First report of the kdr pyrethroid resistance mutation in a French population of the English grain aphid, Sitobion avenae

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ABSTRACT

The lack of recent insecticide innovations and the withdrawal of efficient, but ecotoxic molecules (e.g. neonicotinoids) may lead to a shift to older insecticidal active substances, particularly pyrethroids. The evolution of resistance to this family of insecticides has long been recognized, including in many aphid species such as the grain aphid *Sitobion avenae*. The target-site resistance mechanism involved, named *kdr* (knock-down resistance), was first identified in the United Kingdom in 2014. In this study, we screened for the presence of this mutation in 25 French populations of *S. avenae* sampled between 2017 and 2021 on various cultivated cereals. Aphids were genotyped individually for the *kdr* mutation using PCR-RFLP or partial sequencing of the voltage-gated sodium channel gene. Of the 400 *S. avenae* individuals tested, one carried a heterozygous *kdr* mutation. This *S. avenae kdr* mutant was sampled in Northern France, the French region closest to the United Kingdom. The low frequency of the detected mutation and the geographical proximity to the UK suggest that it arose from a

migration event and not an independent convergent mutation event, although this hypothesis remains to be confirmed. Our study also reveals that partial sequencing of the voltage-gated sodium-channel gene can be used for another cereal aphid species, *Rhopalosiphum padi*.

Keywords: insecticide resistance monitoring, cereals, aphids

INTRODUCTION

The English grain aphid, *Sitobion avenae* (Fabricius, 1775) is one of the major cereal aphid species in the temperate areas of the Northern Hemisphere. This aphid can infest a wide range of cultivated or wild cereals (wheat, barley, oat, maize, rice, cocksfoot grass, etc.). It causes direct damage by sucking sap from the host, which can lead to senescence of the flag leaf and reduced grain weight (Wratten et al. 1975). This species is also a vector of viruses, such as barley yellow dwarf virus (BYDV), which causes a reduction in yield and grain quality (Edwards et al. 2001, Gray et al. 1991). Controlling this pest generally involves the use of insecticides in conventional agriculture. Repeated application of pesticides exerts selection pressure on pests, which can lead to the evolution of resistance within the species with — in the long run — the risk of losing treatment effectiveness (Hawkins et al. 2019). *S. avenea* has great evolutionary potential, as demonstrated by the multiple types of resistance that have evolved against several active substances, including target-site resistance mechanisms (Foster et al. 2014) and non-target site resistance mechanisms (Zang et al. 2017,Gong et al. 2021). Some French populations of *S. avenea* can reproduce both sexually and asexually (Simon et al. 1999, Dedryver et al. 2001), thereby allowing genetic recombination and rapid dispersal of competitive clones by asexual multiplication. Although other active substances have since been developed, pyrethroids remain one of the main insecticide families used to control aphid

46 populations on cereal crops in France. In Europe, pyrethroid-resistant individuals of *S. avenae*
47 were first reported in 2014 on populations collected in 2011 in England and Scotland (Foster
48 et al. 2014). The resistance mechanism involves a target-site mutation, resulting in the amino-
49 acid substitution of a leucine by a phenylalanine on codon 1014 (L1014F) of the voltage-gated
50 sodium channel, the pyrethroid target. This mutation, named *kdr* (knock-down resistance), is
51 one of the most common pyrethroid-resistance mechanisms in insects (Dong *et al.*, 2014).
52 Several other mutations affecting the voltage-gated sodium channel have also been described,
53 especially in aphid species. They are generally located at position 918 and they can be found
54 with or without *kdr* (Eleftherianos et al. 2008, Fontaine et al. 2011, Panini et al. 2015, Carletto
55 et al. 2010). Mutations affecting this codon are called *skdr* (super knock-down resistance),
56 because they most often lead to very high level of resistance to pyrethroids (Eleftherianos et
57 al. 2008, Fontaine et al. 2011).

58 Considering the pyrethroid spray failures caused by a resistant *S. avenae* clone in the UK
59 (Foster et al. 2014) and the wide use of pyrethroids on cereals in France, we screened for the
60 presence of *kdr* between 2017 and 2021, as part of the French system for monitoring the
61 adverse effects of plant protection products.

62 MATERIALS AND METHODS

63 Aphid sampling

64 Field samples were done from 2017 to 2021 by harvesting 30 to 40 randomly distributed cereal
65 plants infested with *S. avenae*. Live aphids were collected with a brush and individually placed
66 in 2 mL Eppendorf tubes with a sterile stainless-steel bead of 4.76 mm diameter. Aphids were
67 stored at -80°C until RNA extraction.

RNA Purification

Samples were ground in a TissueLyser II (Qiagen, Hilden, Deutschland) with two cycles of 2 min at 20 Hz after adding 100 µL of buffer RA1 with 2% TCEP provided in the Nucleospin RNA Mini kit (Macherey Nagel, Düren, Deutchland). RNA was then purified according to the manufacturer's instructions. Final elution was done in 10 µL of RNase-free water. Purified RNA was stored at -80°C until use in a reverse-transcription polymerase chain reaction (RT-PCR).

Pyrethroid resistance genotyping

PCR-RFLP genotyping for *kdr* mutation

From 2017 to 2018, the presence of the *kdr* mutation in the voltage-gated sodium-channel gene was determined using the RT-PCR method described in Foster *et al.* (2014). This method requires reverse transcription of RNA into DNA, followed by PCR with the Sa1 and Sa3 primers. RT-PCR was performed with the Superscript One Step RT-PCR system with Platinum Taq kit (Invitrogen, city, CA, USA). The reaction mix contained 1x reaction buffer, 400 µM of each primer and 0.25 µL of RT/Platinum Taq mix. PCR products were digested with the enzyme *Bss*SαI (New England Biolabs, Ipswich, United Kingdom) at 37°C for at least 4 h. This enzyme cuts the sequence '5'-CACGAG-3', which can differentiate the wild-type sequence (a single restriction site) from the mutated sequence (no restriction site).

Partial sequencing of the voltage-gated sodium channel

In addition to the PCR-RFLP method, the voltage-gated sodium-channel mRNA was partially sequenced in 2019–2021 samples. This method has the advantage of including codon 918 and codon 1014 of voltage-gated sodium channels, both known to be implicated in pyrethroid resistance, i.e. the *skdr* and *kdr* mutations, respectively. The sequencing approach also provides the opportunity to investigate the few atypical digestion profiles that were obtained

due to incorrect aphid species identification during sampling (see below). RT-PCR products were directly sequenced by Genewiz (South Plainfield, NJ, USA) or Genoscreen (Lille, France) using the Sanger method with the forward primer.

RESULTS

A total of 25 populations (from 1 to 9 per year) were sampled in small grain cereals (24) and in a maize field (1) (Figure 1). Field sampling was carried out mostly in conventional fields (18 vs. 7 from trial fields). The objective was to collect 30 aphids in each sampled field, but this goal was not always reached due to the low infestation rate of some samples. In total 482 out of 501 aphids sampled (mean per sample = 20 +/- 10.75 – Supplementary Data 1) were successfully analyzed. Over the four years of survey, almost two thirds of aphids were sampled in 2019 and 2021. The amplification failure rate of the analyzed aphids was low (3.6%) and related to low-quality RNA extracts.

During the two first years of the survey, resistance genotyping was performed using the PCR-RFLP method on 103 aphids; 98 of them showed a digestion profile with two fragments (one of 74 bp and one of 397 bp) corresponding to susceptible individuals. Five aphids showed atypical band profiles consisting of two bands at approximately 230 bp and 240 bp. These PCR fragments were sequenced. A BLAST analysis showed that the highest identity score (98.97%) of these fragments matched with the *Rhopalosiphum padi* sodium channel 1 mRNA sequence (accession number: MN972451.1). The result of the Blast analysis as well as the frequent co-occurrence of *R. padi* and *S. avenae* on straw cereals in France make us think that an error of identification took place during the sampling in the field. Interestingly, this result reveals that the primers used in this study can also amplify the voltage-gated sodium channel from RNA extracted from *R. padi.* In these five aphids, no mutations were identified at codon 918 or

1014, known to be implicated in pyrethroid resistance. The sequence was deposited in GenBank (accession number OM585630).

From 2019 to 2021, to avoid genotyping failure due to incorrect species identification, RT-PCR was directly followed by sequencing. The analysis revealed that one aphid had been wrongly identified and belonged to *R. padi*. Among 385 *S. avenae* successfully analyzed during the last three years of the survey, only one aphid was heterozygous for the *kdr* mutation (L1014F) (Figure 2). This *S. avenae* individual was sampled in 2020 in a straw cereal crop field, located in the located in one of the northernmost plots in this study (Figure 3). None of the analyzed aphids showed a mutation at codon 918. The partial the voltage-gated sodium-channel gene sequences of a wild and mutant clone were deposited in GenBank (accession numbers OM585631 and OM585632 for the wild and kdr-mutated sequence, respectively).

DISCUSSION

This is the first report of *S. avenae* with a *kdr* mutation in France. Using bioassays, heterozygous *kdr* shows resistance level that is 30–40 times greater than that of the homozygous wild-type and is associated with reports of pyrethroid spray failure (Foster *et al.*, 2014). In the UK, only heterozygous aphids have been detected and all of them have belonged to a unique clonal lineage (Sa3). In French *S. avenae* populations, the *kdr* mutation appears to be present at a very low frequency, which contrasts strongly with the situation observed in the UK, where more than 50 % *S. avenae* collected were resistant (Walsh et al. 2020). We could not determine whether the clone identified in France is identical to that detected in the UK. Given the geographical proximity between the UK and Northern France where the resistant aphid was detected, a migratory event (natural or related to human activity) is the

most likely origin of the mutation. However, we cannot completely rule out independent emergence of the *kdr* mutation. Genotyping neutral markers is required to determine whether the resistant French aphid is related to the Sa3 clone. Our study was conducted on RNA and not on DNA, precluding DNA analysis. Recent studies have reported a fitness trade-off in *S. avenae* clones with *kdr*, such as lower population growth and lower individual relative growth as well as a greater rate of parasitization by *Aphidius ervi* (Jackson et al. 2020) and later onset of male forms (Walsh et al. 2021). Although the very low *kdr* allele frequency in our survey (0.2%) can be suggestive of fitness trade-offs, this finding must be interpreted with caution. The situation in the UK has shown how a resistant clone can spread rapidly across a large area due to selection pressure in the presence of pyrethroids.

Interestingly, the primers published by Foster et al. (2014) and used in this study were able to amplify the sodium channel from RNA of another aphid species living on cereal crops. Cereals are often co-infected with different aphid species at different stages of development, which can complicate aphid identification. Adult aphids can be easily identified, but species determination can be more difficult at the larval stage. Therefore, some identification errors (1%) can occur during aphid sampling of infested cereals. Both aphid species experience comparable insecticide treatments, and pyrethroid resistance involving voltage-gated sodium-channel mutations has been recently reported in Chinese populations of *R. padi* (Wang et al. 2020). Therefore, the availability of a tool for the characterization of the *kdr* mutation compatible with *S. avenae* and *R. padi*, two aphids widespread on cereal crops, offers the advantage of detecting resistance-related genotypes even if the exact identification of the species is uncertain. This tool could be easily used in the monitoring framework planned to early detection and following of pyrethroid resistance involving target site modification

specially, in countries where the withdrawal of neonicotinoids has occurred or where cases of resistance are suspected in these species of cereals.

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Figure 1: Map of fields sampled from 2017 to 2021. The pie charts indicate the proportion of small-grain cereal (yellow) and maize (green) crop fields (populations) sampled in each administrative division ("*département*") included in the survey. The size of the pie charts is proportional to the number of populations sampled (i.e.: field plot sampled).

Figure 2: Chromatographs of wild type and kdr heterozygous *S. avenae* sequences around the position 1014 of the para-type sodium channel gene. The 1014^{th} codon is indicated by a red rectangle. Y corresponding to T and C pics according the IUPAC code. CTC and TTC are coding for leucine and phenylalanine, respectively.

Figure 3: Map of the distribution of *kdr*, the target site of pyrethroid resistance, in populations of *Sitobion avenae* sampled in French cereal crop fields from 2017 to 2021. The pie charts indicate the proportion of populations (i.e.: field plot sampled) in which the *kdr* mutation (L1014F) was detected (red) or not detected (blue) in each administrative division ("*département*") included in the survey. The size of pie charts is proportional to the number of populations sampled.

Figure(s)

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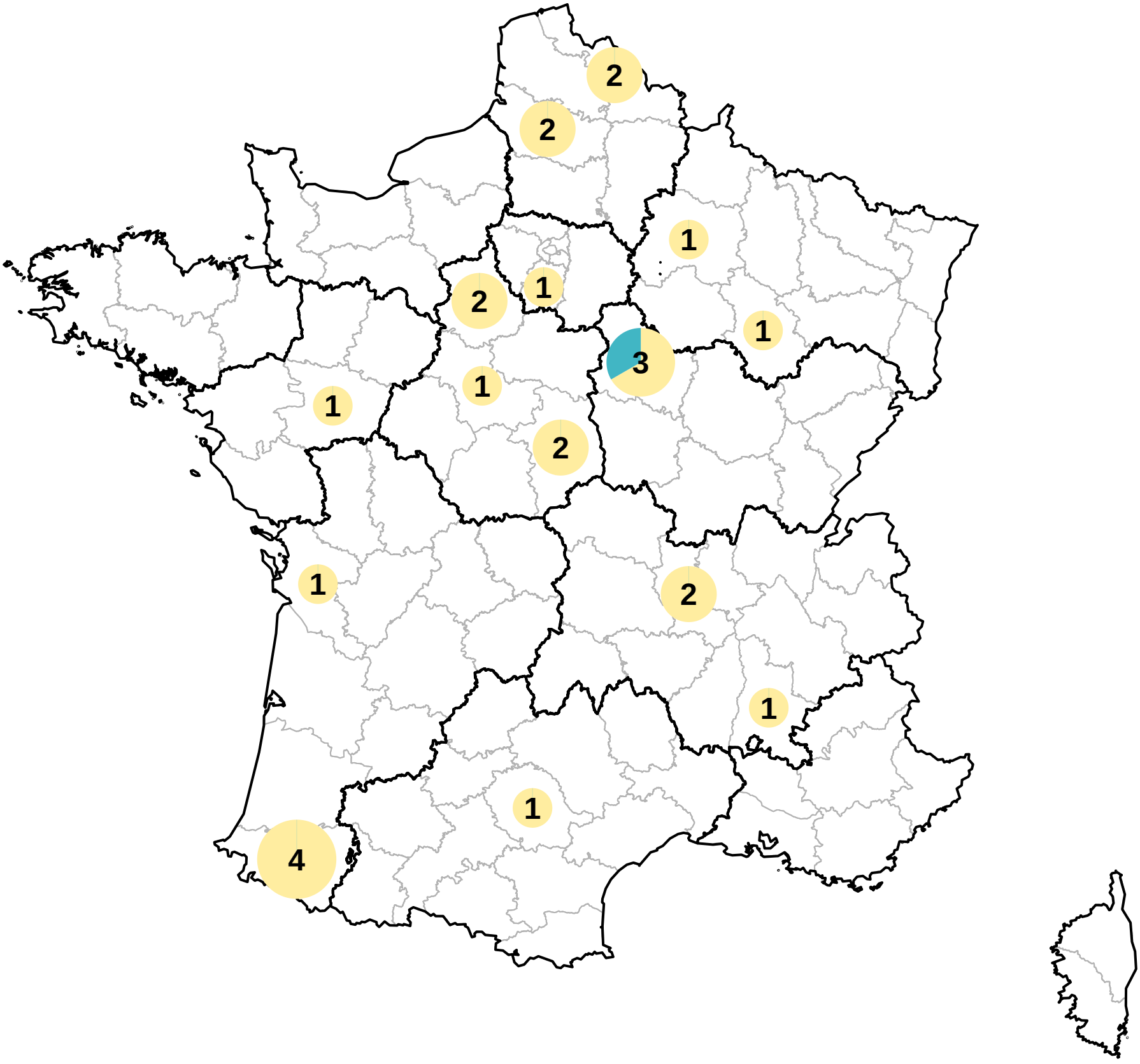

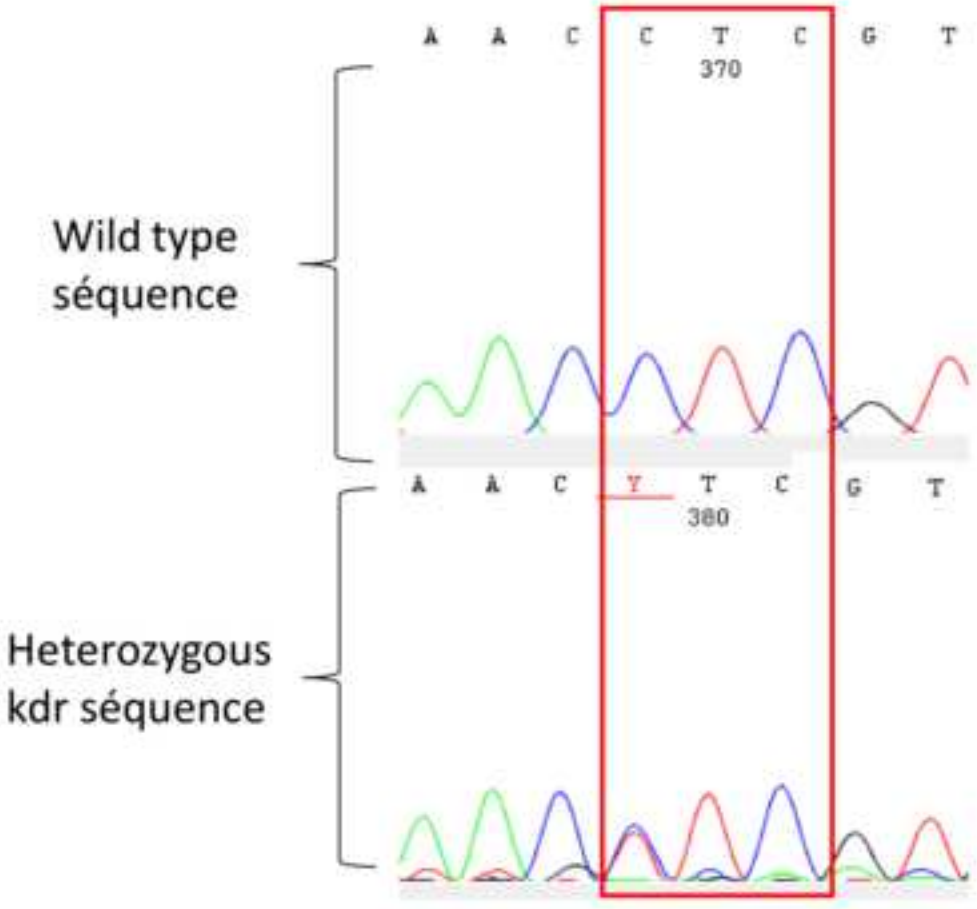

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