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Originalarbeit

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Detection of Iris yellow spot virus (IYSV) in selected Allium species and overwintering hosts in Austrian onion-producing areas

Nachweis von Iris yellow spot virus (IYSV) an ausgewählten Allium-Arten und dessen Überwinterungswirten in österreichischen Anbaugebieten

Abstract

Iris yellow spot virus (IYSV) has been reported for the first time in USA in 1989. Rapid spread of this viral pathogen has occurred in the western United States. IYSV has been frequently reported from most onion-production regions of the United States and many areas of the world in recent years. In 1998 it has been reported for the first time for Europe, in the Netherlands on *Iris hollandica.* Shortly afterwards it could be detected on onion in Israel. In Austria the first report of IYSV occurred in 2009. It is not seed born and only vectored by onion thrips (*Thrips tabaci)*.

As a result of our investigations all onion-producing areas in Austria tested positive for IYSV with DAS-ELISA in 2010. Leek has been tested IYSV-positive for the first time in Austria. Additionally IYSV could be detected in onion bulbs, which represents the third report worldwide and the second for Europe. The influence of weed hosts for the overwintering and distribution of the virus are undetermined to date. Eleven weed species have tested positive using DAS-ELISA, six species have been found out to be new hosts for IYSV according to this method. Nevertheless, none have been confirmed using the RT-PCR.

Key words: *Thrips tabaci*, *Allium* spp., tospovirus, detection in onion bulbs, first report on leek, Austria

Zusammenfassung

Das *Iris yellow spot virus* (IYSV) wurde erstmals 1989 in den USA nachgewiesen. IYSV konnte in den letzten Jahren in den meisten Zwiebelanbauregionen der Vereinigten Staaten und vielen anderen Ländern nachgewiesen werden. Der wirtschaftlich bedeutendste Wirt ist die Zwiebel (*Allium cepa*).

Der Erstnachweis in Europa und eine detaillierte Beschreibung erfolgte 1998 an *Iris hollandica* in den Niederlanden. Kurz danach konnte ein Befall an Zwiebel in Israel nachgewiesen werden. In Österreich wurde das IYSV erstmals im Sommer 2009 nachgewiesen.

Das Virus wird nicht mit dem Saatgut übertragen, konnte jedoch in Zwiebelbulben nachgewiesen werden. Während der Vegetation erfolgt die Übertragung durch den Zwiebelthrips (*Thrips tabaci*).

In Österreich konnte das IYSV in allen Zwiebelanbauregionen mit dem DAS-ELISA nachgewiesen werden. Des Weiteren erfolgte ein Erstnachweis von IYSV an Porree für Österreich. Darüber hinaus konnte auch ein Befall in einer Zwiebelbulbe nachgewiesen werden (es ist dies weltweit der dritte und für Europa der zweite derartige Nachweis).

Der Stellenwert von infizierten Unkräutern als Überdauerungs- oder Verbreitungsorgane ist bis heute ungeklärt. Das Virus konnte an elf Unkrautarten mit dem DAS-ELISA-Verfahren bestätigt werden, davon stellen sechs

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Arten nach dieser Methode neue Wirte von IYSV dar. Mit der RT-PCR konnte es an Unkräutern nicht nachgewiesen werden.

Stichwörter: *Thrips tabaci*, *Allium* spp., Tospovirus, Nachweis in Zwiebelbulben, Erstnachweis an Porree, Österreich

Introduction

Iris yellow spot virus (IYSV) is one of the economically most important plant diseases which affect onion production in United States (Pappu and Bag, 2010; Gent et al., 2004). It was first observed in the USA in 1989 (Hall et al., 1993) and then in the Netherlands in 1998 (Cortês et al., 1998), where the virus was described and characterized. In the year 2009 *Iris yellow spot virus* was for the first time detected in Austria on *Allium cepa* in two onion production areas, the analyses being carried out using ELISA and RT-PCR (Plenk and Grausgruber-Gröger, 2011).

There is evidence of a wide and constantly increasing range of weed species that serve as hosts of the virus, but little is understood regarding their relationship to the development of IYSV outbreaks (Evans and Frank, 2009). In 2006, at least 47 plant species were reported to be infected naturally by IYSV under field conditions (Gent et al., 2006). Major transmission of the virus is due to *Thrips tabaci* and the transport of infected plant material. The virus will probably spread further into uninfected regions, because the control of the vector is problematic. Therefore quality loss in spring onion and leek production, as well as yield loss in onion production due to smaller bulb sizes are possible in the future.

In Austria, a cropland of 2600 ha of onion and 140 ha of leek is exposed. Onion and leek, with a production volume of 145 000 t, constitute the most important sector of Austria's entire vegetable growing, followed by carrot with 85 000 t (Anonymous, 2010).

Symptoms

Symptoms caused by IYSV are mostly unspecific (Fig. 1). Infected onions show white, straw- to tan-coloured necrotic spots and diamond-shaped lesions on leaves. Symptoms caused by IYSV may be confused with those caused by thrips, herbicide-incompatibility, hard rain, hail storm or the beginning infection with other bacterial or fungal pathogens (e.g.: *Cladosporium*). Those different symptoms are likely to be influenced by cultivation method, time of infection, amount of virus inoculated during puncturing, amount of viruliferous thrips feeding, period of feeding, nutrient availability for the host plant and climate factors (Evans and Frank, 2009).

The most specific symptoms are green islands (Fig. 2 and Fig. 4). In these lesions, an island of green plant tissue develops in the centre of the necrotic area. Continuous attack can make the lesions grow and coalesce, girdle a leaf and snap and kill it. This is of high relevance for seed propagation. More symptoms occur when plants are older and can lead to a complete dieback.

Transmission

Iris yellow spot virus is only transmitted by *Thrips tabaci* (Kritzman et al., 2001). *Thrips tabaci* is polyphagous, feeding on a wide range of vegetable- and ornamental crops in the field and in the greenhouse (Rueda and Shelton, 1995). The exact period of incubation has not been reported so far (Evans and Frank, 2009). An early infection leads to a reduction in plant population and therefore also in yield. However, a later infection of the plants hardly ever causes quality and yield losses (Culbreath et al., 2003). *Thrips tabaci* is likely to survive on hardy weeds, seed stock and other plants or in the soil, using these structures as a source to affect its preferred host plants in the following year.

Due to anatomical facts, virus acquisition is only possible in a short time frame. For a successful acquisition there

Fig. 1. Unspecific symptoms of *Iris yellow spot virus* on onion.

Fig. 2. "Green islands" caused by IYSV on onion.

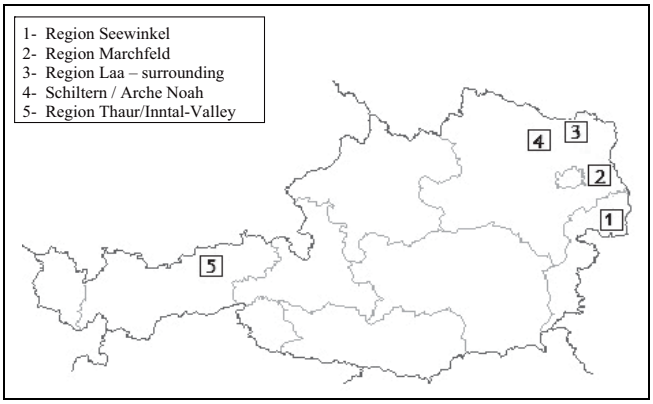

Fig. 3. Areas of onion sampling in Austria.

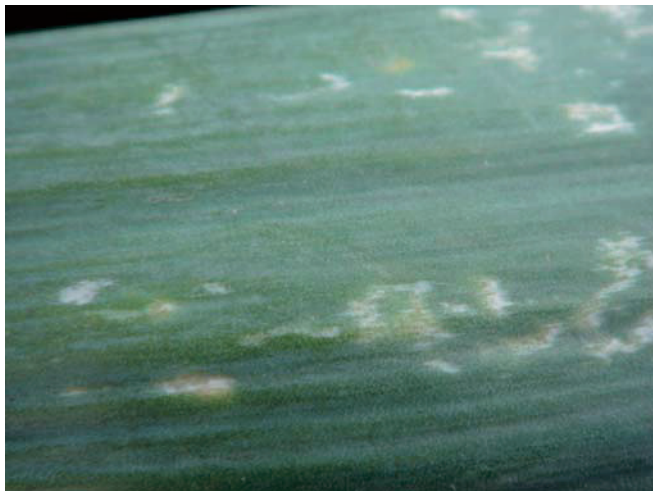

Fig. 4. "Green islands" of IYSV on leek.

has to be a circulation of the virus to the salivary gland. For reaching this aim the virus has to overcome four important barriers. This uninhibited path to the salivary gland is given exclusively in the first and the beginning of the second larvae stage, through a direct contact between midgut and salivary gland (Plumb and Callow, 2002). Therefore a successful virus distribution can only happen if oviposition occurs in infected plant tissue.

Beside thrips, there is no evidence of virus transmission through seed (Kritzman et al., 2001; Hoepting, 2005; Gent et al., 2006; Evans and Frank, 2009; Pappu et al., 2009; Pappu and Bag, 2010). Bulbs of *Allium cepa* tested IYSV positive (Robéne-Soustrade et al., 2006; Anonymous, 2007). Only *Nicotinia benthamiana* and *Eustoma resselianum* became systemically infected with IYSV after mechanical inoculation (Kritzman et al., 2001).

Purpose of the study

Because of the virus's rapid spread over the world and the induced yield loss, a reduction of *Iris yellow spot virus* infestation is gaining in importance. For this purpose only preventive measures are at hand. One measure is to stop the transmission by controlling the vector (*Thrips tabaci)* during the vegetation period. An effective prevention of IYSV could be to control their overwintering hosts.

In which organism IYSV is overwintering has not been explored so far. In this paper, the overwintering of IYSV in weeds, *Thrips tabaci* and volunteer onion will be studied. Another aspect is a monitoring of the occurrence of *Iris yellow spot virus* in Austrian onion growing areas.

Material and methods

The four most important onion growing areas were selected for the IYSV-monitoring. Two areas are in Lower Austria, one in north of Burgenland and one in Tyrol (Fig. 3). Samples were also taken at Schiltern.

Burgenland. The region Seewinkel is a major young onion and spring onion growing area in Austria. The variety 'Tonda Musona' was studied. Number of samples taken is shown in Tab. 1.

Lower Austria. The regions Marchfeld and Laa ("surrounding of Laa") are the major onion growing areas in Austria. In those regions the varieties 'Laaer Gelbe', 'Laaer Rote', 'Schoderleer', 'Drago', 'Mustang', 'Pandero', 'Spirit', 'Taresco', 'Crockett' and 'Gunnison' were studied.

The village Schiltern is the home of Arche Noah, the "Society for Preserving and Developing the Diversity of Cultural Plants". The Schiltern area has a special impor-

Tab. 1. Samples numbers of plants and varieties by region

Location	Region	Young Onion	Onion	Leek	Chives
1	Seewinkel	115	–	–	–
2	Marchfeld	–	74	–	–
3	Laa – surrounding	–	76	–	–
4	Schiltern	2	–	–	–
5	Thaur/Inntal-Valley	18	–	27	5
	Total	**135**	**150**	**27**	**5**

tance because of its absolute isolation from other onion growing areas and a long lasting tradition of independent organic seed propagation. The varieties 'Laaer Gelbe' and 'Ailsa Craig' were studied.

Tyrol. The region Thaur in the Inntal-Valley has a great importance for growing vegetables, including leek, chives, spring- and young onions. The leek varieties 'Belton' and 'Easton', chives varieties 'Fitlau' and 'Polycross' and the onion varieties 'Tonda Musona', 'Baja Verde' and 'Scopina' were studied. Sample species are shown in Tab. 2.

Overwintering

Trial site. The studies concerning overwintering of IYSV were made on a field on which the virus was diagnosed in 2009 (Plenk and Grausgruber-Gröger, 2011). This field is located in northern Burgenland in a region called Seewinkel, in the market town Andau. Andau is situated in the Pannonian lowland at 116 meters above sea level. From March 29th to August 25th 2010 samples were regularly taken from the trial field and adjoining field borders and waysides.

Thrips were collected with white- and blue bowls in the period from April 1st to May 4th 2010.

Overwintering in volunteer onion. The start of both the vegetation period and the sampling was marked by March 29th. Volunteer onions were regularly sampled from this time onwards.

The end of sampling was induced by the succeeding cultivation of potatoes and marked by the point of time when the fast growing potato plants had covered the soil and overgrown the onions. At beginning of June the last sampling on this field in Andau was carried out. Another sampling was done in Marchfeld, in Lower Austria on June 10th. Altogether 47 samples were examined.

Overwintering in weed. One of the field borders was especially suited for sampling due to its naturally speciose vegetation. Here the samples were taken over a longer period (March 29th to August 25th), to get more species of weed. Collection included annual, biennial and perennial weeds to study the overwintering in different species. A total of 125 samples of plants were taken, from 58 species belonging to 24 families (Tab. 3).

Overwintering in Thrips tabaci. The overwintering thrips population was sampled on the trial field, and tested for infection by *Iris yellow spot virus*. The collection was done at three sites, each equipped with one white and one blue bowl. The bowls were half filled with water, a detergent in order to reduce the surface tension was added. After 2 to 3 days the bowls were emptied and refilled.

Simultaneously the occurrence of *Thrips tabaci* was studied on the sampled plants. This was done by tapping the plants above a light-coloured surface, and transferring the sloping *Thrips tabaci* (thrips and larvae) with a fine hair brush into a 1.5 ml reaction tube. Those thrips were frozen at −20°C until RNA extraction was done. *Thrips tabaci* were found on *Allium cepa*, *Lamium purpureum*, *Taraxacum officinale*, *Cardaria draba*, *Bromus tectorum*, *Senecio vulgaris*, *Silene alba* and *Datura stramonium*.

Tab. 2. *Allium* species studied

Species	
Allium cepa	Onion
Allium schoenoprasum	Chives
Allium ampeloprasum ssp. *porrum*	Leek

Virus detection

The virus assay of *Iris yellow spot virus* in *Allium* sp. and weeds was carried out using DAS-ELISA (Agdia) and RT-PCR. *Iris yellow spot virus* detection in *Thrips tabaci* was done with RT-PCR.

RNA extraction from thrips. Reaction tubes containing thrips were centrifuged to collect the insects at the bottom of the tubes. A small amount of autoclaved sea sand and 140 µl DEPC treated water were added to each reaction tube. The thrips were ground using micro pestles. The RNA extraction was done using the QIAamp Viral RNA Kit (Quiagen®, Hilden, Germany) following the manufacturer´s instructions.

For positive control thrips larvae grown on infected plant material were used. The RNA was solved in water and stored at −20°C.

RNA extraction from plants. Frozen plant samples were put into extraction bags (BIOREBA, Reinach, Switzerland) and homogenized. For plant samples containing less plant sap (for example grasses) 1−2 ml RLT lysis buffer (Quiagen, Hilden, Germany) was added before homogenization. For each sample 50 µl of the plant sap was mixed with 500 µl RLT buffer, 5.5 µl β Mercaptoethanol and 55 µl 20% N-Lauroylsarcosine. The samples were incubated for 10 minutes at 70°C and further extracted using the RNeasy Plant Mini Kit (Quiagen®, Hilden, Germany) according to the manufacturer´s instructions.

For positive control freeze-dried samples from infected plant material (strain PV-0528, DSMZ, Braunschweig, Germany) were used. The RNA was solved in water and stored at −20°C.

One step RT-PCR. For one step RT-PCR the QIAGEN® OneStep RT-PCR Kit (Quiagen, Hilden, Germany) and the primer pair IYSV-f (5´-ATT CTT AGG AAC AGA GCA G-3´) and IYSV-RT-r (5´-TTG AAT TCC TTT GCT GCC AT-3´) designed by Krauthausen et al. (2012) was used. One step PCR was carried out in a final volume of 15 µl containing 2 µl RNA and 400 nM of each primer. A Biometra T3000 thermocycler (Biometra GmbH, Göttingen, Germany) and a thermal profile of 50°C for 30 min (reverse transcription), 95°C for 15 min (activation of the DNA polymerase), 40 cycles of 94°C for 30 sec, 45°C for

Tab. 3. Species of weed studied

	Sample		Sample
LILIOPSIDA		***Chenopodiaceae***	
Liliaceae		*Chenopodium album*	3
Gagea villosa	3	***Convolvulaceae***	
Poaceae		*Convolvulus arvensis*	4
Arrhenatherum elatius	1	***Fabaceae***	
Bromus hordeaceus	1	*Onobrychis viciifolia*	2
Bromus tectorum	2	*Robinia pseudacacia***	1
Calamagrostis epigejos	1	***Geraniaceae***	
Dactylis glomerata	1	*Erodium cicutarium*	2
Echinochloa crus-galli	2	***Hyacinthaceae***	
Elymus repens	2	*Ornithogalum umbellatum*	1
Poa sp.	1	***Lamiaceae***	
Setaria pumila	1	*Ballota nigra*	2
ROSOPSIDA		*Lamium purpureum*	4
Asteraceae		*Salvia pratensis*	2
Achillea millefolium	5	***Malvaceae***	
Arctium lappa	2	*Malva neglecta*	5
Arctium tomentosum	1	***Papaveraceae***	
Artemisia vulgaris	4	*Papaver rhoeas*	1
Carduus acanthoides	1	***Plantaginaceae***	
Cichorium intybus	1	*Plantago lanceolata*	2
Conyza canadensis	1	***Polygonaceae***	
Matricaria chamomilla	2	*Polygonum aviculare*	1
Erigeron annuus	1	*Rumex crispus*	2
Senecio vulgaris	4	*Rumex obtusifolius*	2
Sonchus arvensis	2	***Resedaceae***	
Taraxacum officinale	6	*Reseda lutea*	1
Tripleurospermum inodorum	2	***Rubiaceae***	
Amaranthaceae		*Galium aparine*	2
Amaranthus retroflexus	3	*Galium mollugo*	1
Apiaceae			
Carum carvi	2	***Scrophulariaceae***	
Daucus carota	2	*Veronica hederifolia*	3
Scandix pecten-veneris	1	*Veronica persica*	1
Boraginaceae		***Solanaceae***	
Lithospermum officinale	1	*Datura stramonium*	2
Brassicaceae		*Solanum tuberosum**	3
Brassica napus	2	***Urticaceae***	
Capsella bursa-pastoris	7	*Urtica dioica*	2
Cardaria draba	4	***Vitaceae***	
Caryophyllaceae		*Vitis vinifera***	2
Silene alba	2	**total**	**125**
Silene vulgaris	1	* succeeding crop of onions	
Stellaria media	2	** plants surrounding the trial site	

30 sec and 72 °C for 45 sec concluding with 72 °C for 7 min was used. DEPC-treated water was used as negative control.

Visualization of amplification products. The amplification products were separated on 1.5% agarose gels and visualized using ethidium bromide staining.

Results

Monitoring

Tab. 4 gives an outline of onion, chive and leek samples studied, the number of samples and the samples tested positive at different growing regions. In those regions in which IYSV had been proven in 2009 (Seewinkel and Marchfeld), it could be confirmed in 2010. In the region Seewinkel *Iris yellow spot virus* was confirmed in 47% of all young onion samples tested. This might indicate that there is already a strong distribution in this region.

In the region Laa – surrounding the virus could be confirmed in two samples.

In the region Thaur/Inntal Valley in Tyrol one sample of leek and one sample of young onion was tested positive, respectively. In this study, we have for the first time documented the occurrence of IYSV in leek in Austria. The virus could not be confirmed in chives.

The virus could be confirmed in the two tested onion samples from Schiltern.

21 bulbs were tested for occurrence of IYSV. One sample from Seewinkel of variety 'Tonda Musona' could be confirmed positive by ELISA.

Overwintering of IYSV

Volunteer onion. In seven volunteer onions out of 47, we confirmed IYSV.

Thrips tabaci.

- Colour bowls
  The colour bowls were positioned and emptied four times. On the last date, May 4th 2010, the absolute maximum of thrips was collected. Considering the weather and implantation, a second generation of thrips could have partly accounted for the high number. As this enormous number of thrips also included individuals other than overwintering ones, this last date of sampling was cancelled for interpretation. All *Thrips tabaci* were tested negative with PCR.
- Collection of thrips from plants
  All collected thrips (of weeds and volunteer onion) were tested with PCR. The virus could not be confirmed in one of the thrips, whether or not the host plant was virus positive or negative. So there is no direct link between overwintering thrips and the occurrence of IYSV in plants and volunteer onion.
- Weeds
  In Tab. 3 all weeds are listed which have been sampled from the trial field. Altogether eleven weedspecies were confirmed positive by ELISA. These include *Arctium tomentosum*, *Taraxacum officinale*, *Tripleurospremum inodorum*, *Rumex crispus*, *Senecio vulgaris*, *Dactylis glomerata*, *Poa* sp., *Convolvulus arvensis*, *Artemisia vulgaris*, *Cichorium intybus* and *Capsella bursa-pastoris*.

Discussion

Monitoring

The results are showing a regionally varying distribution of *Iris yellow spot virus* in all important onion growing areas in Austria. Those significant pathogens could increase in the future.

A first report of IYSV on *Allium ampeloprasum* ssp. *porrum* (leek) was done for Austria, in Tyrol. However, leek has not yet gained the importance and distribution of *Allium cepa* as an IYSV-host. An increasing importance as potential host cannot be excluded. Leek as IYSV-host has so far been confirmed in the United States (Pappu et al., 2007; Gent et al., 2007) in Spain (Córdobe-Sellés et al., 2007), in Greece (Chatzivassiliou et al., 2009), in the Netherlands (Anonymous, 2007), in Sri Lanka (Widana-Gamage et al., 2010) and in Slovenia (Anonymous, 2007).

There is some doubt about the way of virus infiltration into the Schiltern region, especially, when the isolation from all other growing areas is taken into consideration. Seeds of variety 'Ailsa Craig' were purchased from a seed company in Great Britain in the year 1995, seeds, of variety 'Gelbe Laaer' from an Arche Noah seed preserver from the Weinviertel region.

The US example shows the possibility of the virus to enormously distribute in short time and to simultaneously cause disastrous yield loss. Although it is not possible to prognosticate the effects for Europe, the aggressiveness of this pathogen should not be underestimated. Which resources the virus is using for that rapid spreading is un-

Tab. 4. Tested *Allium*-species per region and respective results

Location	Region	Samples	ELISA positive	Species
1	Seewinkel	115	55	*Allium cepa*
2	Marchfeld	74	1	*Allium cepa*
3	Laa – surrounding	76	2	*Allium cepa*
4	Schiltern/Arche Noah	2	2	*Allium cepa*
5	Thaur/Inntal-Valley	27	1	*Allium ampeloprasum* ssp. *porrum*
5	Thaur/Inntal-Valley	18	1	*Allium cepa*
5	Thaur/Inntal-Valley	5	0	*Allium schoenoprasum*
Samples total		**317**	**62**	

clear to date. *Thrips tabaci*, the only vector, has a high reproduction potential and mobile activity. But there is no transovarial transmission, so every generation of thrips has to be newly infected. "The onion thrips will not be the one and only carrier, there is likely to also be an anthropogenic way" (Kahrer, 2011). An effective anthropogenic way is the trade and transport of young onions. *Thrips tabaci* is located mainly on the stem base between the leaves. This place is on the one hand protected from external influences and gives constant food supply, and on the other hand it is the plant region with most virus content. The thrips can adapt to cold storage conditions for long time. By subsequent temperature increase the thrips can acquire full activity again. This can be an important way for quick distribution. Robéne-Soustrade et al. (2006) could confirm the IYSV in the onion bulb. In Austria the second Europe-wide proof in bulbs and the third worldwide was carried out. Based on this result a distribution via infected onion bulbs cannot be excluded. This could be relevant in production of ornamental and bulb-onions.

The trade with infected seeds could also have lead to a rapid spreading. However, IYSV could not be confirmed in the seeds until today (Kritzman et al., 2001; Hoepting, 2005; Gent et al., 2006; Evans and Frank, 2009; Pappu et al., 2009; Pappu and Bag, 2010).

Iris yellow spot virus has a long incubation time in *Allium* spp. Characteristic symptoms of IYSV in 2010, were noticeable from August onwards. An infection in autumn could cause an earlier outbreak of visible symptoms and a higher yield loss. Gent et al. (2006) reported an infection shortly after autumn sowing and consecutively extremely lower stand density. The areal and temporal behaviour pattern for distribution of IYSV in onion production remains unexplained (Gent et al., 2006).

Overwintering

For the study about overwintering in *Thrips tabaci* on the trial field six colour bowls were used. The virus was not confirmed in thrips by PCR. Gent et al. (2006) say that overwintering infected thrips could infect the volunteer onion in spring. An overwintering of IYSV in thrips could not be confirmed. Larentzaki et al. (2007) say that overwintering thrips are mainly in or close to an onion field. So there is a possibility of sucking first on infected volunteer onion in the spring. Evans and Frank (2009) characterize volunteer onion as overwintering host and as reservoir for the next season. The first volunteer onion which was tested positive was taken from the trial field on March 29th 2010. Altogether the virus was confirmed in 7 out of 47 volunteer onions. In this way volunteer onion is a potential inoculum close to the overwintering place of thrips, at the beginning of vegetation. The infectiousness of *Thrips tabaci* gets lost during the overwintering, for unexplained reason. Therefore a renewed outbreak of IYSV depends on an oviposition in infected plant tissue. An effective dissemination can occur from the second thrips-generation.

Studies from Israel have shown that there is no positive relationship between occurrence of *Thrips tabaci* and *Iris yellow spot virus* in onion growing fields (Kritzman et al., 2001).

Future studies, especially those done in regions with increased virus incidence, should focus on overwintering weeds, which can also serve as an inoculum. In our study eleven weed species were tested positive by DAS-ELISA. Six of those were considered to be new hosts. Gent et al. (2006) say that weeds could be overwintering hosts. Those positive weeds which were sampled at the two first dates (March 29th, April 1st) were of special interest.

It might be possible that perennial weeds are potential inoculums not only for the coming season, but also for the following years.

Even after a cultivation break of several years a renewed outbreak could occur, because of a remaining source of virus in vegetative storage organs in weeds. The influences of weed as hosts are undetermined to date (Evans and Frank, 2009). No transfer of IYSV from a weed host to onions (*Allium* spp.) has been documented to date, although this is suspected to occur (Evans and Frank, 2009; Gent et al., 2006).

Another question is whether there is a natural occurrence of IYSV in wild plant-species, which leads to infestation of crops which were newly planted in such regions, thus creating host plants of IYSV?

Based on the constantly expanding number of IYSV-host plants, an infestation of new important crop plants cannot be excluded. *Solanum tuberosum*, *Capsicum annum* and *Solanum lycopersicum* were confirmed IYSV positive in Tunisia (Ben Moussa et al., 2005). Also, some species of *Poaceae* are hosts of IYSV, which might lead to an infestation of important grain plants in the future.

All weed species tested negative when using polymerase chain reaction (RT-PCR). Nischwitz et al. (2007) could not confirm IYSV on weeds by RT-PCR, either. Gent et al. (2006) describe a positive testing of weeds exclusively by ELISA. So there is a possibility that inhibitors negatively affect the RT-PCR process. Those samples which were negative by RT-PCR and positive by ELISA were spiked. It shows there were no inhibitors in the samples. Some samples were made with BSA by PCR, which showed no other result.

Sampagni et al. (2007), Evans and Frank (2009) say that even with a positive ELISA the virus could not be confirmed with RT-PCR in most weed species. With both methods the virus could be confirmed in *Amaranthus retroflexus, Tribulus terrestris, Kochia scoparia, Lactuca serriola* and *Chenopodium album*.

This means that, at least in weeds, a sole confirmation by ELISA is not enough. Does this imply that the positive results, which several authors have found in weeds using ELISA testing, cannot be supported any longer?

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