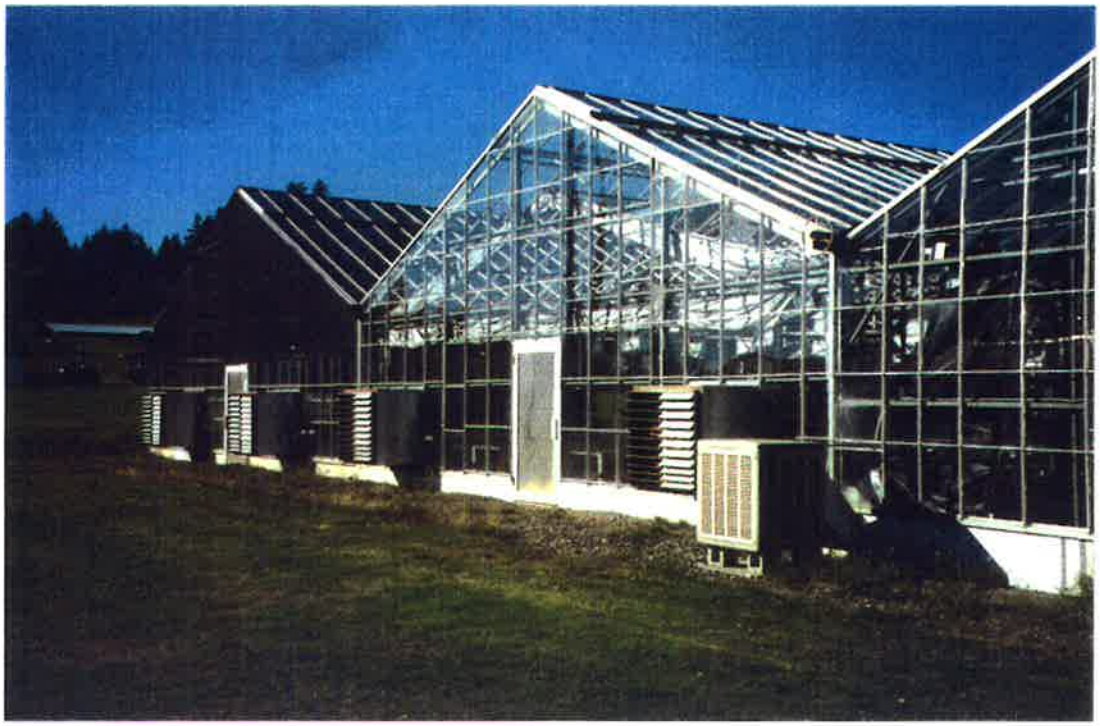


Figure 3 : Aspect général des serres

Figure 5 : Aménagement intérieur des tunnels

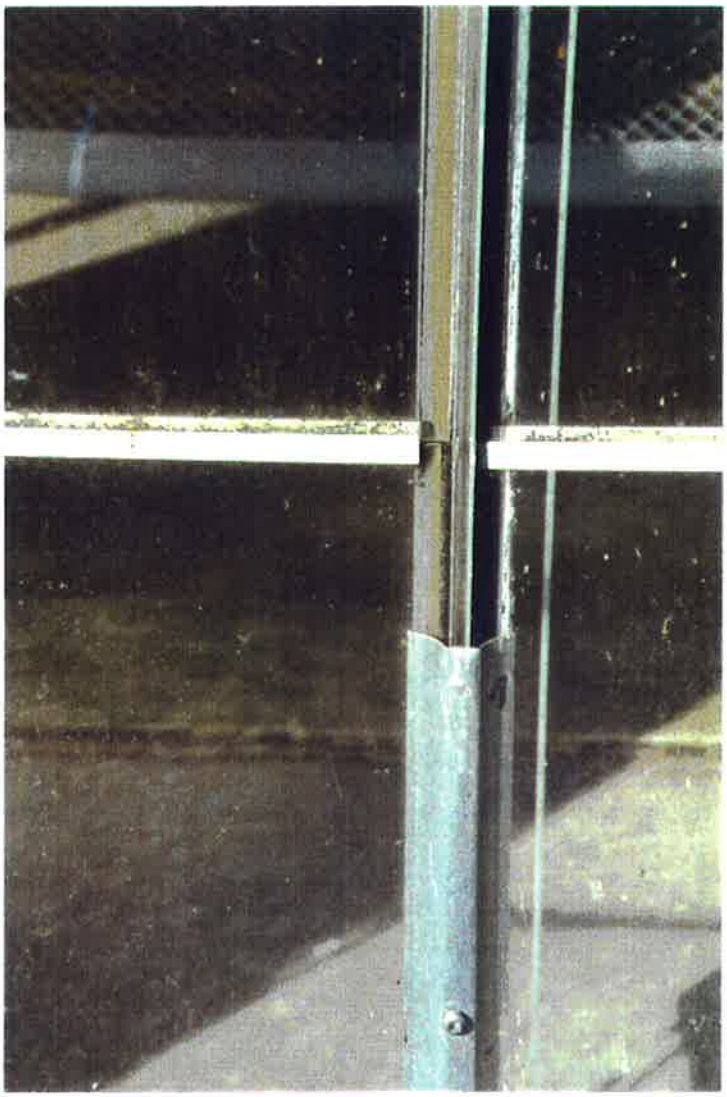


Figure 4 : Détail de l'assemblage des serres

Les conservatoires sont tous "insect-proof" et dotés de sas (figure 6). Le sol y est traité comme dans les tunnels.

Quatre chambres de thermothérapie et plusieurs chambres froides sont disponibles.

Les pièces laboratoires sont dans l'ensemble assez vétustes et exiguës. Par contre, elles disposent d'un équipement moderne et performant (figures 7 et 8).

La culture in vitro se développe car elle permet la multiplication rapide des plantes. Pour éviter les déconvenues avec les milieux gélosés, une méthode de culture en milieu liquide agité a été mise au point. La figure 9 montre une table oscillante utilisée pour cela.

La préparation des échantillons pour le test ELISA est effectuée avec un broyeur à billes multiposte (figure 10). Le traitement des échantillons ligneux à l'aide de ce broyeur est rendu possible grâce à un appareil mis au point à la station. Il s'agit d'un taille-crayon muni d'un moteur (figure 11). Les virus peuvent être extraits des fins copeaux de bois ainsi obtenus.

Tous les effluents solides, quelle que soit leur origine, sont détruits par autoclavage.

5.4.2 Le projet de construction

Prenant en compte l'évolution des besoins de la station et des techniques utilisées, la vétusté des locaux, les problèmes de sécurité pour l'environnement...etc., Agriculture Canada projette de construire de nouveaux locaux à la station. On en trouvera le plan ci-après (document 13) et quelques données techniques se trouvent en annexe 7.

Ce projet a été évalué à 7 millions de dollars soit environ 28 millions de francs. Les dépenses se répartissent de la façon suivante : terrassements 2%, structures 21%, partition intérieure 5%, aménagements intérieurs 4%, fluides 43%, études et plans 10%...

Les options techniques suivantes ont été choisies :

- pas d'incinération sur place, les déchets seront autoclavés puis transformés en compost;
- les déchets chimiques seront traités à l'extérieur par une entreprise spécialisée;
- les pathogènes à haut risque seront pris en compte :
  - espace clos pour la réception des colis,
  - autoclavage de tous les déchets,
  - laboratoires de types L3, maintenus en dépression par rapport à l'extérieur;
- tous les laboratoires seront maintenus en dépression;
- une seule grande pièce laboratoire et une salle de préparation, plutôt que plusieurs petits laboratoires;
- les serres seront de type S2 (c'est-à-dire sans traitement des effluents gazeux).

Les surfaces suivantes ont été retenues :

- laboratoire de reception 20m^2,
- laboratoire de diagnostic 61m^2,
- salle de préparation 20m^2,
- quatre chambres froides de 15m^2 chacune,
- trois salles de vitroculture de 31m^2 chacune,
- bureaux 11m^2 chacun,
- archives 15m^2.

Les règles de sécurité canadiennes imposent des fenêtres qui ne s'ouvrent pas et deux portes par laboratoire.

Figure 6 : Monsieur Ray JOHNSON à l'entrée d'un tunnel conservatoire

Figure 7 : Vue générale d'un laboratoire

Figure 8 : Exemple d'équipement moderne, un thermocycleur

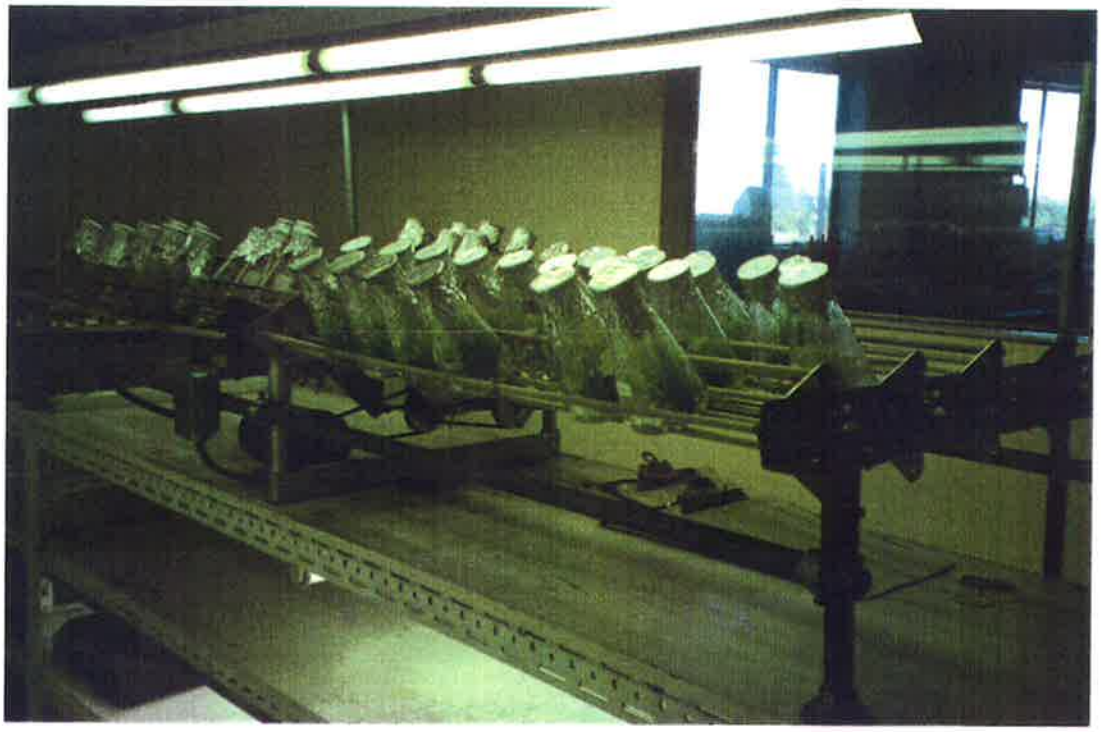


Figure 9 : Table oscillante utilisée pour la culture de vitroplants en milieu liquide

VISITING SPECIALIST
STUDENTS
SHOWER
TECHNICIAN
LABORATORY
WC
AGRONOMIST
COMPUTER ROOM
WORKROOM / LIBRARY
AGRONOMIST
RECEPTION / OFFICE MANAGER
WAITING
ELECTRICAL ROOM
EMERGENCY GENERATOR
BOILER PLANT
WORKSHOP
WASTE COLLECTION
COLD STORAGE
COLD STORAGE
PROPAGATOR'S WORKROOM
PROPAGATION ROOM
HEAT-THER. PRE-COND.
HEAT-THER. PRE-COND.
HEAT-THER. PRE-COND.
HEAT-THER. PRE-COND.
HEAT-THER. PRE-COND.
HEAT-THER. PRE-COND.
CHEMICAL TREATMENT
VENTED LOCKERS
COLD STORAGE
COLD STORAGE
ROOTING BENCH
ROOTING BENCH
OPEN AREA GROWTH CHAMBERS
MALE
LUNCH ROOM
FEMALE CHANGE
MALE CHANGE
RECEPTION / UNPACKING
LOBBY
FEMALE
CUSTODIAL STOR.
TISSUE CULTURE GROWTH ROOM
TISSUE CULTURE TRANSFER ROOM
TISSUE CULTURE GROWTH ROOM
E.M. LAB
DARKROOM
FEED / BEDDING
GENERAL STORAGE
SCIENTIST OFFICE
PHOTOGRAPHY STUDIO
CASE WASHING
MOUSE ROOM
SCIENTIST OFFICE
STAIRS DOWN TO GARAGE BELOW
RECEPTION / WAITING
MANAGER
COATS
MAIL ROOM
CLERICAL
BOARD ROOM
EQUIP.
EQUIPMENT
EQUIP.
EQUIPMENT
EQUIPMENT
EQUIPMENT
EQUIPMENT
SAMPLE PREP.
DIAGNOSTIC LAB
GENERAL LAB
MONOCLONAL LAB
SCIENTIST OFFICE
E.M. PREP
G & O LAB
TREE FRUIT LAB
MANAGER
GENERAL WORK AREA
OPEN OFFICE WORK AREAS TECHNICIANS / SPA
ADMIN. FILES / STOR
DIRECTOR
DIAGNOSTIC FILES
CLERK
LIBRARY
BIOLOGIST
BIOLOGIST
COMPUTER ROOM

5.5 Le point sur quelques maladies et sur les recherches en cours

5.5.1 La maladie du Little Cherry

Le Little Cherry est connu depuis longtemps en Ontario où il ne cause pas de dégâts majeurs. Introduit en Colombie Britannique dans les années 1950, il a détruit les cerisiers de toute une vallée. Depuis lors une autre vallée de production est traitée en zone protégée où toute introduction extérieure est interdite. La maladie est causée par un virus filamenteux de 2000nm. Il peut être mis en évidence sur indicateurs Sam et Canindex. Les hypothèses pour expliquer l'importance des dégâts constatés en Colombie Britannique sont les suivantes :

- le climat plus chaud des vallées de la Colombie aurait permis l'extériorisation des symptômes;
- les cultivars présents à l'Ouest seraient plus sensibles;
- les porte-greffes différents auraient sensibilisés les plantes;
- il existerait des vecteurs (non précisés) de ce virus en Colombie.

Mais aucune explication prouvée ne peut être apportée.
La mise au point de méthodes de détection est actuellement orientée vers la production d'anticorps monoclonaux et la recherche d'amorces pour l'amplification génique.

5.5.2 Le virus du Tomato Ring Spot

Le Tomato Ring Spot Virus a été détecté sur vigne dans le Sud de l'Ontario. Des mesures d'éradication ont été prises, et les cultures de la vigne et des arbres fruitiers ont été interdites dans la zone concernée. En Colombie Britannique, les contrôles sont fréquents car le nématode vecteur est présent. Potentiellement, le pêcher et la vigne sont les plantes présentant les plus gros risques. La maladie s'exprime mieux sous des climats plus chauds qu'au Canada. Il y a donc un risque potentiel important pour la France. De plus, la spécificité de transmission par les nématodes nord-américains semble remise en cause (cf. document 14, résumé 17.1.22)

5.5.3 Les maladies du pêcher et du cerisier

Il existe sur les pêchers beaucoup de maladies mal identifiées et difficiles à différencier.

- Le Peach Rosette serait causé par un MLO, mais l'agent n'a jamais été clairement identifié. Il est transmis par des cicadelles. On le met en évidence par indexage sur GF305 et Alberta. Cette maladie cause des dégâts importants, notamment en réduisant la durée de vie de l'arbre.
- Le Peach Rosette Mosaïc Virus est un Nepovirus transmis par nématodes. Il existe un sérum permettant de le détecter. Cependant aucune recherche particulière n'est conduite à la station sur ce sujet. Aucune information intéressante n'a donc pu être recueillie.
- Le Peach Latent est un viroïde.
- Le Cherry Twisted Leaf est un virus flexueux.
- Le Peach Mosaïc Virus est un virus flexueux sur lequel les recherches se poursuivent. Un antiserum produit contre le Cherry Mottle Leaf Virus (autre virus flexueux infectant le cerisier) reconnaît également le Peach Mosaïc. Les deux agents pathogènes sont transmis par des acariens. Il pourrait s'agir du même virus mais cette hypothèse n'a pas encore été prouvée (cf. document 14, résumé 17.1.20) . D'autres observations suggèrent que les souches américaines du Cherry Mottle Leaf Virus sont différentes des souches européennes. Pourrait-il s'agir de deux virus différents ? C'est l'un des sujets de recherche de M JAMES, pour lequel il souhaite une coopération avec la France.

17.1.18

CYTOPATHOLOGY AND ULTRASTRUCTURAL EVIDENCE THAT AN ISOLATE OF TOMATO SPOTTED WILT VIRUS IS A MEMBER OF BUNYAVIRIDAE. K.S. Kim, R. Gergerich and S.C. Goeke. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, USA.

Tomato spotted wilt tospoviruses (TSWV) have recently been recognized as related to the Bunyaviridae, an established group of animal viruses, due to their similarities in particle morphology, molecular composition, mode of transmission and genome structure. Ultrastructural study of an isolate of TSWV from yellow ironweed (Verbesina alternifolia), which has no serological relationship to common isolates of TSWV and impatiens necrotic spot virus, revealed that the cisternae of Golgi apparatus were involved in virus assembly. Although the involvement of Golgi body in virus replication has not been demonstrated in known tospoviruses, the occurrence of virions within Golgi vesicles has been used as a reliable characteristic in the classification of viruses in the Bunyaviridae in animals. This and other cytopathic effects and serolgical data presented in this study further support the argument that plant tospoviruses have a taxonomic relationship with the Bunyaviridae, and that the yellow ironweed isolate is a distinct strain from known tospoviruses.

17.1.19

A FLEXUOUS VIRUS ISOLATED FROM CAULIFLOWER PLANTS IN APULIA (Southern Italy) C.Vovlas, A.di Franco, F. Grieco. Dipartimento di Protezione delle Piante dalle Malattie, Università degli Studi di Bari, Italy.

From cauliflower plants, showing necrotic streaks of older leaves and stunting a virus with flexuous particles c-600 nm. in lenght was isolated. The virus was purified from artificially infected Nicotiana occidentalis plants and an antiserum was raised with a titre of 1:256. Purified particles were tested with different potexvirus antisera in IEM decoration experiments. The cauliflower isolate showed a mild serological relation with viola mottle potexvirus only.

Cytopathological alterations of Chenopodium amaranticolor cells, were similar to those causing by potexvirus. The symptoms of the disease were reproduced by artificial inoculation under natural conditions but no transmission was obtained using aphids under glasshouse conditions. Experiments are in progress to determine the physicochemical and molecular properties of the virus.

17.1.20

COMPARISONS OF CHERRY MOTTLE LEAF VIRUS AND A VIRUS ASSOCIATED WITH PEACH MOSAIC DISEASE. [1]D. James, and [2]W.E. Howell. [1]Saanichton Plant Quarantine Station, Agriculture Canada, 8801 East Saanich Road, Sidney, B.C., Canada, V8L 1H3, and [2]IR-2/NRSP5, Washington State University, Prosser, Washington, USA, 99350.

A closterovirus-like particle was isolated from a peach mosaic affected tree. Monoclonal antibodies developed against cherry mottle leaf virus (CMLV) detected the peach mosaic associated particles in both ELISA and Western blot tests. In Triple Antibody Sandwich ELISA using a rabbit polyclonal antibody to CMLV as the trapping antibody, a CMLV monoclonal as the second antibody, and rabbit anti-mouse conjugate, higher absorbance values were observed for CMLV. In Western blot analysis the virus coat protein associated bands migrated at identical rates, suggesting that the coat protein subunits are of similar sizes, 20.5 kilodaltons. Some differences in symptomatology were observed. The peach mosaic associated particles induced no symptoms on the CML indicator Bing cherry. The peach mosaic associated virus, but not CMLV, was transmitted to Nicotiana occidentalis '37B'. Also, Chenopodium amaranticolor is a symptomatic host for both viruses, but CMLV produces more severe symptoms. It appears that these may be strains of a single virus.

17.1.21

A DOUBLE VIRUS INFECTION OF HYDRANGEA IN BASILICATA (ITALY). M. Nuzzaci, I. Camele, G.L. Rana and A. De Stradis. Department of Biologia, Difesa & Biotecnologie Agro-Forestali, Faculty of Agriculture, University of Basilicata, Potenza, Italy.

Some plants of Hydrangea macrophylla, showing general stunting, chlorotic or brown ringspot of basal leaves and reddening and rolling of apical leaves, were observed in some gardens of Potenza in Basilicata region (southern Italy).

To discover which virus/es infected the above plants, sap extracted from their leaves in presence of 0.1 M neutral phosphate buffer containing 10 g/l sodium ascorbate, was subject to observation at electron microscope, mechanical inoculation to herbaceous plants (Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Nicotiana glutinosa) and immuno sorbent electron microscopy followed by decoration using antisera to some elongated and spherical viruses comprehensive of hydrangea ringspot (HRSV) and tomato spotted wilt virus (TSWV).

The results of these tests showed that plants of H. macrophylla with the above symptoms consistently harboured a double infection by HRSV and TSWV. This seems the first report of a double infection by TSWV and HRSV in hydrangea in Italy.

17.1.22

APPARENT LACK OF SPECIFICITY IN TRANSMISSION OF N. AMERICAN NEPOVIRUSES BY *XIPHINEMA* NEMATODES. A.T.Jones & D.J.F.Brown. Scottish Crop Research Institute, Dundee, UK; T.C.Vrain. Agric. Canada, 6660 N.W. Marine Drive, Vancouver, Canada; J.M.Halbrendt, Dept. Pant Pathology, Pennsylvania State University, Biglerville, USA; R.T.Robbins, Nematology Laboratory, University of Arkansas, Fayetteville, USA.

Nepoviruses occur world-wide but are most common in Europe and N. America where their effects on crops can be very damaging. About 30% of recognised members of the nepovirus group have been shown to be transmitted by species of *Longidorus* and *Xiphinema* nematodes. Early studies reported that a high level of specificity occurred between nepoviruses and their nematode vector and, in some instances, between virus strains and specific nematode populations. This specificity is determined by RNA-2 which, in nepoviruses, carries the determinant for the virus coat protein (CP). However, recent studies in Scotland on three unrelated nepoviruses from N. America have indicated a comparative lack of specificity in their transmission by nematodes. Thus, populations of *X. americanum* from Arkansas, California and Pennsylvania, *X. californicum* from California, and *X. rivesi* from Pennsylvania, each transmitted tomato ringspot (TomRSV), tobacco ringspot and cherry rasp leaf viruses. This indicates a lack of specificity in transmission of these viruses by different species and geographical populations of nematode vectors. Furthermore, a population of *X. bricolensis* from a raspberry plantation in Washington State, was found naturally transmitting at least three distinct serotypes of TomRSV. This and other information indicates considerable variability in the TomRSV-CP gene both in isolates at, and between, geographical sites, apparently without affecting greatly its transmissibility by vector species. Studies on raspberry ringspot virus in Scotland have also detected a wide range of serological variants, both at, and between geographical sites. Molecular analysis of this variability in the CP gene of nepoviruses is being studied to understand the basis for the attachment and release of virus particles in nematode vectors.

17.1.23

GENOMIC DIVERSITY AND CROSS PROTECTION BETWEEN AND WITHIN SUBGROUP I AND II CUCUMBER MOSAIC VIRUS ISOLATES FROM PEPPER. G. Rodriguez-Alvarado and J. A. Dodds. Department of Plant Pathology, University of California, Riverside, CA 92521, U.S.A.

Isolates of cucumber mosaic virus (CMV) from pepper in California were found to belong to either subgroup I or II based on subgroup specific cDNA probes, dsRNA analysis, and host range studies. Naturally mild strains were only found in the collection of type II isolates. 19 isolates from one field were all subgroup II types, but could be subdivided into additional categories by RNAse protection assays using minus-sense RNA transcript probes representing 3 different lengths of the 3' part of RNA 3 of one type II strain. The longest probe included the entire capsid protein sequence. The results indicated a considerable degree of genomic heterogeneity for a single virus within a single field. A mild strain of subgroup II was able to offer cross protection against replication of severe strains from either subgroup I (determined by symptoms, virion and dsRNA analysis) or from subgroup II (determined by symptoms and RNAse protection assay). RNAse protection was shown to be a useful tool for in field genome diversity and cross protection studies involving closely related genomes of CMV.

5.5.4 Le recherches en cours sur les maladies de la vigne

L'essentiel des efforts de recherche sur la vigne concerne le virus de l'enroulement. La quantité de sérotypes que présente ce virus constitue un obstacle majeur au développement de méthodes sérologiques de détection. Le projet est de préparer, dans un délai de quatre ans, des amorces pour l'amplification génique. De plus des travaux sont conduits pour finaliser le postulat de Koch. Pour cela des techniques de vitroculture de plants infectés ont été mises au point. Le virus est en plus grande quantité et mieux réparti dans les vitroplants. Pour infecter des cellules saines plusieurs techniques pourraient être utilisées : bombardement de protoplastes avec des particules d'or recouvertes d'ARN viral ou insertion de l'ARN viral à l'aide d'un surfactant lipophile, puis régénération in vitro de plants de vigne.

Les travaux concernent également le Corky Bark, pour lequel des méthodes de détection par amplification sont espérées.

5.5.5 L'apport des biotechnologies

Les recherches en biotechnologie concernent à la fois la biologie moléculaire (ADN, ARN, amplification) et la sérologie (anticorps monoclonaux). Les principaux agents pathogènes étudiés sont des parasites de quarantaine non transmissibles. Des travaux sur l'identification variétale par hybridation moléculaire ("DNA fingerprinting") sont également conduits.

La spécificité des réactifs et notamment des sérums ne peut être garantie qu'après le testage de nombreuses souches du pathogène considéré. Les anticorps monoclonaux s'avèrent parfois trop spécifiques. Des procédures écrites et détaillées de vérification de la qualité des réactifs sont en cours d'élaboration sous l'impulsion d'Agriculture Canada. Les réactifs sérologiques sont réévalués à chaque changement de lot, ce qui incite la station à produire ses propres sérums.

Le premier problème rencontré en amplification génique est la préparation de l'échantillon. Les tissus ligneux produisent beaucoup de polyphénols qui inhibent les réactions. L'extraction à l'aide du kit de purification Quiagen™ donne de bons résultats. Elle est un peu chère mais permet de s'affranchir des risques liés à la manipulation du phénol et du chloroforme. C'est la seule méthode réellement efficace actuellement pour travailler avec les plantes ligneuses.

Un nouveau thermocycleur de conception simple est utilisé avec satisfaction. Les réactions sont réalisées dans des tubes capillaires qui autorisent des échanges de température rapides et des volumes réactionnels réduits. Les changements de température sont effectués par ventilation d'air ce qui assure une grande homogénéité entre les tubes. Le document 15 présente cet appareil. Une lettre d'information est diffusée entre les utilisateurs de ce système. Elle permet des échanges d'informations pratiques (annexe 8).

La séparation des pièces utilisées pour la réalisation de l'amplification n'est pas obligatoire dès lors qu'il y a peu de mouvements dans le laboratoire et que les personnels sont conscients des risques de pollution.

Le meilleur rapport qualité-prix est obtenu avec la Taq polymérase commercialisée par GIBCO-BRL.

The 1605
Air Thermo-Cycler

Light Speed!

IDAHO TECHNOLOGY
149 CHESTNUT ST.
IDAHO FALLS, ID. 83402
1-800 735-6544
208 524-6354
FAX: (208)524-1605

Idaho
Technology

Air Thermo-Cycler

Light Speed Thermo-Cycling

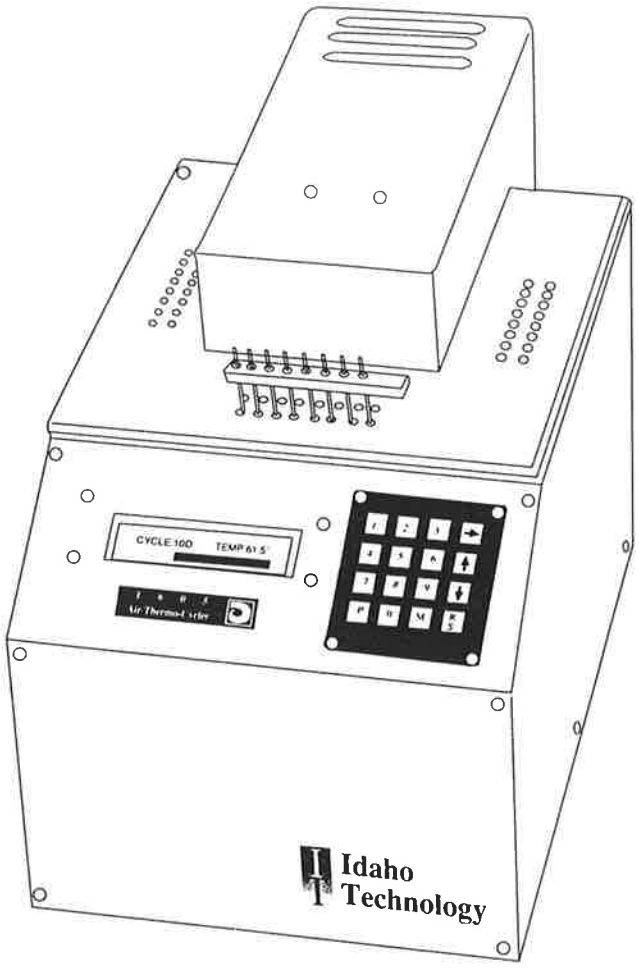


Figure 1. The 1605 Air Thermo-Cycler (ATC).
patent pending

Fastest Temperature Cycling Available

Traditionally, 30 cycle reactions have taken half a day to run. The 1605 Air Thermo-Cycler (ATC) can complete a 30 cycle reaction in as little as 10 minutes. If it is possible to run a reaction so quickly, why are other thermal cyclers so slow?

Temperature cyclers generally use micro-centrifuge tubes or modified microtitre plates. These plastic containers insulate the sample. The temperature of a 100 µl sample within a microfuge tube lags 20-40 seconds behind the block temperature. This, combined with sluggish temperature ramp rates of heating blocks, explains why they are so slow.

There is another major factor - temperature hold times. A common protocol for temperature cycling is 1 minute at 94° C, 2 minutes at 55 ° C, and 3 minutes at 72° C. Even if instantaneous temperature transitions were possible, one cycle would take 6 minutes. The actual enzymatic extension occurs in about 10 seconds for a 500 b.p. fragment. This leaves 97% of the total time wasted, not even counting ramp time. What are these machines doing? They are waiting for steady-state conditions to bring all the samples to roughly the same temperature. Unfortunately, because of the geometry of the heating elements and metal blocks, even the steady-state condition may not produce uniform temperatures across all samples. At best, these machines suffer from temperature imbalance during the long wait for equilibrium conditions. At worst, the imbalance is permanent.

Various manufacturers have partially addressed these issues, by reducing the mass of the block, producing thin-walled tubes or microtitre plates, and by designing systems which achieve more uniform steady-state conditions. With the ATC we have gone far beyond these efforts.

Light Speed!

Maximum Speed

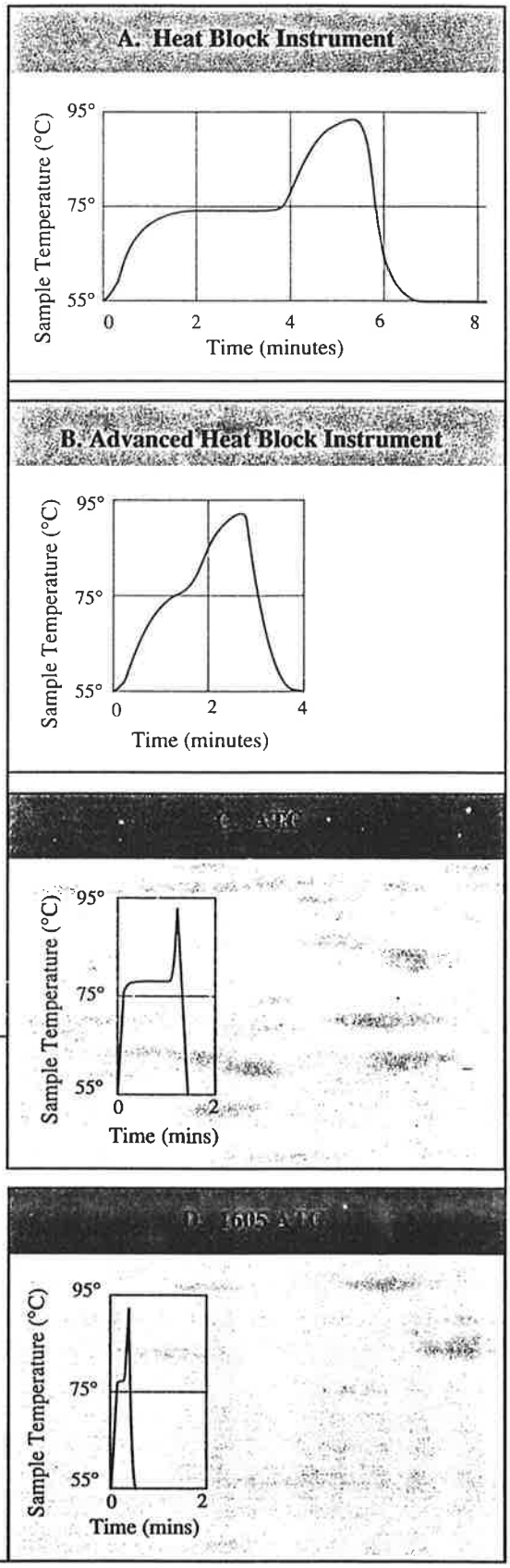
The ATC uses micro-capillary tubes which are ideally suited to temperature cycling since they have a very thin wall and a high surface area to volume ratio. Capillary tubes cut sample temperature lag to less than a second. We combine the ideal sample container with the ultimate heating and cooling medium - high velocity air. The use of air as the medium gives high temperature ramp rates. Tornado-like conditions inside the cylindrical reaction chamber ensure both temperature uniformity and rapid heat exchange with the sample. This allows us to spend less than one second at the high and low temperatures and still be certain that each sample has reached the target temperature. A fraction of a second is adequate for the reaction.

Maximum Homogeneity

The ATC features dynamic but uniform conditions inside the reaction chamber. The radial symmetry of the fan blade, halogen light bulb and air inlet (see Fig. 3) all ensure that the samples arranged within the chamber are at the same temperature.

Better Reactions

With fast cycle times came an unexpected result - cleaner reaction products (see Fig. 2). Short cycle times have been shown to greatly increase product purity (Wittwer, C.T., Garling, D.J. 1991. Biotechniques. 10:76-83.) The amount of nonspecific product is directly related to the time spent near the lower temperature. Faster cycling produces cleaner electrophoresis gels. If low specificity is desired, the ATC can be programmed for hold times. If you want maximum specificity, the ATC is the only choice.



Temperature Profile	Time for 30 Cycles (min)
A. Heat Block Instrument	240
B. Advanced Ht. Blk. Instr.	120
C. ATC	40
D. 1605 ATC	15

Figure 2. Temperature profiles (right) and photograph (above) compare product specificity in samples run on a common heat block instruments and on the ATC. Samples were cycled 30 times through the temperature cycles indicated at right.

Simple Procedure

Sample Preparation and Handling

The 1605 ATC is now adapted for use with positive displacement pipette tips. Samples must be mixed, loaded into pipette tips, or glass capillary tubes, sealed, temperature cycled, and finally analyzed. To prepare for cycling, samples are mixed in a 96 well microtitre plate, or in microfuge tubes with 5X buffer optimized for rapid cycling. Capillary tubes are loaded by capillary action either individually or eight at a time using an optional capillary rack. The ends of each tube are sealed with a cigarette lighter or Bunsen burner in about the same time that it takes to overlay the sample with mineral oil and close a microfuge tube. Up to 48 samples can be loaded into the reaction chamber and cycled.

Reduced Chance of Sample Contamination

After amplification, the ends of the glass capillaries can be quickly scored with a file (provided in the Start-up Kit) and snapped off. Pre-scored capillary tubes are also available. The capillary tubes serve both as a transfer pipette and container for temperature cycling. The reaction product, optionally containing Ficoll and an electrophoresis indicator dye, can be directly emptied into a gel well without exposure to an intermediate pipette tip or to extraction procedures. Using the capillary tube as a transfer pipette minimizes the risk of aerosolization and contamination.

Convenient User Interface

The single screen user interface allows for easy user programming. Two types of tests may be run, holding one temperature or cycling between two or three temperatures. One may program an exact protocol in either of these two modes or tailor a protocol linking the two modes. Parameters may also be set for larger samples (25 and 50 µl). Multiple user programmable protocols can be stored in each mode and linked in any combination.

Save Money on Reagents

The ATC will pay for itself by savings on expensive enzymes and reagents. Typically, reactions are done in 100 µl volumes. The ATC is optimized for 10 µl samples, saving you tenfold on reagents. A 10 µl reaction yields enough product to give a strong band on an ethidium bromide-stained agarose gel. With 10 µl samples and quick runs, the required parameters for any specific reaction can be easily and inexpensively optimized.

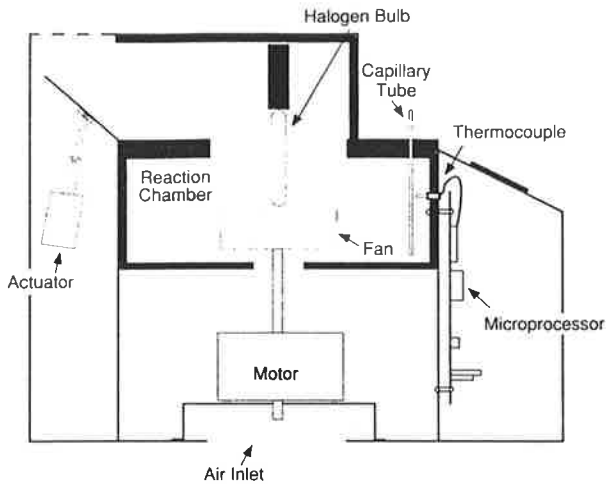


Figure 3. Interior view of the ATC. Air flow through the machine is indicated by the colored arrows.

Specifications of the 1605 Air Thermo-Cycler : patent pending

Sample vessel:	Positive displacement pipette tips or micro-capillary tube, 5-50 µl capacity. A total of 48 samples may be run simultaneously.
Cycle times:	Between 30-60 seconds for most applications.
Temperature Ramp Rates:	>10°C/sec.
Temperature Uniformity:	Calibrated to ± .5° C. Rapid temperature transfer by high velocity air guarantees exceptional temperature uniformity.
Display:	Forty character LCD (2 x 20). Cycle number and temperature are displayed and continuously updated during each run. All profile parameters are user programmable from the keyboard.
Keypad:	A sixteen key (real button) keypad allows numerical protocol entry.
Dimensions:	Ht: 10.5" ,W: 9.25", Depth: 15", Wt: 26.5 lbs.

Your Canadian Distributor

Professional Diagnostic Inc

Telephone: 1-800-661-4556
Facsimile: 1-800-661-4557

4-1605

La digestion du produit de l'amplification par une enzyme de restriction adaptée n'est pas considérée obligatoire. Cela dépend de l'organisme testé, de sa complexité et des risques de faux négatifs et de faux positifs. Avant tout la qualité de l'extraction et des amorces influencent le résultat de l'analyse.

Pour apporter la preuve d'une infection il serait idéal d'utiliser deux méthodes d'analyse différentes qui donnent le même résultat. En tout état de cause, il convient de toujours conserver des extraits pour éventuellement répéter les tests.

6. CRITIQUE DU PROJET FRANCAIS

Après présentation du projet français, M LANTERMAN a estimé que les réflexions et choix opérés allaient dans le même sens que le projet canadien. Il a insisté sur l'importance de la procédure, de la traçabilité et de la rigueur expérimentale. Il a proposé un échange régulier d'informations et des collaborations dans le domaine scientifique. j'ai accepté ces propositions en précisant que la coopération scientifique ne pourrait commencer que quand un outil fonctionnel aurait été créé à Clermont-Ferrand.

M JOHNSON a estimé que l'option française qui consiste à créer plusieurs petits laboratoires pour mieux gérer les risques en cas de contamination fortuite présentait des avantages. Le projet canadien pourrait être revu dans ce sens.

M l'Attaché agricole a insisté sur l'importance des implications de l'analyse des risques dans la mise en oeuvre des accords du GATT. Il faut apporter la preuve du risque d'où l'intérêt d'une station de quarantaine qui ne ferme pas complètement la porte.

M l'Ambassadeur s'est tout d'abord étonné que la France ne possède pas déjà de station de quarantaine, puis s'est félicité du projet en gestation. Il a insisté sur deux points :

- l'éthique des stations de quarantaine qui ne doivent pas faire commerce des produits qu'elles analysent et conservent (cf. problèmes franco-canadiens concernant la vigne),
- les nécessaires coopérations à instaurer en Europe d'une part et avec le continent américain d'autre part.

7. CONCLUSION

Les buts fixés pour cette mission ont été atteints, à l'exception des informations techniques sur la maladie du Peach Rosette Mosaïc Virus. Des greffons infectés par divers virus ont été rapportés et greffés aussitôt sur des porte-greffes sains à la Station de Quarantaine de Clermont-Ferrand (cf. document 16). Ces plantes serviront à familiariser les agents avec les symptômes causés par ces maladies, à réaliser des études sur ces maladies, et elles seront utilisées comme témoins dans les indexages pratiqués à la Station.

Il apparaît que dans son ensemble, le projet de construction d'installations de quarantaine à Clermont-Ferrand répond aux contraintes d'une station de quarantaine. Il conviendra néanmoins de prendre en compte la conduite des indexages et analyses relatifs à la vigne. Il se pourrait que dans ce domaine, le projet ait été sous-évalué. Il apparaît donc nécessaire de prendre rapidement contact avec les intervenants de ce secteur professionnel : ONIVINS, ANTAV, GRISP et INRA de Colmar.

Agriculture Canada

Food Production and Inspection Branch | Direction générale, Production et inspection des aliments

Kanadisches Bundesministerium für Landwirtschaft
Amt für Nahrungsmittelerzeugung und - Kontrolle

PHYTOSANITARY CERTIFICATE PFLANZENGESUNDHEITSZEUGNIS

No. — *Nr.* 854897

To: Plant Protection Organization of (Country of Destination)
An den Pflanzenschutzdienst des Bestimmungslandes France 940809

DESCRIPTION OF CONSIGNMENT — BESCHREIBUNG DER SENDUNG

Name and Address of Exporter — *Name und Anschrift des Exporteurs*
Agriculture Canada, Centre for Plant Health, 8801 E. Saanich Rd., Sidney, B.C., Canada V8L 1H3

Declared Name and Address of Consignee — *Name und Anschrift des Empfängers*
Mr Chauveau, DRAF-SRPV auvergne, BP 45 - RN 89 - Marmilhat, 63370 LEMPDES

Number and Description of Packages — *Zahl und Beschreibung der Packungen*	Distinguishing Marks — *Unterscheidungsmerkmale*
1 package	As addressed

Place of Origin — *Ursprungsland*	Declared Means of Conveyance — *Transportmittel laut Zollerklärung*	Declared Point of Entry — *Grenzübergangsstelle laut Zollerklärung*
Sidney, B.C.	By hand	

Name of Produce and Quantity Declared — (Botanical Name of Plants)
Name und deklarierte Menge des Erzeugnisses — (Botanischer Name der Pflanzen)

Malus sp. infected with Tomato Ringspot Virus (roots)
2 cuttings of Prunus sp. infected with Cherry Rasp Leaf Virus
2 cuttings of Prunus sp. infected with Cherry Twisted Leaf
2 cuttings of Prunus sp. infected with Line Pattern
2 cuttings of Prunus sp. infected with Little Cherry
2 cuttings of Malus sp. infected with Tomato Ringspot Virus

- This is to certify that the plants or plant products described above have been inspected according to appropriate procedures and are considered to be free from quarantine pests, and practically free from other injurious pests; and that they are considered to conform with the current phytosanitary regulations of the importing country.
- Hiermit wird bescheinigt, daß die oben genannten Pflanzen bzw. pflanzlichen Erzeugnisse sachgemäßen Kontrollverfahren unterzog wurden und als frei von quarantäne-bedürftigen Schädlingen, sowi praktisch frei von sonstigen Schädlingen gelten, und daß angenomm wird, daß sie den gegenwärtigen Pflanzengesundheitsbestimmunger des Einfuhrlandes entsprechen.

DISINFESTATION AND/OR DISINFECTION TREATMENT — ENTSEUCHUNGS — BZW. DESINFEKTIONSMETHODE

Date — *Datum*	Treatment — *Methode*	
Chemical (Active Ingredient) — *Mittel (Wirkstoff)*	**Duration and Temperature — *Dauer und Temperatur***	**Concentration — *Gehalt***

Seal — *Dienstsiegel*

ADDITIONAL DECLARATION — ZUSÄTZLICHE ERKLÄRUNG

Place of Issue — *Ort der Ausstellung*	Name of Authorized Officer — *Name des bevollmächtigten Beamten*
Sidney, B.C.	Ray Johnson
Date — *Datum*	**Signature — *Unterschrift***
September 23, 1994	Ray Johnson

- No liability attached to Agriculture Canada or any of its officers in respect of this certificate
- *Ausstellung dieses Zeugnisses durch das Bundesministerium für Landwirtschaft bzw. durch seinen Beamten erfolgt ohne Gewähr*

AGR 1327 (82/12) Canada 7530-21-036-6045

En dehors des équipements, les points les plus importants de la conduite d'une station de quarantaine sont la méthodologie utilisée et la traçabilité. Il conviendra d'être particulièrement attentif à ces aspects lors du développement de la station. Les moyens pour cela pourraient être :

- un système d'enregistrement et d'archivage cohérent et transparent,
- un système de repérage physique des échantillons adapté,
- la description et le respect des procédures,
- et une grande discipline du personnel impliqué.

Le rôle du responsable sera, dans ces domaines, prépondérant.

La proposition de futures collaborations doit être retenue comme une opportunité de placer la station de quarantaine de Clermont-Ferrand dans un contexte mondial. Il conviendra, en même temps, de trouver des partenaires européens (station de Valence ?) pour la nécessaire coopération européenne.

ANNEXE 1

DOSSIER ADMINISTRATIF

préfecture de la région
AUVERGNE

direction régionale de l' **agriculture** et de la **forêt**

service **Régional de la Protection des Végétaux**
notre référence DEMATISC.DOC
votre référence
dossier suivi par JF CHAUVEAU
téléphone 73421480
objet demande autorisation importation à titre scientifique

Ministère de l'Agriculture
DGAL / SDPV / SPRI
175, rue du Chevaleret
75646 PARIS CEDEX 13

(à l'attention de M LELION)

Lempdes, le 11 août 1994

Lors de mon prochain déplacement au Canada et à la Station de Quarantaine de Vancouver notamment, il serait souhaitable que je puisse collecter et rapporter des échantillons de plants fruitiers (greffons) infectés par divers parasites de quarantaine. Ces introductions seront ensuite maintenues dans la serre de quarantaine et utilisées comme témoins infectés lors des analyses.

La liste des parasites de quarantaine concernés (voir ci-après) a été établie avec M Jardillet et elle va être transmise au Dr Ray Johnson. Il est possible que tous ne soient pas disponibles. Néanmoins, si vous acceptez le principe d'une introduction de plants témoins dans la station de Clermont-Ferrand, je vous demanderai de bien vouloir établir une autorisation d'importation à titre scientifique pour tous les parasites.

Par ailleurs, j'ai bien noté que l'attaché agricole à Ottawa souhaitait que je ne me rende pas au Canada sans avoir été au préalable bien informé sur les problèmes bilatéraux concernant la vigne. L'agenda du mois de septembre est déjà bien rempli. Néanmoins, je trouverai une journée pour me rendre à Paris, y discuter avec vous de cette question. Les dates auxquelles je suis disponible sont le vendredi 9 et le jeudi 15 septembre.

LE CHEF DE SERVICE

JF CHAUVEAU

B P. 45 R. N. 89 Marmilhat 63370 LEMPDES
Standard : 73. 42. 14. 14 - Télécopie : 73. 90. 83. 70

LISTE DES PARASITES A INTRODUIRE A LA STATION DE QUARANTAINE EN PROVENANCE DE LA STATION DE VANCOUVER

- American Plum Line Pattern = lignées en arabesques du prunier. Greffons de prunier, cerisier et pêcher.
- Peach Yellows et Peach Rosette (MLO) = jaunisses du pêcher. Greffons de pêcher.
- Peach X Disease MLO. Greffons de pêcher.
- Peach Asteroid Spot = Taches astéroïdes du pêcher. Greffons de pêcher.
- Little Cherry = Petite cerise. Greffons de cerisier.
- Apple Mirion Necrosis = Tomato Ringspot Virus. Greffons de pommier (Red Delicious).
- Cherry Rasp Leaf Virus (Flat Apple). Greffons de pommier (delicious).
- Cherry Twisted Leaf. Greffons de cerisier.

FONDATION NATIONALE ENTREPRISE ET PERFORMANCE

Tour EP 2 Place de la Coupole - Cedex 45 - 92078 Paris La Défense

FNEP - 94.364

Monsieur Jean-François CHAUVEAU
Chef du service régional de la protection
des végétaux
Direction régionale de l'Agriculture et de la Fôret
Préfecture de la région Auvergne
B.P. 45
63370 LEMPDES

Paris, le 30 août 1994

Objet : mission spéciale Fonction Publique 1994
Constitution d'une station de quarantaine pour les végétaux ligneux - Canada

Monsieur,

Nous vous prions de bien vouloir trouver sous ce pli vos titres de transports et bons de réservation d'hôtels pour votre mission au Canada du 18 au 25 septembre 1994.

Compte tenu du forfait de 12.000 FF alloué par la FNEP pour cette mission et des factures de frais de voyages et d'hébergement suivantes, réglées par la fondation :

- facture transport AIR FRANCE 6.909 FF
- facture d'hôtels HAVAS VOYAGES 2.060 FF
- total 8.969 FF

nous vous versons le complément d'allocation accordée, soit 3.031 FF par chèque sur la BNP n° 4 834 746, ci-joint.

Nous joignons également à cet envoi une attestation en double exemplaires dont un est à nous renvoyer dûment signé par retour du courrier.

Nous vous rappelons que le rapport de votre mission devra nous parvenir en 5 exemplaires dans les deux mois qui suivent votre retour

La protection de l'environnement est un thème qui concerne vos activités. Nous avons donc le plaisir de vous adresser le dernier rapport publié par la FNEP et rédigé par un groupe de jeunes fonctionnaires et cadres d'entreprises,dans le cadre de la mission annuelle FNEP 1993 sur le thème "Entreprise et environnement", activité également financée par la fondation.

Nous vous souhaitons un bon séjour au Canada et nous vous prions d'agréer, Monsieur, l'expression de nos salutations distinguées.

Claude POSTEL
Délégué Général

préfecture de la région
AUVERGNE

direction régionale de l' agriculture et de la forêt

service Régional de la Protection des Végétaux

notre référence

votre référence

dossier suivi par JF CHAUVEAU

téléphone 73421480

objet

Dr Ray JOHNSON
Centre for Plant Health
8801 East Saanich Road
SIDNEY BC
V8L 1H3
CANADA

Lempdes, le 12 septembre 1994

Dear Dr Johnson,

Concerning my next visit to the Quarantine Station, I am now able to precise more details.

In practice, I will land in Victoria on Tuesday 20 September at 14.45 by flight AC1513 from Vancouver. I should spend three nights in Sidney. On Friday 23 September, I should go back to Vancouver by ferry, and then leave Canada on Sunday morning.

I plan to go straight to the Station after landing (on 09/20). Is this possible ? Could you manage to book a room for me somewhere in Sidney ?

In addition to my letter dated 11/05/94, I would like to precise the items which could be discussed during my visit. They can be split into three main topics :

1. The design of the buildings in connection with safety for the environment : technical choices for glasshouses, tunnels, laboratories...and prospects for future buildings in relation with safety rules (engineered plants, good laboratory practice...). And discussion of our own project.

2. The procedures used in the Station : from the general way of checking a sample to more detailed laboratory and glasshouse procedures.

B P. 45 R. N. 89 Marmilhat 63370 LEMPDES
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3. Technical points
   - The quarantine pests and pathogens searched on vine.
   - The most important quarantine organisms as considered in the station.
   - How to handle vine in a quarantine station ?.
   - The Little Chery disease
   - The Peach Rosette Mosaic Virus and its vector Longidorus diadecturus
   - Samples of diseased budwood for which I have obtained an import permit.

Would you mind to suggest a draft agenda for my visit, since I am asked by French authorities to give one before I take off to Canada ? I apologize asking this so late, I have been away from the desk a quite long time.

Looking forward to meeting you,
Sincerely

LE CHEF DE SERVICE

JF CHAUVEAU

préfecture de la région
AUVERGNE

direction régionale de l' **agriculture** et de la **forêt**

service PROTECTION DES VEGETAUX

notre référence

votre référence

dossier suivi par J.F. CHAUVEAU

téléphone 73.42.14.80

objet

Monsieur BRADNOCK
Directeur Division Protection
des Plantes CANADA

Lempdes, le 13 Septembre 1994

Monsieur le Directeur,

Suite à mes télécopies des 24/06 et 22/07/94 concernant la possibilité de me rendre en visite d'études à la Station de Saanitchon, j'ai le plaisir de vous confirmer que cette mission aura bien lieu.

D'une durée d'une semaine, elle est divisée en 2 parties : tout d'abord une rencontre avec vos Services à OTTAWA, puis la visite de la Station proprement dite.

Dans la pratique, j'arriverai à OTTAWA le dimanche 18/09 à 18 h 09 par le vol AC 164 en provenance de TORONTO. Je serai logé à l'Hôtel des Gouverneurs – 111, Rue Bellehumeur à OTTAWA – Tél. 568–52–52.

La journée du lundi 19/09 devrait être consacrée aux rencontres avec vos Services.

L'Ambassade du Canada en France a été informée sur cette mission. A la suite de plusieurs contacts avec la Direction de la Station de Quarantaine, M. LANTERMAN a également laissé entendre que nous pourrions nous rencontrer à OTTAWA ce jour là.

Les motifs essentiels de ma visite à vos Services sont de vous présenter dans le détail les buts de ma mission et d'être informé sur l'organisation générale de vos Services et l'intégration de la Station de Quarantaine dans les procédures de contrôle que vous utilisez.

Vous remerciant par avance pour l'aide que vous pourrez m'apporter, je vous prie de croire, Monsieur le Directeur, à l'assurance de mes sentiments les meilleurs.

LE CHEF DU SERVICE REGIONAL
DE LA PROTECTION DES VEGETAUX,

p/o

J.F. CHAUVEAU.

B P. 45 R. N. 89 Marmilhat 63370 LEMPDES
Standard : 73. 42. 14. 14 - Télécopie : 73. 90. 83. 70

MISSION SPECIALE 1994

Nom du voyageur : **Mr Jean-François CHAUVEAU**

Objet mission : **Constitution d'une station de quarantaine pour les végétaux ligneux**

Dates : **18 au 25 septembre 1994**

N° dossier AF :

DATES	ITINERAIRES	VOLS/TRAINS	HORAIRES	HOTELS
Dimanche 18 septembre	PARIS CDG2A -> TORONTO	AF 044	D : 13h20 A : 15h45	
	TORONTO -> OTTAWA	AC 164	D : 17h15 A : 18h09	HOTEL DES GOUVERNEURS 111 rue bellehumeur - Gatineau J8T 6K5 OTTAWA Tél. : 19.1.819.568.5252
Mardi 20 septembre	OTTAWA -> VANCOUVER	AC 177	D : 09h40 A : 12h51	
	VANCOUVER -> VICTORIA	AC 1513	D : 14h20 A : 14h45	HOTEL A SIDNEY (réservé par les Canadiens)
Vendredi 23 septembre	VICTORIA -> VANCOUVER	Ferry		QUALITY INN 1335 Howe Street VANCOUVER BC Tél. : 19 1 604.687.0575
Dimanche 25 septembre	VANCOUVER -> TORONTO	AC 136	D : 08h00 A : 15h19	
	TORONTO -> PARIS	AF 045	D : 18h45 A : 07h00 le 26/09	

direction générale
de l' **Alimentation**
service
de la **Qualité alimentaire**
et des **Actions vétérinaires et phytosanitaires**
sous-direction
de la **Protection des végétaux**

bureau Règlementation Phytosanitaire
notre référence SPV 4-DF n° 940810
dossier suivi par Mme Françoise PETTER
poste 81.88
objet Autorisation d'importation à titre scientifique

Direction Régionale de l'Agriculture
et de la Forêt
Service Régional de la Protection
des Végétaux "***Auvergne***"

A l'attention de Mr CHAUVEAU

◆ RÉPUBLIQUE FRANÇAISE
ministère de l'**agriculture** et de la **pêche**

Paris, le

19 AOUT 1994

175, rue du Chevaleret
75646 PARIS Cedex 13
tel : **49 55 + n° de poste**
fax : 49 55 59 49

Vous avez sollicité auprès du Service de la Protection des Végétaux une autorisation d'importation de greffons de prunier, cerisier et pêcher en provenance du Canada.

J'ai l'honneur de vous faire savoir que je donne une suite faborable à cette demande, sous réserve que ce matériel soit exclusivement réservé à des essais dans un but d'expérimentation et de recherche scientifique.

A cet effet, vous trouverez ci-joint l'autorisation d'importation qui devra être présentée au Receveur des Douanes du lieu d'arrivée.

Mme PETTER Françoise
Ingénieur d'Agronomie

Chauveau
Copie - Jardillet.

direction générale
de l' **Alimentation**
service
de la **Qualité alimentaire**
et des **Actions vétérinaires et phytosanitaires**
sous-direction
de la **Protection des végétaux**

bureau Règlementation Phytosanitaire -
notre référence SPV 4-DF nº 940809
dossier suivi par Mme Françoise PETTER
poste 81.88
objet Autorisation d'importation à titre scientifique

Direction Régionale de l'Agriculture
et de la Forêt
Service Régional de la Protection
des Végétaux "***Auvergne***"

A l'attention de Mr CHAUVEAU

◆ RÉPUBLIQUE FRANÇAISE

ministère de l'**agriculture** et de la **pêche**

Paris, le 19 AOUT 1994

175, rue du Chevaleret
75646 PARIS Cedex 13
tel : **49 55 + nº de poste**
fax : 49 55 59 49

Autorisation d'importation à titre scientifique

En application de l'article 348 du Code Rural,
de l'article 10 de l'arrêté du 2 septembre 1993,

Une autorisation est accordée à : Mr CHAUVEAU
DRAF-SRPV Auvergne
BP 45 - RN 89 - Marmilhat
63370 LEMPDES

Pour importer :

.Greffons de prunier, cerisier et pêcher porteurs de American Plum Line Pattern. plum
.Greffons de pêcher porteurs de Peach Yellows et Peach Rosette (MLO).
.Greffons de pêcher porteurs de Peach X Disease MLO.
.Greffons de pêcher porteurs de Peach Asteroid Spot.
.Greffons de cerisier porteurs de Little Cherry.
.Greffons de pommier (Red Delicious) porteurs de Apple Virion Necrosis. apple
.Greffons de pommier (Delicious) porteurs de Cherry Rasp Leaf Virus. cherry.
.Greffons de cerisier porteurs de Cherry Twisted Leaf. apricot

En provenance de : Vancouver - CANADA

La totalité de ce matériel est exclusivement destinée à des expérimentations au laboratoire.

Cette autorisation est valable ***jusqu'au 31 octobre 1994.***

Mme PETTER Françoise

PV

MINISTÈRE DE L'AGRICULTURE

ORDRE DE MISSION A L'ÉTRANGER

A REMPLIR PAR LE FONCTIONNAIRE QUI PART EN MISSION

NOM : CHAUVEAU Prénoms : Jean François

Grade : Ingénieur d'Agronomie Indice : 579 Groupe : ...

Emploi : Chef du SRPV Auvergne

Adresse : BP. 45 R.N. 89 MARMILHAT 63370 LEMPDES

Se rendra à : OTTAWA - VANCOUVER (Canada)

Objet de la mission : visite station quarantaine végétaux ligneux

Durée prévue : 7 jours Départ le 18/09/94 Retour le 25/09/94

Moyen de transport utilisé : Avion Réduction % ...

Imputation budgétaire : FNEP-FRADEC Chapitre ... Article ...

Visa du Ministre
ou du fonctionnaire ayant reçu délégation,

Visa du Contrôleur financier,

A REMPLIR PAR LE CONTRÔLEUR FINANCIER

ALLOCATION DE FRAIS DE MISSION A L'ÉTRANGER

Indemnités journalières : dans la limite de : ... jours, sur la base d'un taux journalier de : ...

Montant des frais : de représentation ...
de transport dans le pays où s'effectue la mission ...

Service chargé de délivrer les devises :

(Application de l'Instruction du 5 mars 1957)

Vu pour autorisation
d'engagement de dépenses à l'étranger
Pour le Ministre de l'Economie et des Finances,
et par autorisation,
Le Contrôleur Financier,

CF 887

N.B.– Ce document est à établir en cinq exemplaires :

- 1 à conserver par le Contrôle des dépenses engagées
- 1 à conserver par l'Agent payeur à l'étranger - le verso devant être complété par le missionnaire avant son retour en France.
- 1 à remettre, au retour, au bureau chargé, dans chaque Service, de suivre les missions.
- 2 à adresser, pour liquidation, au gestionnaire des crédits « missions à l'étranger » après avoir eu soin de compléter le verso.

MINISTERE DE L'AGRICULTURE Dossier N° 454
ET DE LA PECHE
Direction de la Production et des Echanges
Service des Relations internationales
Avis d'opportunité favorable
Paris, le 22/8/94
Le Chef du Service des Relations internationales

MM. 520.803

ANNEXE 2

DOSSIER FINANCIER

FONDATION NATIONALE ENTREPRISE ET PERFORMANCE

NOTE DE FRAIS DE : JF CHAUVEAU

Pays : CANADA

Dates mission : 18 - 25 Septembre 1994

Date	Départ de	Arrivée à	Transport	Hôtel	Repas	Taxis	Divers	Total
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17	CLERMONT-F	PARIS						
18	PARIS	OTTAWA			26 $	35 $	40 F	
19	OTTAWA	OTTAWA			40 $	100 $	1 $	
20	OTTAWA	SIDNEY		24,84 $	22,5 $	30,25 $	6,19 $	
21	SIDNEY	SIDNEY			33 $		22,96 $	
22	SIDNEY	SIDNEY			34,14 $		7,53 $	
23	SIDNEY	VANCOUVER	14,05 $	362,21 $				
24	VANCOUVER	VANCOUVER						
25	VANCOUVER	PARIS						
26	PARIS	CLERMONT						
27								
28								
29								
30								
31								
Total								

	FF	Devises
Total Dépenses :	40 F	765,73 $
~~Acompte perçu~~ :		x 3,94
~~Rendu avance~~ :		
Net à payer :	40 F	3016,97

← cours Trésor Public le 26/10/94

Date et signature	26 octobre 1994

FONDATION NATIONALE ENTREPRISE ET PERFORMANCE

NOTE DE FRAIS DE : J-F CHAUVEAU

Pays : CANADA

Dates mission : 17 - 26 Septembre 1994

Date	Départ de	Arrivée à	Transport	Hôtel	Repas	Taxis	Divers	Total
1								
2	Base de calcul : pour la FNEP qui a payé la							
3	majeure partie de frais. -							
4								
5	Frais réels avec justificatifs d'un moins 30% de							
6	déjeuner							
7								
8	Le Clar Canadien au cours Trésor Public du 26/10/94							
9								
10								
11								
12								
13								
14								
15								
16								
17	Clermont-Fd	PARIS	446 F		32 F	75F	8 F	
18	PARIS	OTTAWA						
19	OTTAWA	OTTAWA						
20	OTTAWA	SIDNEY					3,81 $	
21	SIDNEY	SIDNEY						
22	SIDNEY	SIDNEY						
23	SIDNEY	VANCOUVER			30,29 $	7 $	135,66 $	
24	VANCOUVER	VANCOUVER		290,42 $			120,69 $	
25	VANCOUVER	PARIS	10 $			20 $	53,95 $	
26	PARIS	Clermont-Fd	35 F		35F			
27								
28								
29								
30								
31								
Total								

partie prise en charge par la FRADEC

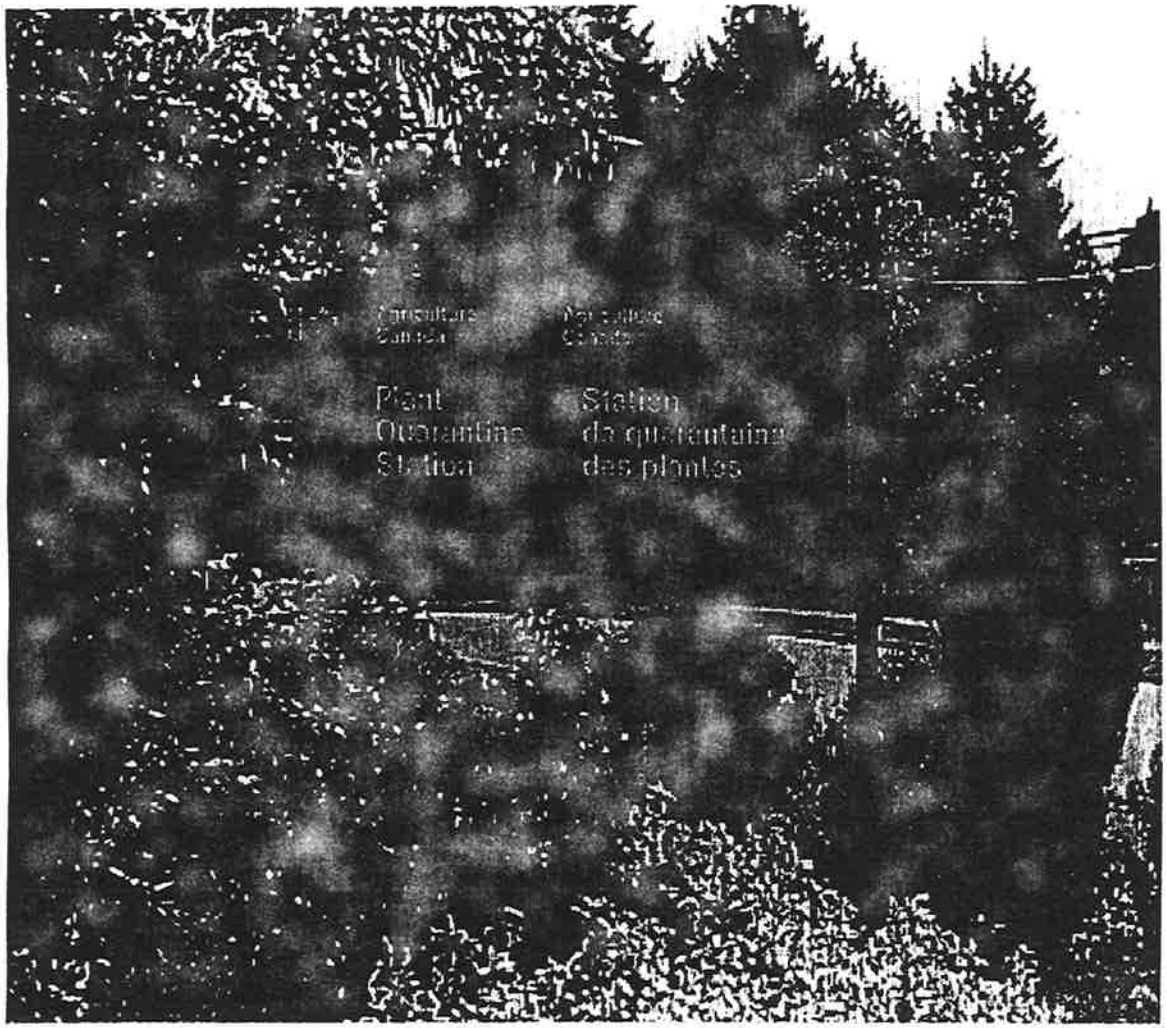
	FF	Devises
Total Dépenses :	631 F	671,82 $
~~Acompte perçu~~ :		x 3,94
~~Rendu avance~~ :		
Net à payer :	631 F	2646,97 F = 3277,97 F

Date et signature 26 octobre 1994

ANNEXE 3

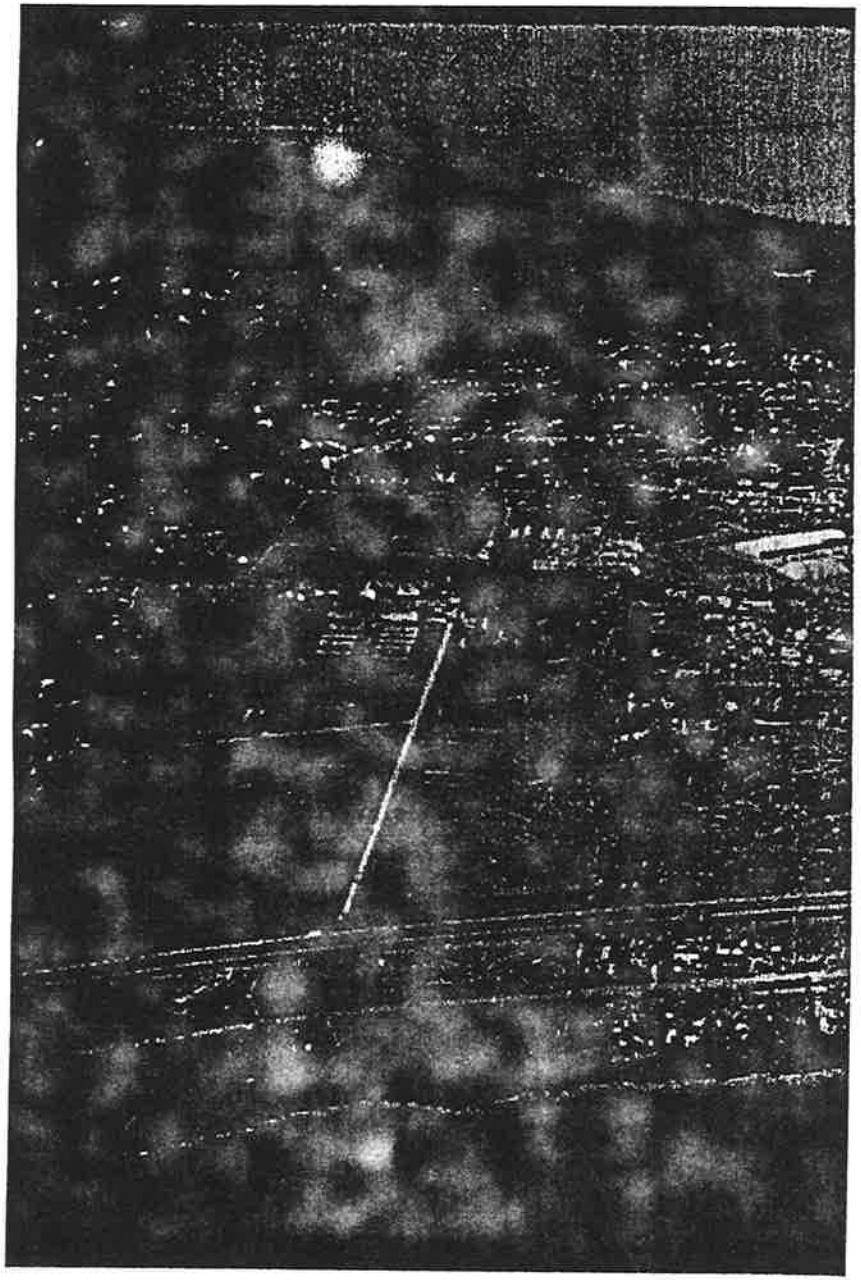
PRESENTATION DE LA STATION DE SAANICHTON

Centre de Protection Phytosanitaire de Saanichton



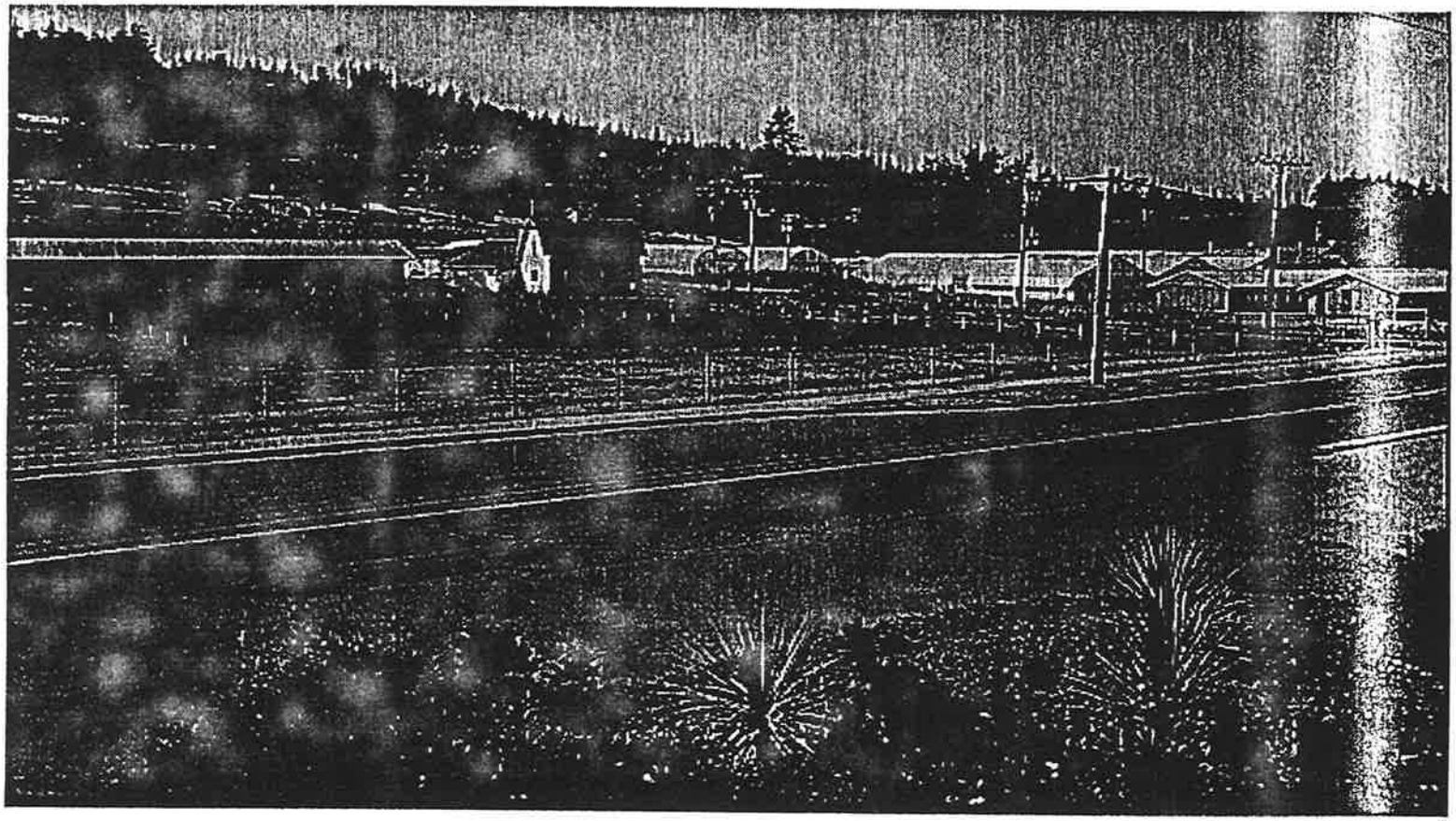
- **C'est le laboratoire national de diagnostic des virus et l'installation de quarantaine à l'entrée des arbres fruitiers, des vignes et d'autres plantes ligneuses**

SAANICHTON



- Le Centre occupe une parcelle de 48 ha située à 22 km au nord de Victoria dans la péninsule Saanich de l'Ile de Vancouver, près de Sidney
- Cet emplacement a été choisi à cause de son isolement des zones de production fruitière commerciale, ce qui empêche la propagation de maladies

SAANICHTON

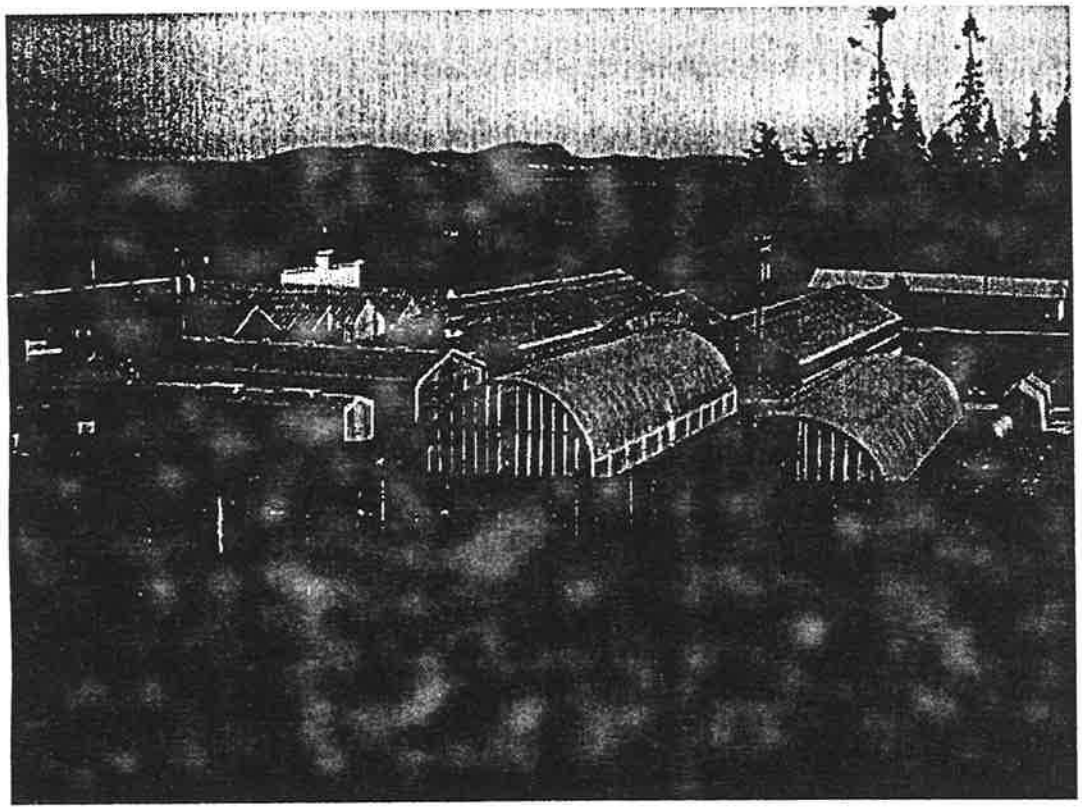


- Le sol propice et le climat doux de la péninsule convenaient à la production de toutes les cultures fruitières et plantes ornementales canadiennes
- Le Centre exerce ses activités de diagnostic et de quarantaine depuis 1965

SAANICHTON

- **Le Centre avid d'abord pour objet de permettre l'introduction de variété d'arbres fruitiers ou de matériel généalogique exotiques**

SAANICHTON



- Les responsabilités du Centre se sont rapidement élargies pour inclure la vigne
- Depuis 1990, les installations de quarantaine et les fonctions de diagnostic du Centre ont servi à introduire sans danger tout autre matériel de pépinière qui ne peut être importé directement au Canada à cause de la possibilité d'infection par des maladies exotiques graves

SAANICHTON

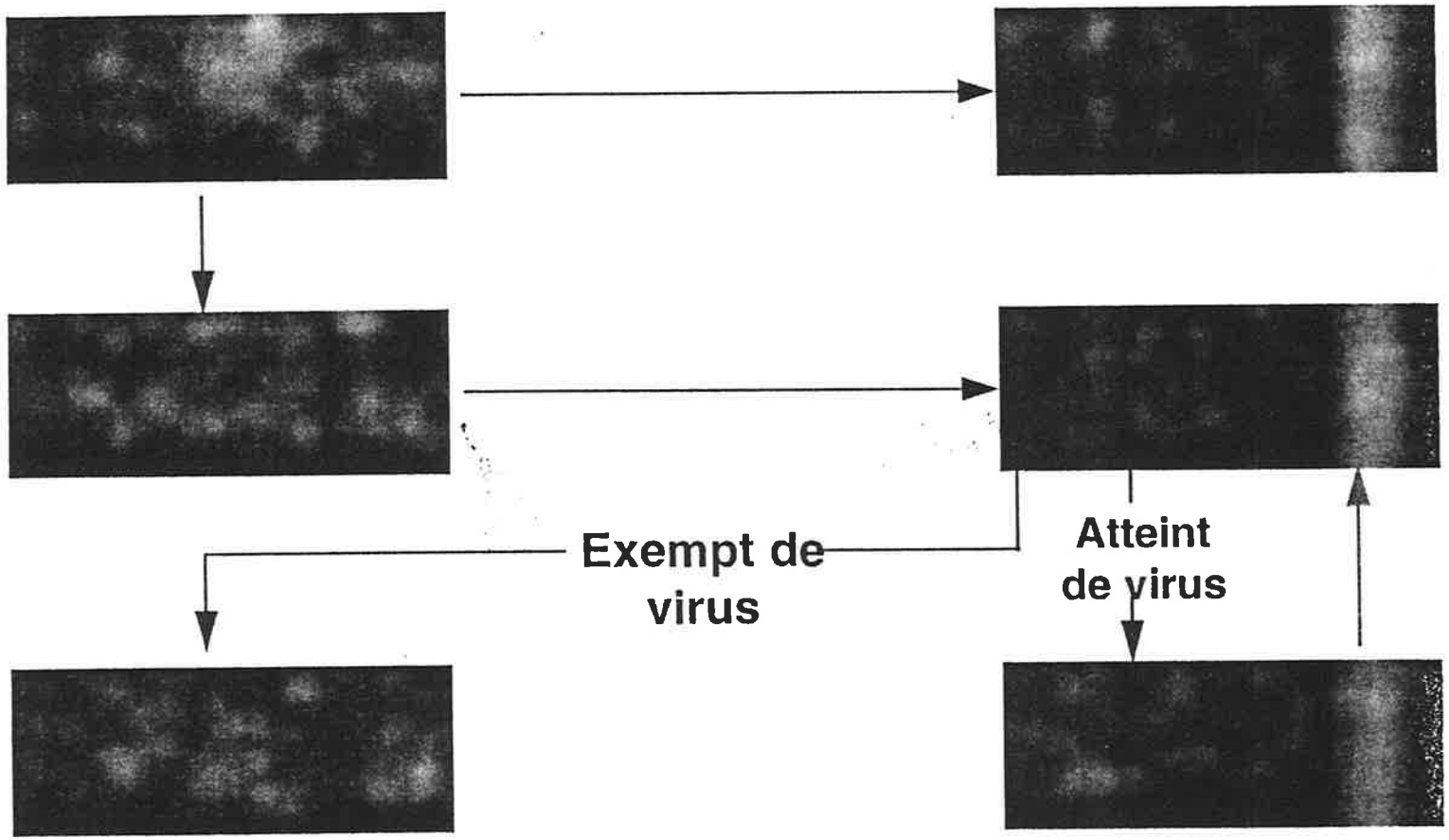
Les fonctions du personnel comprennent :

- Le Centre contrôle et met au commerce des végétaux d'origine étrangère qui ne peuvent être importés directement au Canada à cause des restrictions phytosanitaires à l'importation
- Le Centre contrôle et met au commerce des végétaux canadiens. Il offre ses services aux sélectionneurs et aux pépiniéristes canadiens qui veulent présenter des échantillons au contrôle phytosanitaire avant la mise au commerce

SAANICHTON

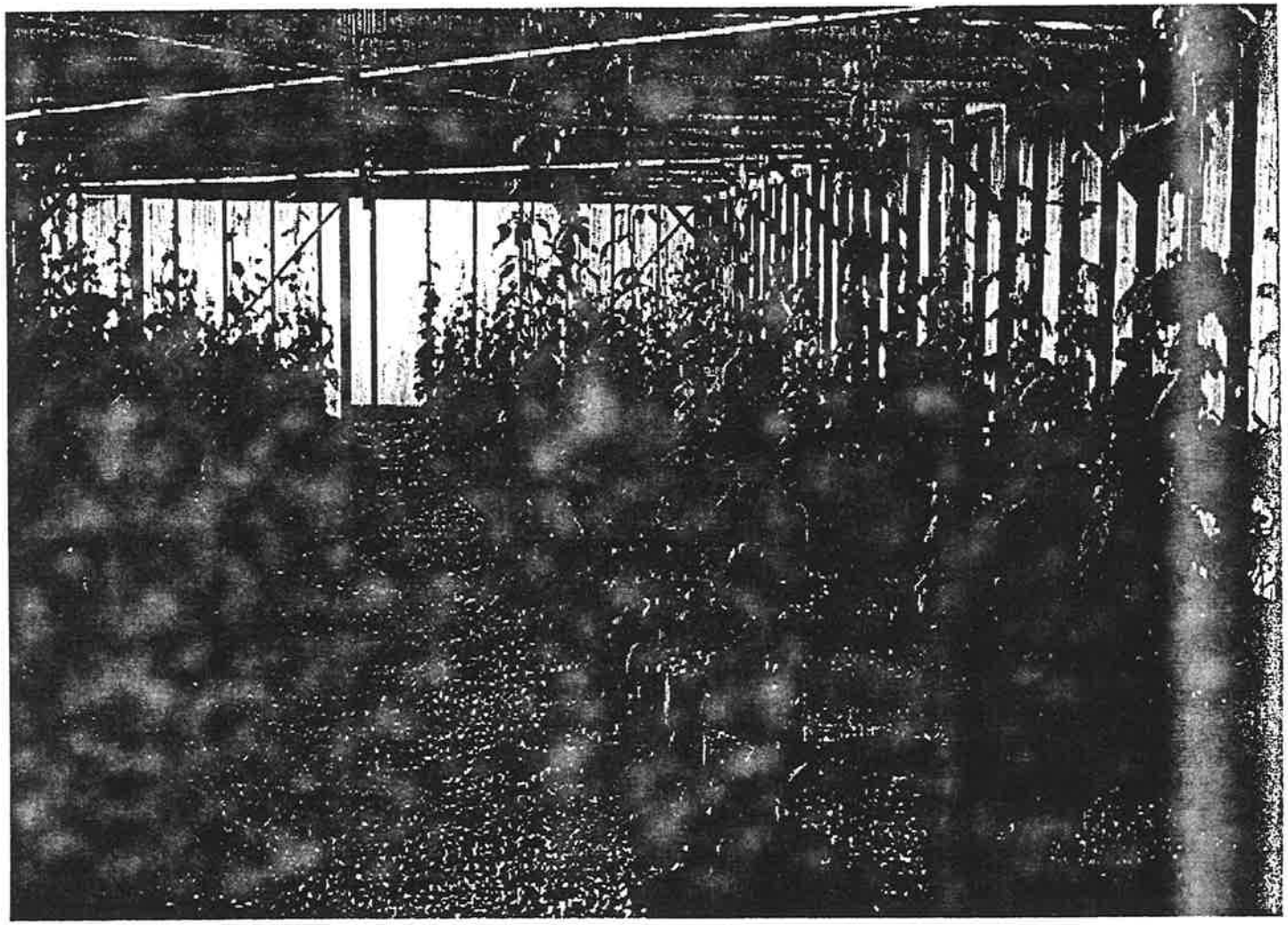
- Après avoir réussi les épreuves, les végétaux peuvent servir à multiplier des plants exempts de virus dans le cadre du programme canadien de certification pour exportation ou pour utilisation intérieure
- Élimination des viroses. Les plants atteints de viroses peuvent être soumis à des méthodes d'élimination des virus
- Après les contre-épreuves obligatoires, les plants sains sont remis à l'importateur ou au producteur canadien qui a présenté la variété

SAANICHTON



- Le Centre vérifie l'exactitude des programmes de certification étrangers et s'assure de leur fiabilité; les échantillons de produits commerciaux importés, prélevés par les bureaux de douances, sont contrôles pour la présence de maladies justiciables de quarantaine

SAANICHTON

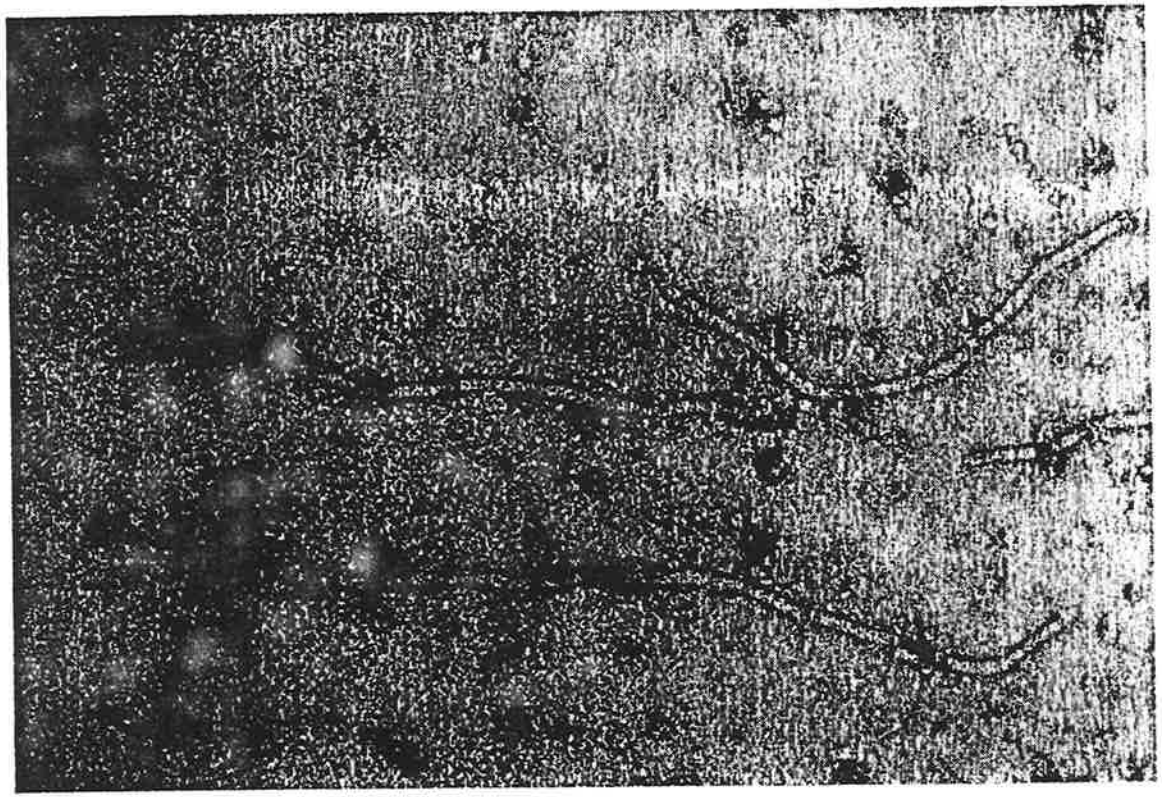


- **Le Centre conserve et distribue du matériel ayant fait l'objet d'un contrôle phytosanitaire**

- Le Centre maintient une banque de plus de 1 513 variétés et sélections de vignes et d'arbres fruitiers
- Le matériel de multiplication provenant de cas végétaux est distribué aux responsables de programmes de certification au Canada et à l'étranger, à des producteurs, à des pépiniéristes et à des établissements de recherche

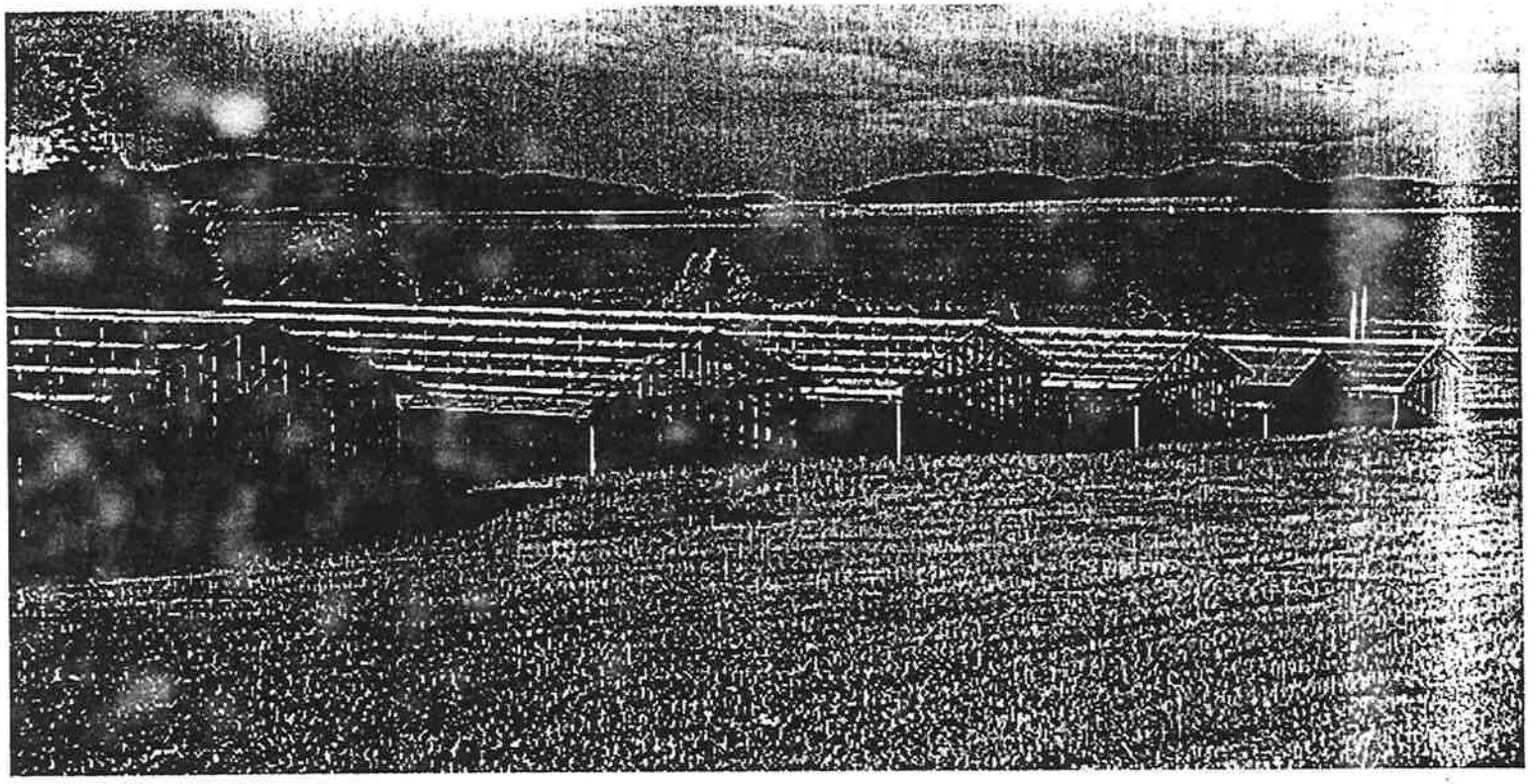
- Mise au point de nouvelles méthodes de détection

SAANICHTON



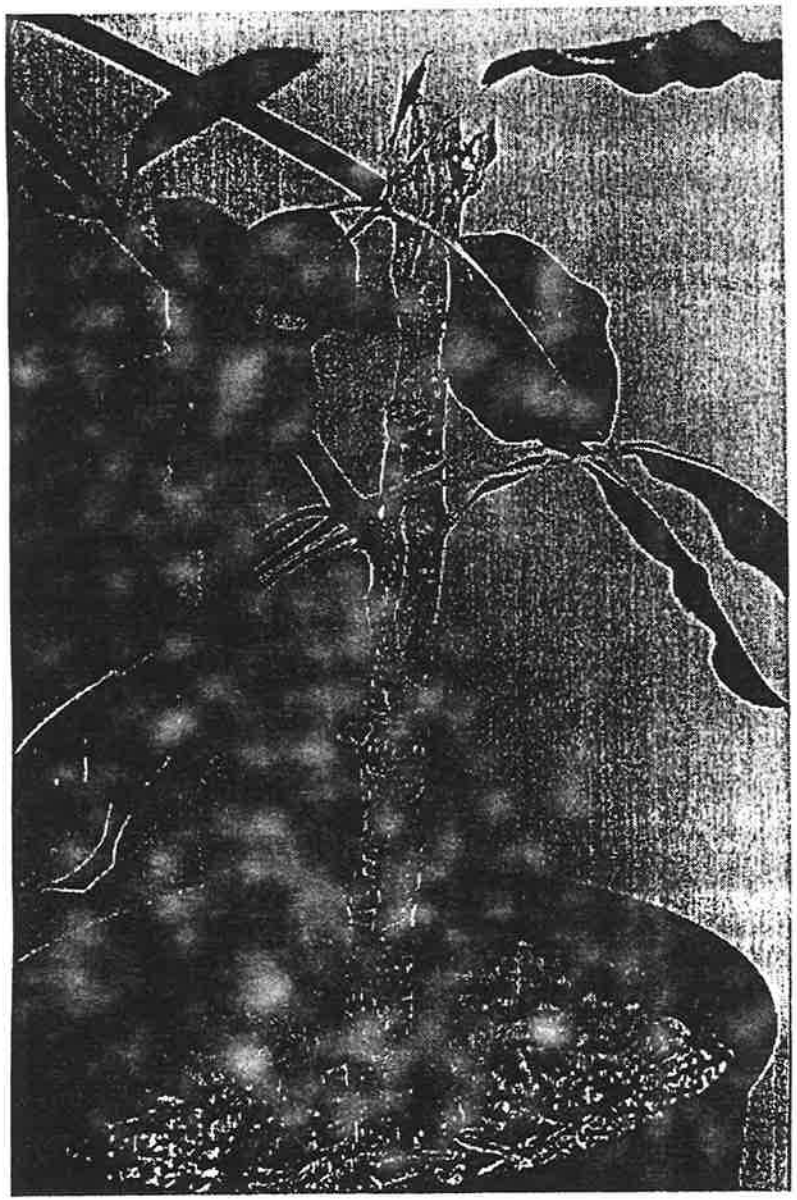
- Les chercheurs étudient de nouvelles méthodes de purification et d'isolement des particules virales responsables des viroses
- Les chercheurs appliquent les plus récentes méthodes de détection pour mettre au point des épreuves de détection plus rapides et plus sensibles

SAANICHTON



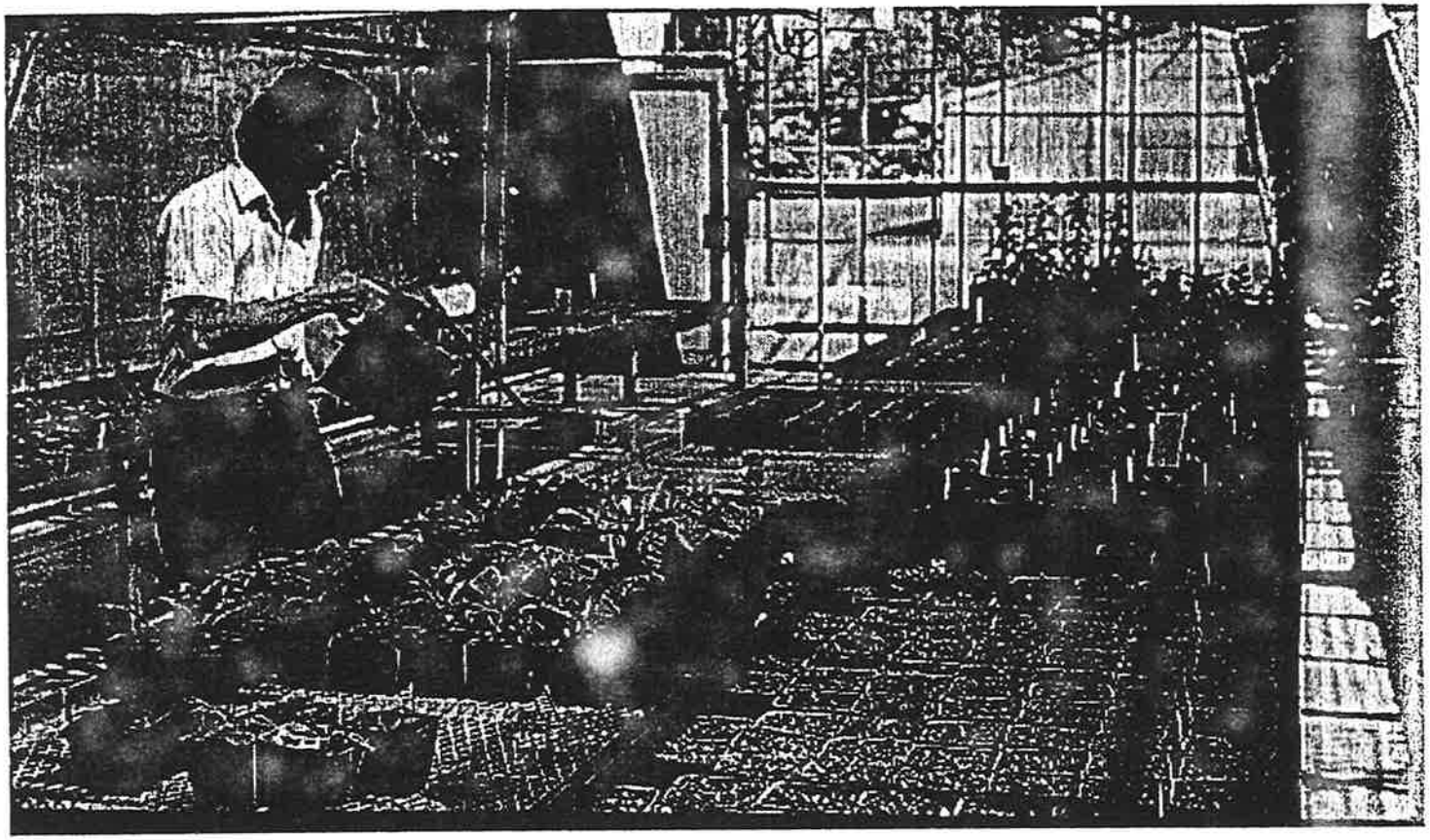
- Les végétaux importés sont isolés dans les installations de quarantaine du Centre pendant qu'ils font l'objet d'épreuves à l'égard des maladies
- Après avoir subi une batterie d'épreuves, le matériel de multiplication est remis à l'importateur

SAANICHTON



- Les phytoviroses sont détectées au Centre grâce à l'utilisation d'épreuves biologiques et de techniques de laboratoire
- L'épreuve biologique est une méthode permettant de détecter un virus en observant les symptômes qui se manifestent sur une plante indicatrice sensible en ambiance contrôlée

- Les plantes indicatrices herbacees sont cultivees en serre



- Selon le virus en question

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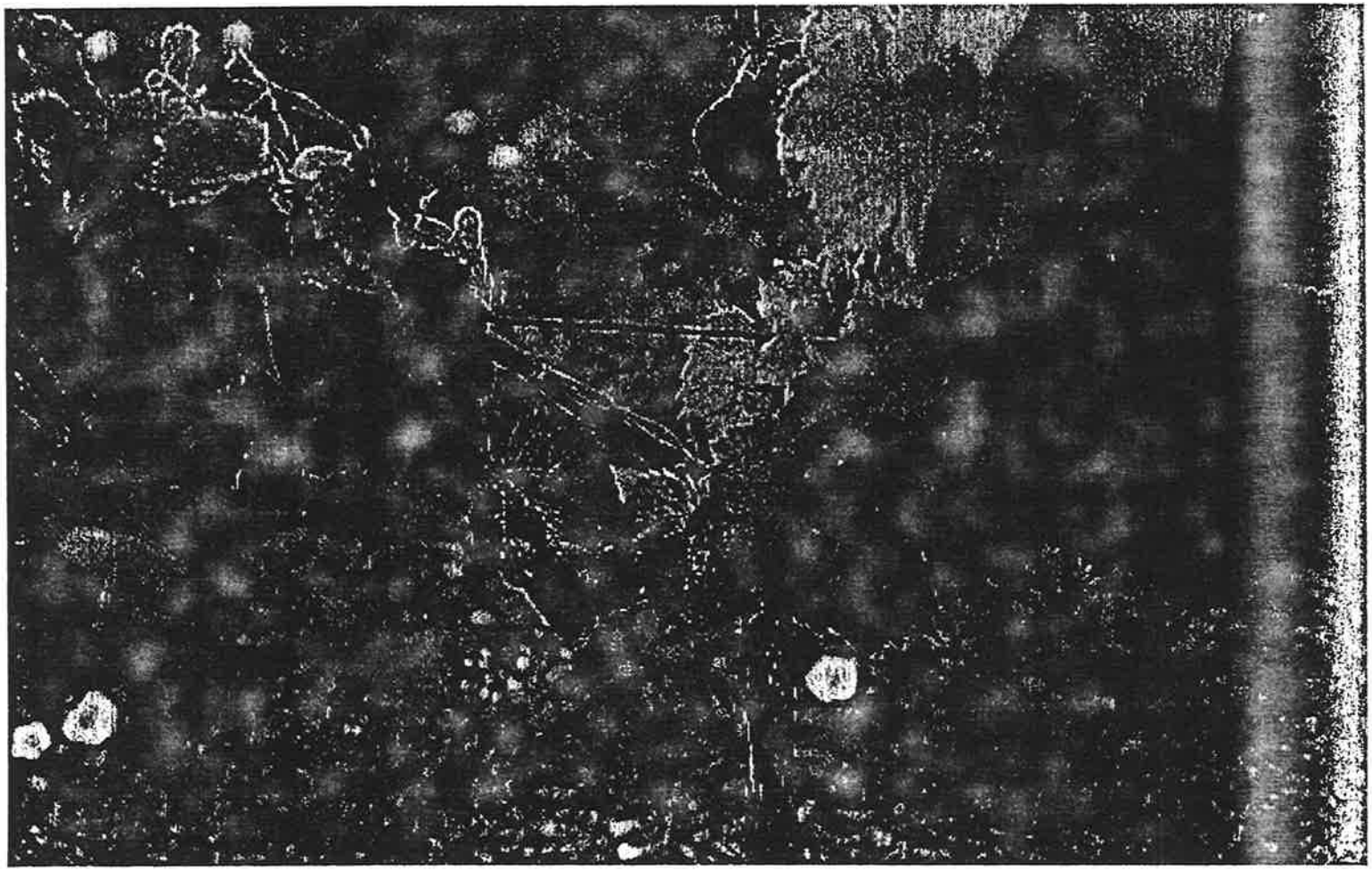

- **Les symptômes de viroses peuvent se manifester sur le feuillage,**

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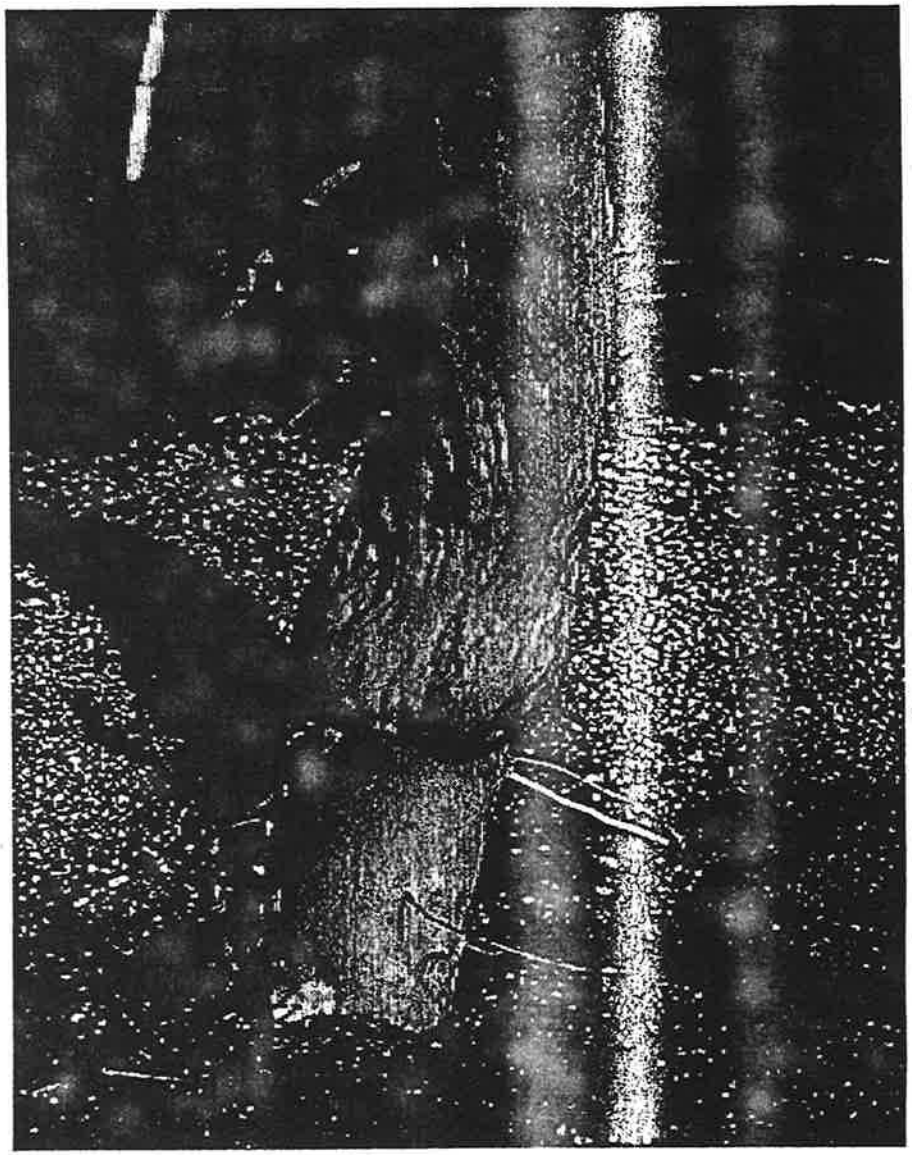
- sur le bois,



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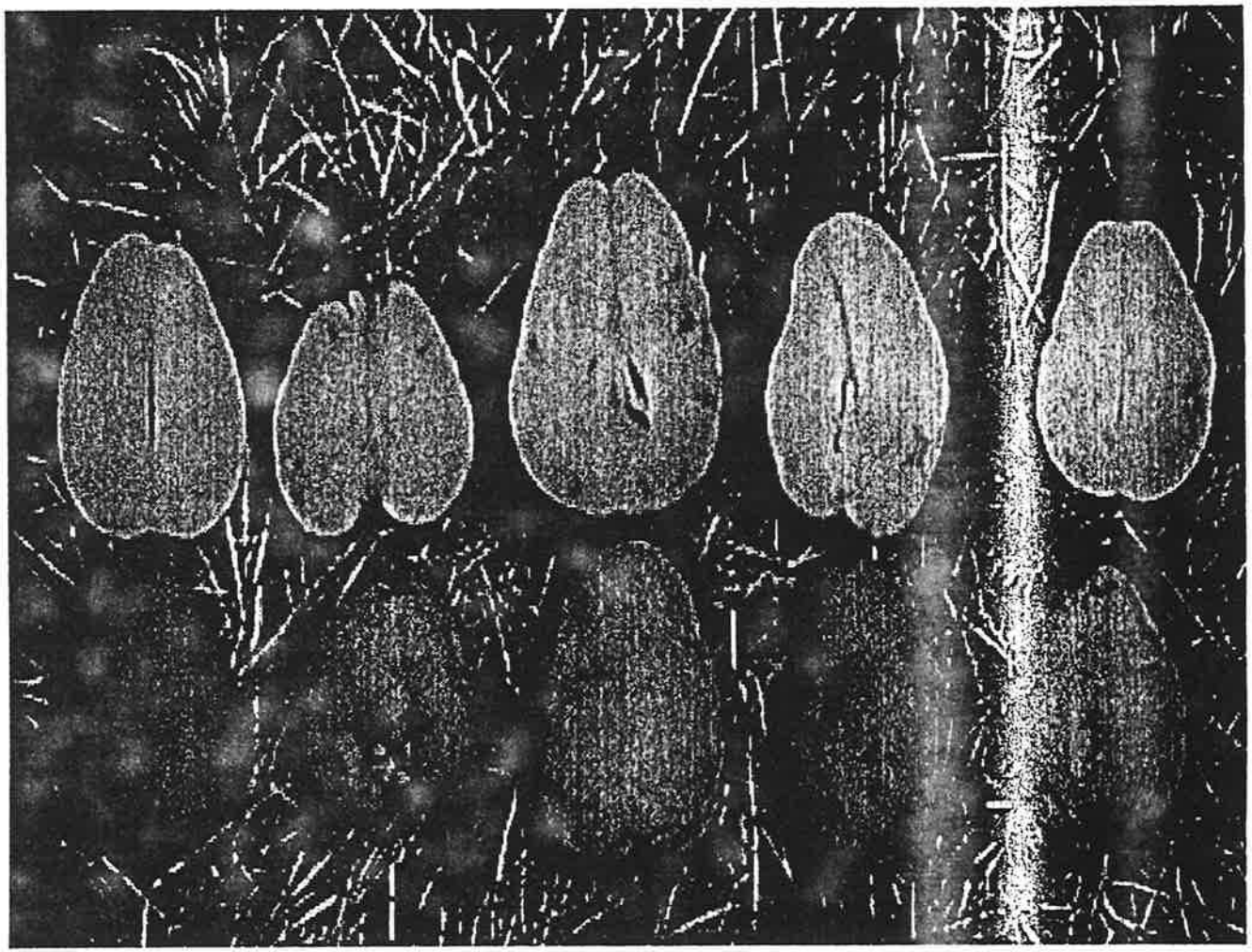
- ou sur les fruits

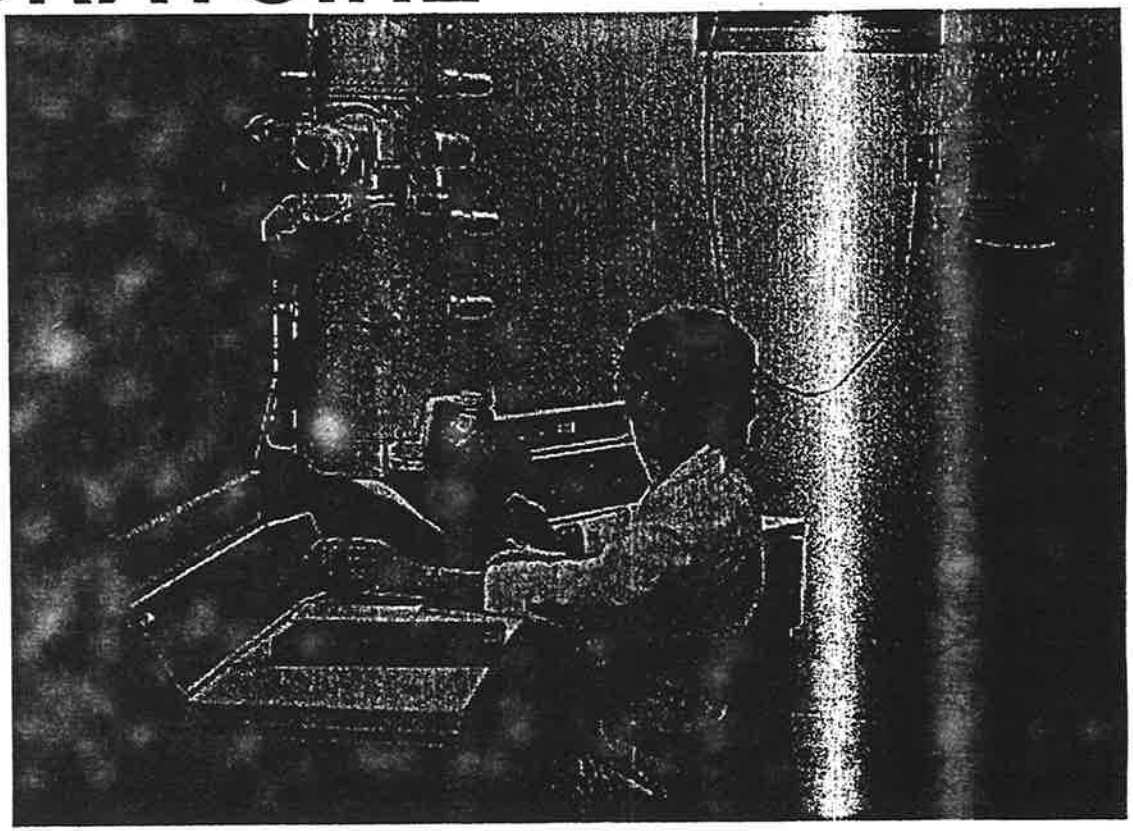


SAANICHTON - TECHNIQUE DE LABORATOIRE

Sérologie

- **Au laboratoire, la technique immunocenzymatique ELISA sert régulièrement à diagnostiquer les virus**

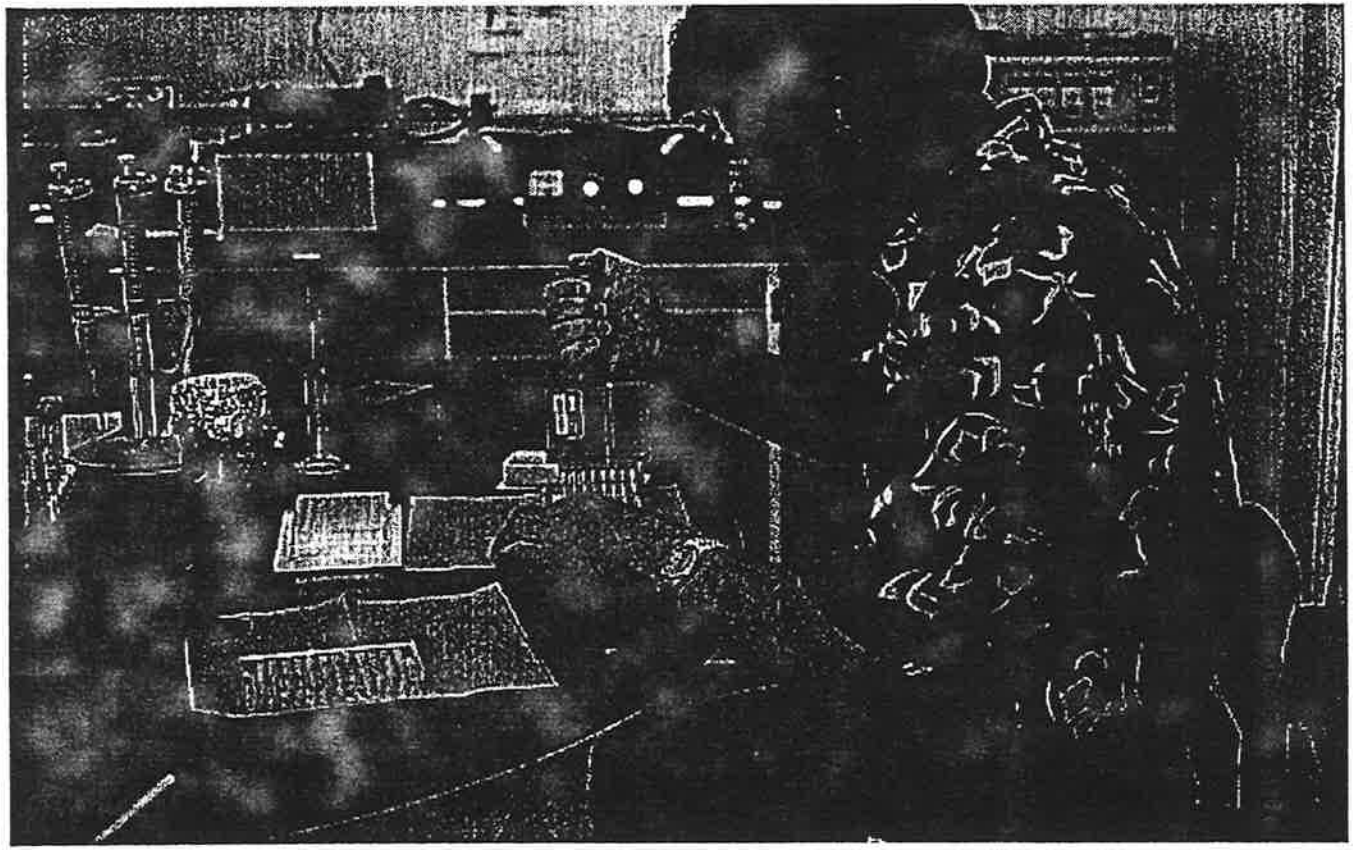
SAANICHTON - TECHNIQUE DE LABORATOIRE



Microscrope électronique

- C'est un outil inestimable pour confirmer la présence de certains virus
- Il fait partie intégrante du programme de recherche du Centre

SAANICHTON - TECHNIQUE DE LABORATOIRE



- Les chercheurs ont acquis une réputation internationale pour leurs travaux d'isolement et de purification de nouveaux virus pathogènes

SAANICHTON - TECHNIQUE DE LABORATOIRE

- Les plus récentes technologie de purification et de détection sont évaluées et appliquées dans le cadre des travaux visant à mettre au point des épreuves de diagnostic plus rapides et plus sensibles

Centre de Protection Phytosanitaire de Saanichton

Pour en savoir davantage au sujet du Centre, communiquez avec :

RAY JOHNSON
Centre de protection phytosanitaire de Saanichton

8801 East Saanichton Road

Sidney, B.C.

V8L 1H3

Téléphone Number: (604) 363-6650

ANNEXE 4

LES MISSIONS DE LA STATION DE SAANICHTON

CENTRE FOR PLANT HEALTH

The Centre for Plant Health is operated by Agriculture and Agri-Food Canada under the Food Production and Inspection Branch. The Centre is located on approximately 48 hectares of land south of Sidney on Vancouver Island. It was first established in 1912 as a Dominion Experimental Farm. In 1950 it was developed into a research facility and in 1965 a plant quarantine function was added. In 1987 all functions were directed toward the national plant health program and specifically the exclusion from Canada of virus diseases affecting tree fruit and grapevines. There are 29 full time employees on staff.

The Centre is isolated enough from commercial tree fruit and grape plantings to prevent possible spread of infection, yet the climate is suited to the culture of all Canada's fruit crop and ornamental plants. Originally the Centre was responsible only for quarantine of tree fruits, grapes and related ornamentals. Since its establishment, its functions have been expanded considerably and now include:

Quarantine and testing of all imported tree fruits, grapevines and related ornamentals, and all other ornamental genera such as Corylus, Juniperus, Ulmus that are not accompanied by an acceptable certificate of good health from the exporting country.

Testing promising selections from Canadian tree fruit and grape breeders to ensure that original releases to industry are free from detectable viruses.

Eliminating virus infections and other diseases from valuable fruit, grape and ornamental clones by heat therapy.

Verifying the reliability of recognized foreign certification programs by testing plant samples from imported commercial shipments for virus infection and other diseases.

Maintenance of valuable virus-tested tree fruit (1062 varieties) and grape clones (451 varieties) in a repository for distribution to Canadian certification programs, fruit industries and research programs.

Performing laboratory diagnostics for the detection of Golden Nematode in the western region.

Performing program support research by developing rapid, sensitive diagnostic techniques.

Because of the long lengths of time required to field test woody plants for virus diseases, much applied research is directed at developing laboratory methods to shorten these times. Some of these methods include serology, electrophoresis, cDNA cloning and immunosorbent electron microscopy. Substantial emphasis is also placed on development of tissue culture media and techniques for rapid propagation of plants and in the conventional and in-vitro virus elimination program.

Besides the applied research directed at developing rapid diagnostic techniques, other research and observational trials are currently underway in the following areas.

1) Classification of symptom expression of corky bark virus on grapevine indicators.

2) Quantification of virus transmission through chip-bud grafting.

3) Transfer of technology for heat therapy elimination of Arabis mosaic virus and grapevine fanleaf virus.

4) Measurement of the effects of rupestris stempitting (RSP) virus-like agent on grapevine growth.

5) Development and implementation of greenhouse and field indexing trials to refine procedures used to shorten indexing times.

6) Lab trials on tissue culture regimes for in-vitro production of rootstock clones.

7) Tests to determine whether virus-like particles can be detected in grapevine leafroll and RSP infected grapevines.

8) Lab trials to improve virus identification following inoculation onto herbaceous plants using:

   - virus concentration and purification techniques.
   - direct visualization by electron microscopy combined with immunosorbent electron microscopy.
   - gel double diffusion and other serological techniques.

9) Production of cDNA hybridization probes for grapevine viruses.

Up to seventy-five (75) tree fruit and fifty (50) grapevine varieties are received from non-certified sources for indexing each year. The audit program for indexing varieties from certified sources tests up to seventy (70) tree fruit and fifty (50) grapevine samples per year. Virus indexing is performed on a full range of woody and herbaceous indicator plants in the field and greenhouses. Routine serological testing is carried out by ELISA for four grapevine and four tree fruit viruses.

The Centre has a large holly collection and kiwi fruit demonstration orchard. The greenhouse technology emphasizes energy conservation through the use of solar energy, thermal blankets and computerized climate controlled systems. The Centre maintains close contact with fruit grower's associations across Canada and technical liaison with the British Columbia Nursery Trades Association and the West Coast Greenhouse Growers Co-op. Requests for information, lectures, tours or fee schedules for virus indexing and elimination should be addressed to:

Dr. William S. Lanterman
Director
Centre for Plant Health
8801 East Saanich Road
Sidney, B.C. V8L 1H3
Phone: (604) 363-6650 Facsimile: (604) 363-6661

ANNEXE 5

UN EXEMPLE DE DESCRIPTION DE PROCEDURE D'ANALYSE

Bovine Viral Diarrhea (BVD) Virus: Serum Neutralization (SN) Test for the Detection of Bovine Antibody to BVD Virus

HALD Standard Protocol

Research and Development
Dr. Dirk Deregt
Virology Section
ADRI, Lethbridge

Protocol Version	:	1.1*
Contact	:	Ms Siv Smithson Virology Section ADRI, Lethbridge
Date	:	15-MAR-90
Revision Due	:	15-MAR-91
Biocontainment	:	Level 2

*Version 1.0 (1047F/69F) revised MAR 1987 by Dr. A. Bouffard.

Director, Diagnostic Services

TABLE OF CONTENTS

1. INTRODUCTION

Bovine viral diarrhea virus (BVDV) is currently classified in the Togaviridae in the genus Pestivirus. The pestiviruses, BVDV, border disease virus (BDV) and hog cholera virus are closely related serologically. In fact, the BVDV SN test is often used for BDV. BVDV causes enteric disease, abortion, teratogenic defects, persistent infection and fatal mucosal disease. Two viral biotypes exist: cytopathic (CP) and noncytopathic (NCP). The later produces no overt cytopathic changes in tissue culture and can only be detected in tissue culture by immunological methods (i.e. by the indirect immunoperoxidase test).

Infection with NCP BVDV *in utero* can result in immunological tolerance and persistent infection (Baker, 1987). The calf may appear normal but is born viremic and antibody negative. These persistently infected animals continually shed virus and are thought to be the major source of infection in a herd. Most importantly, these animals can only be identified by virus isolation. These animals eventually succumb to mucosal disease when they are superinfected with an antigenically similar cytopathic strain of BVDV. If they reach breeding age, they will produce persistently-infected offspring. Persistently-infected animals are immune tolerant only to the infecting (or similar) strain of BVDV; if vaccinated with an heterologous BVDV strain they can become serologically positive for BVDV antibody.

Due to fetal infections resulting in virus persistence, contamination of fetal bovine and calf serum as well as primary fetal bovine cell lines is a common phenomenon. Commercial sources of fetal bovine serum, even irradiated serum, are invariably contaminated with BVDV. If possible, utilize a BVDV-free herd for a source of BVDV-free calf serum and primary (bovine kidney) cell lines for the SN test.

2. EQUIPMENT/INSTRUMENTATION

2.1. Titertek Multi-(8)-channel pipetter with tip ejector or equivalent (range of 5-50 µl).

2.2. Sterilized (autoclaved) (Bio-Rad Lab.) disposable pipet tips, type BR-35 or equivalent.

2.3. Costar sterile disposable reservoirs for multichannel pipetters or equivalent.

2.4. Titertek Motorized Multidilutor - (12 well, 25 µl capacity) or equivalent.

2.5. Falcon Micro Test III 96-well (flat bottom) tissue culture plates and lids, or equivalent.

2.6. Gilson Pipetman (p200), or equivalent.

2.7. Titertek Reusable dropping-Pipet with a 6 mL capacity and drop volume of 50 µl, or equivalent.

2.8. Corning disposable sterile glass pipettes (1, 5 and 10 mL), or equivalent.

2.9. Falcon sterile polystyrene snap cap (12 x 75 mm) dilution tubes or equivalent and rack.

2.10. Falcon sterile disposable square (100 x 100 x 15 mm) petri dishes or equivalent.

2.11. Sterilized blotting paper: autoclaved Whatman filter paper or equivalent.

2.12. Absorbent paper (nonsterile).

2.13. Freezers (-20°C for storage of sera, -70°C for stock virus)

2.14. Centrifuge.

2.15. Water bath.

2.16. CO_2 Incubator.

2.17. Inverted light microscope.

3. REAGENTS

3.1. Test Sera -Preferably collected in Vacutainer (Becton Dickinson) or equivalent tubes.

3.2. Positive serum control of known BVDV antibody titre.

Note: Titer of positive serum control should be at least 1:8 and not more than 1:64.

3.3. Negative serum control (no antibody titre to BVDV).

Note: Positive and negative serum controls should be heat-inactivated (see 4.1.). Serum controls should then be aliquoted in amounts sufficient for a single or a few (2-3) test(s) to avoid repeated freeze-thawing. Do not use frost-free freezers for prolonged storage as these freezers fluctuate in temperature.

3.4. Stock of BVD virus of known titre (NADL cytopathic strain). Based on the results of a previous titration, the virus stock is diluted (in Diluent) to contain 100 $TCID_{50}$/0.025 mL (the challenge virus dose) for the test (see Appendix 2).

3.5. BVDV-free bovine fetal kidney (BFK) cells or calf kidney (BK) cells - Use primary to the 5th-8th passage level depending on cell viability. Check for BVDV-free status with the immunoperoxidase test (HALD Standard Protocol) through to the 5th-8th passage (through to the last passage that will be used for the test).

3.6. Fetal bovine serum (FBS) or calf serum (CS) - heat inactivated (see 4.1.) and free of BVD virus and BVDV antibodies (see Appendix 3).

3.7. Diluent - Minimal Essential Medium (Gibco) with Earle's salts, L-glutamine and nonessential amino acids containing: 0.195% w/v sodium bicarbonate*, 25 mM Hepes and Gentamycin (50 µg/mL).

*or 0.075% w/v sodium bicarbonate without Hepes. Penicillin at 200 µg/mL and Streptomycin at 200 I.U./mL can be added if desired.

3.8. Growth Medium for BK cells - Diluent (see 3.7.) containing 20% FBS or CS.

3.9. Phosphate-buffered Saline (PBS) (ie. Oxoid Ltd.), pH 7.2-7.4.

3.10. Trypsin-EDTA solution (Gibco) diluted in PBS (0.05% trypsin, 0.53 mM EDTA, final concentration).

3.11. Sterile distilled water.

4. PREPARATION FOR THE TEST

4.1. Sorting and preparation of specimen sera

- Upon arrival, enter submitted blood samples onto diagnostic record sheet and give samples laboratory numbers.

- Mark blood samples with laboratory numbers.

- Separate serum from the clot by centrifugation at 2000 rpm for 10 min.

- Pour or pipette sera into sterile snap cap tubes labelled with laboratory numbers.

- Heat-inactivate sera at 56 ± 1°C for 30 ± 5 min in a water bath (to destroy complement). The level of water in the water bath should be sufficient to reach a height covering at least one-half the volume of serum in the tubes. CCP #1

QC: One may expect a reduction in SN antibody titre of the test sera if the upper limits placed on heat-inactivation are greatly exceeded. If the limits are not kept the actual temperature and duration of heat-inactivation is recorded and the supervisor notified before proceeding further with the test.

The supervisor will decide whether the test can proceed or may ask for sera to be resubmitted (see Appendix 4).

- Use in test immediately or store at -20°C.

Note: Do not use frost-free freezers for samples and do not freeze-thaw samples repeatedly.

4.2. Labelling 96-well microtitre plates (lids) to be used (see Appendix 1).

4.2.1. Test samples

- Label with laboratory numbers.

- For each specimen 3 rows of eight dilutions (four test samples per plate). The first 2 rows are used for the test proper and the third row is used for a serum toxicity control.

4.2.2. Controls

- A separate plate is reserved for positive, negative, virus and cell controls and back titration of challenge virus.

- For positive and negative BVD serum controls, 3 rows of eight dilutions each.

- Cell controls, 1 row of eight wells.

- Challenge virus controls (4 wells) and back titration of challenge virus, 3 ten-fold dilutions, 4 wells each.

5. PERFORMANCE OF THE TEST

5.1. Test samples (refer to Appendix 1)

- Add 25 µl of Diluent to all rows of the microtitre plate except the first row of 12 wells.

- Add 50 µl of test serum to each of three wells in the first row (4 test samples per plate).

- Using 25 µl dilutors, take up 25 µl of serum from the first row and transfer to the following row, mix 3-4 seconds (with motorized dilutors) or by rotating 6-10 times (with manual dilutors) making a two-fold dilution, continue the same making two-fold dilutions for the remaining rows.

Note: Flame microdiluters, quench in sterile petri dish containing sterile distilled water and blot to remove water on sterile blotter paper before making the first two-fold dilution. Between each dilution, blot microdilutors onto absorbent paper, rinse microdilutors in water, flame microdilutors, then quench in sterile water and blot to remove water on sterile blotter paper.

- Add 25 µl of challenge virus (100 $TCID_{50}$) from the sterile reservoir to the first two rows (of eight dilutions) for each test sample using a multi-(8)-channel pipettor. CCP #2

QC: See 6.1.

- To the third row of each test sample add 25 µl of Diluent for a serum toxicity control.

5.2. Controls (refer to Appendix 1)

- For positive and negative serum controls follow the test sample procedure in 5.1.

- The seventh row of 8 wells will serve as cell controls. Place 50 µl of Diluent in these wells.

- The next four rows are used for virus control and back titration. Add 25 µl of Diluent to first 4 wells of each row, then add 25 µl of challenge virus to top four wells, and 25 µl of 10-fold dilutions (10^{-1} to 10^{-3}) of challenge virus to subsequent wells.

To make 10^{-1} to 10^{-3} dilutions add 100 µl of challenge virus with a pipetman to 0.9 mL of Diluent in a dilution tube, mix and add 100 µl to the next dilution tube, etc. using a new sterile pipet tip for each dilution.

5.3. Incubation of Plates

- Incubate test sample and control plates for 1 hour ± 5 min at 37 ± 1°C in a humidified 5% CO_2 incubator. CCP #3

QC: If limits are not kept, record actual time and temperature and notify supervisor before proceeding.

The supervisor will decide whether the test can proceed or may ask for sera to be resubmitted. (see Appendix 4).

5.4. Preparation of Cell suspension

Note: one Corning 490 cm^2 roller bottle of confluent BK cells can seed 20 microtitre plates (ie. seed at 40,000 ± 10,000 cells/well sufficient to produce confluent cell monolayers).

- Pour or pipette off media and rinse with PBS before adding 10 mL of trypsin-EDTA solution.

- Leave on roller 2-5 minutes until cells detach.

- Add 90 mLs of growth medium (see 3.8) and resuspend cells.

5.5. Addition of Cells to Microtitre Plates

- Add 50 µl of cell suspension to all wells with a 50 µl dropper pipet.

5.6. Incubation of Plates

- Incubate microtitre plates at 37 ± 1°C in a humidified 5% CO_2 incubator for 5 days at which time the test is read.

6. INTERPRETATION OF THE TEST

6.1. Controls

- Cell controls must appear normal.

- The challenge virus control wells must show complete cytopathic effect (CPE) characterized by vacuolation of cells, cell swelling and eventually destruction of the cell sheet.

- The back titration of the challenge virus dose must be within ± 0.5 log_{10} of 100 $TCID_{50}$ (Reed & Muench method, Dulbecco, 1980; also see Appendix 2). CCP #4

QC: If the challenge virus titre is below or above these limits, the titre must be adjusted appropriately and the test repeated.

- The negative control serum must not inhibit the CPE from the challenge virus otherwise the test must be repeated.

- The titre of the positive control serum must be within ± one 2-fold dilution of the initial titre after production (recalibration is done if necessary) otherwise the test must be repeated.

6.2. Test samples

- The first row of samples is considered a 1:2 dilution, thus dilutions of 1:2 to 1:256 are represented in the test.

- The serum toxicity control wells are first examined for serum toxicity (cells do not appear normal as compared to cell controls). If cells show toxicity, mark the plate wells with a "T" that show toxicity.

- In the virus-inoculated serum test wells, the last dilution of serum inhibiting CPE for each row is marked on the plate.

- In the event toxicity is observed in the serum toxicity control wells the sample is reported as "toxic" unless CPE is inhibited at higher dilutions in virus-inoculated rows and a titre can be read without ambiguity.

Note: If toxicity interferes with reading the test, the test can be repeated to determine if toxicity was due to another cause such as a problem with the plastic in certain wells of the microtitre plate. If this is not suspected or if on retesting samples are still toxic ask for sera to be resubmitted.

- When there is CPE in all the virus-inoculated wells, the serum sample is reported as "negative" for antibody to BVD virus.

- When CPE is inhibited in one or more wells, the titre of the serum sample is determined and reported.

Note: When screening undiluted sera this result is recorded and reported as "positive" for antibody to BVD virus.

- The 50% endpoint (titre) occurs at the last dilution where CPE is inhibited in one or two of the 2 replicate wells of a particular serum dilution where there is CPE in all higher dilutions of serum.

- In the event that CPE is inhibited in only one of the two replicate rows for the last two (inhibiting) dilutions the custom is to move and add the positive result to the other row and calculate the titre as above. For example, if one row shows inhibition to 1:32 and the

other row shows inhibition only to 1:8 the final calculated titre is 1:16.

- Enter results into the diagnostic record and onto the serum test report.

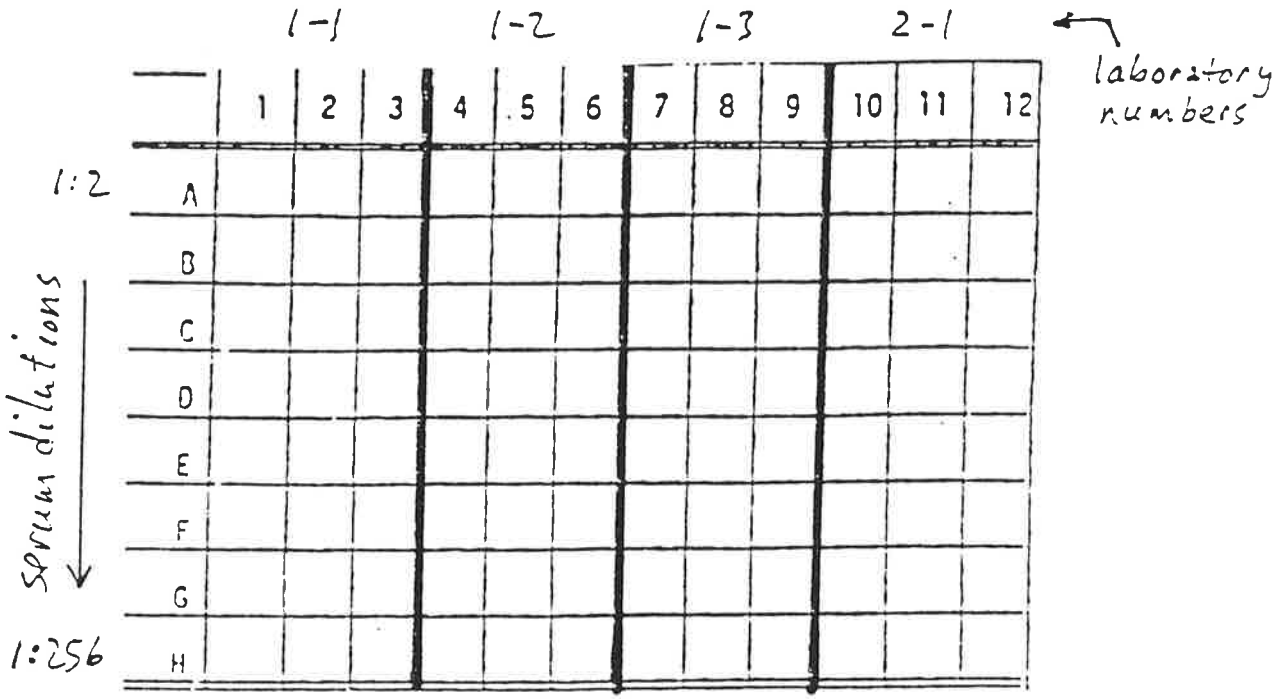
7. REFERENCES

1. Baker, J.C. (1987). Bovine viral diarrhea virus: a review. J. Am. Vet. Med. Assoc. 190: 1449-1458.

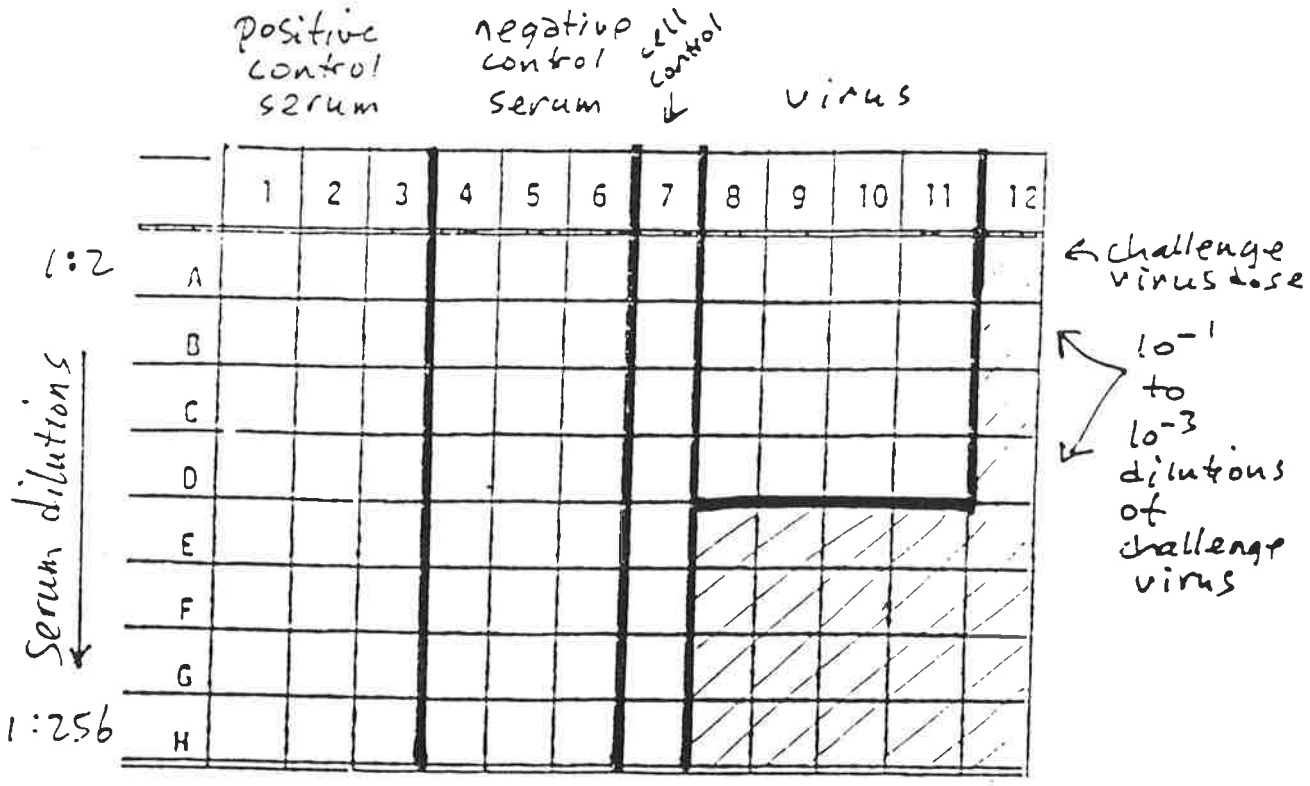
2. Dulbecco, R. (1980). The nature of viruses. In: Microbiology. Davis, B.D., Dulbecco, R., Eisen, H.N., and Ginsberg H.S., Eds. Third Edition. Harper & Row, Philadelphia.

Appendix 1: Set up and labelling of Test and Control Plates

Test plate:



Control Plate:



Appendix 2: Acceptable limits of the virus challenge titre

Challenge virus and back titration (10^{-1} to 10^{-3}):

X=CPE, O=No CPE

	Ideal (100 $TCID_{50}$)				Lower limit				Upper limit			
Virus	X	X	X	X	X	X	X	X	X	X	X	X
10^{-1}	X	X	X	X	X	X	X	X	X	X	X	X
10^{-2}	X	X	O	O	O	O	O	O	X	X	X	X
10^{-3}	O	O	O	O	O	O	O	O	O	O	O	O

Appendix 3

Notes on section 3.6.

1. Test for BVD SN antibody:

Undiluted serum is checked for serum neutralizing (SN) antibody yielding a negative reaction at 1:2 final dilution using the HALD Standard BVD Virus SN Protocol (this protocol).

2. Test for BVD virus:

When using a BVD virus-free herd for a source of calf serum it is sufficient to test the serum of each calf before the pooling of sera using the HALD Standard BVD Virus Indirect Immunoperoxidase Test. After each serum has been tested and found to be negative for BVD virus (and SN antibody) the sera can be pooled and used for the BVD virus SN test.

If commercial sources of serum (fetal bovine or calf) must be used (see Introduction) it will be probably be necessary to check a number of lots before finding a lot free of BVD virus. Since commercial lots of serum are pooled sera, sera containing virus originating from BVDV persistently-infected animals will be diluted. Therefore, it is usually more difficult to determine the presence of virus and amplification procedures are required for virus detection. For the detection of BVD virus in commercial serum refer to the appropriate section in the HALD Standard BVD Virus Indirect Immuno-peroxidase Test.

Appendix 4

Note on QC for CCP's #1 and #3:

The upper and lower limits are those considered proper for this test, however, minor deviations from these limits will not be expected to result in a significant change in SN titre. The supervisor's decision to proceed with the test instead of repeating the test with resubmitted samples should be an informed one based on experience. Otherwise and if time allows, the supervisor may elect to proceed with the test and ask for a partial resubmission of samples (a minimum of 5 samples with SN titres) to determine whether the test was valid using the following procedures:

Heat-inactivate (for CCP #1) one-half of each resubmitted sample with the recorded (improper) temperature and/or duration and the other half using the proper temperature and time for heat-inactivation. Likewise for CCP #3, use resubmitted samples in tests employing the recorded (improper) incubation time/temperature and in tests employing the proper time/temperature. If any resubmitted sample shows a greater than 2-fold difference in SN titre between these conditions of heat-inactivation (CCP #1) or incubation (CCP #3) the entire test is deemed invalid and repeated with new samples. Further, if there appears to be a deleterious change; for example, if the majority of resubmitted samples show a 2-fold difference (up or downward trend) in SN titre between these conditions of heat-inactivation (CCP #1) or incubation (CCP# 3) the entire test is deemed invalid and repeated with new samples.

ANNEXE 6

LES COLLECTIONS DE VIGNES ET D'ARBRES FRUITIERS DE LA STATION DE SAANICHTON

Centre for Plant Health
8801 East Saanich Road
Sidney, B.C.
Canada
V8L 1H3

Tel: (604) 363-6650
Fax: (604) 363-6661

GRAPEVINE REPOSITORY LIST - DECEMBER, 1993

The following list contains grapevine varieties, selections and rootstock available for distribution from the repository at the Centre for Plant Health. Propagative material is distributed in the form of dormant cuttings unless otherwise requested. A limited quantity of cuttings are available for distribution as only one or two mother plants of each variety are maintained. Orders for cuttings are compiled in February and cuttings distributed on a priority basis. Priority is given to organizations and nurserymen producing plants within a certification scheme, research breeders and evaluators and other commercial plant propagators. Distribution will start in late February unless otherwise requested.

Most cuttings supplied will measure 25 to 37.5 cm. (10 to 15 inches) in length and contain at least 2 buds with a caliper of approximately 1/4 to 3/8 inches.

Distribution Charges

All shipping charges and fees will be paid by the receiver. Cuttings will be sent by either courier or air freight collect, as requested. Please indicate the desired courier and account number with your order. This information is critical for foreign shipments.

Ordering Procedures

Please ensure that all orders are received at the Centre before **February 28** for dormant cuttings. When making requests, quote the variety name and inventory number.

Foreign requests (including U.S.A.) for cuttings should include import permits where applicable. Please enquire with regulatory agencies in your country for import requirements. Phytosanitary certificates will be issued as required. Material will not be shipped unless foreign import requirements have been met.

Liability

Unless otherwise indicated, all material leaving the Centre for Plant Health is apparently free from viruses and other diseases detectable by the methods listed below. **Her Majesty the Queen in right of Canada, Her officers, servants and employees and the Centre for Plant Health cannot be held responsible for virus freedom of any variety or selection of plant material supplied by the Centre.**

Although an effort is being made to have our material examined for trueness-to-type, identification of some of the foreign material is extremely difficult. **Her Majesty the Queen in right of Canada, Her officers, servants and employees and the Centre for Plant Health assume no responsibility for and do not guarantee the identity of each variety, selection or rootstock supplied**

by the Station and cannot be held responsible for varietal trueness and performance of any such plant material so supplied or obtained. Material received from us should be examined closely for identification discrepancies. We would appreciate any variety evaluation reports in order to upgrade our information.

Virus Indexing

The repository accessions have been indexed by chip bud grafting onto the grapevine virus indicators *Vitis rupestris* c.v. St. George, *V.* hybrid LN33, *V. vinifera* Pinot Noir and Baco 22A, by mechanical inoculation onto the herbaceous host *Chenopodium quinoa* and *C. amaranticolor* and by serology.

Explanation of Codes

The source country of each variety is indicated after each variety.

(RSP) Indicates that the variety is infected with grapevine rupestris stem pitting disease. This virus-like agent is not quarantinable in Canada. The disease is not known to have detrimental effects on grapevines other than those with *V. rupestris* parentage. It is only known to be transmitted by vegetative propagation. In the interest of providing completely virus-free stock, all accessions with this designation are presently in our virus elimination program. Varieties infected with rupestris stem pitting disease may not be eligible for export to other countries including certain U.S. states.

(HT) Indicates that viruses have been eliminated from this variety by heat therapy.

NOTE: Some varieties may also be infected with grapevine fleck disease, which is not quarantinable in Canada and in many other countries. This information will be supplied upon request.

FRUITING VARIETIES AND SELECTIONS

Varieties	Q Number	Source
7542	Q1386-01	France (RSP)
Agria	Q1235-02	Hungary
Alicante	Q 632-01	Netherlands
Aligote	Q 637-02	France (RSP)
Alphonse Lavallee	Q1144-02	France (HT)
Alwood	Q 193-32	New York
Aris (SB 1 2-19-58)	Q 168-09	Germany (RSP)
Ark 1708	Q1264-03	Arkansas
Arnsburger	Q 625-05	Germany (RSP)
Aurore	Q 193-03	New York
Auxerrois Cl 56	Q 658-01	France (RSP)
Auxerrois Cl 56	Q 658-04	France (RSP)
Auxerrois Cl 57	Q1018-01	France
Auxerrois Traminer	Q 231-03	France (RSP)
Bacchus	Q 168-03	Germany (RSP)
Baco 1	Q 3-01	B.C., Can.
Bath	Q 227-04	B.C., Can.
Bergonia	Q 91-02	B.C., Can.
Bianca	Q1179-03	Hungary (RSP)
Black Muscat	Q 685-01	Washington (HT)
Blanca de Maria	Q 466-05	Argentina
Cabernet franc Cl 313	Q 944-02	France (HT)
Cabernet Sauvignon	Q 172-05	California
Cabernet Sauvignon	Q 390-05	France
Campbell's Early	Q 395-06	California
Canadice	Q 8-10	New York
Cardinal	Q 390-13	France (RSP)
Cascade	Q 5-02	Illinois
Castel 19637	Q 284-13	New York (HT)
Cayuga White	Q 133-01	New York
Challenger	Q1338-01	Missouri (RSP)
Chambourcin	Q 158-04	France
Chardonnay	Q 233-03	Ontario
Chardonnay	Q 390-09	France (HT)
Chardonnay	Q 661-04	France (RSP)
Chardonnay Cl 75	Q1236-16	France
Chardonnay Cl 76	Q 949-03	France (RSP)
Chardonnay Cl 76	Q1341-09	France (RSP)
Chardonnay Cl 95	Q1361-01	France (RSP)
Chardonnay Cl 96	Q1346-02	France (RSP)
Chardonnay Cl 277	Q 949-08	France (RSP)
Chasselas 1921	Q1043-02	Switzerland (RSP)
Chasselas blanc	Q 535-01	Switzerland (RSP)
Chasselas HS3	Q1043-03	Switzerland (HT)
Chasselas Dore	Q 97-12	California
Chenin blanc	Q 191-03	Washington
Chenin blanc	Q 446-03	California
Clinton	Q 357-02	Ontario
Concord	Q 222-02	California
Concord Seedless	Q 193-28	New York
Dalniewostoznyd Ramning	Q 2-03	Poland
De Chaunac S9549	Q 659-01	New York (RSP)
De Chaunac V120P2AA	Q 856-05	Ontario
Deckrot Cl 12-3	Q 650-07	Germany
Delaware V668	Q 856-04	Ontario
Delight	Q 97-10	California
Diamond	Q 227-08	B.C., Can.
Dornfelder	Q1052-02	Germany (HT)
Dunkelfelder	Q 401-07	Germany (HT)
Dutchess	Q 233-05	Ontario
Early Wonder	Q 487-02	Norway (RSP)
Ehrenfelser	Q 778-12	Germany (HT)
Faber	Q 382-01	Germany (HT)(RSP)
Favorit	Q 31-01	Hungary

Varieties	Q-Number	Source
Festival	Q 487-03	Norway (RSP)
Festivee	Q1166-05	Ontario
Flora	Q 624-02	California
Florental	Q1170-05	Ontario (RSP)
Foch	Q 193-01	New York (RSP)
Frankuthaler	Q 632-03	Netherlands
Fredonia	Q 357-08	Ontario
Friesamer	Q 6-04	Switzerland
FS 4	Q 4-02	Germany
Galea	Q 45-16	Italy
Gamay Beaujolais	Q 396-01	California (RSP)
Gamay Chaudenay	Q1257-01	Switzerland (RSP)
Gamay Freaux	Q1257-02	Switzerland (RSP)
Gamay noir	Q 390-19	France (RSP)
Gamay noir	Q1311-04	Ontario (RSP)
Gamay noir Cl 282	Q1351-01	France
Gamay noir Cl 356	Q1236-12	France (RSP)
Gamay noir Cl 509	Q1233-01	France (RSP)
Gamay Rouge de la Loire	Q 370-01	Switzerland (RSP)
Garonet	Q 106-02	France (HT)
Gewurztraminer	Q 819-02	Washington
GL 30-5-82	Q 168-01	Germany
GL 31-17-115	Q 168-07	Germany (RSP)
Gloria	Q 168-02	Germany
GM 49-84	Q 401-09	Germany
Golden Muscat	Q 193-14	New York
GR 1 (NY34803)	Q 193-13	New York
GR 3 (NY34981)	Q 193-15	New York
GR 7 (NY34791)	Q 193-26	New York
Gray Riesling	Q 446-05	California
Gruner Veltliner	Q 544-06	Austria (HT)
Gutedel Weis (Chasselas)	Q 78-03	Austria (RSP)
Gutedel x Sylvaner	Q 6-05	Switzerland
GW8 (NY44968)	Q 193-18	New York
GW10 (NY451981)	Q 284-08	New York
Horizon (GW7)	Q 193-04	New York
Huxel-rebe	Q1047-03	Germany
Huxel-rebe	Q 382-02	Germany (HT)
Itchkimar	Q 484-05	U.S.S.R. (HT)
Illinois 172-3	Q 7-05	Illinois
Illinois 316-3	Q 7-03	Illinois
Interlaken	Q 363-01	B.C., Can. (HT)
Italian Riesling	Q 395-03	California
Johannesburg Reisling	Q1311-03	Ontario
Kee-Wah-Din	Q1278-01	Ohio (RSP)
Keknyelu	Q 23-04	Hungary (HT)
Kerner	Q 631-01	Germany (HT)
Koret	Q1179-07	Hungary (RSP)
Kouldjinski	Q 484-03	U.S.S.R. (HT)
Lady Patricia	Q 193-07	New York
Lagrain	Q1186-07	California (RSP)
Landot 244	Q 284-14	New York (RSP)
Leon Millot	Q 15-01	France
Limberger	Q 588-03	Germany (HT)

Madeleine Angevine 7672	Q 56-03	United Kingdom
Madeleine Sylvaner 2851	Q 56-07	United Kingdom
Malbec	Q 494-05	California
Marechal Foch	Q 661-02	France (RSP)
Mars	Q1264-01	Arkansas
Mars	Q1337-02	Arkansas
Merlot	Q 45-14	Italy
Merlot	Q 172-04	California
Merlot Cl 184	Q1236-14	France (RSP)
Merlot Cl 447	Q1236-17	France (RSP)
Miczurinoweic	Q 2-06	Poland (HT)
Morio Muscat	Q 377-01	Germany
Moscatel Rosado de Blas	Q 466-02	Argentina
Mosel Riesling	Q1049-01	B.C., Can. (HT)
Muller-Thurgau	Q 494-07	California
Muller-Thurgau (Weis)	Q1299-01	Germany (RSP)
Muscat	Q 487-01	Norway
Muscat blanc	Q 685-08	Washington
Muscat D'Alsace	Q 330-25	France (RSP)
Muscat of Alexandria	Q1071-01	California (RSP)
Muscat De Hamburg	Q 390-06	France (HT)(RSP)
Muska	Q 328-02	South Africa (HT)
Naples	Q 193-30	New York
New York 17452	Q 8-09	New York
New York 25542	Q 193-10	New York
New York 33998	Q 8-03	New York
New York 34762	Q 147-01	New York
New York 36661	Q 193-09	New York
New York 36806	Q 8-11	New York
New York 43096	Q 8-05	New York
New York 46065	Q 8-06	New York
New York 63.944.1	Q 560-07	New York
New York 65.533.13	Q1140-01	New York (RSP)
New York Muscat	Q 227-03	B.C., Can.
Nimrang	Q 484-06	U.S.S.R (HT)
Norakert PGR 2224	Q 834-01	U.S.S.R
Okanagan Riesling	Q 361-01	B.C., Can.
Ontario	Q 193-05	New York (RSP)
Optima	Q 168-05	Germany
Oraniensteiner	Q 650-09	Germany (HT)(RSP)
Osteiner	Q 650-08	Germany (HT)
Perle	Q 54-02	Germany
Perle of Csaba (Csabagyongye)	Q 806-01	Austria (RSP)
Perle of Zala (Zalagyongye)	Q1133-01	Hungary (HT)
Perle of Zala (Zalagyongye)	Q1179-06	Hungary
Pinot blanc	Q1043-04	Switzerland (HT)
Pinot blanc (Melon)	Q1186-02	California
Pinot gris (Rulander)	Q 650-02	Germany (RSP)
Pinot gris Cl 53	Q 658-02	France (RSP)
Pinot noir	Q 396-02	California
Pinot noir	Q 390-02	France (RSP)
Pinot noir (Hurliman 2/45)	Q1043-07	Switzerland(HT)(RSP)
Pinot Noir (Mariafeld M3)	Q1349-01	Switzerland
Pinot noir (Spatburgunder)	Q1073-01	Germany
Pinot noir Cl 91 (Spatburgunder)	Q1267-06	Germany (RSP)
Pinot noir Cl 164	Q1351-05	France (RSP)
Pinot noir Cl 375	Q1361-06	France (RSP)
Pollux (B-6-18)	Q1014-03	Germany (RSP)
Portugieser Blau	Q 806-03	Austria (HT)
Pslanka	Q 23-10	Hungary (RSP)
Queen	Q 97-02	California

Rabaner	Q 393-04	Germany
Rabaso Piave	Q 45-08	Italy
Ranny Vira	Q 484-02	U.S.S.R. (HT)
Ravat 6	Q 317-01	France (HT)(RSP)
Ravat 578	Q 284-12	New York (HT)(RSP)
Regner	Q1047-08	Germany (HT)
Reichensteiner	Q 393-06	Germany (RSP)
Reliance	Q1337-01	Arkansas
Riesling	Q 83-04	France (RSP)
Riesling	Q 357-03	Ontario
Riesling	Q 233-04	Ontario
Riesling Cl 21B	Q 840-02	Germany
Riesling Cl 239	Q 829-01	Germany (HT)(RSP)
Riesling Muscat	Q 231-06	France (RSP)
Rkatzitelli	Q 395-02	California (RSP)
Romulus	Q1186-08	California
Rotberger	Q 604-04	Germany (RSP)
Sauvignon blanc	Q 97-20	California (RSP)
Sauvignon blanc	Q 390-10	France (RSP)
Sauvignon blanc Cl 378	Q1387-01	France (RSP)
Scheu-rebe	Q 778-06	Germany (RSP)
Schonburger	Q 393-07	Germany (HT)
Schonburger	Q 711-03	Germany (RSP)
Schuyler	Q1186-01	California (RSP)
Seibel 10076	Q 193-23	New York (RSP)
Seibel 70790	Q 367-02	Germany
Semillon	Q 97-01	California
Semillon	Q 819-03	Washington
Semillon	Q 830-04	New York
Seneca	Q 494-02	California
Sev Lernatu PGR 2223	Q 834-02	U.S.S.R.
Seyval Blanc (SV5-276)	Q 683-01	New York
Seyve-Villard 5.247	Q 193-17	New York (RSP)
Shimek	Q 238-01	B.C., Can.
Siegerrebe	Q 56-08	United Kingdom
Siegfried V607	Q 857-01	Ontario
Siewiernyj	Q 2-05	Poland (HT)
Sovereign Charter	Q 716-01	B.C., Can.
Sovereign Coronation	Q 716-05	B.C., Can.
Sovereign Noir	Q 716-03	B.C., Can.
Sovereign Tiara	Q 716-04	B.C., Can.
Sovereign Rose	Q 716-02	B.C., Can. (HT)
St. Pepin (ES 282)	Q 567-01	Minnesota
Steuben	Q 193-16	New York
Strawberry Grape	Q 89-01	United Kingdom
Suffolk Red	Q 8-01	New York
Suputinsky Bietyz	Q 2-01	Poland (HT)
Swenson Red	Q 567-04	Minnesota
Sylvaner	Q 535-04	Switzerland (RSP)
Sylvaner Red	Q 64-03	Germany
Sylvaner White	Q 64-04	Germany
Tajoznyt Izumrud	Q 2-02	Poland (RSP)
Tchilar	Q 484-07	U.S.S.R. (HT)
Thurling	Q1047-01	Germany (HT)(RSP)
Titan	Q1235-01	Hungary (RSP)
Totmur (Baco 2-16)	Q 284-01	New York
Trollinger (Black Hamburg)	Q 64-01	Germany
Van Buren	Q 193-11	New York
Vanessa	Q1166-04	Ontario
Veeblanc	Q1166-08	Ontario
Veeport	Q1166-06	Ontario
Venus	Q1337-03	Arkansas
Ventura	Q1166-02	Ontario
Verdelet (S 9110)	Q 395-05	California (RSP)
Vidal blanc	Q 450-01	Ontario
Vignoles (Ravat 51)	Q 284-04	New York
Villard blanc	Q 564-01	France

Villard noir	Q 317-03	France (HT)
Vincent	Q1166-07	Ontario
Vineland 37023	Q 228-02	Ontario
Vineland 37034	Q 228-01	Ontario
Vineland 49063	Q 92-01	Ontario
Vinered	Q1166-01	Ontario
Vivant	Q1166-03	Ontario
V. vinifera (Red)	Q 571-01	Hungary
Welschriesling (Italian Riesling)	Q 544-04	Austria (HT)
Welschriesling (Italian Riesling)	Q1186-04	California (RSP)
West Freesia	Q 632-02	Netherlands
White Riesling	Q 494-06	California
Wurzer	Q1047-09	Germany (HT)
Yates	Q 193-06	New York
Zengo	Q1179-01	Hungary
Zinfandel	Q 861-05	California

ORNAMENTALS:

V. betufolia	Q 280-02	United Kingdom
V. coignetiae	Q 280-03	United Kingdom
V. piasezkii	Q1078-03	United Kingdom

ROOTSTOCKS:

101-14		
Q 390-17	France (RSP)	
125-AA	Q 626-02	Germany (HT)
125-AA	Q 782-01	Germany (RSP)
125-AA Cl-1	Q 649-04	Germany (RSP)
161-49C Cl 176	Q1400-03	France
1613 C	Q 177-03	California
1616 E	Q 186-01	New York (RSP)
3306 C	Q 186-02	New York (RSP)
3309 C	Q 177-06	California
3309 C	Q 330-04	France (RSP)
3309 C	Q1260-01	France
3309 C Cl 143	Q1134-02	France
41 B Millardet	Q 504-01	California
420 A Cl 11	Q1236-08	France
44-53 M	Q 98-02	France
5 A	Q 186-04	New York
5 BB	Q 97-07	California
5 BB	Q 98-03	France
5 BB	Q 330-10	France
5 BB	Q 233-02	Ontario
5 BB	Q 516-01	Germany
5 BB	Q 649-05	Germany
5 BB Cl 13-45-5	Q 649-02	Germany
5 BB Cl 114	Q1236-02	France (RSP)
5 C	Q 45-06	Italy
5 C	Q 67-02	France
5 C	Q 516-02	Germany
99 R	Q 177-08	California
Brant	Q 56-09	United Kingdom
Cosmo 2	Q 45-01	Italy
Cosmo 10	Q 45-02	Italy
Dogridge	Q 177-07	California
Fercal	Q1336-01	France (RSP)
Ganzin 1	Q1186-03	California
Gravesac	Q1386-02	France (RSP)
Harmony	Q 177-04	California
Salt Creek	Q 177-02	California
SO4	Q 186-03	New York
SO4	Q 649-07	Germany
SO4	Q 820-03	France (RSP)
SO4	Q 861-06	California
SO4	Q1132-02	France
SO4 Cl 5	Q 776-02	France (HT)
SO4 Cl 5	Q1400-02	France
USDA 4801	Q 9-02	California
V. riparia Gloire	Q 330-12	France

INDICATORS:

Baco 22A	Q1067-02	California
Cabernet franc	Q1187-01	California
LN 33	Q1067-01	California
Richter 110	Q 554-01	Switzerland (HT)
St.George Cl 15	Q 990-01	California
Siegfried V607	Q 857-01	Ontario

RESTRICTED DISTRIBUTION

The distribution of the following accessions is RESTRICTED. However, if you still wish to request any of this material, please contact the original consignee. The material will be distributed upon consent.

Consignee: Association of B.C. Grape Growers
#5 - 1864 Spall Road
Kelowna, British Columbia
V1Y 4R1

Auxerrois Cl 21 Gm	Q 782-22	Germany (HT)
Auxerrois Cl 22 Gm	Q 782-41	Germany (HT)(RSP)
Ehrenfelser	Q 782-02	Germany
Gm 3-46	Q 782-14	Germany
Gm 311-58	Q 782-15	Germany (RSP)
Gm 312-53	Q 782-16	Germany (RSP)
Gm 316-57	Q 782-17	Germany
Gm 318-57	Q 782-18	Germany (RSP)
Gm 322-58	Q 782-19	Germany (RSP)
Gm 323-58	Q 782-20	Germany (HT)(RSP)
Gm 324-58	Q 782-21	Germany (HT)
Gm 4-46	Q 782-10	Germany (RSP)
Muller-Thurgau (11 Heinz)	Q 782-35	Germany
Oraniensteiner	Q 782-31	Germany (RSP)
Ortega	Q 275-01	Germany
Osteiner	Q 782-30	Germany (RSP)
Riesling (110-06 Gm)	Q 782-32	Germany (RSP)
Riesling (198-16 Gm)	Q 782-33	Germany (RSP)
Riesling (239-13 Gm)	Q 782-34	Germany
Rulander (2/15 Gm)	Q 782-37	Germany (RSP)
St. Laurent	Q 782-27	Germany (RSP)
Weiser Burgunder (5 Gm)	Q 782-40	Germany (RSP)
Wittberger	Q 782-08	Germany (HT)

Rootstock

125 AA	Q 782-01	Germany (RSP)

Consignee: Joachim Hollerith
Rt. 1, Box 172
Reva, VA
USA 22735

Rootstock:

5 BB Cl 13-5 Gm	Q1440-01	Germany
5 C Cl 6-13 Gm	Q1440-03	Germany
Boerner	Q1440-02	Germany (RSP)
SO 4 Cl 47 Gm	Q1440-04	Germany (RSP)

Consignee: Hedrick Hall
New York Agricultural Experimental Station
Department of Pomology & Viticulture
Geneva, N.Y. 14456

GR	Q 560-04	New York
New York 63.944.1	Q 560-07	New York

Consignee: Prof. Dr. E. Ruhl
Institut Fur Rebenzuchtung und Rebenveredlung
6222 Geisenheim/RH., Den, Germany

Gm 318-57	Q 604-06	Germany (RSP)
Gm 323-58	Q 604-07	Germany
Gm 324-58	Q 604-08	Germany (RSP)
Gm 6494-5	Q1362-09	Germany
Gm 6495-3	Q1362-05	Germany
Gm 6495-4	Q1362-08	Germany (RSP)
Gm 7116-10	Q1362-04	Germany (RSP)
Gm 7742-8	Q1362-02	Germany
Gm 7742-21	Q1362-03	Germany
Gm 7742-29	Q1362-07	Germany
Gm 7746-6	Q1362-06	Germany (RSP)

Consignee: Dr. A. Reynolds
Agriculture Canada, Research Station
Summerland, British Columbia, Canada
V0H 1Z0

Selection 16	Q 872-03	B.C., Can.
Selection 25	Q 872-04	B.C., Can.
Selection 65	Q 872-05	B.C., Can.
Selection 82	Q 961-01	B.C., Can. (HT)(RSP)
Selection 88	Q1074-03	B.C., Can.
Selection 105	Q 872-09	B.C., Can.
Selection 117	Q 872-01	B.C., Can.
Selection 166	Q 872-02	B.C., Can.
Selection 223	Q 716-06	B.C., Can.
Selection 240	Q 716-10	B.C., Can.
Selection 245	Q 872-10	B.C., Can.
Selection 347	Q 872-11	B.C., Can.
Selection 364	Q 872-13	B.C., Can.
Selection 370	Q 872-14	B.C., Can.
Selection 378	Q 872-16	B.C., Can.

Consignee: Agriculture Canada
Saanichton Plant Quarantine Station
8801 E. Saanich Rd.
Sidney, B.C., Canada V8L 1H3

AZ-64-2-254	Q1334-01	Germany
Badacsony-10	Q1407-01	Hungary (RSP)
Bianca	Q1371-01	Hungary
Blauer Spatburgunder	Q1378-01	Germany (RSP)
Cantaro	Q1373-01	Germany
CSFT 194	Q1283-04	Hungary (RSP)
CSFT 2423	Q1377-06	Hungary (RSP)
CSV 525	Q1283-03	Hungary (RSP)
CSV 525	Q1377-03	Hungary (RSP)
CSVT 55	Q1283-01	Hungary (RSP)
Dan Ben Hanna	Q1310-01	South Africa
Egri Csillagok-26	Q1407-02	Hungary (RSP)
Egri Csillagok-30	Q1407-03	Hungary (RSP)
Erlihane	Q1310-02	South Africa
Ezerfurtu	Q1371-02	Hungary (RSP)
Faberrebe Cl 22-60	Q1334-02	Germany (RSP)
Fontanara	Q1335-02	Germany (RSP)
GF III-28-51	Q1357-09	Germany (RSP)
Gm 6414-11	Q1347-04	Germany
Gm 6414-26	Q1347-05	Germany
Gm 6414-36	Q1347-06	Germany (RSP)
Gm 6417-7	Q1347-07	Germany (RSP)
Gm 6421-15	Q1347-08	Germany (RSP)
Gm 6423-3	Q1347-09	Germany
Gm 6423-12	Q1347-10	Germany
Gm 6427-5	Q1347-11	Germany (RSP)
Gm 643-20	Q1347-01	Germany (RSP)
Gm 646-02	Q1347-02	Germany (RSP)
Gm 6493-3	Q1347-12	Germany
Gm 6495-3	Q1347-13	Germany
Gm 6497-4	Q1347-14	Germany
Gm 676-2	Q1347-03	Germany
Gm 7116-26	Q1347-18	Germany (RSP)
Gocseji Zamatos	Q1371-03	Hungary (RSP)
Gyongyrizling	Q1407-04	Hungary (RSP)
Helfensteiner	Q1350-02	Germany (RSP)
Heroldrebe	Q1350-05	Germany (RSP)
Heroldrebe	Q1318-02	Germany
Hoelder	Q1350-03	Germany
Jewel (WE S378)	Q1350-04	Germany
K-13	Q1345-02	Hungary (RSP)
K-13	Q1371-04	Hungary (RSP)

Korente	Q1310-03	South Africa
Kozma Pal Muskotaly	Q1283-02	Hungary (RSP)
Kozma Pal Muskotaly	Q1377-05	Hungary (RSP)
NF 10-126	Q1357-08	Germany
Orion (GA 58-30)	Q1357-03	Germany (RSP)
Perle (Stein)	Q1335-04	Germany
Perle of Zala (Zalagyongye)	Q1407-05	Hungary
Phoenix (GA 49-22)	Q1357-01	Germany (RSP)
Pollux (B-6-18)	Q1357-04	Germany (RSP)
Reflex (RF5)	Q1265-01	Hungary
Regent (GF 67-198-3)	Q1357-06	Germany
Regner	Q1334-03	Germany (RSP)
Rieslaner Cl 3	Q1335-01	Germany (RSP)
Samtrot	Q1418-01	Germany (RSP)
Silva (GA 54-14)	Q1357-02	Germany (RSP)
Sirius (GA 51-27)	Q1357-05	Germany
Turan 208/Agria	Q1371-06	Hungary (RSP)
Zefir	Q1371-07	Hungary (RSP)
Zengo	Q1371-08	Hungary
ZNR 73-2	Q1318-03	New Zealand (RSP)
Zweigelt-rebe	Q1371-09	Hungary (RSP)

Agriculture Canada

Food Production and Inspection Branch

Direction générale. Production et inspection des aliments

Centre for Plant Health
8801 East Saanich Road
Sidney, B.C.
Canada
V8L 1H3

Your file Votre référence

Our file Notre référence

Tel. (604) 363-6650
Fax. (604) 363-6661

AVAILABLE TREE FRUIT CULTIVARS - JANUARY, 1994

The virus-tested tree fruit cultivars and rootstocks on the attached list are available for distribution from a repository maintained at the Centre for Plant Health. A limited quantity of budwood is available for distribution as only one or two plants of each cultivar are being maintained. Priority is given to organizations and nurserymen producing plants within a certification scheme, research breeders, evaluators and other commercial propagators.

Distribution Charges

As of December, 1991, fees will no longer be charged for propagative material distributed from the repository. However, the limited number of cuttings available may not allow us to supply all requests. **All shipping charges and fees will be paid by the receiver. Cuttings will be sent by courier or air freight collect as requested by the receiver.**

Ordering

Please ensure that all orders are received at the Station before **January 15** for dormant scions and rooted plants and **July 15** for summer budwood. Persons requesting rooted plants should first enquire as to their availability. When making requests quote the cultivar and inventory number. Foreign orders should include an import permit if applicable. Please enquire with the regulatory agencies in your country for import requirements. Phytosanitary certificates will be issued as required. Material will not be shipped unless foreign import requirements have been met.

Liability

Unless otherwise indicated, all material leaving the Centre for Plant Health is apparently free from detectable, known viruses. **However, Her Majesty the Queen in right of Canada, Her officers, servants and employees and the Centre for Plant Health assume no responsibility for and cannot be held responsible for virus freedom of any variety, clone or selection of plant material supplied by the Centre.**

Although an effort is being made to have our material examined for trueness-to-type, identification of some foreign material is extremely difficult. **The Centre for Plant Health does not guarantee the identity of each variety, selection or rootstock and cannot be held responsible for varietal trueness and performance.** To assist us, would you examine our material closely for any identification discrepancies. We would appreciate any evaluation reports concerning the accessions in order to upgrade our information.

P - No distribution to USA because of Patents.
C - These cultivars are registered with the Canadian Ornamental Plant Foundation. Commercial Propagators must be a member to receive budwood. Royalties are payable directly to the foundation.
(Ind.) - Variety is used as an indicator for virus testing.
(X) - Indicates varieties to be removed from the repository in 1995.

M A L U S

Indicators used for Malus are Russian R12740-7A, Virginia Crab, Spy 227, Lord Lambourne, Gravenstein, Golden Delicious, Spartan Robusta #5 (B.C. material only), Cox's Orange Pippin (European material only), Granny Smith (Asiatic material only) and Chenopodium quinoa. All newly released material was also tested by ELISA (ACLSV).

	Varieties	QNumber	Source
	Akane (IRA225-1)	Q1150-01	Washington
	Alkmene	Q1217-05	Netherlands
X	Altaiski Sweet	Q 818-01	USSR
	Anaros	Q 469-01	Manitoba
X	Anis Aliy K-32	Q 583-09	USSR
	Battleford	Q 469-02	Manitoba
X	Beacon (IRA180-1)	Q1045-01	Washington
	Beautiful Arcade	Q 69-09	Nova Scotia
	Belle de Boskoop	Q 364-01	Switzerland
	Belle de Boskoop Red (Schmid Hubsch)	Q1217-07	Netherlands
X	Bessemianka micuzina (Seedless of Michurin)	Q 415-01	USSR
	Bonza	Q1319-02	Australia
	Braeburn	Q1285-01	Switzerland
	Bramley's Seedling	Q 537-02	England
	Breakey	Q 59-09	Manitoba
	Britegold	Q1175-01	Ontario
	Burgundy	Q 491-01	New York
X	Caravel	Q 43-06	Ontario
	Carroll	Q 19-02	Manitoba
X	Charlotte	Q 815-01	Nova Scotia
	Collet	Q 19-03	Manitoba
	Cortland	Q 69-04	Nova Scotia
	Cortland 1-5-1	Q 982-04	Nova Scotia
	Cortland 1-14-4	Q 982-08	Nova Scotia
	Cortland 3-5-3	Q 982-05	Nova Scotia
	Cortland 4-10-3	Q 982-06	Nova Scotia
	Cortland 7-10-1	Q 982-11	Nova Scotia
	Cortland (78-1203)	Q 956-01	Quebec
	Cortland Nova Red	Q 16-01	Nova Scotia
	Cortland Spur	Q 348-01	Denmark
	Cox's Red #7	Q1319-04	Australia
	Cox's Orange Pippin (HT68A)	Q 259-01	England
	Crofton	Q1319-05	Australia
	Delia	Q1262-11	Romania
	Delicious		
P	Ace Spur Red	Q 971-03	British Columbia
X	Chelan Red	Q 507-01	Ontario
X	Gardner Red	Q 70-06	Quebec
X	Imperial Red	Q 73-03	British Columbia
	Oregon Spur Red	Q 971-01	British Columbia
	Red Chief Spur	Q 971-02	British Columbia
X	Redspur	Q 122-05	Switzerland
X	Royal Red	Q 70-05	Quebec
X	Thorough Red	Q 70-08	Quebec
X	Topred	Q 73-02	British Columbia
X	Vance Red	Q 70-11	Quebec
	Delikates	Q 970-01	Poland
	Discovery (IRA380-1)	Q1329-05	Washington
X	E 12/14	Q 351-01	South Africa
	Earliblaze	Q 969-01	Missouri
	Early Blaze	Q1228-01	Switzerland
X	Early McIntosh (Rob Roy)	Q 474-02	Washington
X	Elita 20 Marculesti	Q 107-10	Romania
P	Elstar	Q1289-01	Netherlands
	Empire 13-12-23	Q 142-01	New York
X	F 12 A28	Q 159-02	Europe
X	F 14 A15	Q 159-01	Europe
	Fall Red	Q1313-02	Manitoba
X	Fantazja	Q 970-03	Poland
P	Freedom (NY 58553-1)	Q 96-02	New York
	Frumos de Voinesti	Q 633-05	Romania
	Fuji Irradiated Strain	Q1286-06	Japan
	Fuji Red Sport Type 2	Q 264-03	Japan
	Gala	Q 475-03	New Zealand
	Glenorchie	Q 371-02	Manitoba
	Gloster 69	Q 100-01	West Germany
	Golden Delicious	Q 341-02	British Columbia
X	Golden Delicious	Q 480-01	Quebec
	Golden Precoce	Q 93-02	France
	Golden Russet	Q1247-02	England
	Goldgelb 55544	Q 100-02	West Germany
	Goodland	Q1276-01	Manitoba
	Granny Smith Standard	Q 475-01	New Zealand
	Granny Smith Spur-type	Q 475-02	New Zealand
	Gravenstein (IRA46-1)	Q 984-02	Washington
	Gravenstein		
	Henry	Q 982-03	Nova Scotia
	Kozlowski	Q 982-02	Nova Scotia
	Kraus	Q 982-01	Nova Scotia
	Teal Crimson	Q 219-01	Nova Scotia
	Washington Red	Q 69-13	Nova Scotia
	3/1	Q1423-02	Norway
X	H 53-F15-6	Q 107-12	Romania
X	H 55-109-149	Q 107-13	Romania
X	Hamilton	Q 70-01	Quebec
	Haralson	Q1121-01	Washington
	Harcourt	Q 709-01	Saskatchewan
	Heyer #12	Q 71-01	Ontario
	Idared (IRA50-1)	Q 591-04	Washington
	Jerseymac	Q 862-01	Ontario
	Jonagold	Q1217-03	Netherlands
	Jonamac (NY44428-5)	Q 956-02	Quebec
	Jonathan Red	Q1217-06	Netherlands
	Jubilee (IRA91-1)	Q1150-04	Washington
	Kestrel	Q 815-02	Nova Scotia
	Kerr	Q 59-07	Manitoba
	King	Q 69-06	Nova Scotia
	Kinsei	Q 264-05	Japan
	Kogetsu	Q1286-03	Japan
X	Korichnoye Polosatoye	Q 590-03	USSR
	Liberty (IRA314-1)	Q1161-03	Washington
X	Lindel (T397)	Q 43-12	Ontario
	Lobo	Q 475-04	New Zealand
	Lodi (IRA54-1)	Q 591-05	Washington
	Lombart's Calville	Q1217-08	Netherlands
	Lord Lambourne 64A	Q 122-03	England
	Loyalist	Q1139-01	Ontario
	Luke	Q 371-03	Manitoba

Malus continued

	McIntosh		
	1st Generation	Q 74-02	Quebec
	Alix	Q 117-01	Quebec
X	Blackmac	Q 130-01	Washington
X	Bright	Q 807-01	British Columbia
	Imperial (IRA199-1)	Q1206-03	Washington
	Mac Spur	Q 492-04	British Columbia
	Marshall 59-25-3	Q1099-01	Nova Scotia
	Rogers Red (IRA220-1)	Q1206-02	Washington
X	Rougemont	Q 70-07	Quebec
X	St. Hilliare	Q 70-03	Quebec
X	Smithfield	Q 52-11	Ontario
	Summerland Red	Q 467-01	British Columbia
X	Vermont Spur	Q 346-01	Nova Scotia
P	Wijcik Spur	Q 73-15	British Columbia
	Macfree	Q1139-03	Ontario
	Maigold (Schin-H-6)	Q 26-01	Switzerland
P	McShay	Q1424-01	Oregon
	Malling Kent	Q 266-01	England
	Manitoba Spy	Q 502-01	Manitoba
	Mantet	Q 482-01	West Germany
	Melred	Q 70-04	Quebec
	Melrose	Q 142-03	New York
	Moira	Q1139-02	Ontario
	Morden 359	Q 170-02	Manitoba
	Murray	Q 347-01	Ontario
	Muscadet de Dieppe (cider)	Q 623-01	France
	Mutsu	Q1206-04	Washington
	Noran	Q 692-06	Alberta
	Norcue	Q 692-03	Alberta
	Norda	Q 692-07	Alberta
	Noret	Q 692-02	Alberta
	Norhey	Q 692-04	Alberta
	Norland	Q 975-04	Manitoba
	Norson	Q 692-05	Alberta
	Northern Spy	Q 43-13	Ontario
	Northwest Greening (IRA298-1)	Q1271-03	Washington
	Nova Easygro	Q 815-03	Nova Scotia
X	Novosibirski Sweet	Q 818-02	USSR
X	OBR Rome	Q 69-17	Nova Scotia
X	Oranzhevoye	Q 590-02	USSR
	Orenco	Q1168-07	British Columbia
	Orin	Q1192-01	Japan
X	Ottawa 271	Q 121-01	Ontario
X	Ottawa 292	Q 121-02	Ontario
X	Ottawa 546	Q 268-08	Ontario
	Ottawa 654	Q1385-01	Ontario
X	Ottawa 5210	Q 52-09	Ontario
	Parkland	Q 975-02	Manitoba
	Patterson	Q 66-01	Manitoba
X	Pepping Chernenko	Q 633-06	Romania
	Priam	Q 521-01	France
	Puritan	Q1253-01	Ontario
X	Quinte	Q 43-10	Ontario
X	Ranger	Q 43-08	Ontario
X	Raritan	Q 119-01	Quebec
X	Reinette Simirenko	Q 633-04	Romania
	Rescue	Q 59-06	Manitoba
	Rhode Island Greening (IRA303-1)	Q1161-08	Washington
	Ribston	Q 69-01	Nova Scotia
	Richelieu	Q1222-02	Quebec
X	Roda Mantet	Q 606-01	Netherlands
	Rome Beauty Cowin	Q 38-03	British Columbia
	Rome Beauty Law	Q 73-06	British Columbia
X	Rossoshanskoje (Striped of Rossosh)	Q 583-02	USSR
	Rosu de Cluj	Q 633-03	Romania
	Rouville	Q1222-01	Quebec
	Russel Red	Q1319-01	Australia
X	Sandel	Q 43-15	Ontario
X	Scotia	Q 69-18	Nova Scotia
	September Ruby	Q1313-01	Manitoba
X	Severny Sinap K-2139	Q 583-01	USSR
X	Shafer	Q 371-01	Manitoba
	Shamrock	Q1252-07	British Columbia
X	Snow	Q 859-10	Ontario
	Spartan	Q 334-01	British Columbia
	Spartan Summerland Spur	Q1129-01	England
	Spigold	Q 142-06	New York
	Spy (Double Red)	Q 69-19	Nova Scotia
	Starkspur Golden Delicious	Q 492-02	British Columbia
	Sterappel	Q1217-04	Netherlands
	Sumac	Q1252-06	British Columbia
	Summerred	Q1324-02	British Columbia
X	T3913	Q 43-04	Ontario
	Trail	Q 59-05	Manitoba
	Trailman	Q 692-01	Alberta
	Trent	Q 268-07	Ontario
	Tydeman Early Worcester	Q 372-01	England
	Tydeman Red (Cooper)	Q 73-08	British Columbia
X	Uralskoye Nalivnoye (Juicy of the Ural)	Q 583-05	USSR
	Vista Bella (NJ 36)	Q 956-03	Quebec
	Wayne	Q 70-12	Quebec
	Wealthy	Q 479-01	Ontario
X	Wellington	Q 142-05	New York
	Westland	Q 975-03	Manitoba
	Winter Banana (IRA48-1)	Q 247-16	Washington
	Yellow Transparent	Q 73-10	British Columbia
	Z61 (scab resistant)	Q 12-13	Netherlands

MALUS ORNAMENTALS

	Adams	Q 816-01	Massachusetts
	Almey	Q 71-02	Ontario
	Athabaska	Q1199-03	Illinois
	CC-14-45	Q 59-02	Manitoba
C	Cameron	Q 18-01	Ontario
	Candied Apple	Q1174-01	Oregon
	Charlottae	Q 473-03	British Columbia
	Crab 1 (pollinator)	Q1284-07	British Columbia
	Crab 2 (pollinator)	Q1284-08	British Columbia
	Crab 3 (pollinator)	Q1284-09	British Columbia
	Crab 4 (pollinator)	Q1284-10	British Columbia
	Crab 5 (pollinator)	Q1284-11	British Columbia
	Crab 6 (pollinator)	Q1284-12	British Columbia
	Crab 7 (pollinator)	Q1284-13	British Columbia
	Dolgo	Q 473-05	British Columbia

Malus Ornamentals continued

	Dorothea	Q 473-02	British Columbia
	Earl	Q 594-01	Saskatchewan
	Eleyi	Q 285-04	Netherlands
	Evelyn (IR169-1A)	Q 849-02	Washington
	Floribunda Rosea	Q 473-01	British Columbia
	Florentina	Q 279-02	Ontario
	Garry	Q 59-11	Manitoba
	Guiding Star	Q1220-01	Ontario
	Hopa (IRA138-1)	Q1244-01	Washington
X	Hoser	Q 24-06	Netherlands
	John Downie	Q 419-04	Netherlands
	Kelsey (IRA260-1)	Q1206-01	Washington
X	Kobenza	Q 24-08	Netherlands
	Liset	Q 162-02	Netherlands
	M. baccata Mandshurica #6114	Q 502-03	Manitoba
	M. Floribunda (IRA361-1)	Q1443-01	Washington
	M. hupehensis	Q 490-01	Pennsylvania
X	M. kansuensis	Q 279-05	England
	M. platycarpa (Ind.)	Q 587-04	Ontario
	Makamik	Q 473-04	British Columbia
C	Maybride	Q 18-03	Ontario
	Nova	Q1131-01	British Columbia
	Ormiston Roy	Q 721-01	British Columbia
C	Prince Charming	Q 18-02	Ontario
	Profusion	Q 473-06	British Columbia
	Purple Wave (IRA101-1)	Q 230-01	Washington
	Radiant	Q 59-16	Manitoba
	Red Splendor	Q 371-06	Manitoba
	Rosthern	Q 371-04	Manitoba
	Royalty	Q 59-14	Manitoba
X	Royalty	Q 270-01	Netherlands
	Robinson	Q 804-01	Indiana
	Rudolph	Q 59-15	Manitoba
	Selkirk	Q 59-12	Manitoba
	Springtime	Q 500-01	Ontario
	Sundog	Q1316-01	Manitoba
C	Thunderchild	Q 189-04	Saskatchewan
	Van Eseltine	Q 473-08	British Columbia
	Virginia Crab	Q 334-03	British Columbia
	White Angel	Q1224-01	British Columbia
	Winter Gold	Q 298-02	Netherlands

ROOTSTOCKS AND MISCELLANEOUS

	62-396	Q1328-01	USSR
	ASG 13-20 (Ind.)	Q1330-01	Washington
	Alnarp 2	Q 37-01	British Columbia
	Antonovka Debnicka	Q 12-04	Poland
	Bedford	Q 59-04	Manitoba
	Budagovsky 9	Q 307-01	USSR
	Budagovsky 9	Q 25-01	USSR
	Budagovsky 54-118	Q 33-02	USSR
	Budagovsky 54-146	Q 585-02	New York
	Budagovsky 57-233	Q 548-01	USSR
	Budagovsky 57-490	Q 548-02	USSR
	Budagovsky 57-491	Q 33-01	USSR
	Budagovsky 490	Q 622-04	USSR
	Columbia	Q 59-03	Manitoba
X	Dab 97	Q 12-09	West Germany
X	Dab 100	Q 12-10	West Germany
X	Dab 183	Q 12-12	West Germany
X	Dab 325	Q 12-11	West Germany
X	Demir	Q 12-03	Turkey
	J-TE-B	Q1303-01	Czechoslovakia
	J-TE-C	Q1303-02	Czechoslovakia
	J-TE-D	Q1360-01	Czechoslovakia
	J-TE-E (15704)	Q1239-01	Czechoslovakia
	J-TE-F	Q1303-04	Czechoslovakia
	J-TE-G (15706)	Q1239-03	Czechoslovakia
	J-TE-H (15707)	Q1239-04	Czechoslovakia
X	Korichnoye Novoye K23938	Q 590-01	USSR
	M.1	Q 653-04	England
	M.2 (IRA89-3)	Q 476-01	Washington
	M.2 Jansen Strain	Q 733-01	Netherlands
	M.3	Q 653-02	England
	M.4	Q 366-01	Netherlands
	M.7	Q 373-01	New York
	M.8	Q 653-01	England
	M.9	Q 365-03	England
	M.9	Q 703-01	West Germany
	M.9 (T.337)	Q 188-01	Netherlands
X	M.9 (T.338)	Q 493-01	Netherlands
	M.9 (T.339)	Q 492-03	Netherlands
X	M.9 (T.340)	Q 188-04	Netherlands
X	M.9 (Troch strain)	Q 42-02	Belgium
	M.20	Q 653-03	England
	M.25	Q 509-01	England
	M.26 (3436)	Q 365-06	England
	M.27	Q 365-08	England
	MM.104	Q 365-18	England
	MM.106	Q 365-02	England
	MM.111	Q 365-01	England
	Nertchinsk	Q 30-01	Manitoba
X	Osman	Q 82-02	Ontario
	Ottawa 3	Q 710-01	Saskatchewan
	Ottawa 4	Q 52-08	Ontario
	Ottawa 5	Q 946-02	Saskatchewan
	Ottawa 7	Q 946-03	Ontario
	Ottawa 8	Q 946-04	Ontario
	Ottawa 11	Q 52-05	Ontario
	Ottawa 12	Q 946-06	Ontario
	Ottawa 13	Q 52-07	Ontario
	P.2	Q 555-02	Poland
	P.16	Q 555-03	Poland
	P.18	Q1062-01	Poland
	P.22	Q 555-04	Poland
X	Patul	Q 107-11	Romania
	Robusta #5 (Ind.)	Q 334-12	British Columbia

ROOTSTOCKS AND MISCELLANEOUS continued

	Russian R12740-7A (Ind.)	Q 334-07	British Columbia
	Spy 227 (Ind.)	Q 334-02	British Columbia
X	Stahls Prinz (Ind.)	Q 503-01	Nova Scotia
	Trusevitch I-48-46	Q 622-07	USSR
	Trusevitch I-48-41	Q 622-02	USSR
	Trusevitch II-14-50	Q 622-06	USSR
	Trusevitch V-5-38	Q 622-03	USSR
	YP (MB 4)	Q 488-01	Finland
X	Z 71 (scab resistant)	Q 12-14	Netherlands
X	Z 73 (scab resistant)	Q 12-15	Netherlands
X	Z 185	Q 12-16	Netherland

OPEN POLLINATED SEEDLINGS

X	Apxathoc	Q 35-26	USSR
X	Evaria (new Georgian cv.)	Q 35-25	USSR
X	Nbascata x Antonovka	Q 35-32	USSR
X	Slava pobeditelyam (Glory of Conquerors)	Q 35-22	USSR
X	Ukrainian Selection	Q 35-29	USSR
X	Ulichurenski	Q 35-28	USSR

P R U N U S

Indicators used for Prunus are Shirofugen, Kwanzan, Tilton, Luizet (European cvs.) Elberta, Shiro plum, Bing, Sam, Lamber (B.C. cherries), Peach and apricot seedling, herbaceous indicators and ELISA (PNRS, PD). All newly released material was als tested by ELISA (ACLSV, PPV).

	Varieties	QNumber	Source
	Almond		
	Early 1	Q1110-02	Czechoslovakia
	Hustopecka 7	Q1223-02	Czechoslovakia
	Tetenyi Kedvenc	Q1180-05	Hungary
	Tetenyi Kemenyheju	Q1180-04	Hungary
	Tetenyi Rekord	Q1180-03	Hungary
	VA 4/8	Q1223-03	Czechoslovakia
	VA 5/8	Q1223-09	Czechoslovakia
	Apricot		
	Beograd 'I' Tanja Pavlovic	Q 972-03	Yugoslavia
	Bilida 8	Q1194-01	Spain
	Bolton	Q 963-03	New Zealand
	Budapest	Q1180-18	Hungary
	Cacak's Gold	Q 967-10	Yugoslavia
	Cegledi Bibor	Q1180-14	Hungary
	Cegledi Hajnalpir	Q1180-21	Hungary
	Cegledi Orias	Q1180-20	Hungary
	Debbie's Gold	Q1314-02	Manitoba
	Dundonald	Q 963-05	New Zealand
	Erevan	Q 967-11	Yugoslavia
	G-15	Q1185-02	Korea
	Goldcot (IR740-1)	Q1368-05	Washington
	Gonci Magyar Kajszi	Q1180-16	Hungary
	H XIII/10	Q 967-09	Yugoslavia
	Harcot	Q 165-23	Ontario
	Harglow	Q1205-01	Ontario
	Hargrand	Q1098-06	Ontario
	Harlayne	Q 165-26	Ontario
	Harogem	Q 165-20	Ontario
	Harval	Q1154-02	Ontario
	HL 1/28	Q1297-06	Czechoslovakia
	HL 1/34	Q1297-08	Czechoslovakia
X	HO XIV/I	Q 967-15	Yugoslavia
	Kecskei Rozsa	Q1180-19	Hungary
	Ligeti Orias	Q1180-15	Hungary
	Luizet (Ind.)	Q 72-02	California
	M604	Q 125-02	Manitoba
	Madarska	Q1109-07	Czechoslovakia
	Mandshurica	Q 979-02	USSR
	Mari De Cenad	Q 123-01	Romania
X	Melitopoeskii Early	Q 36-06	USSR
	Morocco	Q 963-04	New Zealand
	Nigataomi	Q1185-01	Korea
	Paviot	Q1223-05	Czechoslovakia
	Rakovsky BU 33	Q1180-17	Hungary
	RRS-1A	Q 962-06	New Zealand
	RRS-9	Q 962-02	New Zealand
	RRS-57	Q 963-01	New Zealand
	Royal (IR267-1)	Q 131-10	Washington
X	Samarkand (Early)	Q 36-07	USSR
	Scout (IR24-1)	Q 131-14	Washington
	Skaha (4E-28-14)	Q 131-13	Washington
X	Stepynak	Q 1-06	USSR
	Stevens Favourite	Q 962-07	New Zealand
	Sundrop (4E-55-9)	Q 73-30	British Columbia

Apricots continued

	Tilton (IR5-1) (Ind.)	Q 131-11 Washington
	Timpurii de Arad	Q1211-02 Romania
X	Timpurii de Chisonau	Q 123-05 Romania
	VA 13/5	Q1075-13 Czechoslovakia
	Veecot	Q1167-03 Ontario
	Velkopavlovicka	Q1075-05 Czechoslovakia
	Velvaglo	Q1167-02 Ontario
	Viceroy	Q1167-04 Ontario
	Vivagold	Q1167-01 Ontario
	Wenatchee (IR95-2)(Ind.)	Q 131-15 Washington
	Westcot	Q1314-01 Manitoba

Cherry - Sour

	Cigany 7	Q1180-06 Hungary
	Cigany 59	Q1180-08 Hungary
	Cigany C404	Q1227-04 Hungary
	Crisana 15/10	Q1211-31 Romania
	Early Richmond (IR239-2)	Q 859-02 Washington
	English Morello (IR93-3)	Q 576-02 Washington
	Erdi Botermo	Q1180-09 Hungary
	Meteor	Q1365-02 British Columbia
	Montmorency Hobbs LBC	Q 73-35 British Columbia
	Nefris	Q 970-02 Poland
	North Star	Q1365-01 British Columbia
X	Otechestvennaja	Q 581-02 USSR
	Pandy 279	Q1180-13 Hungary
	Pandy 48	Q1227-01 Hungary
	Pandy 88 119	Q1227-02 Hungary
	Schattenmorelle	Q1372-01 Poland
	Scharo	Q 846-01 West Germany
	Ujfehertoi Furtos	Q1227-03 Hungary
X	Vladimirskaia	Q 123-10 Romania

Cherry - Sweet

X	Beta	Q 770-04 Denmark
	Big Burlat (69-1-1188L)	Q1177-01 Italy
	Bing (Milton IBC)	Q 73-31 British Columbia
X	Cananski Rubin	Q 249-05 Yugoslavia
	Canindex I (Ind.)	Q 316-01 British Columbia
X	Cherna Konyavsna	Q 242-01 Bulgaria
	Dawson	Q 693-01 New Zealand
X	Early Rivers	Q 770-03 Denmark
	Kootenay Bay Lambert 103-10	Q 331-01 Washington
X	Krasarica	Q 194-02 Poland
	Lambert (VF-24)	Q 468-01 British Columbia
	Lambert Compact	Q1287-01 British Columbia
	Lapins	Q1217-01 British Columbia
	Merchant	Q1126-01 England
	Merpet	Q1126-02 England
X	'Patricia's cherry'	Q 756-02 Indiana
X	Princess (Ocksenherz)	Q 770-05 Denmark
	Rainier (IR144-1)	Q1329-07 Washington
	Salmo	Q 383-02 British Columbia
	Sam (IR24-1-5)	Q 360-01 Washington
X	Schmidt (IR311-1)	Q 247-04 Washington
X	Schneiders Sildige	Q 770-07 Denmark
	Star (IR488-1)	Q 476-03 Washington
	Stella Compact	Q 383-01 British Columbia
	Stella (Standard)(SC-6-7)	Q 356-01 British Columbia
X	Stella (Standard) (IR538-1-1)	Q 576-03 Washington
	Summit	Q1324-01 British Columbia
	Sunburst (IR1254-1)	Q1329-06 Washington
	Sylvia	Q1252-01 British Columbia
X	Teichnus Schwarze	Q 770-08 Denmark
	Van I-BC	Q 73-34 British Columbia
	Van (Compact)(IR763-1)	Q1271-02 Washington
	Valeska	Q 770-09 Denmark
	Vega (IR602-1-1)	Q 576-05 Washington
X	Velvet (E204)	Q 859-03 Ontario
	Vernon	Q 79-07 Ontario
	Vic	Q 642-01 Ontario
X	Vic (IR671-3)	Q 984-04 Washington
	Victor (IR495-1)	Q 247-01 Washington
	Viscount	Q1167-16 Ontario
	Vista	Q 642-03 Ontario
	Vittoria	Q 241-01 Italy
	Viva	Q 164-01 Ontario
X	Vogue	Q 164-02 Ontario
X	Windsor (IR150-3)	Q 247-02 Washington

Nectarines

	Anderson	Q1294-04 Italy
	Andromeda	Q1294-01 Italy
X	Braundy Morton	Q 214-16 France
	Early Sungrand	Q1040-06 British Columbia
	Fantasia (IR801-1A)	Q1368-02 Washington
	Harblaze	Q1274-01 Ontario
	Hardired	Q1188-01 Ontario
	Harko	Q1098-05 Ontario
X	Nectared	Q 214-15 France
	Nectared 6	Q 139-05 France
	Nectared 4	Q 139-06 France
	New Haven (IR1153-1)	Q1368-04 Washington
	Redgold (IR1114-1)	Q1271-01 Washington
	Ruby Gold	Q1040-02 British Columbia
	Starks Earliblaze	Q1040-07 British Columbia

Peaches

	Albatros	Q 329-02 South Africa
	Babygold #5	Q 139-11 France

Peaches continued

X	Babygold #6	Q 139-07	France
	Babygold #7	Q 139-10	France
	Biscoe (IR745-2)	Q1206-05	Washington
	Canadian Harmony (H1748)	Q 35-02	Ontario
	Candor	Q1366-05	Ontario
	Champion TS 159	Q1180-01	Hungary
	Chui Lum Tao	Q 1-02	USSR
	Correll (IR816-12)	Q1368-03	Washington
	Cresthaven	Q1366-06	Ontario
X	Dorata Tardiva	Q 727-02	Italy
X	Earlibelle	Q 329-05	South Africa
	Earliglo	Q1149-02	Ontario
X	Earlyred	Q 139-09	France
	Early Redhaven	Q 823-01	Washington
	Elberta (IR3-5)	Q 333-17	Washington
	Emilia	Q1296-07	Italy
	Envoy	Q1208-01	Ontario
X	Erlyvee	Q 859-07	Ontario
	Ernared (Galway Bay)	Q 116-02	Ontario
	Fairhaven (IR198-1)	Q 131-06	Washington
X	Favorita I	Q 727-06	Italy
X	Favorita II	Q 727-05	Italy
	Federica	Q1296-08	Italy
X	Ferganensis (PI 113455)	Q 375-05	USSR
X	Fertilia II Morettini	Q 578-02	Italy
	Flaminia	Q1296-01	Italy
X	Fortuna	Q 139-03	France
X	Gabriella	Q 727-01	Italy
X	Gabriella	Q 578-03	Italy
	Gang Shan Zao Sheng	Q1288-02	China
	Garnet Beauty	Q 139-08	France
	Glohaven	Q1224-03	British Columbia
X	Golden Amber	Q 329-03	South Africa
	Halford (IR31-1)(Ind.)	Q 247-07	Washington
	Harbelle C298	Q 587-03	Ontario
	Harbinger	Q 165-12	Ontario
	Harbrite	Q 165-08	Ontario
	Harcrest	Q1205-04	Ontario
	Harken (H 2066)	Q 35-10	Ontario
	Harrow Beauty	Q1154-06	Ontario
	Harrow Diamond	Q1205-03	Ontario
	Harson (H 781)	Q 165-16	Ontario
X	Ingwe	Q 329-04	South Africa
X	Kakamas	Q 541-01	South Africa
	Leaf Curl Resistant Peach	Q 457-01	British Columbia
	Loring (IR200-3)	Q 131-07	Washington
	Madison	Q1366-04	Ontario
X	Mao Tao (PI 240928)	Q 375-07	China
	Mariska	Q1180-02	Hungary
	Merrill 49'er	Q1040-03	British Columbia
	N.J.N. 21	Q1040-10	British Columbia
	Newhaven (1R1153-1)	Q1368-04	Washington
X	Olinda	Q 116-03	Ontario
X	Oom Sarel	Q 541-02	South Africa
	Orion	Q 329-01	South Africa
X	Pats Haven	Q 412-01	British Columbia
X	Prodigiosa	Q 727-07	Italy
	Redhaven (IR62-2)	Q 131-09	Washington
	Redkist	Q1366-08	Ontario
	Redskin (IR204-01)	Q 131-08	Washington
	Reliance	Q1366-07	Ontario
	Rogani - Gow (PI 113452)	Q 375-25	USSR
X	Royalvee	Q1149-06	Ontario
X	Safari	Q 329-07	South Africa
	Sdlg. Red Haven type (leaf curl resistant)	Q 376-01	British Columbia
X	Selection (PI 95501)	Q 375-17	China
X	Selection Sdlg.		
X	(PI 134400)	Q 375-21	China
X	(PI 146137)	Q 375-19	USSR
X	(PI 134401)	Q 375-22	China
	Sha Zi Zao Sheng	Q1288-01	China
X	Sihung Chui Mi	Q 1-03	USSR
	Silver Gold	Q1040-09	British Columbia
	Somervee	Q1208-02	Ontario
	Springold	Q1040-04	British Columbia
	Starking Delicious	Q1258-01	Australia
X	Sunray	Q 329-06	South Africa
	Valiant	Q1149-05	Ontario
	Vanity	Q1072-04	Ontario
	Veecling	Q1072-03	Ontario
	Veeglo	Q1167-15	Ontario
	Villa Ada	Q1296-09	Italy
	Villa Adriana	Q1296-05	Italy
	Villa Doria	Q1296-03	Italy
	Vivid	Q1273-01	Ontario
X	XIII/60BR	Q 967-02	Yugoslavia
X	XVI/8D	Q 967-01	Yugoslavia
X	Yugoslavia	Q 1-04	USSR
X	Yunnan (PI 55776)	Q 375-16	China
	Zin Dai Jiu Bao	Q1288-05	China

Plum

X	II/II/80/59	Q 967-04	Yugoslavia
X	Abundance (IR91-1)	Q 333-01	Washington
X	Battema	Q 34-02	USSR
X	Blufre (IR305-3)	Q 333-03	Washington
	Bounty	Q 84-05	Manitoba
	Bradshaw	Q 73-22	British Columbia
	Brookgold (IR575-3)	Q 131-17	Washington
	Brookred (IR576-1)	Q1292-02	Washington
	Burbank (IR106-1)	Q 333-05	Washington
	Cacak's Best	Q 967-05	Yugoslavia
	Early Crimea	Q 36-01	USSR
	Early Golden	Q 587-01	Ontario
	Early Italian	Q 826-01	Washington
	Ember (IR230-1)	Q1244-04	Washington
	Ersinger	Q 760-01	Switzerland
	Formosa	Q1161-02	Washington
X	Gras Ameliorat	Q 634-05	Romania
	Green Gage	Q 265-01	British Columbia

Plum continued

	Grenville	Q 84-02	Manitoba
X	Hric Ringlot	Q 116-01	Czechoslovakia
X	H 5/10	Q 634-01	Romania
	Italian Prune (IR16-1)	Q 333-06	Washington
X	Kaga (IR556-2-1)	Q 247-15	Washington
	Krikon Damson (IR227-2)	Q 762-01	Washington
X	La Crescent (IR228-1)	Q 333-07	Washington
X	Lanark	Q 349-03	Quebec
	Mandarin	Q 167-05	Manitoba
	Manor Cherry Plum	Q 167-04	Manitoba
	Merton	Q1161-12	Washington
	Methley (IR387-2)	Q 333-08	Washington
	Mirabelle de Nancy	Q 854-01	France
	Morettini 355	Q 727-08	Italy
	Mount Royal	Q1111-05	British Columbia
	Northern	Q 84-07	Manitoba
	Oneida (IR675-3)	Q1161-04	Washington
	Opal	Q 176-01	Sweden
	Opata (IR554-3)	Q 333-09	Washington
	Peach Plum	Q 73-21	British Columbia
X	Peach Plum	Q 137-01	Oregon
	Pembina	Q 167-08	Manitoba
	Pobeda (Victory)	Q 36-03	USSR
	Pozegaca	Q 338-02	Europe
	Ptitsin #5	Q 167-02	Manitoba
	Ptitsin #9	Q 125-05	Manitoba
	Radisson Hybrid	Q 125-08	Manitoba
	Red June	Q1040-01	British Columbia
	Redglow (IR433-1)	Q 333-10	Washington
	Santa Rosa	Q 823-02	Washington
	Sapa	Q 84-10	Manitoba
X	Sapalta (IR549-1)	Q 333-11	Washington
	Shiro (IR14-4)	Q 333-12	Washington
	Shropshire Damson	Q1113-01	England
X	Songold	Q 343-01	South Africa
	Stanley (IR81-1)	Q 333-13	Washington
	Superior (IR544-2)	Q 333-14	Washington
	Tecumseh (IR548-3)	Q 333-15	Washington
	Temptation	Q 36-05	USSR
X	Toka (IR432-1)	Q 333-16	Washington
	Vanier	Q1253-03	Ontario
	Veeblue	Q 642-02	Ontario
	Verity	Q1366-01	Ontario
	Victoria	Q1153-02	Netherlands
	Victory	Q1432-03	Ontario
	Vinat Romanesc	Q 634-07	Romania
	Voyageur	Q1173-03	Ontario
	Wessex #9	Q 221-09	Saskatchewan
	Yakima (IR1073-01)	Q1244-05	Washington
	Zarya	Q 34-01	USSR

ROOTSTOCKS

Apricot

	Haggith	Q 510-03	Ontario

Cherry - Sour

	Mahaleb 48	Q 73-38	British Columbia

Cherry - Sweet

	CAB 4D	Q 545-01	Italy
	CAB 6P	Q 545-02	Italy
	CAB 9E	Q 545-03	Italy
	Mazzard (seed purpose)	Q 486-02	California
	Mazzard(mildew resistant)	Q 717-04	Washington
	Mazzard F12/1	Q 365-15	England
	Stockton Morello (IR450-1)	Q 984-03	Washington
	Vladimir Piarasus	Q 738-01	California

Peach and Peach X Almond

	B-VA-2	Q1075-15	Czechoslovakia
	BVMA 6/2	Q1223-07	Czechoslovakia
X	Boone County Seedling (IR501-1)	Q 339-02	Washington
	Kando	Q1223-06	Czechoslovakia
	Harrow Blood	Q 1-05	Ontario
	P. mira	Q 966-03	France
	Rutger Red Leaf (IR166-4)	Q 339-01	Washington
	Siberian C	Q 1-01	Ontario
	Tennessee Natural	Q 342-01	British Columbia
	VA 8M 1/40	Q1075-17	Czechoslovakia
	VA 8M 29	Q1110-01	Czechoslovakia
	VA 8M VA 1/31	Q1109-04	Czechoslovakia
	VA RI/50 II	Q1223-04	Czechoslovakia

Plum

	Brompton (IR222-2)	Q 247-14	Washington
	Myrobalan B	Q 365-16	England
	P2038 (P.besseyi x myrobolan)	Q 966-05	France
	Pollizo (Soto 101)	Q1070-03	Spain
X	St Julien A	Q 365-17	England
	St Julien A	Q 509-02	England
	Yuksa	Q 84-11	Manitoba

PRUNUS ORNAMENTALS

Chokecherry

X	Chokecherry Seedling (Shubert Type)	Q 847-01	Ontario
	Shubert Chokecherry	Q 587-02	Ontario
X	Shubert Chokecherry	Q 848-01	Alberta

Cherry - Japanese Flowering

	P. avium 'Plena'	Q 178-07	England
	P. x campanulata 'Shosar'	Q 178-04	England
	P. cerasus 'Rhexii'	Q 178-02	England
	P. hillieri 'Spire'	Q 178-10	England
	P. x incamp 'Okame'	Q 496-01	Washington
	P. x incisa 'Umineko'	Q 178-11	England
	P. lannesiana 'Amanogawa'	Q 178-01	England
	P. sargentii 'Rancho'	Q1320-05	British Columbia
	P. schmittii	Q 178-15	England
	P. serrula "Birch Bark"	Q 178-03	England
	P. serrulata		
	'Benifugen'(IR1090-1)	Q1443-07	Washington
	'Ichiyo'	Q 178-05	England
	'Kiku Shidare Zakura'	Q 340-02	British Columbia
	"Korean Hill Cherry"	Q 178-06	England
	'Kwanzan'	Q 334-08	British Columbia
	'Pink Perfection'	Q 178-13	England
	'Shirofugen' (Ind.)	Q 334-09	England
X	'Shirotae'(Lohbrunner)	Q 736-02	British Columbia
	'Shirotae' (Mt Fuji)	Q 178-12	England
	'Ukon'	Q 178-08	England
	P. subhirtella		
	'Autumnalis'	Q 178-09	England
	"Pendula Double"	Q1320-03	British Columbia
	"Pendula Single"	Q1320-02	British Columbia
X	"Pendula Single" (IR320-1)	Q 576-04	Washington
	'Snowdrop'	Q1376-02	British Columbia
	'Whitcombi'	Q 736-01	British Columbia
	P. subhirtella x yedoensis		
	'Pandora'	Q1320-04	British Columbia
	P. yedoensis		
	'Akebono'	Q 496-02	Washington
	"Yoshino Cherry"	Q1081-01	Netherlands
	'After Glow'	Q1439-01	Oregon

MISCELLANEOUS

X	Open Pollinated Sdlg. (Plum)	Q 35-16	USSR
X	Polish Cultivar (Plum)	Q 35-14	Poland
	P. amygdalopersica		
	'VA BM 29'	Q1110-01	Czechoslovakia
	P. x blireiana	Q 586-02	British Columbia
	P. cerasifera		
	'Atropurpurea'	Q 486-01	California
	'Newport'	Q1111-01	British Columbia
	'Nigra'	Q1151-01	British Columbia
	P. cistena	Q 495-03	Ontario
	P. glandulosa		
	'Albiplena'	Q1282-02	British Columbia
	'Sinensis'	Q1282-03	British Columbia
	P. laurocerasus		
	'Otto Luyken'	Q1106-01	British Columbia
	'Reynvaanii'	Q1106-02	British Columbia
	'Schipkaensis'	Q1106-03	British Columbia
	'Zabeliana'	Q1106-04	British Columbia
	P. lusitanica		
	'Portugal Laurel'	Q1077-02	British Columbia
	P. mume		
	'Chenton'	Q1256-04	Taiwan
	P. padus (IR406-2)	Q 591-06	Washington
	P. padus 'Dropmore'	Q 773-01	Manitoba
	P. persica		
C	'Candifloss'	Q 552-02	Ontario
C	'Frostipink'	Q 552-05	Ontario
C	'Rubirose'	Q 552-03	Ontario
	P. prostrata	Q1282-01	British Columbia
	P. pseudocerasus		
	'Cantabrigiensis'	Q 690-01	England
	P. tenella		
	'(GR #6414)'	Q 975-01	Manitoba
	'Firehill'	Q1214-01	Great Britain
	P. tomentosa (Ind.)	Q 853-01	Washington
	P. tomentosa	Q 921-01	Minnesota
	P. triloba (IR274-1)	Q 476-05	Washington

P Y R U S

Indicators used for Pyrus are Pyronia veitchii, Jules d'Airolles, Lord Lamborne, Bosc, Bartlett and herbaceous indicator Chenopodium quinoa. All newly released material was also tested by ELISA (ACLSV).

	Varieties	QNumber	Source
X	17-4/5-32	Q 107-08	Romania
X	138-5-22	Q 107-06	Romania
X	138-31-38	Q 107-03	Romania
	Aniversarea	Q1211-10	Romania
	Anjou de Beurre (IRP1-1)	Q 591-03	Washington
	Anjou Dwarf (IRP9-1)	Q 591-01	Washington
	Aromata de Bistrita	Q1211-15	Romania
	Aurora	Q 193-52	New York
	Bartlett (IRP20-1)	Q 691-01	Washington
	Bartlett B13 (Ind.)	Q 576-06	Washington
	Bartlett Russet	Q 559-01	British Columbia
X	Bella di Giugno	Q 729-02	Italy
	Bosc (Beurre)	Q 849-03	Washington
	Butirra Precoce Morettini	Q 578-05	Italy
	Chanticleer	Q1376-01	British Columbia
	Chapin	Q 193-46	New York
	Chojuro	Q1254-01	Washington
	Clapp's Favorite	Q 69-07	Nova Scotia
X	Clapp's Favorite	Q 477-01	West Germany
X	Clapp's Favorite	Q 470-01	France
	Conference	Q 139-01	France
	Coscia	Q 729-01	Italy
	Coscia Precoce	Q 729-04	Italy

Pyrus continued

	Doina	Q1211-11 Romania
	Doitsu (IRP187-1)	Q1329-02 Washington
	Doyenne du Comice	Q 470-03 France
	Doyenne Hiver	Q 522-01 France
	Doyenne Perrault	Q 522-05 France
	Dutchess de Mouchy	Q 522-04 France
	Eldorado (IRP10-1)	Q 489-03 Washington
	Fiorenza	Q 578-07 Italy
	Frangipane	Q 522-07 France
	General Tottleben	Q 44-12 England
	Giffard	Q 79-05 Ontario
	Golden Spice	Q 66-06 Manitoba
	Gorham	Q 193-48 New York
	Hardy (Ind.)	Q 338-01 Europe
	Harrow Delight (HW 603)	Q 534-04 Ontario
	Harvest Queen (HW 602)	Q 534-02 Ontario
	Highland	Q 491-02 New York
	Hosui	Q1254-12 Washington
X	Italy 1437	Q 44-19 England
	Jeanne d'Arc	Q 522-06 France
	Jules d'Airolle (Ind.)	Q 226-01 England
	Kieffer	Q 79-01 Ontario
	Kosui (IRP198-1)	Q1428-01 Washington
	Magness	Q 193-49 New York
	Maxine	Q 193-51 New York
	Moonglow	Q 470-05 France
X	New York 2480	Q 193-50 New York
	Nijisseiki (20th Century)	Q1317-01 British Columbia
	Nitaka	Q1254-03 Washington
	Nouveau Poiteau (IRP90-1)	Q 707-01 Washington
	Old Home (IRP14-1)	Q 591-02 Washington
X	Ottawa 291	Q 52-12 Ontario
	Pinggioli	Q1288-06 China
	Pioneer #3	Q 502-05 Manitoba
X	Poirier Fleurissant Tard	Q 522-03 France
X	President d'Osmonville	Q 44-14 England
X	Princess	Q 44-15 England
	Red Fleshed Pear	Q1008-01 Illinois
	Reiny Chaternay	Q 522-02 France
	Republica	Q1211-14 Romania
	Santa Maria	Q 578-06 Italy
	Seigyoku (IRP167-1)	Q1329-03 Washington
	Seuri	Q1254-11 Washington
	Shinko	Q1254-10 Washington
	Shinseiki	Q1254-09 Washington
	Sierra	Q 73-18 British Columbia
	Sirrine	Q 193-47 New York
	Spadona di Salerno	Q 729-03 Italy
	Spina Carpi	Q 729-05 Italy
	Timpurii de Dimbovita	Q1211-16 Romania
	Tsu Li (IRP166-1)	Q1368-01 Washington
	Ure (GG 87A-24)	Q 618-02 Manitoba

	Quince C7/1 (Ind.)	Q 260-01 England
	Quince Kwee Adams	Q 505-01 Netherlands
	Quince S	Q 58-01 Poland

OPEN POLLINATED SEEDLINGS

X	Hua Gez Shureweiz	Q 35-18 Poland
X	Ukrainian Sel	Q 35-20 USSR
	Untoasa de Geoagiu	Q1211-12 Romania
	William Bon Chretian (Bartlett)	Q 362-01 British Columbia
	Winter Nellis	Q 475-05 New Zealand
	Yongi	Q1254-07 Washington
	Zao Su	Q1359-02 China
	Zao Suli	Q1288-07 China

Cydonia

	Le Borgeot	Q1111-03 British Columbia

ORNAMENTALS

	Chaenomeles 'Texas Scarlett'	Q1168-01 British Columbia
	P. calleryana 'Bradford' (IRP133-1)	Q1329-01 Washington
	'Chanticleer'	Q1376-01 British Columbia
P	'Redspire'	Q1168-04 British Columbia
	P. nivalis (Snow Pear)	Q 278-01 England

ROOTSTOCKS AND MISCELLANEOUS

X	Akntubinskaya (Axmyourae)	Q 310-04 USSR
	B 11	Q 456-04 South Africa
	B 22	Q 456-02 South Africa
	B 27	Q 456-06 South Africa
	BAC 29	Q 93-01 France
	BP-1 (813)	Q 456-05 South Africa
	BP-2 (812)	Q 456-01 South Africa
	BP-3 (814)	Q 456-03 South Africa
	Communis LA 62 (Ind.)	Q 122-01 England
	OH x F No. 51	Q 795-02 Ontario
P	OH x F No. 69 (Daynir)	Q 795-01 Ontario
P	OH x F No. 87 (Daytor)	Q1120-01 Ontario
	OH x F No. 206	Q 907-03 Ontario
P	OH x F No. 217	Q1307-01 Oregon
	OH X F No. 267	Q1307-02 Oregon
	OH x F No. 515	Q 907-04 Ontario
	P. betulaefolia (IRP139-1)	Q1250-02 Washington
	P. calleryana	Q1218-01 Montana
	P. serotina 'HN 39' (Ind.)	Q 739-01 Japan
	P. ussuriensis (Ind.)	Q 495-02 Ontario
	Pyronia veitchii (Ind.)	Q 122-02 England
	Quince A	Q 365-10 England
	Quince C	Q 509-04 England

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	Coop 25	Q1348-04 Indiana
	Coop 26	Q1348-05 Indiana

Dr. D. Lane, Summerland Research Station, Summerland, BC, Canada, V0H 1Z0:

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	8C-1-55	Q1252-08 British Columbia
	8C-28-27	Q1429-02 Ontario
	9P-14-32	Q1408-04 British Columbia
	9P-15-30	Q1408-05 British Columbia
	10C-18-33	Q 603-01 British Columbia
	11W-12-81	Q1408-06 British Columbia
	NY 61345-2	Q 997-02 New York
	Sunrise	Q1284-05 British Columbia

Dr. J. Warner, Smithfield Experimental Farm, P.O. Box 340, Trenton, ON, Canada, K8V 5R5:

	Ottawa 637	Q1333-03 Ontario
	Ottawa 662	Q1333-02 Ontario

East Malling Rootstocks: contact Saanichton Plant Quarantine Station (research purposes only):

X	M.3426	Q 365-09 England
X	M.3428	Q 365-04 England
X	M.3430	Q 365-05 England
X	M.3438	Q 365-07 England

Fruit Tree Research Station, Division of Fruit Breeding, Yatabe, Tsukuba, Ibaraki 305, Japan:

	Hac 9	Q1355-02 Japan
	Hac 10	Q1355-03 Japan
	Hokuto	Q1355-01 Japan
	Iwakami	Q1355-05 Japan

Jacob A. Hartenhof, RR#1, 23 Bent Td., Waterville, N.S., B0P 1V0

	Hartenhof McIntosh	Q1243-01 Nova Scotia

Plant Breeding International, Maris Lane, Trumpington, Cambridge, CB2 2LQ England:

	SA 162-130	Q1358-05 England
P	Telamon	Q1358-03 England
P	Tuscan	Q1358-02 England

Nakajima-Tenkoen Co., Ltd., No.34, 1-chome, Higashine City, Yamagata Prefecture, Japan, 999-37:

	Natsuka	Q1286-02 Japan
	Red Jonagold #4	Q1286-11 Japan

Swedish University of Agricultural Sciences, Div. of Fruit Breeding, Balsgard, Fjalkestadsvagen 123-1, 291-94 Kristianstad, Sweden:

	Aroma	Q1000-04 Sweden
P	Bemali	Q1421-01 Sweden
	Katja	Q1000-02 Sweden
	Kim	Q1000-01 Sweden
	Sylvia	Q1000-03 Sweden

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Dr. R. Bernard, Station des recherches d'Arboriculture Fruitiere, La Grande Ferrade, 33140 Pont de la Maye, INRA, Bordeaux, France.
(Information regarding the accessions may be obtained from Dr. R.E.C. Layne, Harrow Research Station, Harrow, ON, Canada, N0R 1G0):

Peach

	G.F. 305	Q 344-01 France
X	S3400 (P.besseyi x P.persica)	Q 966-01 France

Plum

X	Brompton	Q 620-03 France
X	F1 (P.1030X(9R.1xS.557)1)	Q 966-04 France
X	G.F. 1869	Q 580-02 France
X	INRA Prunier (G.F. 43)	Q 620-01 France
X	Ishtara (F1 (322x871))	Q 966-02 France
X	St. Julien G.F. 655-2	Q 620-02 France
X	St. Julien 63	Q 620-04 France
X	St. Julien 53-7	Q 966-06 France

Dr. H. K. Fisher, Ontario, Horticultural Research Institute of Ontario, Vineland Station, ON, Canada, L0R 2E0:

Apricot

	V60031	Q1167-05 Ontario
	V60032	Q1167-06 Ontario
	V60082	Q1167-07 Ontario
	V60083	Q1167-08 Ontario
	V66034	Q1167-11 Ontario
	V66052	Q1167-12 Ontario
	V68031	Q1167-13 Ontario
	V68032	Q1167-14 Ontario
	V66032	Q1432-01 Ontario

Dr. D. Lane, Summerland Research Station, Summerland, BC, Canada, V0H 1Z0:

Cherry

11W-16-16	Q1284-02 British Columbia
11W-15-37	Q1284-03 British Columbia
Sweetheart (13S-22-8)	Q1252-02 British Columbia
13S-24-28	Q1252-03 British Columbia
13S-36-18	Q1408-11 British Columbia
13S-43-48	Q1408-12 British Columbia

Dr. R.E.C. Layne, Harrow Research Station, Harrow, ON, Canada, N0R 1G0:

Apricot

H6302.028	Q 165-29 Ontario
H6303.009	Q 165-27 Ontario
H6624.021	Q 165-30 Ontario
H8201017	Q1395-13 Ontario
H8201109	Q1395-06 Ontario
H8201147	Q1395-14 Ontario
H8202008	Q1395-02 Ontario
H8202108	Q1395-09 Ontario
H8205201	Q1395-15 Ontario
H8205240	Q1395-05 Ontario
H8208295	Q1395-03 Ontario
H8209083	Q1395-16 Ontario
H8210023	Q1395-04 Ontario
H8316051	Q1395-10 Ontario
H8317098	Q1395-07 Ontario
H8412208	Q1395-08 Ontario
H8414271	Q1395-12 Ontario
HW436	Q1205-02 Ontario
HW439	Q1291-01 Ontario
HW440	Q1291-02 Ontario
HW441	Q1384-02 Ontario
HW442	Q1291-04 Ontario
HW443	Q1291-05 Ontario
HW454	Q1431-03 Ontario

Peach

H719	Q 35-04 Ontario
H420/6112	Q 35-07 Ontario
H1102B	Q 165-14 Ontario
H2091	Q 165-17 Ontario
H2219	Q 165-13 Ontario
H4219	Q 165-11 Ontario
H6818023 (ornamental)	Q 552-01 Ontario
H6818066 (ornamental)	Q 552-04 Ontario
H7119032	Q1369-07 Ontario
H7121044	Q1154-10 Ontario
H7121084	Q1205-05 Ontario
H7501037	Q1321-02 Ontario
H7819007	Q1321-03 Ontario
H7938023	Q1398-03 Ontario
H8219033	Q1369-08 Ontario
H8241038	Q1398-04 Ontario
H8412276	Q1431-04 Ontario
HW106	Q1431-01 Ontario
HW107(H7936029)	Q1398-02 Ontario
HW103	Q1291-12 Ontario
HW206	Q1154-08 Ontario
HW226	Q1291-06 Ontario
HW228	Q1291-07 Ontario
HW229	Q1154-04 Ontario
HW233	Q1154-05 Ontario
HW239	Q1291-08 Ontario
HW242	Q1154-12 Ontario
HW244	Q1205-06 Ontario
HW248	Q1321-01 Ontario
HW250	Q1291-10 Ontario
HW252	Q1291-11 Ontario
HW254	Q1369-06 Ontario
HW256	Q1384-05 Ontario
HW257	Q1384-04 Ontario
HW262(H7519238)	Q1398-01 Ontario

Dr. N.W. Miles, Horticultural Research Institute of Ontario, Vineland Station, ON, Canada, L0R 2E0:

Peach

V68051	Q1072-05 Ontario
V68101	Q1117-02 Ontario
V68272	Q1072-06 Ontario
V68275	Q1072-07 Ontario
V72013	Q1117-14 Ontario
V75011	Q1117-03 Ontario
V75013	Q1208-04 Ontario
V75024	Q1208-05 Ontario

Dr. G. Tehrani, Horticultural Research Institute of Ontario, Vineland Station, ON, Canada, L0R 2E0:

Cherry

V69061	Q1173-08 Ontario
V69062	Q1173-12 Ontario
V69068	Q1173-06 Ontario
V690611	Q1173-07 Ontario
V690616	Q1173-09 Ontario
V690618	Q1173-10 Ontario
V690620	Q1173-11 Ontario

Plum

V66071	Q1173-04 Ontario
V68011	Q1173-01 Ontario
V70031	Q1173-05 Ontario
V72333	Q1432-04 Ontario
V72481	Q1432-05 Ontario
V72521	Q1432-07 Ontario

Plant Breeding International, Maris Lane, Trumpington, Cambridge, CB2 2LQ England:

Plum Rootstock

Pixy	Q 881-01 England

Cherry Rootstock

P Colt	Q 757-01 England

Dr. R. Trefois, Station des Cultures Fruitieres et Maraicheres, Gembloux 5800, Belgium:

Cherry Rootstock

P GM 61/1 (Damil)	Q1209-01 Belgium

PYRUS RESTRICTED

Dr. R. Bell, Appalachian Fruit Research Station, Route 2, Box 45, West Virginia, U.S.A

	US309	Q1414-01 West Virginia

Dr. D. Hunter, Harrow Research Station, Harrow, ON, Canada, NOR 1GO:

	H6803/2.25	Q 907-01 Ontario
	H7237-1	Q1393-05 Ontario
	Harrow Sweet (HW609)	Q1122-03 Ontario
	HW607	Q1122-01 Ontario
	HW608	Q1122-02 Ontario
	HW610	Q1122-04 Ontario
	HW611	Q1122-05 Ontario
	HW613	Q1270-02 Ontario
	HW614	Q1270-03 Ontario
	HW615	Q1393-04 Ontario
	HW616	Q1393-03 Ontario
	HW617	Q1393-01 Ontario
	HW619	Q1393-02 Ontario

Fruit Tree Research Station, Yatabe, Tsukuba, Ibaraki 305, Japan:

	Selection 87-8-11	Q1355-11 Japan

I.N.R.A., Station d'Arboriculture Fruitiere, Beaucouze 49000 Angers, France:

X	6.16.85	Q1054-01 France
X	6.17.86	Q 461-01 France
X	6.18.10	Q 461-03 France
X	6.20.91	Q 461-06 France
X	6.21.53	Q 461-04 France
X	6.26.97	Q 461-09 France
X	6.31.18	Q 461-07 France
X	TN 27.19	Q 461-02 France
X	TN 28.75	Q 461-05 France
X	TN 33.31	Q 461-08 France

Swedish University of Agricultural Sciences, Division of Fruit Breeding, Balsgard, Fjalkestadsvagen 123-1, 291-94 Kristianstad, Sweden:

	BP1043	Q1082-03 Sweden
	BP1575	Q1082-01 Sweden
	BP10030	Q1082-05 Sweden

South African Plant Improvement Organization, Private Bag X5023, Stellenbosch 7600, South Africa:

	Bon Rouge	Q 350-01 South Africa

Centre for Plant Health:

X	C 129 (rootstock)	Q 365-11 England
X	C 132 (rootstock)	Q 365-13 England

ANNEXE 7

LE PROJET D'EXTENSION DE LA STATION DE SAANICHTON

SPACE REQUIREMENTS - ROOM BY ROOM

Space	Area Requirements units	nsm/unit	nsm	Ref
1.0 DIAGNOSTIC SECTION				
Administrative/Support Area				
Reception/Unpacking/Shipping Room	1		20.0	1.001
Workstation, Clerk	1		11.0	1.002
File Room	1		15.0	1.003
Office, Biologist	2	12.0	24.0	1.004
Computer Room	1		15.0	1.005
Office Area, Technicians/SPA	1		36.0	1.006
General Work Area	1		8.5	1.007
Subtotal			129.5	
Laboratory Areas				
Laboratory	1		61.5	1.008
Sample Preparation Room	1		20.5	1.009
Subtotal			82.0	
Heat Therapy Area				
Workroom, Propagators	1		10.0	1.010
Heat Therapy & Pre-Conditioning Room	6	10.0	60.0	1.011
Open Area, Growth Chambers	1		100.0	1.012
Rooting Bench Room	2	12.0	24.0	1.013
Propagation Room	1		12.0	1.014
Chemical Treatment Room	1		15.0	1.015
Subtotal			221.0	
Cold Storage				
Cold Storage Room	4	15.0	60.0	1.016
Total, Diagnostic Section			492.5	

Space	Area Requirements units	nsm/unit	nsm	Ref
2.0 RESEARCH SECTION				
Laboratory, Grapevine and Ornamental (G & O)	1		41.0	2.001
Laboratory, Tree Fruit	1		41.0	2.002
Office, Research Scientist	2	12.0	24.0	2.003
Total, Research Section			106.0	
3.0 BIOTECHNOLOGY SECTION				
Office, Research Scientist	1		12.0	3.001
Laboratory, Biotechnology	1		41.0	3.002
Laboratory, Monoclonal	1		20.5	3.003
Total, Biotechnology Section			73.5	
4.0 COMMON LAB SUPPORT AREAS				
Tissue Culture Growth Room	2	31.0	62.0	4.001
Tissue Culture Transfer Room	1		31.0	4.002
E.M. Lab	1		15.0	4.003
E.M. Preparation Room	1		20.5	4.004
Photography Studio	1		12.0	4.005
Dark Room	1		8.5	4.006
Equipment/Machine Area	1		41.0	4.007
General Storage Room	1		10.0	4.008
Small Animal Room				
Mouse Holding/Rearing Room	1		8.5	4.009
Cage Washing/Holding Room	1		9.0	4.010
Feed/Bedding Store	1		6.0	4.011
Total, Common Lab Support Areas			223.5	
5.0 ADMINISTRATION SERVICES				
Reception/Wait	1		11.0	5.001
Mail Room	1		10.0	5.002
Storage Room, Files & Supplies	1		12.0	5.003
Office, Director	1		18.0	5.004
Office, Manager	2	12.0	24.0	5.005

Space	Area Requirements units	nsm/unit	nsm	Ref Gross
Office, Clerical	1		20.0	5.006
Board Room	1		45.0	5.007
Library	1		30.0	5.008
Washroom, Staff	2	2.5	5.0	5.009
Coat Closet	1		2.5	5.010
Total, Administration Services			175.5	
6.0 GENERAL SUPPORT SERVICES				
Main Entrance Lobby	1		(20.0)	
Workshop, Maintenance Technician	1		15.0	6.001
Changing/Washroom, Male	1		20.0	6.002
Changing/Washroom, Female	1		18.0	6.003
Staff Lunch Room	1		57.0	6.004
Custodial Storage Room	1		9.0	6.005
Central Waste Collection Area	1		15.0	6.006
Receiving/Loading Dock	1			
Total, General Support Services			134.0	
7.0 B.C. MINISTRY OF AGRICULTURE				
Office, Receptionist/Office Manager	1		12.0	7.001
Waiting Area	1		3.0	7.002
Office, Agronomist	2	12.0	24.0	7.003
Office, Technician	1		10.0	7.004
Office, Students/Assistants/Visiting Specialist	2	7.5	15.0	7.005
Computer Room	1		10.0	7.006
Laboratory	1		20.5	7.007
Workroom/Library/Publications Holding/Audio Visual Store	1		30.5	7.008
Store Room	1		9.0	7.009
Changing Room/Washroom/Shower	1		8.0	7.010
Total, B.C. Ministry of Agriculture			142.0	

1.0 DIAGNOSTIC SECTION

FUNCTIONAL DESCRIPTION

Service

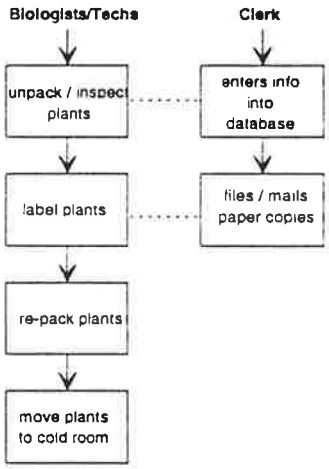
The Diagnostic Section is the main focus of experimental activities at the Centre, performing the principal functions involving plant quarantine, testing, heat therapy, propagation and maintenance of the plant repository.

Facilities will comprise a number of distinct areas including:

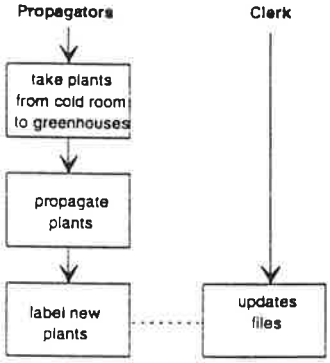
- an Administrative/General Support Area for biologists, technicians and clerks, where computers, printers and hardcopy files will be located
- a Laboratory Area
- a Heat Therapy Area with special environmental requirements
- a Cold Room Area

Workflow within the section comprises 7 distinct activities, as follows:

1. Reception of Plant Material

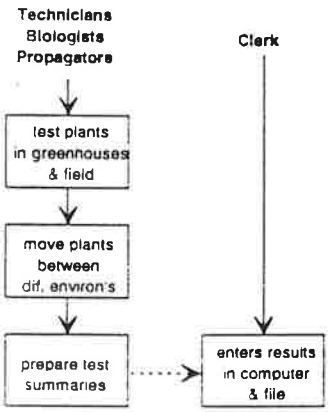


- Mail and packages received from the mail room located in Administration.
- Packages opened in an isolated room and inspected by biologists or technicians. Plants may be dirty or infected with insects or diseases. They may number up to 100 small plants in a sample (4-5 boxes). Some boxes can be large (e.g., 1.2 m^3).
- Samples labelled, repackaged and then moved to cold room storage by biologists, technicians and plant propagators.
- Information entered into the computer database by the program clerk.
- Paper copies placed in files, and copies mailed to consignees and appropriate government offices by program clerk.



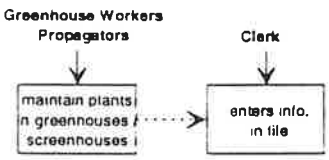
2. Propagation of Plant Material

- Plants propagated by propagators either in greenhouses or screenhouses.
- New plants labelled by propagators either in the greenhouse or the office area.
- Plant information entered into the computer by clerk and files updated if necessary.



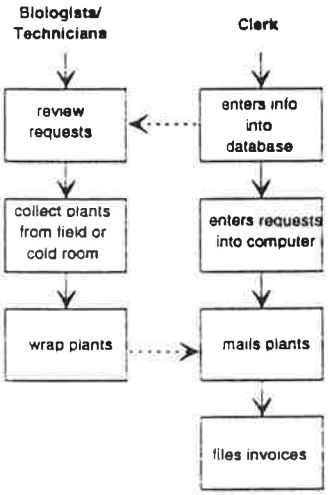
3. Testing of plant material.

- Testing carried out in the greenhouses by technicians, propagators and biologists. Plants moved between greenhouses, cold rooms and screenhouses as required to make use of different environments.
- Testing carried out in the labs or in the field by propagators, technicians and biologists.
- Test results entered into the computer by the clerk and technicians. Paper copies put into files as required.
- Test summaries prepared by biologists for computer entry by clerk and technicians.



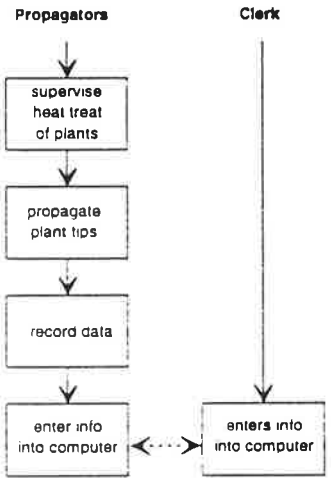
4. Maintenance of Plants

- Samples maintained in screenhouses and greenhouses.
- Information about disposition of plants given to clerk for computer entry.



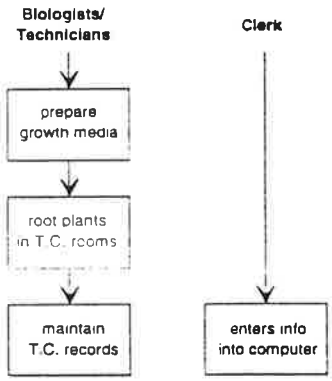
5. Distribution of Plants

- Requests for plants received by clerk.
- Requests given to technicians and biologists for review.
- Requests entered into computer by clerk and technicians and necessary papers generated.
- Plants collected from cold rooms, screenhouses, or field by technicians; information entered into computer by clerk or technicians, wrapped, labelled and delivered by clerk to mail room or reception.
- Completed invoices filed.



6. Disease Elimination

- Heat treatment of plants in the Heat Therapy Area growth chambers or in the tissue culture rooms.
- Propagation of new treated plants and recording of data.
- Plant information entered into computer by clerk or technicians.



7. Tissue Culture

- Preparation of growth media in the Pathology Lab.
- Initiation, proliferation and rooting of plants, utilizing the tissue culture growth rooms and T.C. transfer room when a sterile environment required.
- Maintenance of tissue culture records and update of computer.

Education

All lab sections will be involved in formal and informal educational programs for students from local high schools, colleges and universities. It is currently anticipated that 2 co-op students per year will spend 4 months each in the Centre.

In-service education will occur at regular intervals for all staff.

OPERATIONAL DESCRIPTION

(See Project Parameters section)

STAFFING & OCCUPANCY ESTIMATES

Staff Position	Existing FTE	Future FTE	Total Headcount	Max. Occupancy
Biologist	2.0	2.0	2	2
Plant Propagator	2.0	2.0	2	2
Virology Technician	3.0	3.0	3	3
Program Clerk	1.0	1.0	1	1
Virology & TC Lab Assistant	-	1.0	1	1
Total	8.0	9.0	9	9

Note:
See Common Lab Support Areas for visiting scientists and students.

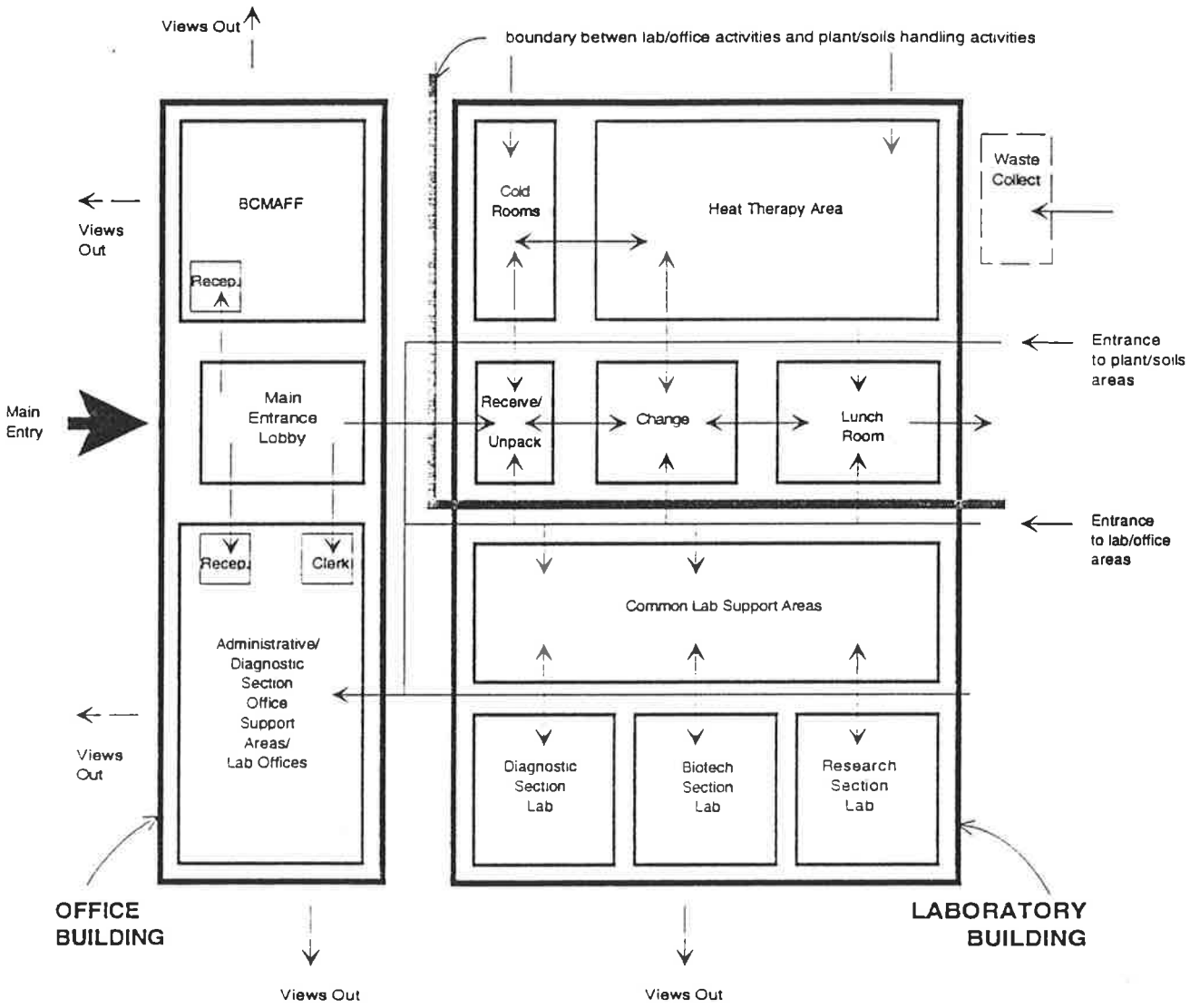
COMPOSITE FUNCTIONAL RELATIONSHIP DIAGRAM

The following diagram, is intended to be an abstract envisioning of program information, illustrating a potential organization of the functional components described in the program. Key organizational goals which are (intended to be) facilitated by this arrangement are as follows:

- the interaction between staff in related sections/functional components
- the movement/workflow of materials (plants, supplies, information) through the Centre
- the segregation of lab/office activities from plant/soils handling activities
- the proximity of work areas to an external location to ensure daylight and view

The diagram illustrates how organization in an "Office Building" zone and a "Laboratory Building" zone might work.

DIAGRAM


Views Out
boundary betwen lab/office activities and plant/soils handling activities
BCMAFF
Recep.
Views Out
Cold Rooms
Heat Therapy Area
Waste Collect
Entrance to plant/soils areas
Main Entry
Main Entrance Lobby
Receive/ Unpack
Change
Lunch Room
Entrance to lab/office areas
Recep.
Clerk
Common Lab Support Areas
Administrative/ Diagnostic Section Office Support Areas/ Lab Offices
Views Out
Diagnostic Section Lab
Biotech Section Lab
Research Section Lab
OFFICE BUILDING
LABORATORY BUILDING
Views Out
Views Out

ANNEXE 8

UN EXEMPLE DE LA LETTRE D'INFORMATION "THE RAPID CYCLIST"

The RAPID CYCLIST

Volume 2 , Number 1 A Newsletter of the Rapid Cyclist Users Group Spring 1994

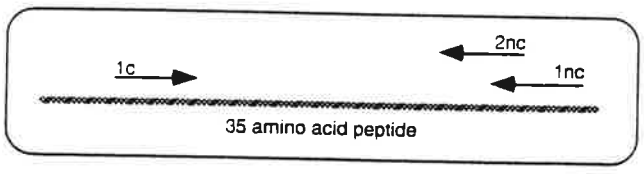
Creating a DNA Probe, Thermal Cycling with Degenerate Primers

Marianne Schroeder
Dept. of Biology
University of Utah
Salt Lake City, UT 84112

We are cloning the DNA from a structural protein in Xenopus leavis to further characterize it. A DNA probe was needed for Southerns, northerns and probing libraries for our gene of interest. The following is our procedure using the Air Thermo-Cycler to clone and amplify a fragment of DNA using degenerate primers. We found increased primer concentration as well as longer annealing times were beneficial in obtaining DNA products from degenerate primers.

Primer Design

The protein of interest was digested with endo-Asp-N to obtain protein fragments for amino acid sequencing. Of these, a 35 amino acid peptide was chosen to design degenerate primers for amplification of the peptide DNA. Coding (1c) and non-coding (1nc) primers were made from terminally located amino acids with minimal codon degeneracy. A third non-coding (2nc) primer was made internal to 1nc primer (see figure).



(turn to CREATING A DNA PROBE, p.2)

INSIDE

Superior Quantitation of Rare mRNA's Using Rapid Cycling

Randy P. Rasmussen
Dept. of Biology
University of Utah
Salt Lake City, UT 84112

After a long period of skepticism, quantitative PCR is finally gaining acceptance in the molecular biology community. No one doubted that PCR could be quantitative in theory, but there was a general consensus that the efficiency of DNA amplification would be too sensitive to interference for practical quantitation. Small effects on the reaction's efficiency, it was argued, would destroy the quantitative value of PCR. Quantitation of mRNA added the additional complication of the reverse transcription step.

Despite these initial concerns, it has now been thoroughly demonstrated that the quantitative power of reverse transcriptase PCR (RT-PCR) is as good or better than the traditional methods of mRNA quantitation such as northern blot[1], dot blot[2], and in situ hybridization[3]. Two recent papers from the John Weis lab report a sensitive RT-PCR assay using rapid air thermocycling[4,5]. The Weis lab was trying to

(turn to SUPERIOR QUANTITATION, p.2)

CREATING A DNA PROBE

(continued from page 1)

Each primer was 26 nucleotides long. All combinations of nucleotides at codon wobble positions were synthesized with the following exceptions: inosines were used for 4-fold degeneracy at the wobble position when appropriate (according to Molecular Cloning, a Laboratory Manual. Sambrook et al., page 11.18); to accommodate a serine in 1c and a leucine in 1nc, primers had to be made in duplicate; for serine, the codons TCI and AGT/C were used; for leucine (1nc, 2nc), IAG and T/CAA were used. Each primer was synthesized with a GGC clamp and an EcoR1 site at the 5' terminus. The degeneracy of the 1c, 1nc, and 2nc primers were respectively 48 fold, 8 fold and 48 fold. The expected size of the product from the 1c and 1nc primers was 100 bp, and from the 1c and 2nc primers, 94 bp.

Reaction Mix	
primers	7 µM working concentration; 1 µl each of 1c and 1nc
template	7.6 ng/µl Xenopus leavis oocyte cDNA; 1µl
$[Mg^{2+}]$	30 mM; 1 µl
dNTP mixture	2 mM each dNTP; 1 µl
10 X buffer	500 mM tris pH 8.3, 2.5 mg/ ml BSA, 1 µl
enzyme	1 µl Taq polymerase diluted 1:12.5 in enzyme dilution buffer (10 mM tris pH 8.3, 2.5 mg/ml BSA)
water	to 10 µl total volume

Thermal Cycling Conditions

Each 10 ul reaction was done in heat sealed glass capillaries.

Initial hold -- 2 minutes 94°C

2 cycles	D: 94°C 0 sec,	A: 40°C 7 sec,	E: 74°C 5 sec
5 cycles	D: 94°C 0 sec,	A: 42°C 7 sec,	E: 74°C 5 sec
23 cycles	D: 94°C 0 sec,	A: 45°C 7 sec,	E: 74°C 5 sec

These conditions produced the expected 100 bp fragment in small amounts as visualized on a 4% Nusieve low melting temperature agarose gel. The band was cut from the gel (approximately 2X2X4 mm chunk) and used as a template in subsequent thermocycling reactions.

Confirmation of the 100 bp product

To confirm the accuracy of our 100 bp product, we attempted to amplify a smaller fragment using the 100 bp cycling product as a template with the internal non-coding primer (2nc) and the original coding primer (1c).

The 100 bp product isolated in agarose was heated at 100°C until melted, and 500 µl TE was added. Two µl of this mixture were used as the template in a 10 µl reaction. One µl each of the 1c and 2nc primers was used, and the other parameters were as described above.

The thermal cycling reaction was run with an initial 2 minute denaturation at 94°C followed by 30 cycles: 0 sec at 94°C (denaturation), 12 sec at 50°C (annealing), 5 sec at 74°C (elongation).

This reaction produced a 94 bp band as seen on a 4% Nusieve agarose gel indicating that the 100 bp fragment was the correct DNA sequence. A duplicate reaction to the one directly above was done with the 100 bp fragment as template and the original outside primers (1c and 1nc) to amplify the 100 bp fragment. One 10 µl reaction gave approximately 30 ng of product.

The 100 bp product was cloned directly into the pCRII™ vector from the Invitrogen TA Cloning Kit. Subsequent DNA sequencing of this vector confirmed that this product coded for the original amino acid sequence and will be used as a probe for subsequent experiments. •

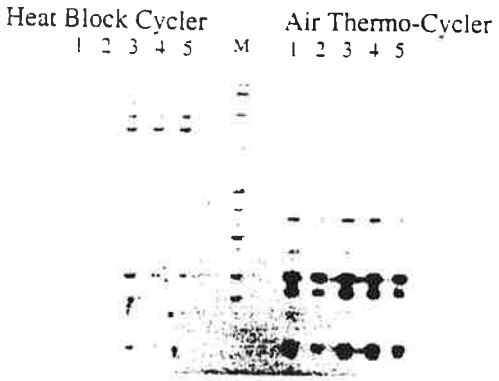
SUPERIOR QUANTITATION

(continued from page 1)

measure mRNA for the complement receptor Cr2, a rare mRNA in mouse spleens. They were unable to quantitate the message when they used slow heat block cyclers because of very low yields of product DNA and highly variable amounts of product. They switched to an Air Thermo-Cycler and solved both of these problems. The amount of DNA produced was at least 100 fold greater in the air cycler than in the heat block instrument and the variability problem disappeared (Figure 1).

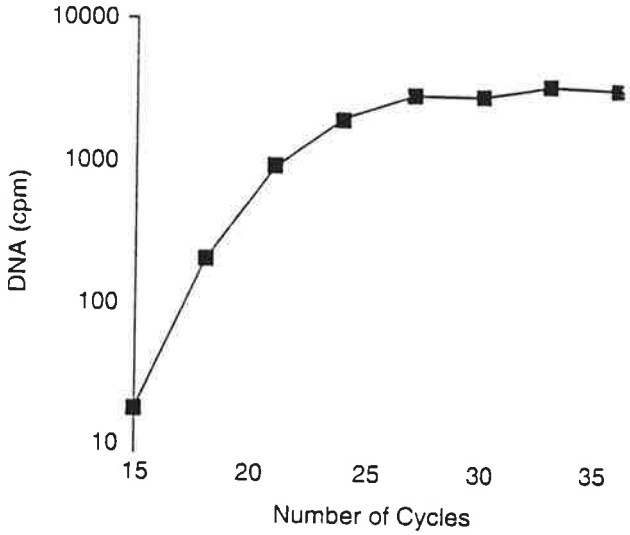
This short review will include some general considerations in quantitative PCR followed by the detailed Weis protocol.

Figure 1



Comparison of RT-PCR products using heat block instrument and the Air Thermo-Cycler (ATC). Five different spleen cDNA samples were set up for PCR amplification and equally split between the standard heat block instrument (first 5 lanes) and the ATC (last 5 lanes) for the same number of cycles. Quantitation of these results (cutting the bands out of the gel and counting incorporated ^{32}P-dCTP) indicated that there was at least 100 fold more product from the ATC than the heat block machine.

Figure 2



Amplification of mouse splenic cDNA with primers complimentary to the complement receptor Cr2. Eight identical samples were prepared with 250 ng of cDNA and removed sequentially every third cycle. After electrophoresis and autoradiography, bands were excised and quantitated by liquid scintillation counting (from Weis 1991).

The Linearity Problem

The amount of DNA produced in a PCR reaction is predicted by the well known equation:

$$y = x(E)^n$$

where **y** is the concentration of DNA produced by the amplification

x is the initial concentration of DNA

E is the efficiency of the reaction. For example, in a reaction where the amount of DNA is doubled every cycle, the efficiency is 2.

n is the number of amplification cycles

This equation can be linearized to:

$$\log(y) = n\log(E) + \log(x)$$

The y intercept of this line gives the log of the starting concentration of DNA while the slope of the line gives the log of the efficiency of the reaction.

When the DNA concentration of an amplification is determined after varying numbers of cycles, the results fit quite nicely to the equation above during the early cycles. Efficiency is reduced during the later cycles of an amplification reaction (Figure 2). This is probably due to primers competing less effectively with templa reannealing and a lower molar ratio enzyme to product. The number of cycles aft which these effects become important depends on th initial concentration of DNA.

When doing a quantitation experiment with the A Thermo-Cycler, a typical experiment would incluc making up a large master mix, filling multipi capillaries from that master mix, and starting all the tubes the same time. As the reaction goes on, tubes are pulle out at increasing numbers of cycles. The amount of DN in each tube can be quantitated in various ways. The point that fall in the log-linear portion of the curve can be used t determine the amount of starting material and the efficienc of the reaction. For Figure 2, the efficiency of the reactio during the log-linear phase was about 1.7 which is typical for a real reaction.

The Quantitation and Detection Problem

The most common technique for detection anc quantitation of DNA is radiolabeling with ^{32}P. Amplifiec products can be labeled by incorporation of radiolabelec nucleotides or by end labeling one of the primers. Enc labeling tends to be more sensitive because a higher fraction of the product carries a label[6], but labeling by incorporation is easier if you don't need the sensitivity. After the

amplification. reactions are size separated by gel electrophoresis. The gels can then be directly quantitated by autoradiography using film or a PhosphorImager type system . The limited linear range of film (usually 3 orders of magnitude or less) makes this approach difficult. PhosphorImager type systems are convenient and have extended linear ranges (5 to 9 orders of magnitude) but are very expensive. The Weis protocol uses labeling by incorporation of ^{32}P-dCTP, location of the product by autoradiography, and quantitation by excision of the band and liquid scintillation counting.

Some users of the Air Thermo-Cycler are hesitant to load glass capillaries with a radioactive reaction mixture because of a fear of breakage. While Weis reports that this has not been a problem, plastic capillary tubes are now available (see "New from Idaho Technology" in this issue).

The Reverse Transcriptase Problem

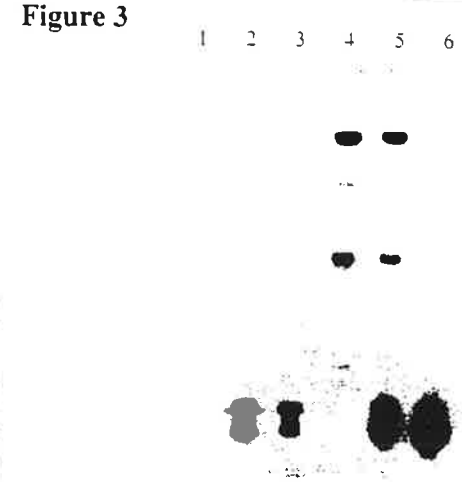
In most protocols the reverse transcription is primed with the same primer that is later used for the amplification. The Weis group uses random hexamers to prime the cDNA synthesis and they report several advantages to this approach. First, it ensures that all RNA's are represented equally in the cDNA pool. Second, as reverse transcription is done at low temperatures, using 20-mers to 30-mers can lead to synthesis of cDNA's from non-specifically hybridized primers. These products might be specifically amplified during the quantitation.

The Relative versus. Absolute Quantitation Problem

When measuring product by radiolabel, it is difficult to convert CPM's to absolute measures of DNA quantity. One solution to this problem is to set up an external standard curve by running known amounts of DNA each in their own reaction tube. Unfortunately this straightforward method has run into trouble due to large variation in the efficiency of different reactions. Further complications arise with RT-PCR because of the desire to control for the efficiency of the reverse transcriptase step. These problems have led to the use of internal standards of various types[7].

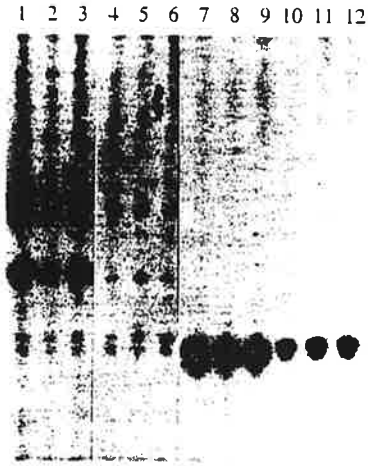
The simplest internal standard is to simultaneously quantitate the sequence of interest with some more or less invariant "housekeeping" mRNA. If the level of the housekeeping gene's message is constant between samples, then the amount of the unknown transcript can be reported in relative terms. Popular genes for

Figure 3



Effect of amplifying two different gene products in one reaction. PCR analysis of 100 ng of splenic cDNA with Cr2 and ß-actin oligos. Lanes 1-3 were done for 18 cycles. Lane 1, Cr2 oligos alone; lane 2, ß-actin oligos alone; lane 3, Cr2 + ß-actin oligos. Lanes 4-6 were done for 24 cycles. Lane 4, Cr2 oligos alone; lane 5, Cr2 + ß-actin oligos; lane 6, ß-actin oligos alone (from Tan 1992).

Figure 4



Reproducibility of PCR amplification for quantitation of products: multiple tissue samples. PCR anaylsis for 24 cycles with Cr2 oligos (lanes 1-6) and ß-actin oligos (lanes 7-12) with 100 ng of cDNA generated from three different spleens (lanes 1-3 and 7-9) and livers (lanes 4-6 and 10-12) (from Tan 1992).

standardization are ß-actin and HLA genes. All of these internal standard methods are based on the presumptions that: 1) the reverse transcription is not biased between the standard and test transcripts, 2) the amplification of the standard and the unknown occur with the same efficiency, and 3) the amplifications do not interfere with each other significantly.

Weis uses ß-actin mRNA as an internal standard (Figure 3). The autoradiograph shown in figure 3 shows that both products can be simultaneously amplified with minimal interference.

The Variability Problem

Sample-to-sample variability has long been a problem with RT-PCR. The efficiency of reverse transcription has been reported to vary from 5% to 90%[3], while the amplification itself may vary up to 200-300% between duplicate reactions. The Weis group reports good reproducibility not only between duplicate aliquots of the same cDNA but also between tissue samples (Figure 4).

The Protocol

1. Total RNA was prepared by the method of Chirgwin *et al.*[9]

2. RNA (5 µgs) was reverse transcribed in 1X RT buffer(GIBCO-BRL), 0.125 mM each dNTP, 0.5 µg random hexamers (New England Biolabs) and 400 units of Moloney virus reverse transcriptase (GIBCO-BRL) in a 50 µl reaction. The reaction was incubated at 37°C for 60 minutes. DNase free RNase was added and incubated for 5 minutes at 37°C. The reaction volume was adjusted to 270 µl with 0.4 M NaCl and was phenol extracted and precipitated with ethanol. cDNA concentration was determined by UV absorbance.

3. The optimal cDNA concentration and number of cycles was determined by a titration from 1 to 500 ng of cDNA and from 18 to 39 cycles. Optimal parameters were 200 ng of cDNA for 20 cycles. Each 10 µl reaction contained 200 ng of cDNA, 70 pmoles of each primer, 50 mM tris pH 8.3, 3 mM $MgCl_2$, 20 mM KCl, 0.5 mg/ml BSA, 0.2 mM each dNTP, 2.5 uCi [^{32}P]dCTP(3000 Ci/mmol; New England Nuclear), 0.72 units AmpliTaq DNA Polymerase (Cetus). To improve reproducibility, a master mix was prepared without primers and then aliquoted to separate tubes containing the different primer pairs. These mixtures were then aliquoted to the cDNA samples. Each 10 µl reaction was loaded into a glass microcapillary tube (Idaho Technology) and the ends were flame sealed. Capillaries were cycled in the 1605 Air Thermo-Cycler (Idaho Technology). Cycling parameters were denaturation, 94°C for 1 sec; annealing, 59°C for 1 sec; elongation, 72°C for 4 seconds (products ranged in size from 80 to 200 base pairs). Total cycle time was 24 seconds.

4. Following amplification the ends of the capillary tubes were scored and the samples removed using a microaspirator and then 5 µl were electrophoresed in a 6% acrylamide gel. Radioactive bands were detected by autoradiography and then the bands were cut from the gel for quantitation by liquid scintillation counting. A ^{32}P-end labeled MspI digest of pBR322 was used as a size standard. •

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Rapid Cycle Amplification of VNTR Loci for Engraftment in Bone Marrow Transplantation.

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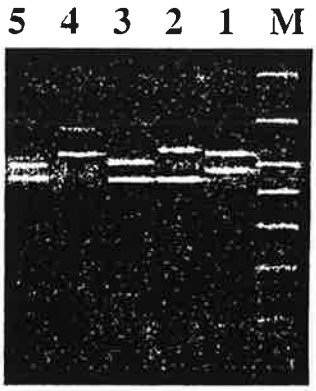
Bone marrow transplantation is now standard therapy for a range of diseases including many hematologic malignancies, some solid tumors, and some acquired or inherited hematologic and immunologic diseases. Many of these disorders result from a malfunctioning bone marrow, and the only cure is to inactivate the diseased bone marrow and replace it with healthy marrow. After the original marrow is destroyed, healthy marrow from a donor is infused into the recipient. Bone marrow transplantation may be: 1) autologous (where healthy stem cells have been previously harvested from the same individual), 2) syngeneic (where the donor is an identical twin), and 3) allogeneic (where the donor is different genetically from the recipient). In allogeneic transplantation, it is possible to determine the success of transplantation by monitoring the genotype of cells appearing in the peripheral blood. If the recipient type converts to the donor type, successful engraftment has occurred.

Variable number of tandem repeat (VNTR) loci are regions in the human genome where a short nucleotide sequence is repeated in tandem for a variable number of times. If flanking primers are placed outside of the repeats, the number of tandem sequences in any particular allele determine the length of the amplified product. Some VNTR loci are highly polymorphic with over 10 different alleles and are very useful for establishing individuality by genotype. For highly polymorphic loci, homozygosity is uncommon and two bands are expected at each locus because of the diploid nature of human cells. VNTR loci are commonly used in forensics to establish identity and can also be used to establish donor vs. recipient type in peripheral blood leukocytes after bone marrow transplantation. Since peripheral blood leukocytes originate in the bone marrow, the type of circulating leukocytes establishes the type of hematopoietic cells populating the bone marrow. An example of DNA amplification of a VNTR locus in 5 unrelated individuals is shown in Figure 1.

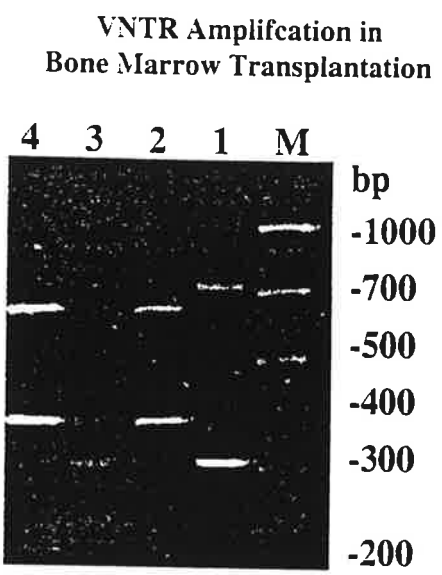
Siblings are often used as donor/recipient pairs in bone marrow transplantation because they may match at HLA loci and have fewer problems with graft/host acceptance. HLA and VNTR loci are not linked and follow classical Mendelian inheritance. If siblings are matched at HLA loci for transplantation, they have a one in four chance of receiving the same parental VNTR alleles at any particular locus. If they do receive the same VNTR alleles at one locus, that particular locus is not useful for distinguishing donor vs. recipient type. However, most of the time, siblings will differ by either one allele (50% of the time) or two alleles (25% of the time). DNA from peripheral blood leukocytes needs to be isolated from donor and recipient before bone marrow transplantation, so that informative VNTR loci can be identified. Since there are many VNTR loci, finding differences between recipient and donor is not difficult, even for siblings. In the case of syngeneic or autologous transplantation, genotyping studies are not informative.

Figure 1



DNA samples from five unrelated individuals amplified with primers for the D1S80 locus[2].

Figure 2



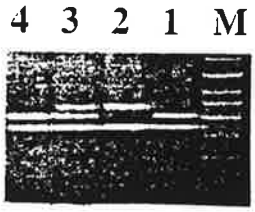
Bone marrow transplant engraftment by 1 month.
The VNTR locus HGM D17S30[1] was used.
Lane 1: Recipient before transplantation.
Lane 2: Donor.
Lane 3: Recipient 2 weeks after transplantation.
Lane 4: Recipient 4 weeks after transplantation.
In lane 3 the patient shows bands from both the recipient and the donor. In lane 4 the patient shows only donor bands suggesting successful engraftment.

The VNTR loci used here are HGM locus D17S30[1] and D1S80[2]. All PCR reactions were run with standard rapid cycling techniques[3-5] in an Idaho Technology 1605 air cycler with buffers and reagents supplied by Idaho Technology (1761 Optimizer Kit). The Mg^{2+} concentration was 2.0 mM. Cycling parameters were denaturation at 94°C for 0 sec, annealing at 55°C for 0 sec, and elongation at 73°C for 20 sec for 30 cycles. The total cycle time was 23.7 min. The samples were loaded directly on a 1.5 % Agarose gel and electrophoresed at 5 V/cm.

Figure 2 illustrates a typical example of engraftment. This is a sibling transplant where all four alleles are different. At 14 days after transplantation, both donor and recipient bands were observed. Residual recipient lymphocytes may circulate for 2-3 weeks after transplantation. However, recipient bands should disappear by 4 weeks if engraftment has occurred.

Figure 3 illustrates a typical example of disease recurrence after bone marrow transplantation. This is a sibling transplant where one allele is shared between donor and recipient types. At 36 days post bone marrow

Figure 3



Disease recurrence after bone marrow transplantation. The VNTR locus HGM D1S80[2] was used.
1: Recipient before transplantation.
2: Donor.
3: 36 days after bone marrow transplantation.
4: 100 days after bone marrow transplantation.
In lane 3, alleles from both the donor and recipient are present at approximately equal amounts. After 100 days (lane 4), the unique donor band has disappeared and only the original recipient alleles are present.

transplantation, both donor-specific and recipient-specific alleles are apparent. This indicates that the donor marrow has not entirely supplanted the recipient marrow at 36 days. At 100 days post bone marrow transplantation only the recipient bands are present, indicating failure of engraftment and recurrence of disease. •

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New From Idaho Technology

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Polycarbonate tubes

There has been a great deal of interest displayed by users of the 1605 Air Thermo-Cycler (ATC) in the possibility of using plastic capillary tubes to augment the glass capillary tubes currently standard in our instrument. The results of our tests on various plastic tubes have been very encouraging. Our selection for final testing is poly carbonate tubing which has thermal response characteristics almost identical to our 10 µl glass tubes. The polycarbonate does not interfere with the reaction and it should be of great help in those situations where the fragility of glass capillary tubes is an excessive hazard. However, those who are interested in using plastic capillary tubes should be aware that plastic tubes are not without their drawbacks.

Glass tubes can be easily loaded either singly or eight at a time by capillary action. However, hydrophobic plastic tubes require a loading mechanism such as a micro-aspirator or a similar device. We are working on ways of loading and sealing eight tubes at a time; but at present it can only be done one tube at a time.

The second drawback is sealing the ends of the plastic tubes. It is tricky but possible to flame seal plastic tubes by intentionally igniting the ends. For many people, this tends to be somewhat disconcerting; therefore, we have developed an electric tip sealer.

The last potential problem with plastic tubes is price. At a cost of approximately $80 per 1000, plastic tubes will be about twice as expensive as similar glass tubes. Even at that price, plastic tubes would still be less expensive than other second generation sample containers. We will know more about pricing after final testing on the tubes is complete.

We hope to be completely finished with the final tests on the plastic tubes and have the tubes and the sealers available in June of '94, barring major catastrophe. (10 µl tubes, part number 1714; tube sealer, part number 1740)

Modular Tops

In other hardware news, some of our earlier customers may be interested in a change made in the design of the 1605 cycler. The plastic top now has removable modules for loading and unloading tubes. The entire module is removable from the rest of the top to allow easier loading and unloading. Each module holds 16 tubes. To help ensure a good fit of all sizes of tube, modules are available in two sizes, 10 µl and 50 µl. An upgrade kit to a modular top is available from Idaho Technology, part number 1869.

Module Racks

A rack for holding the capillary tube modules is also now available. Each rack will hold three filled capillary tube modules. These module racks should help eliminate damage to capillary tubes when filled modules are set down prior to re-insertion into the instrument top. The part number is 1735.

Improved Buffer System

We have made several improvements to the buffers optimized for rapid cycling. Traditionally we have used Ficoll and tartrazine to increase the density of our buffer and make it visible for direct loading of product onto gels. We now recommend substituting sucrose for Ficoll, and cresol red for tartrazine.

For optimizations we have traditionally recommended using a three-by-three matrix of 3 mM, 2 mM, and 1 mM Mg^{2+} run at three annealing temperatures; 40°C, 50°C, and 60°C. However, our experience is that most reactions optimize at the higher end of the Mg^{2+} concentration, therefore we now recommend using 2 mM, 3 mM and 4 mM Mg^{2+} in the high, medium and low buffers.

We will include the new buffers free with all reagent orders for the next few months and if the reaction is positive, we will switch to the new system for individual buffer orders and the Optimizer Kit. As usual we are also publishing the reagent constituents in case you choose to make your own buffers. On the following pages are procedures for running individual reactions, making master-mixes, and making the reaction constituents themselves.

Reaction Mixes and Buffer Recipes

from Carl Wittwer's laboratory

Reaction Constituents for One 10 µl Reaction

Component	[10X]	[Reaction]	Primers Separate	Primers Combined
DNA (human genomic)	50 ng/µL or A_{260} = 1.0	50 ng/10µl	1 µl	1 µl
Primers				
Separate				
Primer 1	5 µM	0.5 µM	1 µl	
Primer 2	5 µM	0.5 µM	1 µl	
or Combined				
Primer 1 + 2	5 µM each	0.5 µM each		1 µl
Nucleotides	2 mM each dNTP	200 µM each dNTP	1 µl	1 µl
Buffer	500 mM Tris, pH 8.3 2.5 mg/ml BSA 20% (w/v) Sucrose 1mM Cresol Red	50 mM Tris, pH 8.3 250 µg/ml BSA 2% (w/v) Sucrose 0.1 mM Cresol Red	1 µl	1 µl
Low Mg^{2+}	20 mM $MgCl_2$	2 mM $MgCl_2$		
Medium Mg^{2+}	30 mM $MgCl_2$	3 mM $MgCl_2$		
High Mg^{2+}	40 mM $MgCl_2$	4 mM $MgCl_2$		
Enzyme	0.4 U/µL	0.4U/10µl	1 µl	1 µl
dH_2O/other			4 µl	5 µl

Amplification Procedure

1. Prepare master mix without DNA and without primers weekly:

For <50 runs at a 10 µl reaction volume:

Dilute Enzyme to 0.4 U/µl

11.5 parts Enzyme diluent
(10 mM Tris pH 8.3, 2.5 mg/ml BSA)
1 part Enzyme (5 U/µl)

For separate 5 uM primers:	For combined 5 uM primers:
4 parts dH_2O	5 parts dH_2O
1 part buffer	1 part buffer
1 part 2 mM dNTPs	1 part 2 mM dNTPs
1 part 0.4U/µl Enzyme	1 part 0.4U/µl Enzyme

Mix and store at 4°C for < 1 week.

For >50 runs at a 10 µl reaction volume:

For separate 5 uM primers:

308 (61.5 parts) dH_2O
63 µl (12.5 parts) buffer
63 µl (12.5 parts) 2 mM dNTPs
5 µl (1 part) 5U/µl Enzyme

For combined 5 uM primers:

370 (74 parts) dH_2O
63 µl (12.5 parts) buffer
63 µl (12.5 parts) 2 mM dNTPs
5 µl (1 part) 5U/µl Enzyme

Mix and store at 4°C for < 1 week.

continued on next page

2. For each run with a specific primer pair, make a primer-specific mix:

For separate 5 µM primers	For combined 5 µM primers
1 part 5 µM primer 1 1 part 5 µM primer 2 7 parts master mix	1 part combined 5 µM primers 8 parts master mix
Mix well by pipetting.	Mix well by pipetting.

3. Add 1 µl of each sample DNA (for genomic DNA, 50 ug/ml or A_{260}=1.0) to individual wells in a microtiter plate. Pipette 9 µl of specific-primer mix into each well and mix by pipetting up and down. Load capillary tubes into the modular tops and aspirate 8 sam at a time by capillary action. Flame seal the loading end of the tubes, then seal other end. Place into the Air Thermo-Cycler and run at des protocol. When reaction is complete, score each end of the glass tubes while still in the modular top, break glass and transfer directly the gel wells.

Working Solutions

1. Primers and DNA are prepared in 1X TE':

50X TE' solution, pH 8.3 (500 mM Tris, 5mM EDTA)

10 m l 2 M Tris, pH 8.3
400 µl 0.5 M EDTA
dH_2O to 40 ml

1X TE' solution, pH 8.3 (10 mM Tris, 0.1 mM EDTA)

200 µl 50X TE' or 10 ml 50X TE'
dH_2O to 10 ml dH_2O to 500 ml

2. Make 50 µM primer stocks with 1X TE'.

Make 10X primers (5 µM) either separately or combined:

For 10X separate primers

40 µl (1 part) 50 µM Primer
360 µl (9 parts) 1X TE'

For 10X combined primers

40 µl (1 part) 50 µM Primer 1
40 µl (1 part) 50 µM Primer 2
320 µl (8 parts) 1X TE'

3. 10X Nucleotides (2 mM each dATP, dCTP, dGTP, dTTP

250 µl 100 mM dATP (Sigma D4788)
250 µl 100 mM dCTP (Sigma D4913)
250 µl 100 mM dGTP (Sigma D5038)
250 µl 100 mM dTTP (Sigma T9656)
to 12.5 ml with dH_2O

4. 10X Buffer

2.5 ml Tris, pH 8.3 (2 M stock)
0.5 ml BSA (50 mg/ml stock)
5.0 ml 40% Sucrose
1.0 ml 10 mM Cresol Red

Low Mg^{2+}	200 µl (1M $MgCl_2$) + 800 µl H_2O
Medium Mg^{2+}	300 µl (1M $MgCl_2$) + 700 µl H_2O
High Mg^{2+}	400 µl (1M $MgCl_2$) + 600 µl H_2O

5. Enzyme diluent (10 mM Tris, pH 8.3, 2.5 mg/ml BSA)

50 µl 2 M Tris, pH 8.3
500 µl 50 mg/ml BSA
9.5 ml dH_2O

Stock Solutions

All solutions are made from deionized, distilled water. No stir bars or pH meters are to be used in the preparation of stock or working solutions. Check pH by withdrawing 10 µl of solution and placing it on pH paper.

2 M Tris, pH 8.3

14.80 g Tris base (Sigma T1503)
12.28 g Tris HCl (Sigma T 3253)
to 100 ml with H_2O

or

27.08 g TRISMA Preset, pH 8.3 (Sigma T5128)
to 100 ml with H_2O

1 M $MgCl_2$

20.3 g $MgCl_2$ (Sigma M9272)
to 100 ml dH_2O

or

Sigma M1028 (ready made)

50 mg/ml BSA

0.50 g BSA (Sigma A2153)
to 10 ml dH_2O (use 15 ml tube)

10 mM Cresol Red

404 mg cresol red (Sigma C9877)
to 100 ml dH_2O

40% (w/v) Sucrose

40.0 g sucrose (Sigma S5016)
to 100 ml dH_2O

0.5 M EDTA, pH 8.3

18.6 g disodium EDTA (Sigma ED2SS)
10 ml 5 N NaOH (Baxter H369-1*NY)
to 100 ml dH_2O

Rapid Cycle DNA Amplification - The 10 Most Common Mistakes

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Mistake #1. Not having bovine serum albumin in the reaction. You will not get amplification in capillary tubes without BSA. Most buffers supplied by manufacturers or the enzyme do not include BSA. BSA is necessary to prevent surface adsorption/inactivation of the DNA polymerase on the large surface area of the capillary tubes. Yield increases with BSA concentrations up to 500 µg/ml in the reaction. Using gelatin gives a poor yield in capillary tubes. You can make up your own buffers. We recommend including 2.5 mg/ml BSA in the 10X buffer and 2.5 mg/ml in a 10X polymerase dilution. The grade of BSA is not critical. We use Sigma #A2153.

Mistake #2. Using acetylated bovine serum albumin. It is expensive and does not work. Presumably, the same sites that are acetylated are those sites necessary to coat the glass walls and prevent polymerase inactivation.

Mistake #3. Using Triton X-100. Some manufacturers of heat stable polymerases state that 0.1% Triton X-100 is needed for enzyme activation. Triton X-100 does activate some enzymes when BSA is not present and amplification occurs in microfuge tubes. However, Triton X-100 is not necessary when BSA is present. Furthermore, if Triton X-100 is added, yield substantially decreases in capillary amplifications that include BSA.

Mistake #4. Adding polymerase to a microtiter plate before BSA. For convenience, many people mix reactions in a microtiter plate so they can be loaded simultaneously by capillary action into tubes already placed in modular tops. However, if the polymerase is added to a microtiter well before BSA, the polymerase can be adsorbed onto the plastic surface and not loaded into the capillaries. To prevent adsorption of polymerase during handling, we recommend diluting the polymerase to a 10X concentration with a diluent that includes BSA at 2.5 mg/ml. In addition,

always block the well surface with BSA by adding the BSA-containing buffer before the polymerase. Microtiter plates that do not absorb protein can also be used and are available from Idaho Technology (microtiter plate, part number 2590; lid, part number 2591) , but BSA is still necessary for the capillary tubes, whether glass or plastic.

Mistake #5. Pulling tubes out near the denaturation temperature. If double stranded product is cooled rapidly (by pulling a tube out of an air cycler that is near denaturation temperatures), not all the product will reanneal and multipl apparent products may appear on gels.

Mistake #6. Using excessive denaturation times. There is no reason for denaturation times longer than "0" sec at 94°C. The Tm of products in amplification buffer is around 85-90°C and complete denaturation of product at 94°C occurs faster than can be measured (< 1 sec. See Wittwer and Garling, 1991, *BioTechniques*, 10: 76-83, or Wittwer et al., 1994, in: The Polymerase Chain Reaction , Mullis, Ferre, and Gibbs, eds., pp. 174-181). The only possible exception is on the first cycle when high quality, complex genomic DNA is used as template. An initial denaturation of 5-15 sec at 94°C on the first cycle may allow more complete initial denaturation. However, extended times at high temperatures degrade DNA, and are particularly harmful in long product amplifications (CE Gustafson et al., 1993, *Gene* 123:241-244, and W.M. Barnes, 1994, *PNAS*, 91: 2216-2220).

Mistake #7. Using nonstandard capillary tubes. The tubular metal-sheathed thermocouple that monitors temperature in the air cycler is precisely matched in thermal response to aqueous samples in the 10 µl capillary tubes sold by Idaho Technology. When nonstandard capillary tubes are used, the temperature of the sample will not correspond to the temperature indicated on the instrument readout. If you optimize a reaction in 10 µl tubes, and later run the reaction in larger tubes, you should not expect similar results. Larger tubes will not reach target temperatures without setting a hold time. If you insist on using larger or nonstandard tubes, you can monitor the sample temperature inside the tube with an IT-23 microthermocouple probe available from Sensortek (Clifton, NJ) and empirically adjust target temperatures and hold times. Be aware that some types of glass interfere with the reaction, presumably because ions on or near the surface of the glass are absorbed into the reaction buffer.

Mistake #8. Forgetting to add a critical component. Accidental omission of polymerase, dNTP's, or buffer components can be avoided by "master mixes" that include everything necessary for amplification except primers and template. Such a master mix, if sterile, lasts for 3-6 weeks at room temperature, >15 weeks at 4°C, and > 26 weeks at -20°C. Master mixes also minimize pipetting errors, particularly with small volumes.

Mistake #9. Inappropriate Mg^{2+} concentration. Rapid cycling generally requires higher magnesium concentrations than slow cycling. For example, whereas 1.5 mM magnesium chloride is standard in slow cycling, 2-4 mM is more typical for rapid cycling. With 2-4 mM magnesium chloride, excellent yield and specificity can be obtained with annealing times of "0" sec. Magnesium chloride is hygroscopic and it may be difficult to prepare accurate solutions from the solid salt. We use a 1 M solution of magnesium chloride available from Sigma (#M1028).

Mistake #10. Poor temperature/time optimization. Rapid cycle temperature/time parameters are very different from slower cyclers. It is a mistake to directly transfer a protocol like, "1 min at 94°C, 2 min at 55°C, and 3 min at 72°C," to a rapid cycler. Denaturation should be set at 94°C for "0" sec. The annealing time should almost always also be set at "0" sec. The extension temperature should be 70-74°C. The extension time should be "0" sec for products up to 100 bp, 5-15 sec for products up to 500 bp, and about 30 sec for a 1000 bp product. Most amplifications with 20-mer primers will work well using 3 mM $MgCl_2$ at an annealing temperature of 50°C. Rapid cycling makes it feasible to rapidly test all combinations of 3 different annealing temperatures (40°C, 50°C, and 60°C) and 3 different Mg concentrations (2 mM, 3 mM, and 4 mM).

The RAPID CYCLIST

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