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Agroscope

A new molecular assay on barley seed to quantify *Ustilago nuda* and predict field infection levels

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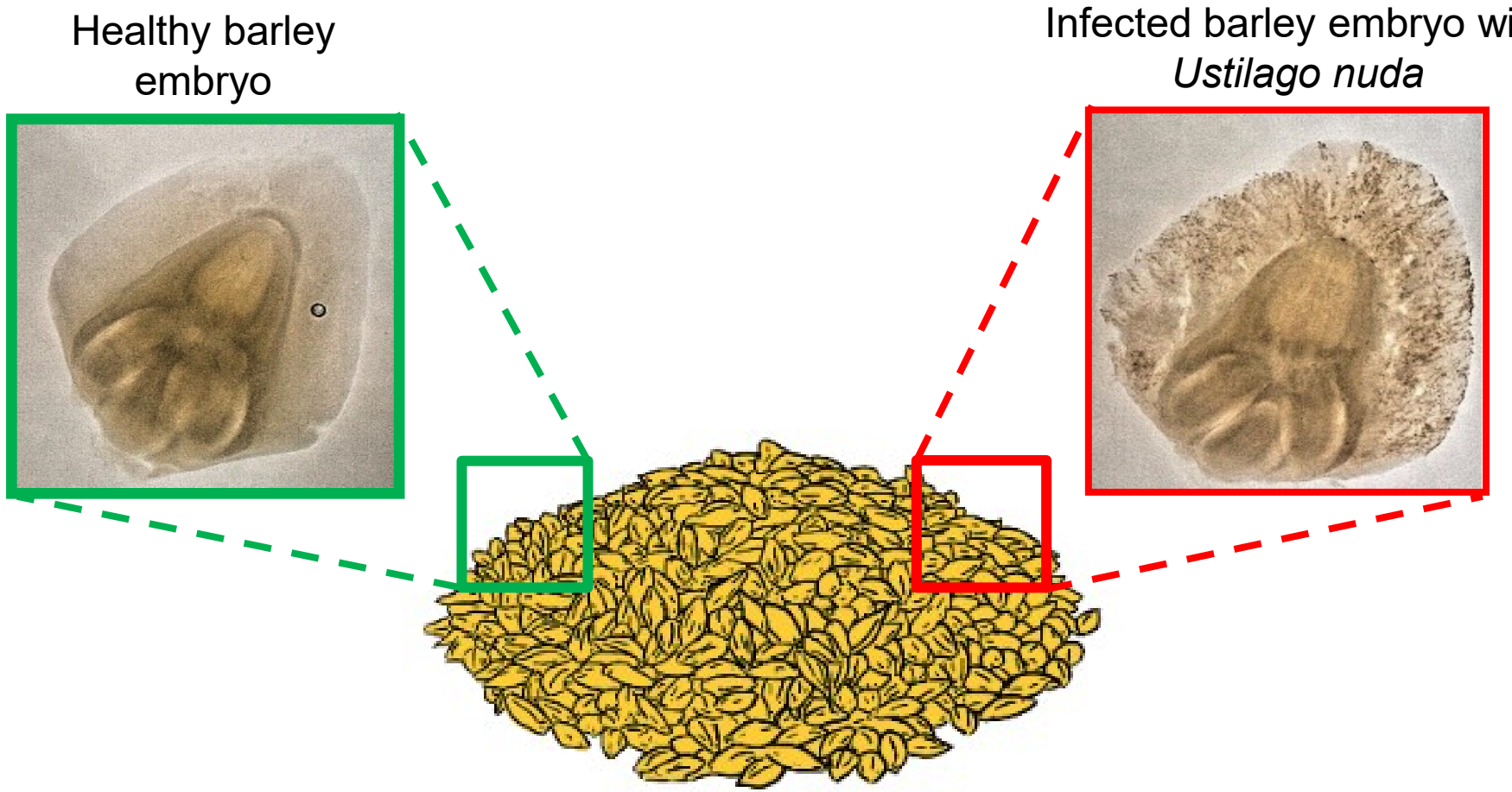
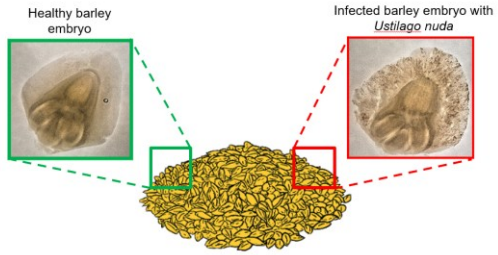
Date: 01.06.2025

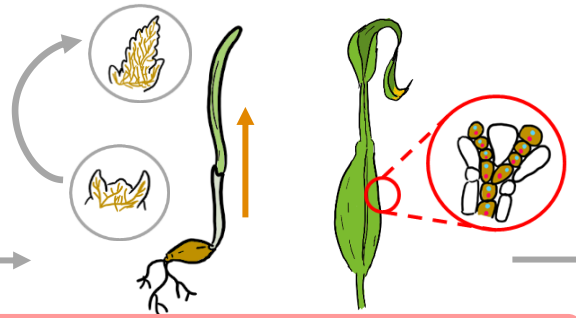
Loose smut (*Ustilago nuda*)



Loose smut (*Ustilago nuda*)

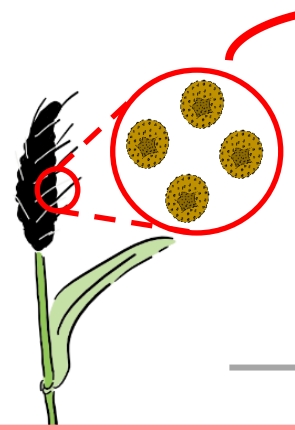


Disease is only transmitted through seeds

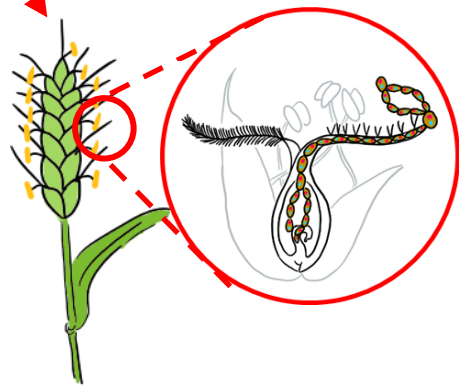


The plants appear healthy

but inside them, the fungus grows asymptomatically



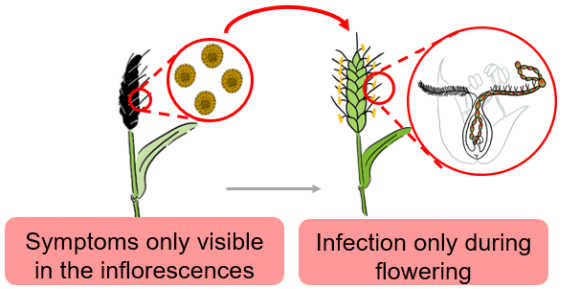
Symptoms only visible in the inflorescences



Infection only during flowering

The infected plant produces seeds that appear healthy

Certified seed and smut infection



Field monitoring

$5 \frac{\text{infected ears}}{100\ m^2}$

Harvested seed

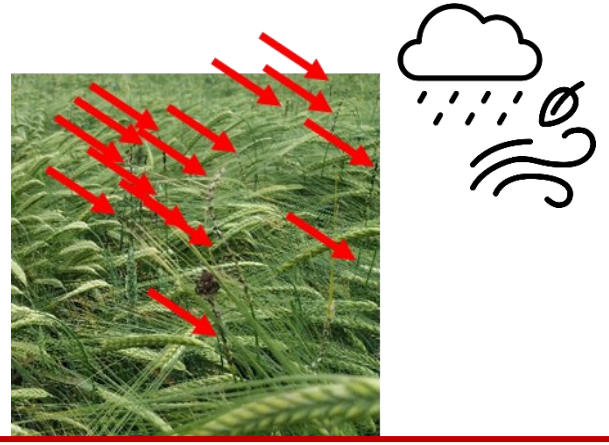
Why?

Unexpectedly high infection rate

Field monitoring problems

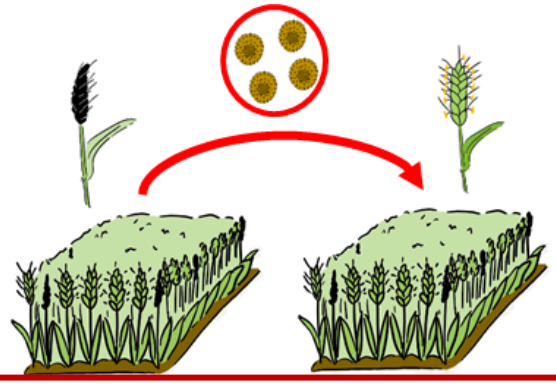
Time-consuming and laborious

Infections are difficult to detect



Sensitive to environmental influences

Infected ears are less visible because the spores are washed away by rain or blown away by the wind



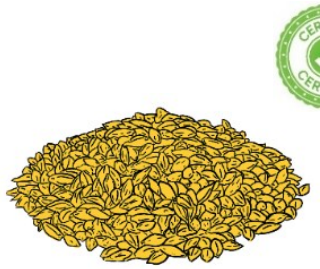
Infection from other fields

A healthy field can be infected by a neighboring, uncontrolled field

Not applicable on a large scale

Solution for field monitoring problems

Field monitoring



Harvested seed

Ensure a tolerable infection level is not exceeded through direct seed testing

Seeds harvested with tolerable *U. nuda* infection levels can be sold as certified seeds

Seeds harvested with *U. nuda* above tolerable infection level should be treated

Direct seed inspection

Ensure a tolerable infection level is not exceeded through direct seed testing

Harvested seed

There is a method, but it is not often used

Visual laboratory examination of extracted embryos (embryo test)

$$1 \frac{\text{infected embryo}}{1000}$$

=

$$35 \frac{\text{infected ears}}{100\ m^2}\ ^*$$

Problems with this method:

Time-consuming and laborious

The extraction of at least 1000 embryos and their individual testing for infections is required

Infections are difficult to detect

The mycelia in the embryo may be small and barely visible

Not applicable on a large scale

An inspector checks an average of 4 samples per day

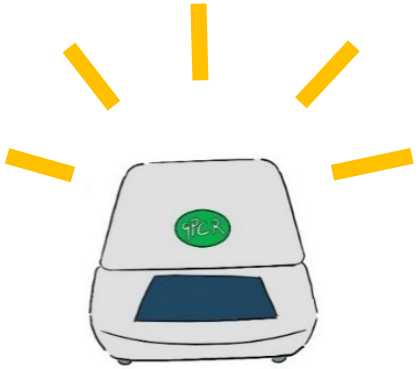
* Sowing density: $\frac{350\ g\ Seed}{m^2}$

Possible solution for direct seed inspection problems

Aims

- Time savings
- Reduction in errors
- Precise detection of infections
- Development of a scalable method

With a molecular method!



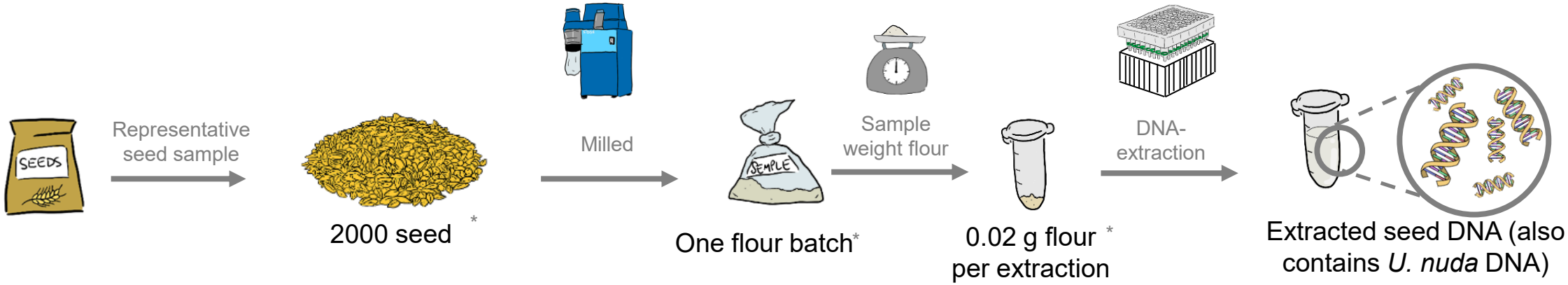
Confirmation of field monitoring certification with *U. nuda* molecular detection on harvested seeds

Advantages of molecular methods:

Detects low levels of infection

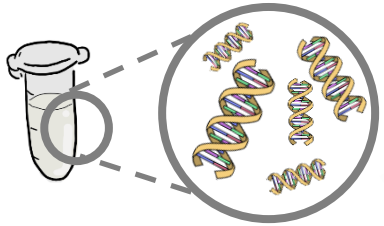
Tests more than a hundred samples in a week

Our *U. nuda* qPCR method



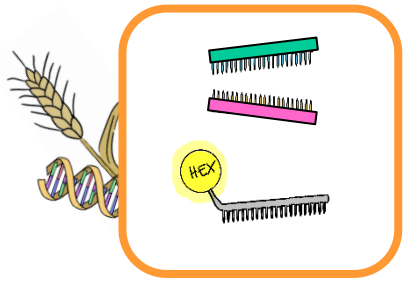
*Quantity/amount determined by tests not shown

Our *U. nuda* qPCR method

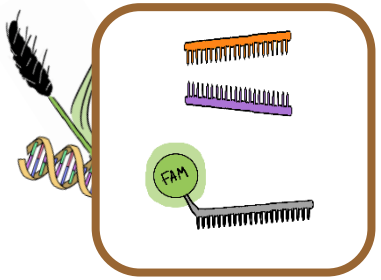


Extracted seed DNA

Hordeum vulgare primers



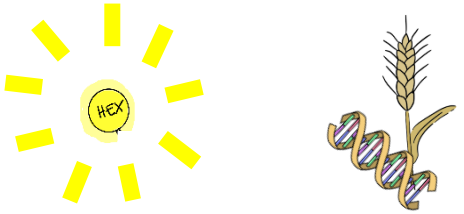
\+ *Ustilago nuda* primers



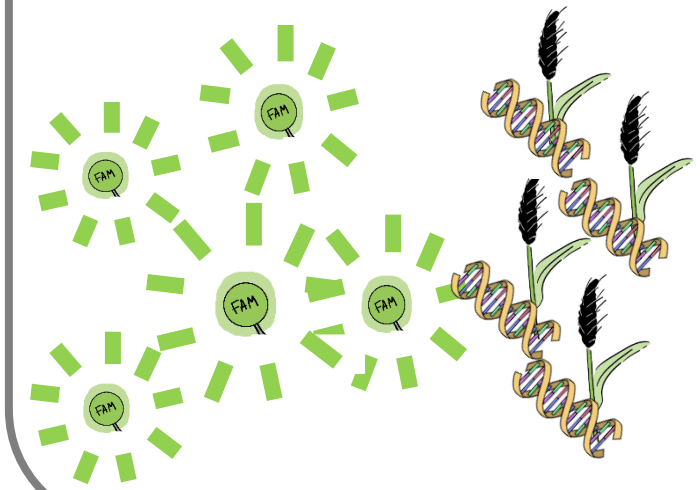
Newly design primers and probes specifically for barley and *U. nuda*

Analysis of extracted seed DNA + primer and probe using multiplex qPCR method

The probes are small glowing “torches” that only switch on when they find the piece of DNA they are looking for



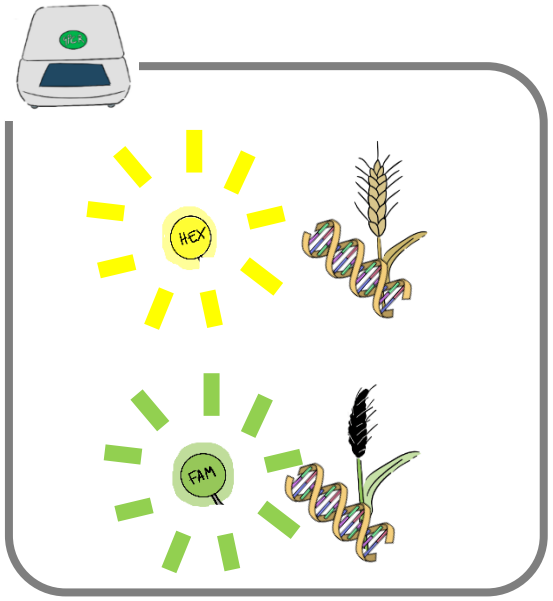
The light emitted by the probes for detecting barley is similar in all samples



The total amount of light emitted by the probes for *U. nuda* varys depending on the degree of infection

More light captured indicates higher infection

Our *U. nuda* qPCR method



The emitted light is used to quantify *U. nuda* and barley DNA in each seed sample

The infection for each sample is quantified by the ratio between the measured *U. nuda* DNA to the measured barley DNA

Check correlation between infection detected by qPCR and field infections in plants from the same seed!

Agroscope

Ustilago nuda seed trial

Aims:
1) Establish the relationship between laboratory detection methods and the field infection level
2) Derive of a qPCR tolerance threshold

"Healthy" seed lot

"Infected" seed lot

10% "infected" seed lot + 90% "healthy" seed lot

1% "infected" seed lot + 99% "healthy" seed lot

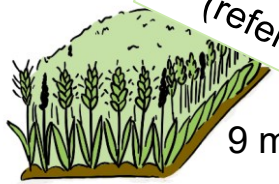
3 cultivars tested

Milled seed sample

? $\frac{\textit{Ustilago nuda}}{\textit{Hordeum vuglare}}$ DNA copies

Establish the relationship

Field evaluation (reference)



9 m²

x 2 growing seasons

35 $\frac{\text{Infected ears}}{100\ m^2}$ *

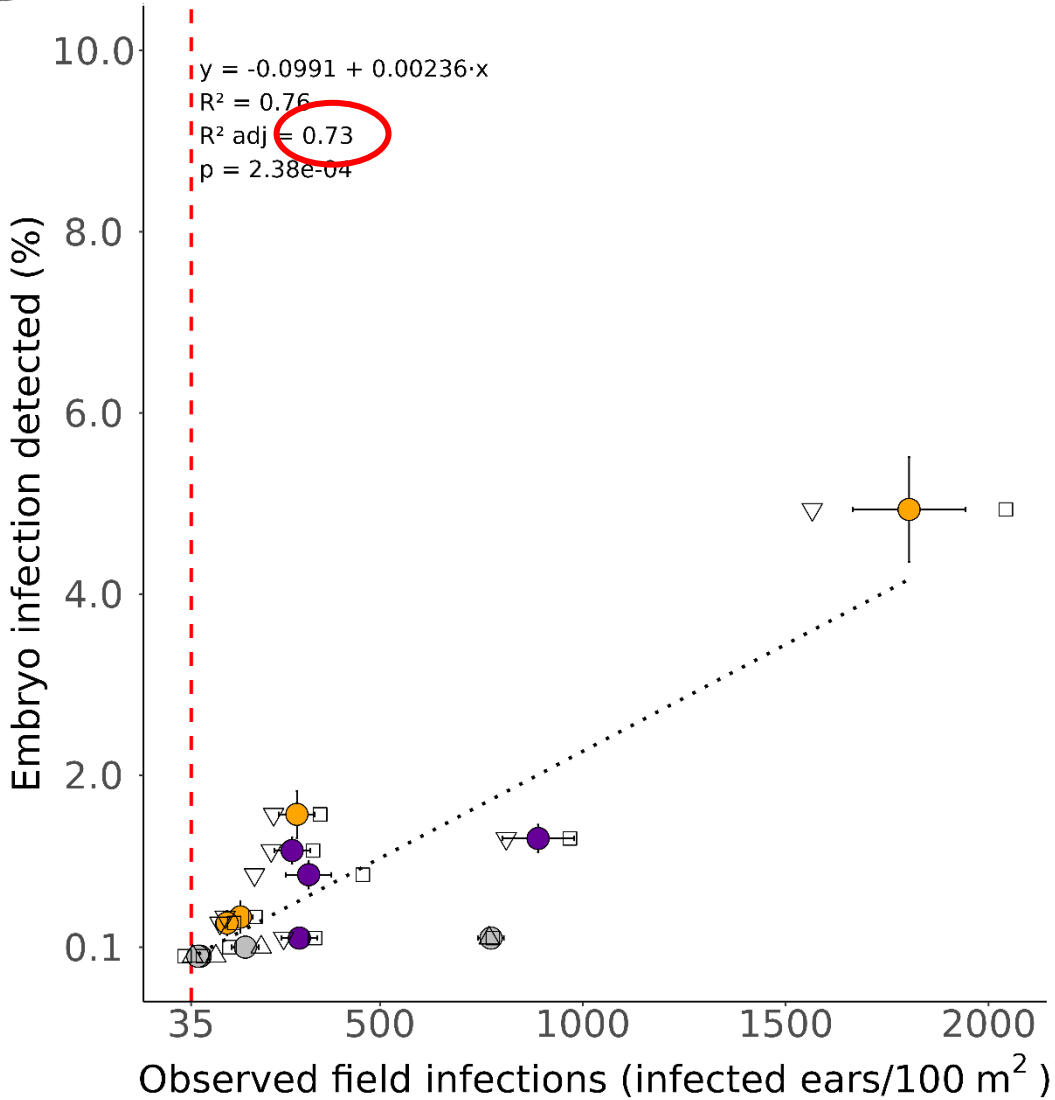
Establish the relationship

External laboratory

1 $\frac{\text{Infected emrbyo}}{1000}$

* Sowing density: $\frac{350\ g\ Seed}{m^2}$

Results



The infection detected by qPCR better reflects the infections observed in the field than the infection determined by the embryo test

Results

Tolerance thresholds

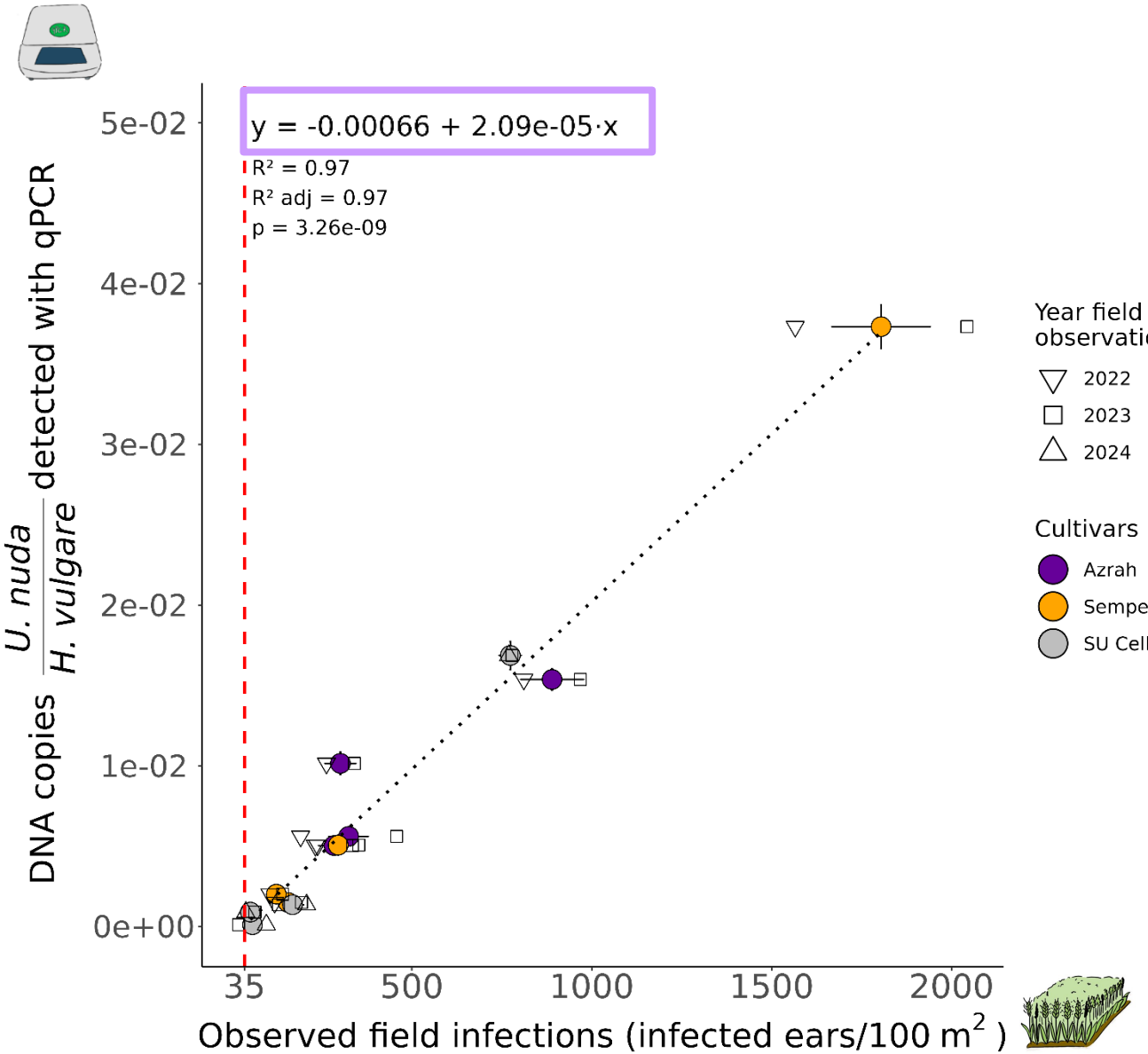
7.5E-05 $\frac{\textit{U. nuda}}{\textit{H. vuglare}}$ DNA copies *

=

35 $\frac{\text{Infected ears}}{100\ m^2}$ *

=

1 $\frac{\text{Infected emrbyo}}{1000}$



* Sowing density: $\frac{350\ g\ Seed}{m^2}$

Ustilago nuda commercial seed trial

Aim: determine the best laboratory detection method

«Healthy» seed lot

«Infected» seed lot

10% «infected» seed lot + 90% «healthy» seed lot

1% «infected» seed lot + 99% «healthy» seed lot

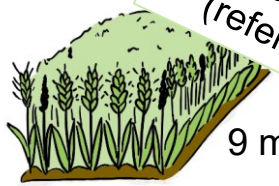
3 cultivars tested

Available on the market

Milled seed sample

$\frac{\textit{Ustilago nuda}}{\textit{Hordeum vuglare}}$ DNA copies

Field evaluation (reference)



9 m²

x 2 growing seasons

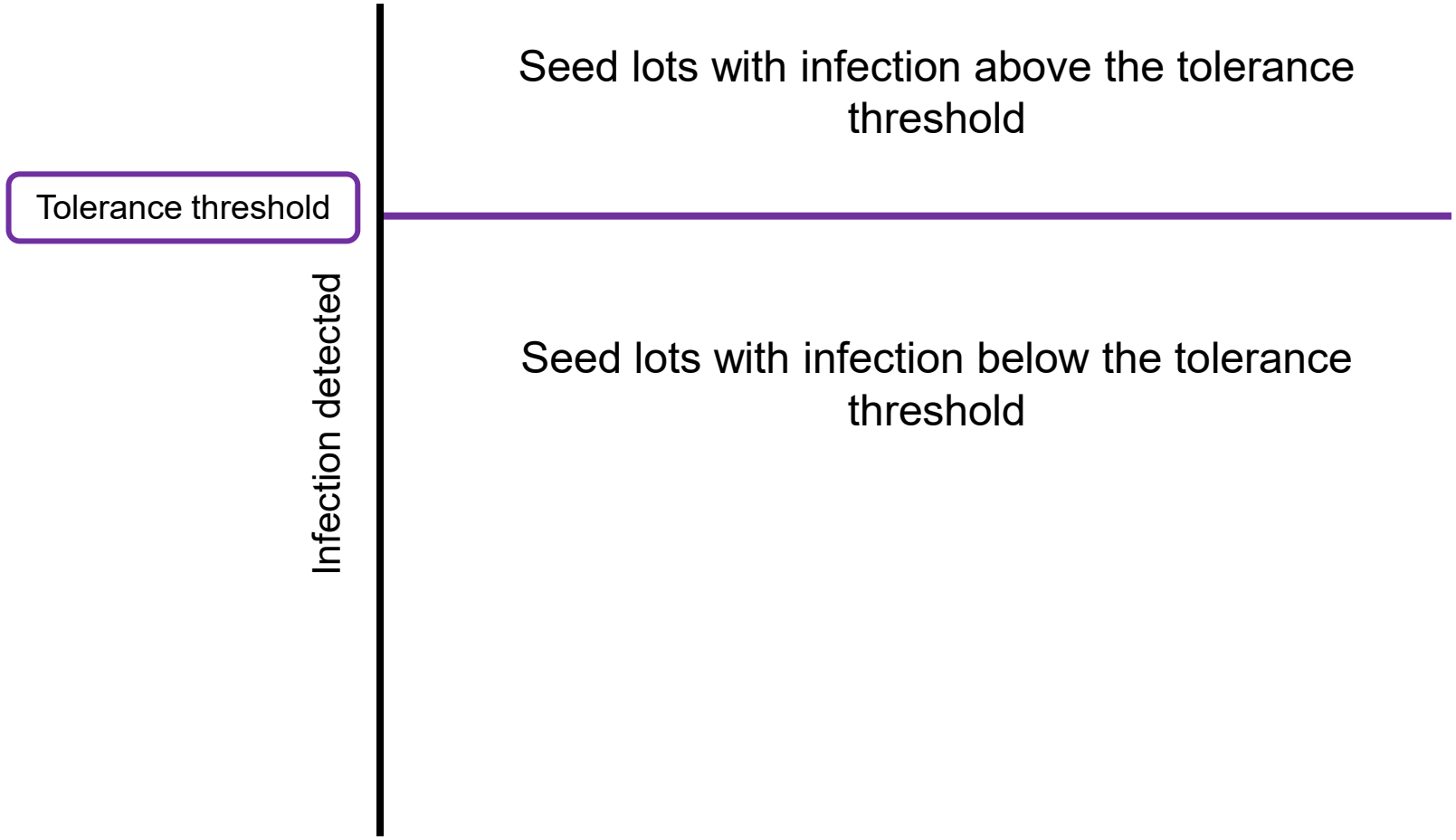
$\#\frac{\text{Infected ears}}{100\ m^2}$

External laboratory

$\#\frac{\text{Infected emrbyo}}{1000}$

compare

Ustilago nuda commercial seed trial: results



Ustilago nuda commercial seed trial: results

Embryo test

Field evaluation (reference)

qPCR method

1 Infected emrbyo / 1000

infected embryo detected

False negative (FN)

True negative (TN)

35 Infected ears / 100 m² *

Observed field infection

7.5E-05 *U. nuda* / *H. vuglare* DNA copies *

Infection detected with qPCR

True positive (TP)

True negative (TN)

Tolerance threshold

Infection detected

True positive (TP)

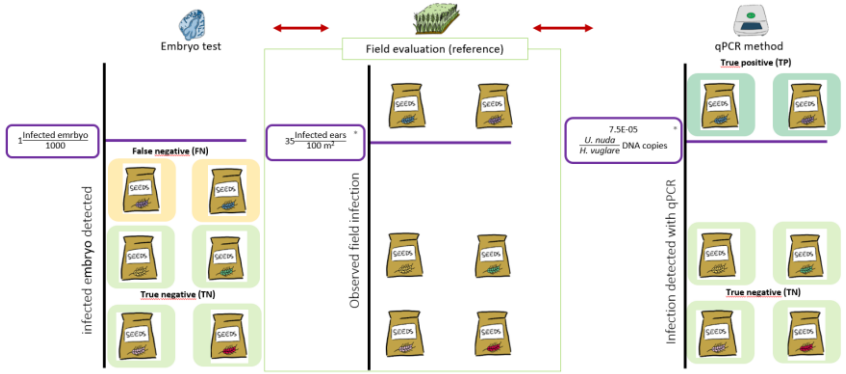
True negative (TN)

False positive (FP)

False negative (FN)

* Sowing density: 350 g Seed / m²

Ustilago nuda commercial seed trial: results



$$\frac{TP}{(TP+FN)} \qquad \frac{TN}{(TN+FP)} \qquad \frac{TP}{(TP+FP)} \qquad \frac{TP+TN}{(TP+TN+FP+FN)}$$

Laboratory detection methods	Sensitivity	Specificity	Precision	Accuracy
Embryo test				
qPCR method				

Sensitivity measures how well a method recognizes infected seed lots

Specificity measures how well a method recognizes uninfected seed lots

Precision measures how likely a positive test result is actually infected

Accuracy measures how well a method correctly classifies both infected and non-infected seed lots

Tolerance threshold

Infection detected

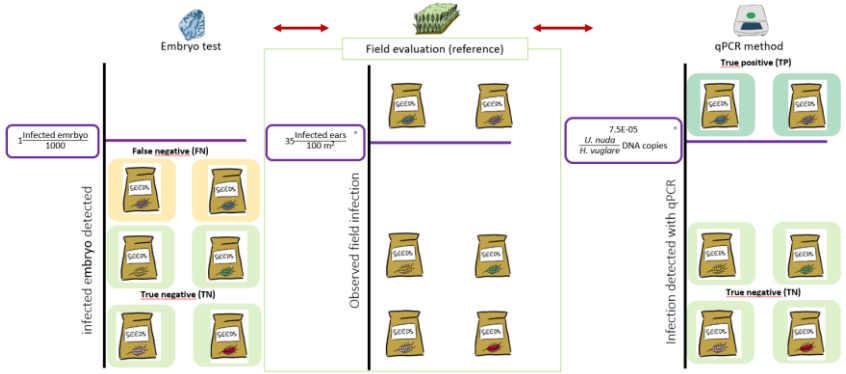
True positive (TP)

True negative (TN)

False positive (FP)

False negative (FN)

Ustilago nuda commercial seed trial: results



$$\frac{TP}{(TP+FN)} \qquad \frac{TN}{(TN+FP)} \qquad \frac{TP}{(TP+FP)} \qquad \frac{TP+TN}{(TP+TN+FP+FN)}$$

Laboratory detection methods	Sensitivity	Specificity	Precision	Accuracy
Embryo test	0%	100%	-	60%
qPCR method	100%	100%	100%	100%
	Sensitivity measures how well a method recognizes infected seed lots	Specificity measures how well a method recognizes uninfected seed lots	Precision measures how likely a positive test result is actually infected	Accuracy measures how well a method correctly classifies both infected and non-infected seed lots

Tolerance threshold

Infection detected

True positive (TP)

True negative (TN)

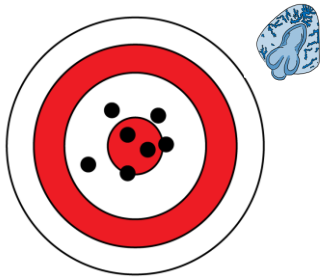
False positive (FP)

False negative (FN)

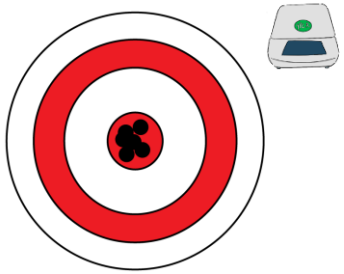
Ustilago nuda commercial seed trial: results

Laboratory detection methods	Sensitivity $\frac{TP}{(TP+FN)}$	Specificity $\frac{TN}{(TN+FP)}$	Precision $\frac{TP}{(TP+FP)}$	Accuracy $\frac{TP+TN}{(TP+TN+FP+FN)}$
Embryo test	0%	100%	-	60%
qPCR method	100%	100%	100%	100%

qPCR method successfully identifies seed lots with Infection either below or above the field tolerance threshold



High accuracy
Low precision

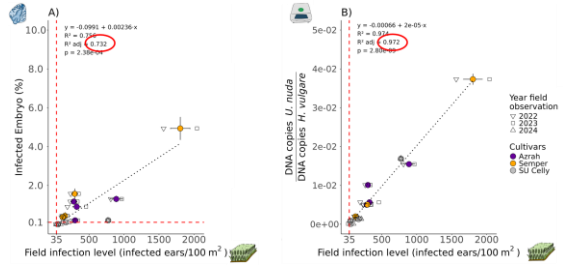


High accuracy
High precision

Richtig Positive (RP) | Falsch Positive (FP)
Richtig Negative (RN) | Falsch Negative (FN)

Summary

- The infection detected by qPCR better reflects the infections observed in the field than the infection determined by the embryo test.



- Our qPCR method accurately classifies samples as either above or below the tolerance threshold matching with the reference

Labor-Methoden	$\frac{RP}{(RP+FN)}$ Sensitivität	$\frac{RN}{(RN+FP)}$ Spezifität	$\frac{RP}{(RP+FP)}$ Präzision	$\frac{RP+RN}{(RP+RN+FP+FN)}$ Genauigkeit
Embryotest	0%	100%	-	60%
qPCR Methode	100%	100%	100%	100%

- Overall, the developed qPCR method is a better predictor of fire blight infections in the field, especially in cases of low infection, compared to the embryo test.

Agroscope

Conclusion

Field monitoring

$5 \frac{\text{infected ears}}{100\ m^2}$

Harvested seed

Recommendation using qPCR method on harvested seeds

$7.5E\text{-}05 \frac{U.\ nuda}{H.\ vuglare}$ DNA copies *

Infection below the tolerance threshold

Seeds can be sold as certified

Infection above the tolerance threshold

Seeds should be treated

* Sowing density: $\frac{350\ g\ Seed}{m^2}$

Acknowledgements

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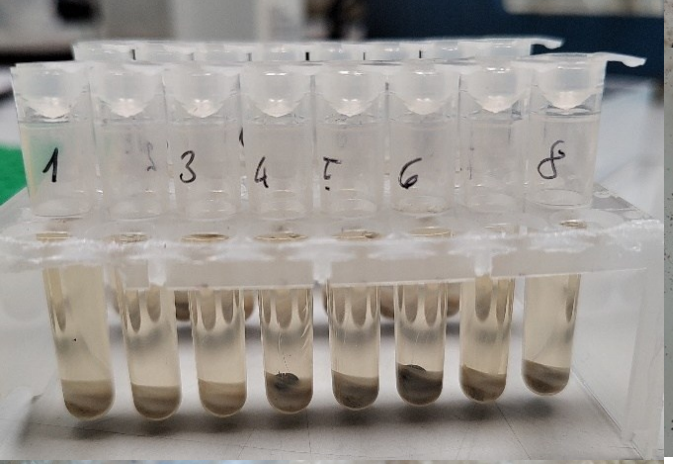

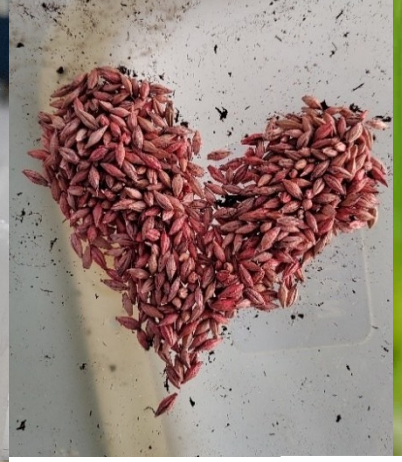

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Thank you for your attention

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