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Potato viruses on cultivated Solanaceae species in mix and sole cropping systems in Cameroon

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Abstract

The role of potato in assuring food security of poor resource farmers in the Western Highlands of Cameroon cannot be over emphasized. Unfortunately, the incidence of potato viruses remains a major limiting factor threatening sustainable potato production in Cameroon. The prevalence of six potato viruses; *Potato virus X* (PVX), *Potato virus A* (PVA), *Potato leaf roll virus* (PLRV), *Potato virus Y* (PVY), *Potato virus M* (PVM) and *Potato virus S* (PVS) was evaluated in the western highlands of Cameroon. Surveys of these viruses were conducted in four *Solanaceae* species; *Solanium tuberosum, S. scabrum, S. capsicum, Lycopersicon esculentum,* in sole and mixed cropping systems. They were identified and assayed by Nitrocellulose Membrane Enzyme Linked Immunosorbent Assay (NCM-ELISA) for the prevalence of viruses. NCM-ELISA tests revealed that the six viruses infect *Solanaceae* species to a varying extent. *Potato virus Y* (PVY) was predominant followed *by Potato virus X* (PVX), *Potato virus A* (PVA), *Potato virus S* (PVS), *Potato virus M* (PVM) and *Potato leaf roll virus* (PLRV). Mixed and single infections were recorded. Our results also showed single, double, triple, quadruple and quintuple infections at 41.89, 21.78, 16.2, 17.87 and 2.23%, respectively. Information obtained from this research will serve as the basis for developing effective control measures against these viruses.

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Introduction

Potato (*Solanum tuberosum* L.) is an herbaceous vegetable crop widely cultivated for its tubers. Tubers are consumed fresh or in powder form. The crop ranks fourth in the world after cereals, rice and wheat (Wilson and Jones, 1993). In Cameroon it is widely cultivated in the western highlands of Cameroon, where other *Solanaceae* species are cultivated, all in mixed and single cropping systems. Potato viruses have been known to infect several *Solanaceae* species in other regions. Recently much has been done on the prevalence of potato viruses in major potato growing regions of Cameroon (Njukeng *et al.*, 2013b). Knowledge of the prevalence and infection of these viruses in cultivated *Solanaceae* species in Cameroon will help guide farmers in adopting efficient control measures to reduce the spread of infection, and facilitate the establishment of seed certification schemes for these important crops.

Tomato (*Lycopersicon esculentum Mill.)* is an herbaceous vegetable crop widely cultivated in large quantities in gardens, especially in the dry season for its fruits. The fruits are widely used to prepare sauces, salad and other dishes. Tomato is the second most important vegetable in the world after potato because of its nutritive value (Abbott, 1999).

Pepper (*Capsicum* ssp) is a perennial plant with a lifespan of 2-3 years, cultivated for its fruits. It is widely used as a spice and a stimulant. It is usually transformed into different forms (powder or liquid) for conservation. In addition, it is used as a carminative and an antiseptic (Bosland *et al.*, 2012; Heiser and Smith, 1953).

Garden huckleberry (*Solanum scabrum L.)* is an important cultivated African vegetable. The leaves are highly consumed and are an important source of income to gardeners in the western highlands of Cameron (Ndamukong *et al.*, 2006; Olet *et al.*, 2006).

These crops are affected either by pests, pathogens or adverse environmental conditions. Bacterial, fungus, virus and mycoplasma cause most pathogenic diseases. Bacterial wilt or brown rot is the most serious bacterial disease of potato (Wilson and Jones, 1993). The most important fungal disease of potato and tomato is late blight caused by *Phytophthora infestans* (Mont) de Barry (Fontem, 2001; Fontem and Aighewi, 1993). Purple top wilt is a disease with mycoplasma pathogen, which is neither viruses nor bacteria; severity affects sprouting, the quality of potato tubers and yield (Banttari *et al.*, 1990; Lee *et al.*, 2006). Insects and nematodes are some of the pests ravaging the production of huckleberry, pepper, potato and tomato in the western highlands of Cameroon (Bridge *et al.*, 1995; Fontem *et al.*, 1999; Matthews *et al.*, 2003).

There are more than 25 viruses and virus-like agents affecting potato either singly or in mixed infections (Salazar, 1996). For the purpose of this work, interest is centered on six potato viruses namely: *Potato virus S*, *Potato virus M (Carlavirus), Potato virus Y*, *Potato virus A (Potyvirus)*, *Potato virus X (Potexvirus)*, and *Potato leaf roll virus (Polerovirus) previousely shown to be present in the Western highlands of Cameroon* (Njukeng *et al.* 2013a; Njukeng *et al.*, 2013b). These potato viruses have been recorded in most developing countries. In descending order of importance; PLRV, PVY, PVX, PVS, PVA and PVM, though in some regions specific strains can be found (Fuglie, 2007; Loebenstein and Thottappilly, 2013). The incidence of PLRV, PVY, PVX and PVS had been reported in the Western Highlands of Cameroon (Fondong *et al.*, 1991).

A virus disease of plants seldom kills the entire plant but reduces plant vigor and yield (Guo *et al.*, 2005). The leaves are the most affected parts of the plants; photosynthetic activities are highly perturbed resulting to loss in yield (Balachandran *et al.*, 1997; Rahoutei *et al.*, 2000). PLRV has caused yield losses of up to 90%, PVY 80% and PVA 40% (Wilson and Jones, 1993; Wright and Bishop, 1981); PVX 15% (Chandra and Mondy, 1981; Delhey, 1981); PVS 10-20% (Delhey, 1981). Little is known of the effects of PVM in single infections, but in combination with other viruses, mosaic symptoms are common (Romancer and Kerlan, 1994). Today viral diseases are a call for concern because of difficulties in

management strategies especially with the appearance of new viral strains, mixed infections and host-range diversity.

Disease symptoms are highly variable depending on the stage of development of the disease, environmental conditions, host cultivar and the strain of the virus involved (Grogan, 1981; Reckhaus and Nienhaus, 1981). The epidemiology of plant diseases is hub on host type, virus type and vector efficiency (Jeger, 2009). Plant virus transmission can be by animal vector, bacterial, fungi, spores, true seeds, tubers, and mechanically (Perez *et al.*, 1995). The symptoms of these six important potato viruses range from mottle, chlorosis, necrosis, defoliation, yellow spotting, streak, and brittle of leaves; stunting, rolling of upper leaves and mosaic of the entire plant (Karyeija *et al.*, 2000; McDonald and Singh, 1996). Necrosis may as well attack the stem and tubers (Boonham *et al.*, 2002). Infected plants (with rare exceptions) produce fewer or smaller tubers than healthy plants (Hooker, 1981; Kaniewski *et al.*, 1990; Whitworth *et al.*, 2006). In most cases, variations exist between primary and secondary infections. *Myzus persicae*, an insect of the family Aphididae is the most efficient vector of PLRV, PVY, PVX, PVS, PVM and PVA. With an exception of PLRV and PVA where transmission is in a persistent non-propagative manner (Mayo and Ziegler-Graff, 1996; Waterhouse *et al.*, 1981), the other four are transmitted in a non-persistent manner (Pattan *et al.*). PVX and PVS can be transmitted mechanically through contact and sap transmission (Pattan *et al.*).

Several techniques are used for the detection of potato viruses. Some include symptomatology, use of detector/indicator (test) plants, electron microscope and diverse serological methods. Symptomatology is less efficient because of interference from mixed infections. Though time consuming, the use of test plants have proven very effective and reliable (Bokx and Ghaffari, 1969; DeBokx and Piron, 1977). Some indicator plants used in potato viruses are of the genera Chenopodium, Datura, Lycopersicon, Physalis, Solanum and Nicotiana (Jayasinghe and Chuquillanqui, 1989). Serological assays are the most efficient used and are based on the specificity of antibodies and antigens (Hill *et al.*, 1984; Salazar, 1996). Here, we report the use of symptomatology and serological ELISA methods for detecting the viruses. The ELISA method is cheap and can easily be carried out in less equipped laboratories.

Any virus based research program, ranging from epidemiology to virus replication studies would not be possible without a reliable assay for detecting and/ or characterizing the pathogen. Potato viruses and potyviruses cause economically significant yield losses in potato, pepper and tomato crops throughout the world (Jeffries, 1998b; Stevenson *et al.*, 2001). They infect plants all over the world originated from Peru through contaminated germplasm (Fribourg and Nakashima, 1984; Hamilton, 1985; Jeffries, 1998b; Jones *et al.*, 1981; Salazar, 1971; Salazar, 1987; Stevenson *et al.*, 2001). The incidence of two PVY strains (PVY^O, PVY^N) has been reported in this agro-ecological zones in Cameroon (Martin *et al.*, 1995). Biological, cultural and chemical mitigation techniques are possible and well developed for the mitigation of pathogenic agents. Unfortunately, it is the contrary for viruses. Studies on the detection and host-range of viruses in different cropping systems are necessary for seed certification programs, and restricting crop choices for mixed cropping among host plant species. A good knowledge of occurrences of the six potato viruses and their host range will enrich potato seed certification programs within these regions. Here, we report work that sort to; detect the presence of *Potato virus S*, *Potato virus Y*, *Potato virus X*, *Potato virus A*, *Potato virus M* and *Potato leaf roll virus*, determine the relative prevalence of these six viruses in single and mixed infections, and determine the host range of these potato viruses amongst *Solanum spp*. in the Western Highlands of Cameroon.

Materials and methods

Host plant cultivation

Solanaceous plants; potato, tomato, pepper and garden huckleberry which are prominent host for the six potato viruses were grown in four locations [(Dschang (1500m.a.s.l), Bambili Upper farm

(1600m.a.s.l), Santa (1700m.a.s.l) and Djuttitsa (2000m.a.s.l)] in the Western Highlands of Cameroon between March to October 2007 and 2008. In each location, combinations of sole cropping and mix cropping treatments were assigned in a complete randomized block design to an area of 50 m^2 planted to potato, tomato, pepper and huckleberry. Each block consisted of four ridges of 3.5/1.5m with in-between lanes of 0.75m. Sole cropping was done on four of these blocks by planting seedlings of potato, tomato, pepper and garden huckleberry, accordingly. On each of the four ridges, for the fifth block, was planted these four crops in an alternating manner (mix cropping).

Sample collection

Potato, tomato, pepper and garden huckleberry leaf samples were collected from Dschang, Bambili, Santa and Djuttitsa. Three leaves (top, middle and bottom) from each plant were harvested separately and directly into specially labelled 10 x 15cm plastic bags and constituted a single sample. Plants were sampled on the basis of systemic symptoms; mottling, mosaic, rugose mosaic, leaf deformation and/or general stunting. Fifty samples were collected from each host plant species per site (25 from mix cropping and 25 from sole cropping). Three samples per host species were equally collected from asymptomatic plants to serve as controls. Samples were transported in a cooler to the Virology Project Laboratory at the University of Dschang for analysis. The samples were kept overnight in a fridge at 4°C.

Virus detection

The incidence of the six potato viruses was determined serologically by testing for the presence of each of the viruses, using Nitrocellulose Membrane Enzyme-linked Immunosorbent Assay (NCM–ELISA) technique. The NCM-ELISA procedure described by Gibbs and Padovan `1993 was followed (Gibb and Padovan, 1993). Tris buffered saline (TBS) containing [0.02 Tris base, 0.5M NaCl, 0.01% NaN_3 pH 7.5] was used as extraction buffer.

A buffer of 1/10(w/v dilution was added into each of the plastic bags containing the leaf samples. Leave sap was then extracted by gently grinding the leaf tissue by rolling a pestle over the plastic bag. The sap was then filtered using a mesh into different test tubes with respect to samples. Sedimentation was done by allowing the tubes containing samples to stand at room temperature for one hour, then 200ml of the supernatant from each tube pipetted into the wells of microtiter plates with appropriate label. The plates were sealed and incubated at 4°C overnight (~16 hours). Grids of 1x1cm were drawn on the NCM with a lead pencil. The NCM was slowly slide at an angle of 45° into distilled water and TBS, respectively, for 5minutes in each. Two sheets of Whatman filter paper were soaked in TBS for 10minutes, and then air-dried for 5minutes. Dilutions of 1/10, 1/100 and 1/1000 Antigen aliquots were prepared per sample. Antigen aliquots (10ul) were pipetted from the clear aqueous supernatant layer and dotted onto each square of the NCM and allowed to dry for (~2 hours).

The membrane was then transferred into a blocking buffer (5% skimmed milk Gloria R) in 60ml TBS and incubated for 1hour. After blocking, the membrane was washed in TBS by soaking 3 times with 3 minutes time intervals. The membrane was transferred to anti – PVY, anti – PLRV, anti – PVX, anti – PVS, anti – PVM and anti – PVA (Gamma globulin diluted to 1ug/ml in buffer (0.05M Na_2CO_3, pH. 9.6) contained in a 9cm diameter glass petri - dish. It was then incubated overnight with gentle rotatory shaking (80 rpm) on Gyratory shaker. The next day the NCM was rinsed (3x3mn) in TBS, incubated with polyclonal anti – PVY, anti – PLRV, anti – PVX, anti –PVS, anti–PVM and anti –PVA (Gamma globulin alkaline phosphatase conjugate at a dilution 1ug/ml in conjugate buffer (6% PVP – 40 000 + 3% NCM) for 1hour, then washed (3x3min). The substrate solution was prepared during the final wash by dissolving 6mg of 5 - bromo - 4 - chloro - 3 - indolyl phosphate (BCIP) (Bio – Rad) in 0.1ml N, N- dimethyl formamide (DMF) and 3mg of nitroblue tetrablue tetrazolium (NBT) (Bio –Rad) in 0.1ml DMF. Substrate buffer (30ml) was prepared by mixing BCIP and NBT solutions (0.1ml Tris base, 0.1M NaCl, 0.005M $MgCl_2.6H_2O$, pH 9.6). The conjugate solution was washed from the NCM with TBS.

The NCM was incubated at room temperature for color development (30min). The color development was keenly followed up and the development of a violet color was considered as being positive. All laboratory manipulation was done under aseptic conditions with all hard ware duly sterilized.

Results

Virus prevalence

Symptomatic potential host plants for potato viruses were sampled and tested for the presence of six potato viruses (PVY, PVX, PVM, PVA, PVS, and PLRV). A total of twenty samples were collected for each of the four host types [(potato (*Solanum tuberosum* L.) tomato (*Lycopersicon esculentum Mill.)*, pepper (*Capsicum* ssp), garden huckleberry (*Solanum scabrum* L.)], and from four locations (Bambili, Santa, Dschang and Djuttisa). For each experimental replicate, a total of 1920 NCM-ELISA tests were performed. The relative prevalence of these potato viruses showed variation with significant differences at $P \leq 0.05$. Results revealed PVY to be the most prevalent among these viruses in the Western highlands of Cameroon. The relative prevalence of the six viruses in decreasing order were: PVY, PVX, PVM, PVA, PVS and PLRV (Table 1).

Table 1. Relative prevalence of the six potato viruses in all samples tested positive for each of the viruses from four host plants in four locations. Bars with the same letter (a, b, c, and d) are not significantly different as revealed by Duncan Multiple Range Test.

	HOST				Total	
	Total	*S. tuberosum*	*S. scabrum*	*S. capsicum*	*Lycopersicon esculentum*	
	S. tuberosum	*S. scabrum*	*S. capsicum*	*Lycopersicon esculentum*		87b
PVX	40	0	19	28	87b	37c
PVA	37	0	0	0	37c	28cd
PLRV	25	3	0	0	28cd	106a
PVY	47	3	24	32	106a	49c
PVM	49	0	0	0	49c	36c
PVS	36	0	0	0	36c	343
Total	234	6	43	60	343	

A greater number of samples from Bambili were positive for the six potato viruses indicating high prevalence. The percentages of positive samples were lower in Bambili. Djuttissa recorded the least prevalence of the viruses, followed by Santa and Dschang. The prevalence of PVY and PVX was significantly higher than that of PVS, PVA, PVM and PLRV (Fig. 1). With respect to cropping systems, mix-cropping revealed a significantly high percentage of infection for PVY, PVX, PVS, PVA, PVM and PLRV as compared to sole cropping, irrespective of the location. Nevertheless, some variation was observed in Dschang where more samples tested positive from the sole cropping system when compared with other locations (Fig. 1 & 2).

The percentage prevalence of potato viruses was significantly lower in samples collected from sole cropping fields and high for those collected from mix cropping fields (Fig. 3). In both cropping systems PVY and PVX were the most prevalence.

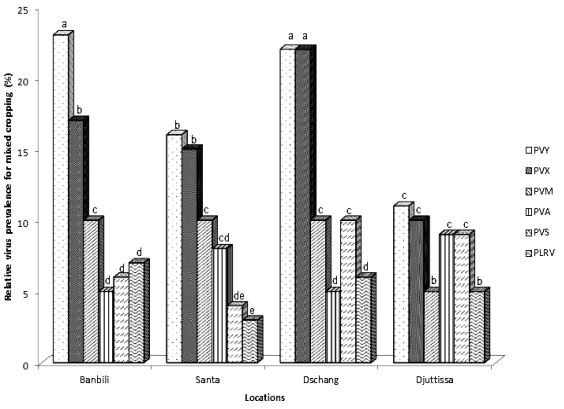

Fig. 1. Mix cropping; Prevalence of six potato viruses tested from each location sampled under mix cropping. Bars with the same letter (a, b, c, d, and e) are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

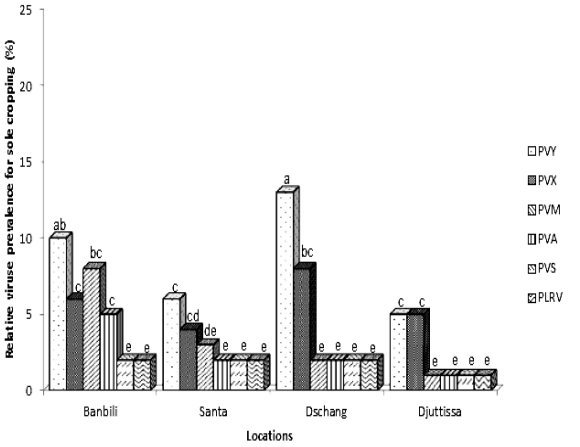

Fig. 2. Sole cropping; Prevalence of six potato viruses tested from each location sampled under mono cropping. Bars with the same letter (a, b, c, d, and e) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test.

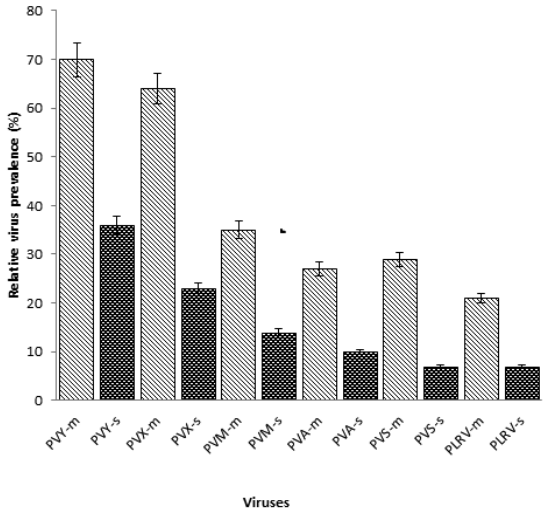

Fig. 3. General relative prevalence of six potato viruses tested from the four locations sampled both under mix cropping and mono cropping systems. Means of independent virus replicates for mix and mono cropping are presented and standard deviations are indicated.

Viruses on different host plant

Six viruses were detected in samples collected from the survey locations. The results show that potato was the most prominent host for the six viruses. The prevalence rate of the viruses infecting potato in a decreasing order was: PVM, PVY, PVX, PVA, PVS, and PLRV. Diseased samples were significant in both cropping systems for all locations. A survey of tomato, pepper and huckleberry for the six potato viruses revealed the presence of some of these viruses in the host. There was significantly high prevalence of PVY and PVX, while no infections of PVM, PVA, PVS and PLRV were recorded for tomato and pepper. In huckleberry, only PVY and PLRV were detected (Fig. 4).

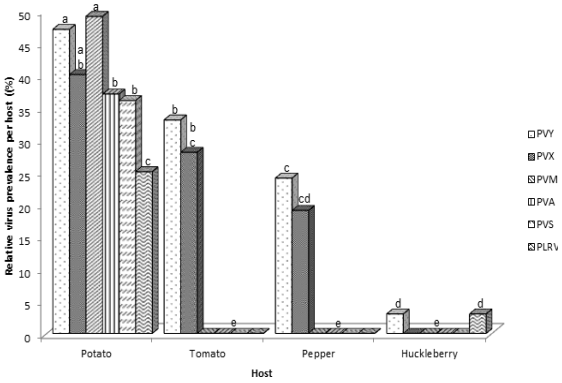

Fig. 4. Prevalence of each of the six potato viruses tested from four locations sampled both under mix and sole cropping on individual host plant, it presents the viruses detected for each host. Bars with the same letter (a, b, c, d, and e) are not significantly different (P≤0.05) according to Duncan's Multiple Range Test.

Discussion

Virus diseases remain a serious limiting factor for the production of some Solanaceous crop species in the North West Region of Cameroon. Six potato viruses are prevalent in the study area and infect potato, tomato, pepper, and huckleberry. Previous research reports have documented the occurrence of these viruses in areas where potential host plants are being grown (Struik and Wiersema, 1999). The presence of these viruses in the surveyed region signifies the urgent need to develop control measures to avoid drastic yield losses that could exacerbate problems of food security that already exist. Given that there are no biochemical control methods available for viruses within a plant, exclusion or eradication of infected materials is the most effective means of controlling plant virus diseases. However, production of resistant cultivar, thermotherapy, chemotherapy, meristem culture and cold treatment techniques have provided promising results for the production of virus-free propagation materials for crops of agricultural and horticultural importance (Ali *et al.*, 2014; Faccioli,

2001; López-Delgado *et al.*, 2004; Šip, 1972; Wang and Valkonen, 2009). These control methods are viable alternatives that could be contemplated in the North Western Highlands of Cameroon. Nevertheless, detection and identification of viruses in host materials constitute a fundamental step in management programs. This can be accomplished by biological indexing, serological and molecular methods. Routine diagnosis of plant virus diseases based on symptom expression in their natural hosts is often difficult. Moreover, plant materials prepared as virus-free stock still require screening for confirmation. Serological techniques are among the most reliable for disease diagnosis and plant virus detection (Van Regenmortel and Dubs, 1993). Enzyme-linked immunosorbent assays (ELISA) techniques were developed (Clark and Adams, 1977). Under circumstances of limited resources, it is the routinely used technique because it is a sufficiently reliable, fast and sensitive approach to detect most of the potato viruses (Petrunak *et al.*, 1991; Wróbel, 2014). The technique is also easy to use, allows viruses to be detected in the presence of host plant material or other impurities, making it possible and very useful for detecting plant viruses in the field (Njukeng *et al.*, 2002; Njukeng *et al.*, 2004). It is important that the government and other stakeholders ensure the training of farmers on basic testing techniques and provide test kits to facilitate early detection and control.

Earlier surveys of potato viruses in the Western highlands of Cameroon reported varied prevalence of these viruses (Fondong *et al.*, 1991; Njukeng *et al.*, 2013a). Fondong and colleagues (1991) surveyed four potato viruses (PVS, PVY, PVX and PLRV) and reported PVS (*Carlavirus*) and PVY (*Potyvirus*) to be the most prevalent. In 2013, PVM (*Carlavirus*) was the most prevalent in potato fields according to a report by Njukeng *et al.*, (2013a) following their survey of six potato viruses (Njukeng *et al.*, 2013a). The present work shows the prevalence of these viruses, with PVY being the most prevalent followed by PVX and PVA. These are indications that the dominance of particular viral strains has changed over the last two decades. The six potato viruses occur throughout the western highlands of Cameroon on tomato, potato, pepper and huckleberry, nevertheless, the prevalence of PVY was significantly high. PVY is a member of the Potyvirus genus, the largest of six genera in the family *Potyviridae*. It is known for its wide host range comprising many crop species from Solanaceae; tomato, pepper, and tobacco (Büchen-osmond, 1987) and infecting species of most angiosperm taxa in both temperate and tropical climates. In a monoculture of potatoes, tomato, pepper or huckleberry, the spread of PVY is favored as viruliferous aphids move from infected to healthy plants without loss of infectivity. This phenomenon is much higher in mix cropping field where all the plants are natural host for PVY. This might be a possible explanation for the relatively high prevalence of PVY in all the localities surveyed. Growing alternative hosts for a particular virus makes it endemic (Zitter, 1977). We reason that, advising farmers who practice mixed cropping to consider planting plants that are not host to PVY and related viruses could be one way of reducing virus incidence.

The type of interaction that usually occurs between the environment (location and time), the vector, viruses and the host plant due to the existence of a favorable environmental condition for epidemic development, widespread occurrence, susceptible of cultivars and the use of non-certified virus infected seed might be possible contributions for discrepancies in the relative prevalence recorded for these viruses in different locations. The high prevalence of the six viruses in Bambili might be due to the presence of a high population of aphids, the planting of already infected tubers coupled with human activity in the farm which helped to increase the infection and spread of these viruses. The prevalence of PVY and PVX was high in Dschang. Both PVS and PVM are members of the genus *Carlavirus* and are spread by aphids in a semi-persistent manner or through seed (Astier *et al.*, 2001) and infect only few hosts. Previous works sampled only a single host (potato), while the present report covers several natural host (pepper, tomato, potato and huckleberry) assessed for the prevalence of six potato viruses. The aforementioned points are

possible explanations accounting for the high prevalence of the six potato viruses in Bambili. The high prevalence of PVY recorded in Djuttitsa and low prevalence recorded for PLRV is in accordance with previous reports (Fondong *et al.*, 1991; Njukeng *et al.*, 2013a). The lowest percentage infection was recorded by PLRV, being generally less than 50%. The relatively low prevalence of PLRV is likely due to the fact that PLRV is not transmitted mechanically, hence farm activities do not help in spreading the virus. PLRV is transmitted by aphids in a persistent manner thus dependent on the timing of sampling with relation to aphid activity. Low temperatures which led to low aphid population and less aphid activity could partly explain the low prevalence of PLRV.

In these locations, mix cropping by planting potential natural host modifies field ecosystems by making it possible for the spread of these viruses. In this case a single host can easily be infected by two or more virus isolate. Sole cropping enhances the spread of viruses of a particular isolate irrespective of the fact that aphids can transmit faster without losing infectivity. The prevalence of PVY and PVX in Dschang can be attributed to the fact that seeds used were not virus-free coupled with the comparably high temperatures that favour the proliferation of aphid populations and activities leading to high virus incidence.

More than 25 different viruses are known to infect potato under natural condition with frequent co-existence of two or more viruses in a single plant. The damage caused by virus infection depends on the particular virus or combination of viruses present, virulence of virus strains, susceptibility of the variety, timing of infection, the abundance of insect vectors and environmental condition. Variations in the relative prevalence of viruses in different host from the different location can be explained by the factors determining virus infection and spread in the locations where samples were collected. These locations were at different geographical regions having varied altitudes which can greatly shape their respective environmental conditions. A report by Wood and Jellis in 1984 described the incidence of viruliferous aphid vectors early in the growing season as the most significant of all interacting factors that enhance virus spread (Ata *et al.*, 1982, Njukeng *et al.* 2013a). The high incidence of PVY, PVX and PVA could therefore partly be explained by the fact that they could be transmitted both mechanically and by insect vectors (Aquino *et al.*, 1996; Fox *et al.*, 2017; Roberts, 1948; Sertkaya and Sertkaya, 2005). Transmision from one host to another is an important stage in the life-cycle of viruses as this ensures spread and maintenance in the host. Previous research reported that transmission mechanisms shape the effect of viruses during host-virus interaction (Gutiérrez *et al.*, 2013; Mauck *et al.*, 2012). Vector-borne pathogens often alter the traits of their hosts (life cycle, population genetics) in ways that influence the frequency and nature of interactions between host and vector (Eigenbrode *et al.*, 2002; Mauck *et al.*, 2012)

There is high specialization in virus-host relationship; viruses are restricted to a particular type of host. However, insect vectors transmitted viruses can replicate within both their host and their vector and can infect plant of closely related taxa (specialist virus), or far relative (generalist or multi-host virus). It has been proposed that plant viruses are host generalists and vector specialists (Power and Flecker, 2003). This was with reference to the analysis of 474 vector-transmitted viruses, with 9.9% having a single host species and 58.4% having a single vector species (Brunt *et al.*, 1996). In addition, data on the natural host range of 29 virus species indicated 17% were restricted to a single genus, while 35% extended over different plant families (García-Arenal and McDonald, 2003). Genetic differentiation of virus populations according to host species or populations may be indicative of host adaptation (Elena *et al.*, 2011; García-Arenal *et al.*, 2001; Moury *et al.*, 2006; Seal *et al.*, 2006). The susceptibility of potential hosts to potato viruses vary enormously, with close relatives of the natural host typically being the most susceptible. For viruses to effectively invade thier host, they must adapt by evolving to use different receptors, to escape the plants immune response. There is ample evidence that among these viruses (PVY, PVX, PVM, PVS, PVA and PLRV), PVY has a naturally wide host range comprising up to nine

families including important crops such as pepper (*Capsicum* spp.), potato (*Solanum tuberosum* ssp. *tuberosum*),tobacco (*Nicotiana* spp.), tomato (*Lycopersicon esculentum*) (Jeffries, 1998a). Potato Virus X (PVX) is known to infect more than 240 species in 16 families with majority of these hosts found in the *Solanaceae* (Purcifull and Edwardson, 1981). Four of these six viruses, PLRV, PVM, PVA and PVS have narrow host ranges with susceptible species belonging mainly to the *Solanaceae* (Hiruki, 1970; MacLachlan *et al*., 1953). Host species equally has an influence on infection with respect to virus strain. Example some species of potato and pepper are useful for distinguishing strains and phenotype among PVY (Gebre Selassie *et al*., 1985; Jones, 1990; Kerlan *et al*., 1999). Host range (the number of plant species exploited by a virus) evolution in plant viruses as predicted by researchers is a key element that shapes virus epidemiology and changes in host range resulting to host range expansions is at the root of virus emergence either in new host species or in host genotypes previously resistant to the virus (Frank, 1996; Lajeunesse and Forbes, 2002; Woolhouse *et al*., 2001). A specific case of host range expansion is resistance breaking, i.e. the acquisition by the virus of the capacity to parasitize host plant genotypes immune or resistant to other virus genotypes. However, this phenomenon threatens the efficiency of the control of viral diseases in crops based on genetic resistance bred into cultivars (Maule *et al*., 2007). The points advanced might help explain the difference in prevalence of these six viruses, which was observed to be high in potato; and the prevalence in tomato of PVY and PVX, pepper PVY and PVX and Huckleberry and the low prevalence of PVY and PLRV. It is worth noting that it is the first time PLRV and PVY have been detected in huckleberry.

Mix-cropping systems have been shown to reduce the incidence of non-persistent insect-borne viruses in other agricultural crops and might be a useful strategy for minimizing the spread of potato viruses in organic potato fields. *Myzus persicae* which is one of the most important crop pest acts as an efficient plant virus vector to plants of the Solanaceae, Chenopodiaceae, Compositae, Cruciferae and Cucubitaceae families (Annis *et al*., 1982). Mix cropping or inter cropping is a measure used to reduce aphid population and subsequently virus transmission and spread (McKinlay, 1985). It is clear that mix cropping with non-susceptible or non-host mimics and reduces/inhibits vector movement. Fereres (2000) successfully used barrier crops (sink) to control viruses by altering vector movement into crop filed thus reducing virus population. Barrier plants act as sinks, for it alters the flight pattern of insect vector (Fereres, 2000). Mix cropping of pepper with maize, okra, sorghum and cotton; and potato with soybean, sorghum and wheat serves as barrier to pepper and potato for virus infection. Relatively, these are better explanations for the high relative prevalence of potato viruses in mix cropping in the present case study. Crops used are all common natural host and thus susceptible host to these potato viruses. Contrary, sole/mono cropping presents limited host range in the same field leading to reduction in virus transmission and spread thus, recorded relatively low percentage prevalence of potato viruses.

Pest and pathogens in *Solanum scabrun* are similar to those of Solanaceae family with fungus responsible for late (*Phytophothora infestans*) and early (*Altenaria solani*) blight being the most devastating. Nematode and bacterial disease on Solanum have been reported in in Kenya and Tanzania. Leaf curl and leaf mosaic are the prominent viral diseases in *Solanum scabrum* inflicted by Tomato yellow leaf curl virus (TYLCV) and Cucumber mosaic virus (CMV) (Garcia-Andres *et al* 2006). Potyvirus-infected huckleberry has been reported in Kenya (Juliane Langer *et al*) and to the best of our knowledge this is the first time Polerovirus (PLRV) have been detected in huckleberry.

Conclusion

The prevalence of the six potato viruses is on a steady increase in the Western Highlands of Cameroon. Mixed viral infections of heterologous Potato viruses [(*Potato virus S and Potato virus M (Carlavirus)*, *Potato virus Y and Potato virus A (Potyvirus)*, *Potato virus X (Potexvirus)*, and *Potato leaf roll virus (Polerovirus)*] are a regular occurrence in the

Western Highlands of Cameroon in potato, pepper, tomato and huckleberry cropping systems.

It may be due to the absence or limited virus control methods applied in field crops and/or ignorance of farmers about symptom or existing strategies to manage plant viruses. The danger of mix cropping using host plants that are susceptible to a range of viruses is equally revealed. Based on our results, we recommend the urgent setting up of a seed certification scheme for Solanaceous crops that are cultivated for seed stock improvement.

References

Abbott JA. 1999. Quality measurement of fruits and vegetables. Postharvest Biology and Technology **15,** 207-225.

Ali M, Nasiruddin K, Haque M, Faisal S. 2014. Virus elimination in potato through meristem culture followed by thermotherapy. SAARC Journal of Agriculture **11,** 71-80.

Annis B, Berry RE, Tamaki G. 1982. Host preferences of the green peach aphid, Myzus persicae (Hemiptera: Aphididae). Environmental Entomology **11,** 824-827.

Aquino V, Espino T, Tisalona L, Flores C. 1996. Isolation, transmission, propagation and purification of major potato viruses: potato virus X (PVX), potato virus Y (PVY) and potato leaf roll virus (PLRV). *27.* Anniversary and Annual Scientific Meeting of the Pest Management Council of the Philippines, Inc., Davao City (Philippines), 7-10 May 1996.

Astier S, Albouy J, Maury Y, Lecoq H. 2001. Principles of plant virology: genome, pathogenicity, virus ecology. Institut National de la Recherche Agronomique.

Ata A, Allen D, Thottappilly G, Rossel H. 1982. Variation in the rate of seed transmission of cowpea aphid-borne mosaic virus in cowpea. Tropical grain legume bulletin.

Balachandran S, Hurry V, Kelley S, Osmond C, Robinson S, Rohozinski J, Seaton G, Sims D. 1997. Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. Physiologia Plantarum, **100,** 203-213.

Banttari E, Orr P, Preston D. 1990. Purple top as a cause of potato chip discoloration. Trans ASAE, **33,** 221-226.

Bokx J, Ghaffari H. 1969. Detection of potato viruses X, Z and A by rubbing infectious foliage onto A6-test leaves, [sn].

Boonham N, Walsh K, Preston S, North J, Smith P, Barker I. 2002. The detection of tuber necrotic isolates of Potato virus Y, and the accurate discrimination of PVY O, PVY N and PVY C strains using RT-PCR. Journal of virological methods **102,** 103-112.

Bosland PW, Votava EJ, Votava EM. 2012. Peppers: vegetable and spice capsicums. Cabi.

Bridge J, Price NS, Kofi P. 1995. Plant parasitic nematodes of plantain and other crops in Cameroon, West Africa. Fundamental and Applied Nematology **18,** 251-260.

Brunt AA, Crabtree K, Dallwitz M, Gibbs A, Watson L. 1996. Viruses of plants. Descriptions and lists from the VIDE database. Cab International.

Büchen-Osmond C. 1987. Potato Y potyvirus in: Plant Virus Online. Descriptions and Lists from the VIDE database (http://biology.anu.au/Groups/ MES/vide/descr652. htm).

Chandra S, Mondy N. 1981. The effect of potato virus X on the nitrogenous constituents of potatoes. Experientia **37,** 577-578.

Clark MF, Adams A. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology **34,** 475-483.

DeBokx J, Piron P. 1977. Effect of temperature on symptom expression and relative virus concentration in potato plants infected with potato virus YN and YO. Potato Research **20,** 207-213.

Delhey R. 1981. Incidence of viruses S and M on potato crops in Argentina. Fitopatologia **16,** 1-5.

Eigenbrode SD, Ding H, Shiel P, Berger PH. 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, Myzus persicae (Homoptera: Aphididae). Proceedings of the Royal Society of London B: Biological Sciences **269,** 455-460.

Elena SF, Bedhomme S, Carrasco P, Cuevas JM, de la Iglesia F, Lafforgue G, Lalić J, Pròsper À, Tromas N, Zwart MP. 2011. The evolutionary genetics of emerging plant RNA viruses. Molecular plant-microbe interactions, **24,** 287-293.

Faccioli G. 2001. Control of potato viruses using meristem and stem-cutting cultures, thermotherapy and chemotherapy. In: Virus and virus-like diseases of potatoes and production of seed-potatoes, Springer, pp. 365-390.

Fereres A. 2000. Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses. Virus research **71,** 221-231.

Fondong V, Ntonifor C, Gass T. 1991. SURVEY AND IDENTIFICATION OF POTATO VIRUSES IN THE WESTERN HIGHLANDS OF CAMEROON. Symposium on Tropical Root Crops in a Developping Economy **380,** pp. 514-514.

Fontem D. 2001. Influence of rate and frequency of Ridomil Plus applications on late blight severity and potato yields in Cameroon. African Crop Science Journal **9,** 235-243.

Fontem D, Aighewi B. 1993. Effect of fungicides on late blight control and yield loss of potato in the western highlands of Cameroon. International Journal of Pest Management, **39,** 152-155.

Fontem D, Gumedzoe M, Nono-Womdim R. 1999. Biological constraints in tomato production in the western highlands of Cameroon. Tropicultura **16,** 89-89.

Fox A, Collins L, Macarthur R, Blackburn L, Northing P. 2017. New aphid vectors and efficiency of transmission of Potato virus A and strains of Potato virus Y in the UK. Plant Pathology **66,** 325-335.

Frank SA. 1996. Models of parasite virulence. The Quarterly review of biology **71,** 37-78.

Fribourg C, Nakashima J. 1984. Characterization of a new potyvirus from potato. Phytopathology **74,** 1363-1369.

Fuglie KO. 2007. Priorities for potato research in developing countries: Results of a survey. American Journal of Potato Research **84,** 353-365.

García-Arenal F, Fraile A, Malpica JM. 2001. Variability and genetic structure of plant virus populations. Annual review of phytopathology **39,** 157-186.

García-Arenal F, McDonald BA. 2003. An analysis of the durability of resistance to plant viruses. Phytopathology **93,** 941-952.

Gebre SK, Marchoux G, Delecolle B, Pochard E. 1985. Variability of natural strains of potato virus Y infecting peppers in south-eastern France. Characterization and classification into 3 pathotypes. Agronomie (France).

Gibb K, Padovan A. 1993. Detection of sweet potato feathery mottle potyvirus in sweet potato grown in northern Australia using an efficient and simple assay. International Journal of pest management **39,** 223-228.

Grogan R. 1981. The science and art of plant-disease diagnosis. Annual Review of Phytopathology **19,** 333-351.

Guo DP, Guo YP, Zhao JP, Liu H, Peng Y, Wang QM, Chen JS, Rao GZ. 2005. Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. tsatsai) after turnip mosaic virus infection. Plant Science **168,** 57-63.

Gutiérrez S, Michalakis Y, Munster M, Blanc S. 2013. Plant feeding by insect vectors can affect life cycle, population genetics and evolution of plant viruses. Functional Ecology **27,** 610-622.

Hamilton R. 1985. Virus transmission. In: The plant viruses, Springer, pp. 245-267.

Heiser CB, Smith PG. 1953. The cultivated Capsicum peppers. Economic Botany **7,** 214-227.

Hill E, Hill J, Durand D. 1984. Production of monoclonal antibodies to viruses in the potyvirus group: use in radioimmunoassay. Journal of general virology **65,** 525-532.

Hiruki C. 1970. Red Kidney Bean, a useful bioassay host for qualitative and quantitative work with Potato virus M. Phytopathology **60,** 739-740.

Hooker WJ. 1981. *C*ompendium of potato diseases. International Potato Center.

Jayasinghe U, Chuquillanqui C. 1989. Use of indicator plants for detection of potato viruses. International Potato Center.

Jeffries CJ. 1998a. Technical guidelines for the safe movement of germplasm. N°19 Potato. Rome **177**.

Jeffries CJ. 1998b. *Potato*. Bioversity International.

Jeger MJ. 2009. Epidemiology of Plant Disease. *eLS*.

Jones R. 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. Annals of Applied Biology **117,** 93-105.

Jones R, Rupert E, Barnett O. 1981. Virus infection of *Trifolium* species in cell suspension cultures. Phytopathology **71,** 116-119.

Kaniewski W, Lawson C, Sammons B, Haley L, Hart J, Delannay X, Tumer NE. 1990. Field resistance of transgenic russeet burbank potato to effects of infection by potato virus X and potato virus Y. Bio/technology **8,** 750-754.

Karyeija R, Kreuze J, Gibson R, Valkonen J. 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology **269,** 26-36.

Kerlan C, Tribodet M, Glais L, Guillet M. 1999. Variability of potato virus Y in potato crops in France. Journal of Phytopathology **147,** 643-651.

Lajeunesse MJ, Forbes MR. 2002. Host range and local parasite adaptation. Proceedings of the Royal Society of London B: Biological Sciences **269,** 703-710.

Lee M, Bottner KD, Secor G, Rivera-Varas V. 2006. 'Candidatus Phytoplasma americanum', a phytoplasma associated with a potato purple top wilt disease complex. International Journal of systematic and evolutionary microbiology **56,** 1593-1597.

Loebenstein G, Thottappilly G. 2013. Virus and virus-like diseases of major crops in developing *countries*. Springer Science & Business Media.

López-Delgado H, Mora-Herrera M, Zavaleta-Mancera H, Cadena-Hinojosa M, Scott I. 2004. Salicylic acid enhances heat tolerance and potato virus X (PVX) elimination during thermotherapy of potato microplants. American Journal of Potato Research **81,** 171-176.

MacLachlan D, Larson RH, Walker JC. 1953. Strain interrelationships in potato virus A.

Martin C, Demo P, Gass T, Fondong V, Koi J. 1995. Development of a seed production system from in-vitro in Cameroon: Experiences from the first two years. American Journal of Potato Research **72,** 299-302.

Matthews G, Wiles T, Baleguel P. 2003. A survey of pesticide application in Cameroon. Crop Protection **22,** 707-714.

Mauck K, Bosque-Pérez NA, Eigenbrode SD, Moraes CM, Mescher MC. 2012. Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Functional Ecology **26,** 1162-1175.

Maule AJ, Caranta C, Boulton MI. 2007. Sources of natural resistance to plant viruses: status and prospects. Molecular Plant Pathology **8,** 223-231.

Mayo M, Ziegler-Graff V. 1996. Molecular biology of luteoviruses. Advances in virus research **46,** 413-460.

McDonald J, Singh R. 1996. Host range, symptomology, and serology of isolates of potato virus Y (PVY) that share properties with both the PVY N and PVY O strain groups. American Journal of Potato Research **73,** 309-315.

McKinlay R. 1985. Effect of undersowing potatoes with grass on potato aphid numbers. Annals of Applied Biology **106,** 23-29.

Moury B, Desbiez C, Jacquemond M, Lecoq H. 2006. Genetic diversity of plant virus populations: towards hypothesis testing in molecular epidemiology. Advances in Virus Research **67,** 49-87.

Ndamukong K, Ntonifor N, Mbuh J, Atemnkeng A, Akam M. 2006. Molluscicidal activity of some Cameroonian plants on Bulinus species. East African Medical journal **83**.

Njukeng A, Atiri G, D'A HUGHES J, Agindotan B, Mignouna H, Thottappilly G. 2002. A sensitive TAS-ELISA for the detection of some West African isolates of Yam mosaic virus in Dioscorea spp. Tropical science **42,** 65-74.

Njukeng A, Atiri G, Hughes JA, Winter S. 2004. Development of serological procedures for rapid, sensitive and reliable detection of yam mosaic virus in yam tissues. Tropical science **44,** 136-147.

Njukeng A, Chewachong M, Chofong G, Demo P, Sakwe P, Njualem K. 2013a. Determination of scanned virus-free potato planting materials by positive selection and screening of tubers from seed stores in the western highlands of Cameroon. International Journal of Biological and Chemical Sciences **7,** 707-716.

Njukeng PA, Chewachong, GM, Sakwe P, Chofong G, Nkeabeng L, Demo P, Njualem K. 2013b. Prevalence of six viruses in potato seed tubers produced in informal seed system in the North West region of Cameroon. Cameroon Journal of Experimental Biology **9,** 44-49.

Olet EA, Heun M, Lye KA. 2006. A new subspecies of Solanum scabrum Miller found in Uganda. Novon: A Journal for Botanical Nomenclature **16,** 508-511.

Perez P, Collar J, Avilla C, Duque M, Fereres A. 1995. Estimation of vector propensity of potato virus Y in open-field pepper crops of central Spain. Journal of economic entomology **88,** 986-991.

Petrunak D, Gildow F, Christ B. 1991. Incidence and distribution of six viruses infecting potatoes in Pennsylvania. Plant Disease **75**.

Power A, Flecker A. 2003. The Importance of Species: Perspectives on Expandability and Triage.

Purcifull D, Edwardson J. 1981. Potexviruses, Vol. 627, Elsevier/North-Holland, Amsterdam.

Rahoutei J, García-Luque I, Barón M. 2000. Inhibition of photosynthesis by viral infection: effect on PSII structure and function. Physiologia Plantarum **110,** 286-292.

Reckhaus P, Nienhaus F. 1981. Etiology of a virus disease of white yam (*Dioscorea rotundata*) in Togo/Untersuchungen zur Ätiologie einer Viruskrankheit an Yam (D*ioscorea rotundata*) in Togo. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 492-509.

Roberts F. 1948. Experiments on the spread of potato virus X between plants in contact. Annals of Applied Biology **35,** 266-278.

Romancer Ml, Kerlan C. 1994. Biological characterisation of various geographical isolates of potato virus Y inducing superficial necrosis on potato tubers. Plant Pathology, **43,** 138-144.

Salazar L. 1971. Estudios sobre metodos de diagnosticos del virus X de papa. Invest Agropecu.

Salazar LF. 1987. La détection des virus dans la production de plants de pommes de terre. LA POMME DE TERRE: BULLETINS D'INFORMATION TECHNIQUE [Perú]. **1,** 123-128.

Salazar LF. 1996. Potato viruses and their control. International Potato Center.

Seal S, Vanden Bosch F, Jeger M. 2006. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Critical Reviews in Plant Sciences **25,** 23-46.

Sertkaya E, Sertkaya G. 2005. Aphid transmission of two important potato viruses, PVY and PLRV by *Myzus persicae* (Sulz.) and *Aphis gossypii* (Glov.) in Hatay Province of Turkey. Pakistan Journal of Biological Sciences **8,** 1242-1246.

Šip V. 1972. Eradication of potato viruses A and S by thermotherapy and sprout tip culture. Potato Research **15,** 270-273.

Stevenson WR, Loria R, Franc GD, Weingartner D. 2001. Compendium of potato diseases. American Phytopathological Society St. Paul^ eMinnesota Minnesota.

Struik PC, Wiersema SG. 1999. Seed potato technology. Wageningen Academic Pub.

Van Regenmortel M, Dubs M. 1993. Serological procedures. Diagnosis of plant virus diseases 159-214.

Wang Q, Valkonen J. 2009. Improved recovery of cryotherapy-treated shoot tips following thermotherapy of in vitro-grown stock shoots of raspberry (Rubus idaeus L.). Cryoletters **30,** 171-182.

Waterhouse B, Moises H, Woodward D. 1981. Alpha-receptor-mediated facilitation of somatosensory cortical neuronal responses to excitatory synaptic inputs and iontophoretically applied acetylcholine. Neuropharmacology **20,** 907-920.

Whitworth JL, Nolte P, McIntosh C, Davidson R. 2006. Effect of Potato virus Y on yield of three potato cultivars grown under different nitrogen levels. Plant Disease **90,** 73-76.

Wilson C, Jones R. 1993. Resistance to potato leafroll virus infection and accumulation in potato cultivars, and the effects of previous infection with other viruses on expression of resistance. Crop and Pasture Science **44,** 1891-1904.

Woolhouse ME, Taylor LH, Haydon DT. 2001. Population biology of multihost pathogens. Science **292,** 1109-1112.

Wright GC, Bishop GW. 1981. Volunteer potatoes as a source of potato leafroll virus and potato virus X. American Potato Journal **58,** 603-609.

Wróbel S. 2014. Modification of ELISA by replacing incubation of microtiter plates in an incubator with their shaking in PVY, PVM and PLRV detection. American Journal of Potato Research **91,** 354-362.

Zitter TA. 1977. EPIDEMIOLOGY OF APHID–BORNE VIRUSES. In: Aphids as virus vectors, Elsevier, pp. 385-412.