Received: 7 January 2019 | Revised: 16 July 2019 | Accepted: 17 July 2019

DOI: 10.1111/jph.12845

ORIGINAL ARTICLE

Journal of Phytopathology WILEY

Development of a bioassay to assess resistance to *Fusarium oxysporum* (Schlecht.) in asparagus (*Asparagus officinalis* L.)

Lena Kathe[1,2] | Reiner Krämer[1] | Holger Budahn[1] | Klaus Pillen[2] | Frank Rabenstein[3] | Thomas Nothnagel[1]

[1]Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural Crops, Julius Kühn-Institut, Quedlinburg, Germany

[2]Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

[3]Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Quedlinburg, Germany

Correspondence
Thomas Nothnagel, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural Crops, Julius Kühn-Institut, Quedlinburg, Germany.
Email: thomas.nothnagel@julius-kuehn.de

Abstract

Fusarium oxysporum is one of the major pathogens causing root and crown rot in asparagus. Breeding of cultivars resistant to *F. oxysporum* would be the most efficient strategy for pathogen control. In this study, a bioassay was developed for screening seedling resistance. The non-destructive bioassay comprises inoculation with a highly aggressive *F. oxysporum* isolate, incubation in a climate chamber and quantification of disease symptoms by a digital image analysing system and a PTA-ELISA. This bioassay is simple to implement and demonstrated high reproducibility. Subsequently, it was used to determine the resistance behaviour of 16 asparagus genotypes to *F. oxysporum*. The asparagus cultivars revealed different levels of susceptibility, whereas the wild relative *A. densiflorus* was confirmed to be resistant.

KEYWORDS

asparagus, bioassay, digital image analysis, ELISA, *Fusarium oxysporum*, PCR, resistance

1 | INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is one of the most popular vegetables. Approximately 8.9 million tons are produced annually from over 1.5 million ha worldwide (FAOSTAT, 2017). The perennial asparagus production hinders an adequate crop rotation as prophylactic plant protection. The common practice of replanting on former asparagus fields due to lack of virgin soils further increases disease pressure. Grogan and Kimble (1959) first defined *Asparagus decline* as a gradual decrease in productivity. After multiple seasons, growth, yield and quality are reduced to such a degree that a commercial asparagus production becomes unprofitable. Among various biotic and abiotic stressors, mainly allelopathy from the asparagus root exudates and virus and fungal diseases were deemed responsible for this decline (Blok & Bollen 1995, 1996; Elmer, 2018; Hung, 1974; Kato-Noguchi, Nakamura, Ohno, Suegaga, & Okuda, 2017; Lake, Falloon, & Cook, 1993).

Crown and root rot caused by *Fusarium oxysporum* (Schlecht.) is one of the most dominant diseases in asparagus (Blok & Bollen, 1996; Goßmann, Büttner, & Bedlan, 2001) and considered a major reason for the *Asparagus decline* (Ellison, 1986; Elmer, 2018; Elmer, Johnson, & Mink, 1996; Lassaga, Camadro, & Babinec, 1998; Schofield, 1991).

Fusarium ssp. cannot be efficiently controlled by plant protection or cropping procedures. Therefore, breeding of cultivars resistant to *F. oxysporum* infection could be the most successful and economically reasonable strategy for controlling crown and root rot (Corpas-Hervias, Melero-Vara, Molinero-Ruiz, Zurera-Muñoz, & Basallote Ureba, 2006; Goßmann, Scholz, Hennig, Bargen, & Büttner, 2011; Stephens, Vries, & Sink, 1989).

The resistance behaviour of asparagus cultivars to *F. oxysporum* was investigated in numerous studies, but only marginal differences in infestation could be determined. Until now, no resistant cultivars were described (Corpas-Hervias et al., 2006; Falavigna, Casali, &

Alberti, 2008; Goßmann, Kleta, Humpf, & Büttner, 2005; Sadowski & Knaflewski, 1990; Schofield, 1991; Stephens et al., 1989; Weber, Karolewski, Irzykowska, Knaflewski, & Kosiada, 2007; Weber et al., 2006). A possible reason could be the narrow genetic basis of garden asparagus (Boonen, 2001; Ellison, 1986).

In previous studies, only few asparagus wild relatives were screened for resistance to *F. oxysporum* (Falavigna et al., 2008; Gonzáles Castanon & Falavigna, 2008; Lassaga et al., 1998; Stephens & Elmer, 1988). Out of the wild relatives, all evaluated *A. maritimus* accessions were found to be susceptible (Ellison, 1986). However, *A. densiflorus* was shown to be a potential source of resistance to *Fusarium* spp. (He, Hsiang, & Wolyn, 2001; Schofield, 1991; Stephens et al., 1989).

As a foundation for improving resistance through breeding approaches, a practical test is required for quantifying resistance to *F. oxysporum* in asparagus genetic resources (Corpas-Hervias et al., 2006; Ellison, 1986; Lassaga et al., 1998; Pontaroli & Camadro, 2001). A non-destructive method like a bioassay offers the possibility of efficient trait detection as well as selection of individual plants with desirable features. The most common approach in numerous studies is the visual symptom scoring of infested plant tissues based on assessment keys (Apaza, Talledo, & Casas, 2018; Blok & Bollen 1995, 1996; Elmer et al., 1996; Goßmann et al., 2011). The aim of the present study was to develop a bioassay for screening juvenile asparagus plants for resistance to *F. oxysporum* applicable in research and breeding.

2 | MATERIALS AND METHODS

2.1 | Plant material

Ten asparagus cultivars and six wild relatives originating from the working collection of the Julius Kühn-Institut (JKI) were utilized for the methodical approaches (Table 1). The seeds were sown into plastic boxes with autoclaved sand. Seedlings were cultivated under climate chamber conditions at a temperature of 20°C/ 25°C N/D, a relative humidity of 60% and a 14-hr photoperiod before application in the tests.

2.2 | Fungal pathogens

Fusarium oxysporum isolate Foxy I was kindly provided by the Humboldt University Berlin, and Foxy II and Foxy III were collected from spears of asparagus plants in experimental fields of the JKI in Quedlinburg. The isolates were identified morphologically as *F. oxysporum* by Goßmann (pers. com., Humboldt Univ.), and all fulfilled Koch's postulates (not shown here). Two monoconidial lines (MCLs) of each of the three strain isolates (Foxy I–Foxy III) and the reference isolate IMB 8445 (in the following called Foxy IV) provided by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) were prepared for the aggressiveness test (Table 2). All isolates were cultivated in Petri dishes (Ø 94 × 16 mm) on potato dextrose agar (PDA) as described by Kathe et al. (2017).

TABLE 1 Plant material used in this study

No.	Asparagus species	Cultivar/Wild relative	Geographic origin	Source [a]	Application[b]
1	*A. officinalis* L.	Andreas	France	INRA	R2
2	*A. officinalis* L.	Pacific Purple	New Zealand	ASP	R1, R2
3	*A. officinalis* L.	Schwetzinger Meisterschuß	Germany	IPK	A, B, R1, R2
4	*A. officinalis* L.	Primaverde	Germany	DSZ	R1, R2
5	*A. officinalis* L.	Mondeo	Germany	DSZ	R1, R2
6	*A. officinalis* L.	Ariane	Germany	DSZ	R2
7	*A. officinalis* L.	Thielim	The Netherlands	LIM	B, R1, R2
8	*A. officinalis* L.	Portlim	The Netherlands	LIM	R1, R2
9	*A. officinalis* L.	Ravel	Germany	SWS	A, B, R1, R2
10	*A. officinalis* L.	Raffaello	Germany	SWS	R1, R2
11	*A. amarus* DC.	Wild relative	Italy	CRA	A, B, R1, R2
12	*A. prostratus* Dumort.	Wild relative	France	VIL	R2
13	*A. verticillatus* L.	Wild relative	unknown	IPK	R1, R2
14	*A. densiflorus* Kunth.	Wild relative	Israel	ISR	R2
15	*A. ramosissimus* Baker	Wild relative	Angola	GRU	R2
16	*A. maritimus* (L.)Mill.	Wild relative	Italy	CRA	R1, R2

[a]ASP—Aspara Pacific (New Zealand), CRA—Research Institute for Vegetable Crops (Italy), GRU—Gruson-Gewächshäuser Magdeburg (Germany), DSZ—Deutsche Spargelzucht (Germany), INRA—Institut National de la Recherche Agronomique (France), IPK—Institut für Pflanzengenetik und Kulturpflanzenforschung (Germany), ISR—Volcani Centre Bet Dagan (Israel), LIM—Limgroup B.V. (The Netherlands), SWS—Süd-West-Saat (Germany), VIL-Vilmorin (France).

[b]A—aggressiveness test, B—bioassay development, R1/R2—resistance assessments.

TABLE 2 Origin and isolation of the *Fusarium* spp. isolates and monoconidial lines

			Isolation		Monoconidial lines
Fusarium spp.	Acronym	Source	Host plant	Tissue	(MCL)
F. oxysporum	Foxy I	HUB[a]	Asparagus	Root	I_8, I_11
F. oxysporum	Foxy II	JKI[b]	Asparagus	Spears	II_11, II_12
F. oxysporum	Foxy III	JKI[b]	Asparagus	Spears	III_3, III_10,
F. oxysporum	Foxy IV	DSMZ[c]	Asparagus	Spears	IV_8, IV_10

[a]*Fusarium oxysporum* isolate Foxy I was kindly provided by M. Goßmann, Humboldt-University Berlin, Germany.
[b]Julius Kuehn-Institute, Germany.
[c]German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany.

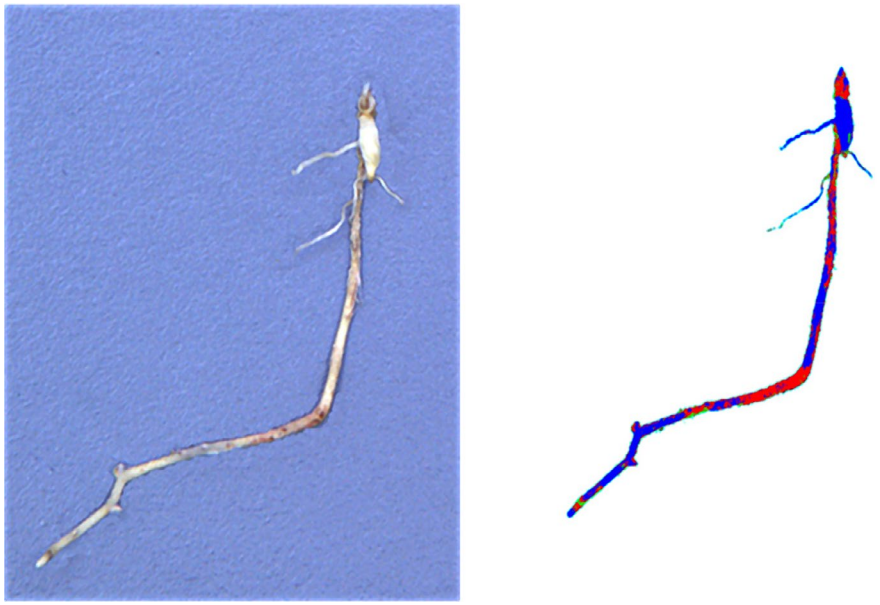

FIGURE 1 Analysis of symptoms caused by *Fusarium oxysporum* on a root of a juvenile asparagus plant using the digital image analysing system (DIAS). Left: original picture; right: symptoms of crown and root rot (red) in the colour class image

2.3 | Development of the bioassay

The development of a practicable, reproducible and non-destructive bioassay comprised the following steps: (a) development of the experimental design, (b) selection of a highly aggressive *Fusarium* isolate, (c) assessment of *Fusarium* symptoms using a digital image analysis system (DIAS) and a PTA-ELISA and (d) screening of *Asparagus* genotypes using the bioassay.

2.3.1 | Experimental design

Each ten 4-week-old plants of four asparagus genotypes (Table 1) were inoculated with 1×10^4, 1×10^5 and 1×10^6 conidia/ml of the isolate Foxy I. The conidial suspensions were established by flooding the agar plates with 2 ml Aqua dest., and the desired conidia concentrations were adjusted using the counting cell chamber. The plants were inoculated by dipping the roots into the conidial suspension on a rotary shaker for 15 min. The controls were inoculated with Aqua dest. Afterwards, the plants were incubated in covered square bioassay dishes (245 × 245 × 24 mm) (Corning, USA) on moistened filter paper for 14 days in a growth cabinet at 22°C and a 16h photoperiod.

2.3.2 | Digital image analysis system (DIAS)

Disease symptoms on asparagus roots were assessed using the Lab Scanalyzer LS10 (LemnaTec, Germany). The phenotyping software LemnaTec-OS was calibrated to discriminate between diseased and healthy tissues. The images were converted into colour class images for visual control (Figure 1). The number of pixel of each calibrated colour class was used for the statistical analysis.

2.3.3 | PTA-ELISA

A semi-quantitative estimation of *F. oxysporum* growth on the asparagus roots was carried out by an indirect plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) according to Rohde and Rabenstein (2005). The PTA-ELISA was performed 14 days postinoculation (dpi) using the polyclonal antiserum FG 18/9 from the JKI. For the detection of *F. oxysporum*, an extraction buffer was added to the roots at a ten-fold ratio (v/w) and roots were crushed using the Precellys 24 (Bertin Instruments). The colour reaction in the PTA-ELISA was determined photometrically by measuring the absorbance at 405 nm using the Microplate Reader Sunrise™ (Tecan

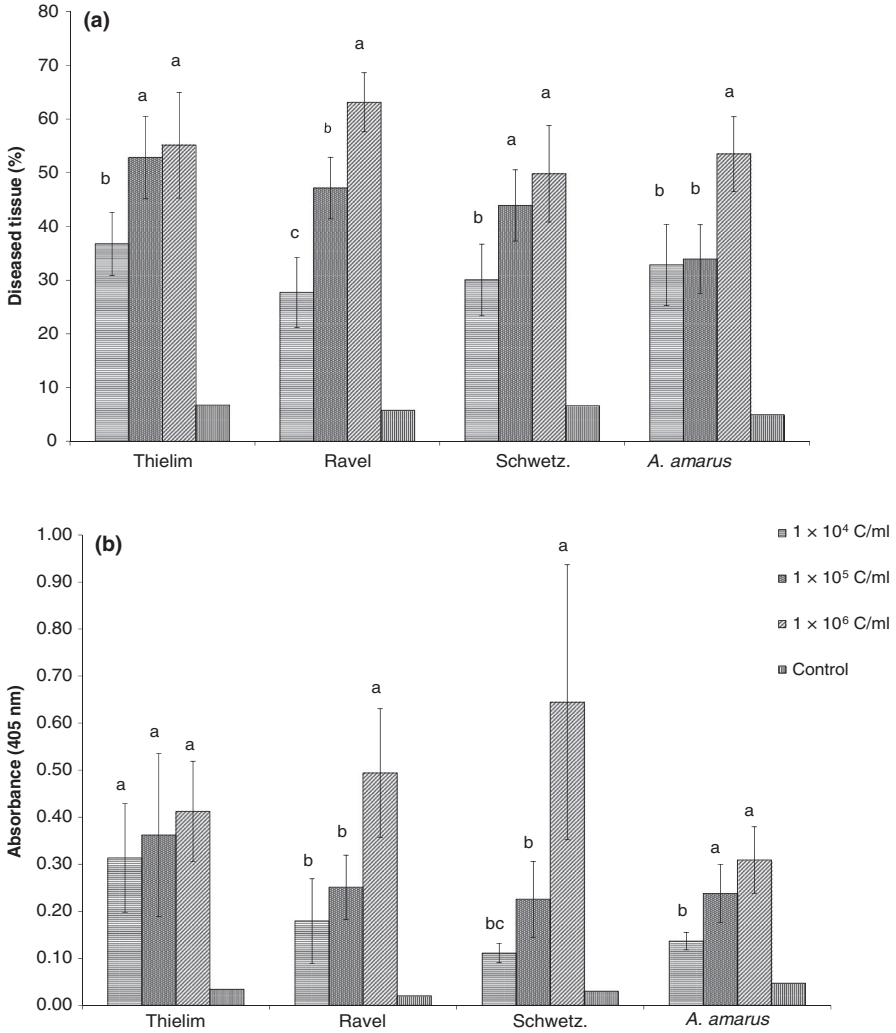

FIGURE 2 Bioassay with different conidia concentrations (C/ml = conidia per millilitre) of *Fusarium oxysporum* isolate Foxy I_1. (a) Diseased root tissue (%) of the cvs. Thielim, Ravel and Schwetzinger Meisterschuß as well as wild relative *Asparagus amarus* (n = 10, 14 dpi). (b) PTA-ELISA extinction values of the *Fusarium*-infected roots (14 dpi). Different letters indicate significance (Tukey, $p < .05$) between the concentrations subdivided for the tested plant genotypes

Trading AG). The data were evaluated by the Magellan™ Software (Tecan Trading AG). The threshold was calculated with the formula: $\bar{x} + 3s$ ($\bar{x}$ = average of the measured data, s = standard deviation of the measured data) (Kegler & Friedt, 1993).

2.3.4 | Aggressiveness test

For determining the aggressiveness of eight *F. oxysporum* isolates (MCLs, Table 2), each ten plants of three genotypes (Table 1) were inoculated with 1×10^6 conidia/ml, incubated for 14 days and evaluated as described for the bioassay.

2.3.5 | Screening for resistance

Two resistance screenings (R1 and R2) were performed to determine the resistance level of a collection of genotypes:

R1—Eleven genotypes were tested with two MCLs, Foxy II_12 and Foxy III_3, estimated as low and highly aggressive, respectively, and scored 14 dpi.

R2—Sixteen genotypes were tested after 10 days of incubation with MCL Foxy III_3.

2.3.6 | Statistical analysis

An ANOVA was performed to prove significances within each experiment followed by a Tukey B test to determine significant differences in means of DIAS and PTA-ELISA values ($p < .05$) using the statistic programme R Version 3.3.3 (R Foundation for Statistical Computing, Austria). Pearson's correlation coefficient was used to evaluate the coherences of the DIAS and ELISA data.

3 | RESULTS

3.1 | Development of the bioassay

3.1.1 | Effect of *Fusarium oxysporum* inoculum concentration

The severity of disease symptoms, for example tissue browning, increased as a function of the conidia concentration in all tested accessions. In the cv. Ravel, 1×10^6 conidia/ml caused more severe symptoms than the two other concentrations (Figure 2a). The ELISA extinction values determined from infested plants exceeded the

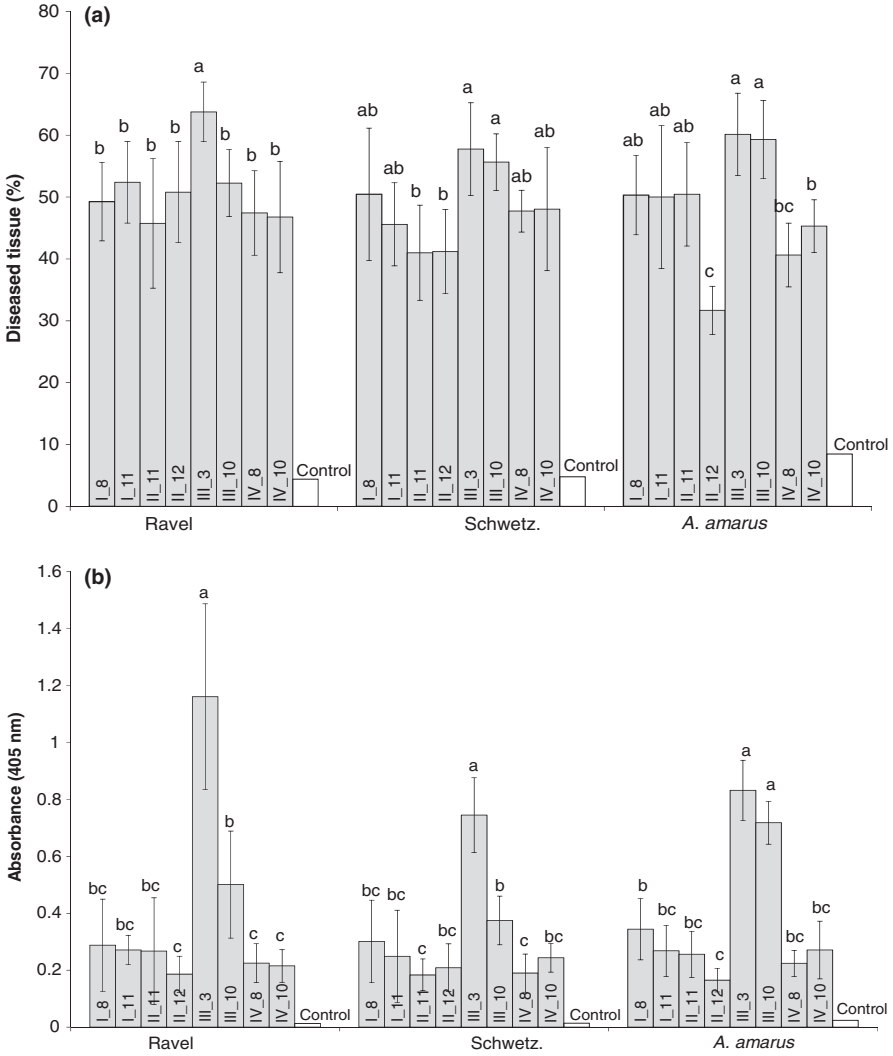

FIGURE 3 Aggressiveness test with eight monoconidial lines of the *Fusarium oxysporum* isolates Foxy I–Foxy IV 14 dpi. (a) Diseased root tissue (%) of two cvs. Ravel and Schwetzinger Meisterschuß as well as the wild relative *Asparagus amarus*, assessed with the digital image analysing system (DIAS). (b) PTA-ELISA extinction values of the *F. oxysporum* infected roots. Different letters indicate significance (Tukey, $p < .05$, $n = 10$) subdivided for each cultivar

calculated threshold of 0.10. The values revealed a significant effect of the inoculum concentration in the cvs. Ravel and Schwetzinger Meisterschuß as well as in *A. amarus*. The highest applied inoculum concentration of 1×10^6 conidia/ml revealed the most distinct differentiation of *Fusarium*-caused effects (Figure 2b). A moderate positive correlation ($r = .49$) between both analyses was found. An inoculum concentration 1×10^6 conidia/ml was chosen for further tests.

3.1.2 | Aggressiveness test

All used MCLs caused visible disease symptoms at the inoculated asparagus roots, for example tissue browning, and exceeded the calculated threshold of 0.08 in the PTA-ELISA. The MCLs are pathogenic for the three tested asparagus genotypes but differed in their level of infestation (Figure 3). The results show that the MCLs of the JKI isolates cause visually the same symptoms as those of the reference Foxy IV. Evaluation of the disease symptoms showed that the MCLs of Foxy III caused the strongest infestation, while both Foxy II MCLs showed a lower symptom severity, comparable to Foxy IV (Figure 3a).

The detection of *F. oxysporum* with the PTA-ELISA confirmed the symptom characteristics caused by the different lines. Thus, Foxy III_3 revealed the highest extinction values in all three cultivars, with the absolute highest value for *A. amarus* (Figure 3b).

3.1.3 | Screening for resistance

R1—The DIAS screening confirmed that the developed bioassay is suitable to reveal differences between genotypes and influences of the isolates. Results obtained in the aggressiveness test were verified. Foxy III_3 caused more visible disease symptoms (DS) (30%–74%) and greater differences between the cultivars than Foxy II-12 (DS 27%–52%). (Figure S1). Cv. Ravel was more infested than Schwetzinger or *A. amarus* (Table 3). The infected root area was already decomposed 14 dpi that an extraction for an ELISA test was impossible. The incubation time of R2 was reduced to 10 days.

R2—The R2 test revealed significant differences between the 16 asparagus genotypes. The disease severity varied between 8% and 65% for the different cultivars and wild relatives (Figure 4a). The PTA-ELISA-determined extinction values between 0.05 and 0.65 for

TABLE 3 Comparison of the *Fusarium oxysporum* disease severity (rank) for three asparagus genotypes tested within six independent experiments

Experiment	Isolate	Incubation (d)	Ravel	Schwetzinger	*A. amarus*
B	Foxy I	14	1	3	2
A	Foxy II_12	14	1*	2	3*
R1		14	1*	2	3
A	Foxy III_3	14	1	3	2
R1		14	1*	3*	2
R2		10	1*	3	2

Note: Values within the genotype columns suggest the rank between the three genotypes in the individual tests. (1—most susceptible;
*Significant Tukey $p < .05$).

the infected roots reflect the infestation severity (Figure 4b). A moderate positive correlation coefficient ($r = .66$) between disease symptoms and the relative pathogen concentration in the immunoassay was determined for the whole experiment (Figure 4c).

The evaluation of the genotypes showed a clear distinction between cultivars and wild relatives based on disease symptoms. With the exception of *A. maritimus* (16), the wild relatives revealed less infestation in comparison with the cultivars. *A. densiflorus* (14) did not show any symptoms. Within the cultivars, the cv. Schwetzinger Meisterschuß (3) showed fewest disease symptoms, whereas the cvs. Pacific Purple (2), Ariane (6) and Ravel (9) revealed the highest susceptibility. (Figure 4a).

Fusarium oxysporum was detected in nearly all inoculated roots by the PTA-ELISA (Figure 4b). No *Fusarium* was detected in *A. densiflorus* (14) which supports the data of DIAS analysis. The high level of susceptibility of the cvs. Pacific Purple (2), Ariane (6) and Ravel (9) was confirmed by increased extinction values in the PTA-ELISA (Figure 4a,b).

3.1.4 | Reproducibility of the bioassay

The cvs. Ravel, Schwetzinger and wild relative *A. amarus* were used in six independent experiments B, A and R1/R2 (Table 1) inoculated with Foxy I, Foxy II_12 and Foxy III_3, respectively, allowing an estimation of reproducibility (Table 3). On cv. Ravel, the most infestation was recognizable in all experiments. Cv. Schwetzinger Meisterschuß showed the lowest disease symptoms. An exception was the experiments with Foxy II_12 where for *A. amarus* the least severity was detectable.

The two-way ANOVA revealed one-sided significant differences between the cultivars and also the isolates. In contrast, the cultivar–isolate interaction was not significant ($p = .052$).

4 | DISCUSSION

The aim of the study was to develop a non-destructive asparagus seedling-resistance test to *F. oxysporum*. In previous studies, it was standard to perform a resistance screening in the greenhouse with artificial inoculation by virulent *Fusarium* strains or by cultivation in contaminated soil followed by visual scoring for disease symptoms using scale 0–3 or scale 1–5 (Apaza et al., 2018; Ellison, 1986; Sonoda, Uragami, & Kaji, 1997; Stephens et al., 1989). Alternatively, laboratory or in vitro tests were developed enabling detection of more detailed differences between the genotypes. However, the transferability of the results to field conditions is often unsatisfactory (Blok & Bollen, 1995; Lassaga et al., 1998; Stephens & Elmer, 1988).

Decisive advantage of our bioassay is the objective determination of damage symptoms by the DIAS. The software allows flexible adaption of selection acridity dependent on the aim of the experiment. The serological *Fusarium* detection was proven to be a sensitive and specific method that ensures a clear differentiation between *Fusarium*-infested and healthy tissues. In view of the moderate positive correlation between DIAS and ELISA values, it is advisable to combine both methods. Although the DIAS was able to show a clearer differentiation between the genotypes in this test, it is advisable to provide immunological pathogen detection with regard to determining possible symptom tolerances or distinct symptom formation due to toxins. The aggressiveness assay revealed diversity between the *F. oxysporum* isolates ranging from low degree (Foxy I_8/I_11 and Foxy II_11/II_12) to high (Foxy III_3/III_10) degree. High aggressiveness means in this case a fast invasion and propagation of the fungus in the plant tissue and an intensive severity.

The developed resistance approach allowed an unambiguous discrimination of the asparagus seedlings regarding the resistance. The distinct resistance in *A. densiflorus*, described by Stephens et al. (1989), was confirmed. All the other tested asparagus cultivars and wild relatives revealed *Fusarium*-associated disease symptoms to a various degree. The susceptibility of *A. maritimus*, described by Ellison (1986), was confirmed.

Advantages of the bioassay for future breeding approaches arise from the possibility of a single-plant evaluation. The comparison of the six experiments showed that depending on the experimental question, the bioassay can be smoothly adjusted by the usage of recent isolates, variation of inoculum concentration or incubation time. The application of the different isolates has shown no isolate–cultivar interaction but a highly similar ranking of comparable genotypes, which suggests a high reproducibility.

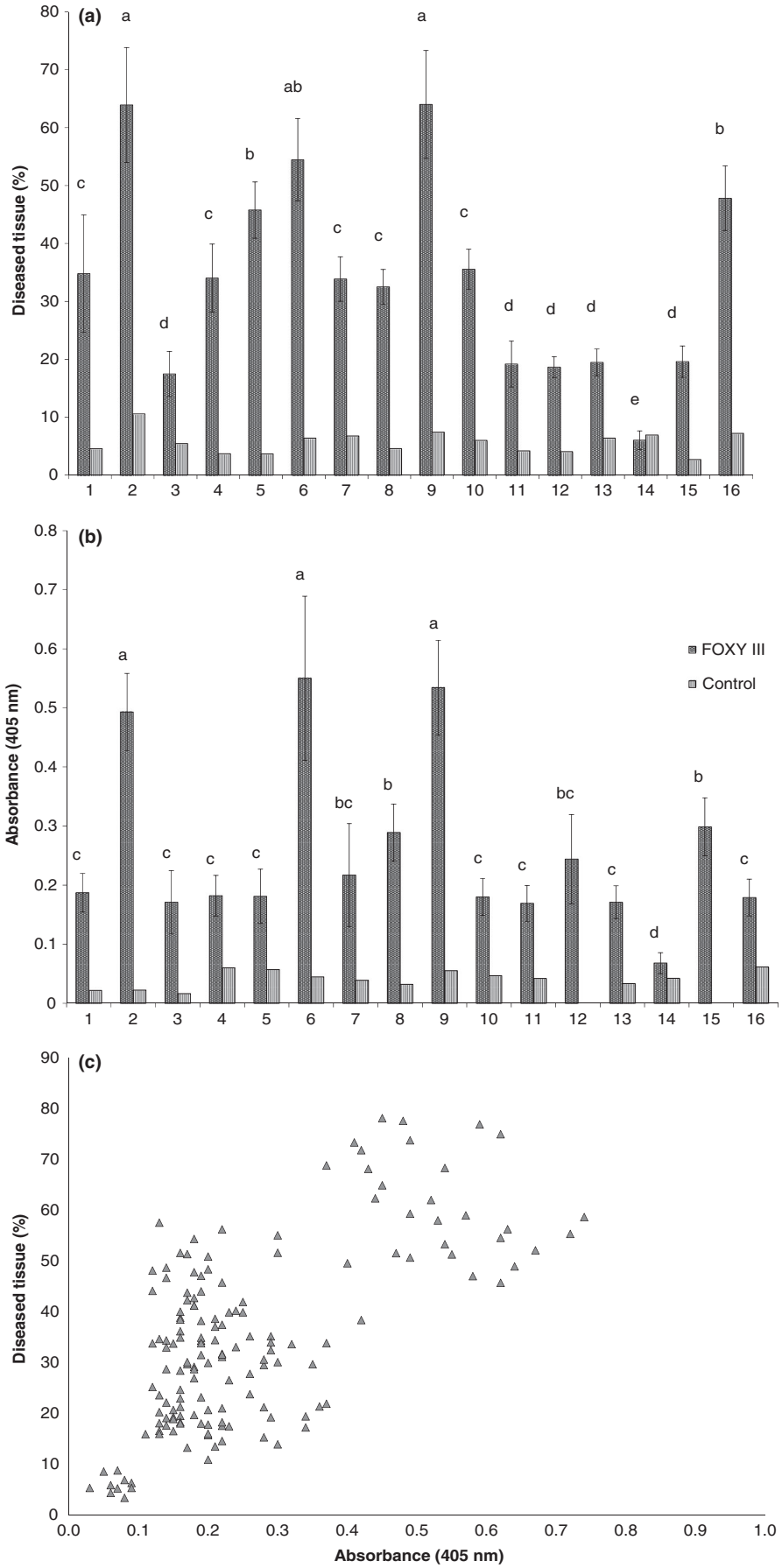

FIGURE 4 Screening of resistance to the *Fusarium oxysporum* isolate Foxy III_3 (Experiment R2): (a) Diseased tissue (%) on roots of the 16 asparagus genotypes (Table 1) 10 dpi, (b) PTA-ELISA extinction values of the *Fusarium*-infected roots (10 dpi) and (c) correlation between disease symptoms and the relative pathogen concentration (r = .66, p < .05). Different letters indicate significance (Tukey, p < .05) between the cultivars

DIAS measurements allow a detailed documentation of the pathogenesis. So it is possible to quantify plant tissue decay over time series and to determine the extent of healthy root formation. Subsequently, specific quantitative pathogen detection could be conducted in the whole infected tissue using the ELISA or qPCR in perspective.

Because plants can be cultivated only for a limited time in the described bioassay, the development of greenhouse approaches for continuing the experiments with later stages of asparagus plants would be very important. In first greenhouse approaches, where plants were cultivated after inoculation in a mixture of sand–humus substrate, the result of DIAS colour analysis was unsatisfactory due to the partial discoloration of roots by soil ingredients. Recent experiments with various substrates are focused to overcome this problem.

The developed non-destructive bioassay can be used to screen seedling resistance to *F. oxysporum* in asparagus, such as evaluation of genetic resources and prebreeding, and to study the genetic inheritance and pathogenesis research.

ACKNOWLEDGEMENTS

The authors thank Dr. M. Goßmann from the Humboldt University Berlin (Germany) for the determination of the *F. oxysporum* isolates. Furthermore, authors thank Martina Malorny and Ursula Apel for their excellent technical assistance.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

ORCID


Thomas Nothnagel https://orcid.org/0000-0001-6723-5283

REFERENCES

Apaza, W., Talledo, A., & Casas, A. (2018). Reaction of 10 Asparagus cultivars to infested soil with *Fusarium oxysporum* f.sp. asparagi and Meloidogyne incognita. *Acta Horticulturae*, *1223*, 233–238. https://doi.org/10.17660/ActaHortic.2019.1223.32

Blok, W. J., & Bollen, G. J. (1995). Fungi on roots and stem bases of asparagus in the Netherlands: Species and pathogenicity. *European Journal of Plant Pathology*, *101*, 15–24. https://doi.org/10.1007/BF01876090

Blok, W. J., & Bollen, G. J. (1996). Inoculum sources of *Fusarium oxysporum* f.sp. *asparagi* in asparagus production. *Annals of Applied Biology*, *128*, 219–231. https://doi.org/10.1111/j.1744-7348.1996.tb07318.x

Boonen, P. (2001). *Spargel von der Heilpflanze zur Delikatesse* (1st edn.) (pp. 53–59). Self-Publishing. ISBN 90-805609-3-6.

Corpas-Hervias, C., Melero-Vara, J. M., Molinero-Ruiz, M. L., Zurera-Muñoz, C., & Basallote Ureba, M. J. (2006). Characterization of isolates of *Fusarium* spp. obtained from asparagus in Spain. *Plant Disease*, *90*, 1441–1451. https://doi.org/10.1094/PHYTO-08-13-0231-R

Ellison, J. H. (1986). Asparagus breeding. In M. J. Bassett (Eds.), *Breeding vegetable crops* (pp. 521–569). Westport, CT: AVI Publishing Co.

Elmer, W. (2018). Asparagus decline and replant problem: A look back and a look forward at strategies for mitigating losses. *Acta Horticulturae*, *1223*, 195–204. https://doi.org/10.17660/ActaHortic.2018.1223.27

Elmer, W. H., Johnson, D. A., & Mink, G. I. (1996). Epidemiology and management of the diseases causal to asparagus decline. *Plant Disease*, *80*, 117–125. ISSN: 0191-2917. https://doi.org/10.1094/PD-80-0117

Falavigna, A., Casali, P. E., & Alberti, P. (2008). Performance of Asparagus Genotypes in Fwsar/Bwi-infested and uninfested soil. *Acta Horticulturae*, *776*, 161–166. https://doi.org/10.17660/ActaHortic.2008.776.19

FAOSTAT (2017). Retrieved from http://www.fao.org/faostat/en/#data/QC;29.04.2018

Gonzáles Castanon, M. L., & Falavigna, A. (2008). Asparagus germplasm and interspecific hybridization. *Acta Horticulturae*, *776*, 319–326. https://doi.org/10.17660/ActaHortic.2008.776.41

Goßmann, M., Büttner, C., & Bedlan, G. (2001). Untersuchungen von Spargel (Asparagus *officinalis* L.) aus Jung- und Ertragsanlagen in Deutschland und Österreich auf Infektion mit *Fusarium*-Arten. *Pflanzenschutzberichte*, *2*, 45–54.

Goßmann, M., Kleta, S., Humpf, H. U., & Büttner, C. (2005). Untersuchungen zum endophytischen Befall von *Fusarium proliferatum* (Matsushima) Nirenberg in geernteten Stangen von Spargel (*Asparagus officinalis* L.). *Gesunde Pflanzen*, *57*, 53–58. https://doi.org/10.1007/s10343-005-0065-z

Goßmann, M., Scholz, A., Hennig, F., Bargen, S., & Büttner, C. (2011). *Fusarium oxysporum*- und *F. proliferatum*-Isolate aus Spargel und deren Pathogenitätsüberprüfung in einer modifizierten *in vitro*-Schnelltestmethode. *Gesunde Pflanzen*, *63*, 175–182. https://doi.org/10.1007/s10343-011-0262-x

Grogan, R. G., & Kimble, K. A. (1959). The Association of *Fusarium* wilt with the asparagus decline and replant problem in California. *Phytopathology*, *49*, 122–125.

He, C., Hsiang, T., & Wolyn, D. J. (2001). Activation of defense responses to *Fusarium* infection in *Asparagus densiflorus*. *European Journal of Plant Pathology*, *107*, 473–483. https://doi.org/10.1023/A:1011218304331

Hung, L. (1974). The asparagus decline and replant problem in Taiwan. *Journal Chinese Society of Horticultural Science*, *20*, 63–70.

Kathe, L., Nothnagel, T., Krämer, R., Budahn, H., Rabenstein, F., & Pillen, K. (2017). Characterisation of *Alternaria radicina* isolates and assessment of resistance in carrot (*Daucus carota* L.). *Journal Für Kulturpflanzen*, *69*, 277–290. https://doi.org/10.1399/JfK.2017.09.01

Kato-Noguchi, H., Nakamura, K., Ohno, O., Suegaga, K., & Okuda, N. (2017). Asparagus decline: Autotoxicity and autotoxic compounds in asparagus rhizomes. *Journal Plant Physiology*, *213*, 23–29. https://doi.org/10.1016/j.jplph.2017.02.011

Kegler, H., & Friedt, W. (1993). Resistenz von Kulturpflanzen gegen pflanzenpathogene Viren. In H. Kegler, & H. Kleinhempel (Eds.), *Virusresistenz der Pflanzen* (pp. 1–53). Jena, Germany: Gustav Fischer Verlag.

Lake, R. J., Fallon, P. G., & Cook, D. W. M. (1993). Replant problem and chemical components of asparagus roots. *New Zealand Journal of Crop and Horticultural Science*, *21*(1), 53–58. https://doi.org/10.1080/01140671.1993.9513746

Lassaga, S. L., Camadro, E. L., & Babinec, F. J. (1998). Assessing genetic variability for *Fusarium* resistance in three asparagus populations with an in vitro assay. *Euphytica*, *103*, 131–136. https://doi.org/10.1023/A:1018335729277

Pontaroli, A. C., & Camadro, E. L. (2001). Increasing resistance to *Fusarium* crown and root rot in asparagus by gametophyte selection. *Euphytica*, *122*, 343–350. https://doi.org/10.1023/A:1012983123410

Rohde, S., & Rabenstein, F. (2005). Standardization of an indirect PTA-ELISA for detection of *Fusarium* spp. in infected grains. *Mycotoxin Research*, *21*, 100–104. https://doi.org/10.1007/BF02954429

Sadowski, C. Z., & Knaflewski, M. (1990). Susceptibility of selected asparagus cultivars to Fusarium spp. under field conditions. *Acta Horticulturae*, *271*, 343–351. https://doi.org/10.17660/ActaHortic.1990.271.49

Schofield, P. E. (1991). Asparagus decline and replant problem in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, *19*, 213–220. https://doi.org/10.1080/01140671.1991.10421803

Sonoda, T., Uragami, A., & Kaji, K. (1997). Evaluation of *Asparagus officinalis* cultivars for resistance to stem blight by using a novel inoculation method. *HortScience*, *32*, 1085–1086. https://doi.org/10.21273/HORTSCI.32.6.1085

Stephens, C. T., De Vries, R. M., & Sink, K. C. (1989). Evaluation of *Asparagus* Species for resistance to *Fusarium oxysporum* f. sp. *asparagi*, and *F. moniliforme*. *HortScience*, *24*, 365–368.

Stephens, C. T., & Elmer, W. H. (1988). An in vitro assay to evaluate sources of resistance in *Asparagus* spp. to *Fusarium* crown and root rot. *Plant Disease*, *72*, 334–337. https://doi.org/10.1094/PD-72-0334

Weber, Z., Karolewski, Z., Irzykowska, L., Knaflewski, M., & Kosiada, T. (2007). Occurrence of *Fusarium* species in spears of asparagus (*Asparagus officinalis*). *Phytopathologia Polonica*, *45*, 9–15.

Weber, Z., Kostecki, M., Bargen, S., Goßmann, M., Waśkiewicz, A., Bocianowski, J., … Golinski, P. (2006). *Fusarium* Species Colonizing Spears and Forming Mycotoxins in Field Samples of Asparagus from Germany and Poland. *Journal of Phytopathology*, *154*, 209–216. https://doi.org/10.1111/j.1439-0434.2006.01085.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kathe L, Krämer R, Budahn H, Pillen K, Rabenstein F, Nothnagel T. Development of a bioassay to assess resistance to *Fusarium oxysporum* (Schlecht.) in asparagus (*Asparagus officinalis* L.). *J Phytopathol*. 2019;167:558–566. https://doi.org/10.1111/jph.12845