Molecular epidemiology of Cercospora leaf spot on resistant and susceptible sugar beet hybrids

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Abstract

Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is a major foliar disease impacting sugar beet production worldwide. The development of new resistant sugar beet hybrids is a powerful tool to better manage the disease, but it is unclear how these hybrids affect CLS epidemiology. We used a molecular epidemiology approach to study natural epidemics of CLS affecting two susceptible and two resistant sugar beet hybrids at two field sites. Infected plants were geotagged on a weekly basis. Isolations of *C. beticola* were made from infected leaves and genotyped using six simple sequence repeat loci to identify clones. We determined that CLS epidemics had a later onset in plots planted to resistant hybrids, but once the pathogen established an infection, there was little difference between resistant and susceptible hybrids in the probability of localized spread and dispersal. We found that different clones often infected the same leaf and that clusters of infected plants were often colonized by a mixture of clones. There was little overall difference in genetic diversity between resistant and susceptible hybrids, however genotypic evenness differed at one site. In this site we found one genotype restricted to the resistant cultivars at a high frequency. At the end of the epidemic infections were not randomly distributed across the fields and we found that a single clone could spread over a distance of 100 m during a growing season.

Keywords: *Beta vulgaris*, *Cercospora beticola*, population genetics, resistant cultivars

Introduction

Sugar beet (*Beta vulgaris* L.) contributes approximately one-fifth of global sucrose production annually (Rangel et al., 2020). The most destructive disease of sugar beet is the foliar disease Cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola*. CLS affects the production of sugar beet in most sugar beet growing regions worldwide (Holtschulte, 2000). Under high disease pressure, the yield loss can exceed 40% (Shane and Teng, 1992). The main method of control is multiple applications of fungicides, however this has resulted in the emergence of widespread fungicide resistance in *C. beticola* populations (Bolton et al., 2013; Kayamori et al., 2021). Resistant cultivars are seen as an important means of control in addition to fungicides, with CLS-resistant cultivars providing protection under low to moderate disease pressure (Francis and Luterbacher, 2003; Vogel et al., 2018; Weiland and Koch, 2004; Wolf and Verreet, 2005). A new resistance gene named *BvCR4* has recently been identified and bred into sugar beets (Törjék et al., 2020). This new gene provides a high level of CLS resistance and could be a useful method of control.

As with the evolution of fungicide resistance, pathogens can evolve to overcome host resistance through selection for virulent strains. How quickly pathogens evolve virulence to resistant cultivars is strongly influenced by the genetic architecture of the resistance – single major resistance (R) genes with large effect are usually less durable compared to quantitative resistance encoded by many genes (Cowger and Brown, 2019; Gou et al., 2023). The evolution of virulence is also influenced by the amount of genetic diversity in the selected pathogen population, the deployment strategies used for the resistance genes, and the distribution of virulent pathogen genotypes across a heterogenous host population (Milgroom and Peever, 2003; Rimbaud et al., 2018; Stam and McDonald, 2018).

The interaction between pathogen genotypes and host genotypes shapes both the development of epidemics at the field scale and the evolutionary landscape of the entire pathosystem at larger spatial and temporal scales. High genetic diversity within a pathogen population can promote the evolution of virulence (McDonald and Linde, 2002; Sacristán et al., 2021), but the type and degree of host resistance can influence the distribution of genetic diversity in the corresponding pathogen population. When a single new resistant cultivar carrying a major R gene is first deployed in the field, the corresponding pathogen

population experiences strong selection and a bottleneck, because only genotypes that carry the compatible virulence factors can infect the resistant cultivar. This selective bottleneck can result in a reduction in genetic and genotypic diversity in the pathogen population. How quickly the pathogen population and the corresponding genetic diversity can recover following this selection bottleneck depends upon the life history and evolutionary potential of the pathogen - in particular the frequency of sexual reproduction and the efficiency of dispersal of virulent genotypes (Barrett et al., 2008; McDonald and Linde, 2002). Though a sexual stage (teleomorph) has not yet been described for *C. beticola*, population genetic evidence is consistent with regular cycles of sexual recombination. This evidence includes the presence of both mating types at the *MAT* locus, often in a 1:1 ratio (Bolton et al., 2012; Groenewald et al., 2008), high genotypic diversity and low clonality assessed with neutral genetic markers, and random associations among neutral loci (Groenewald et al., 2008; Vaghefi et al., 2017b). The rapid evolution of fungicide resistance in CLS indicates that *C. beticola* has a high evolutionary potential, suggesting that *C. beticola* populations could also adapt quickly to deployment of resistant host genotypes.

The epidemiology of CLS has been well studied (Imbusch et al., 2021; Rossi et al., 2000; Tedford et al., 2018; Wolf and Verreet, 2005). *C. beticola* is polycyclic and leaf lesions can sporulate after 3 days under ideal conditions (Rossi et al., 2000). The primary site of infection is leaves, CLS infections did not establish on roots and stems inoculated with spores (Khan et al., 2008). The primary inoculum can come from multiple sources, including overwintering structures called pseudostromata, which can persist on plant debris for 22 months (Khan et al., 2008), conidia produced on alternative hosts growing in or nearby new fields, and infested seeds (Spanner et al., 2022). Previous work in the USA found that even when there is no crop rotation, genotypes were not resampled over time, suggesting that the dominant source of inoculum for an epidemic is external to the field of interest (Knight et al., 2018). Conidia can be dispersed to adjacent plants by rainsplash, wind and insects (Khan et al., 2008; Vereijssen et al., 2007; Weiland and Koch, 2004), with the longest recorded dispersal distance exceeding 45 m (Imbusch et al., 2021). How dispersal is influenced by host genotype has not been experimentally tested, but other traits have been considered. On more resistant hosts, spores were smaller, germinated more slowly, and spore yield was reduced, but latency did not vary with host resistance (Rossi et al., 2000).

96 This finding illustrates that resistant hosts may influence CLS epidemiology, which may in
97 turn affect the emergence and distribution of virulent genotypes.
98
99 Previous work established that *C. beticola* has high genetic diversity and a high evolutionary
100 potential, and that host resistance may influence aspects of CLS epidemiology, but few
101 studies have investigated the evolution of virulence in *C. beticola* populations or used
102 neutral genetic markers to understand how CLS epidemics develop in fields during a growing
103 season. Molecular epidemiology bridges the gaps between disease epidemiology and
104 pathogen population genetics. By including genetic markers into epidemiology studies, new
105 research questions can be addressed around the origins of epidemics, genotype
106 movements, dispersal pathways and the spatio-temporal aspects of disease progression
107 (Archie et al., 2009; Desbiez et al., 2002; Xhaard et al., 2012).
108
109 Here we analyzed the molecular epidemiology of natural CLS epidemics occurring in
110 resistant and susceptible hybrids of sugar beet to better understand the evolutionary
111 potential of this pathogen on newly developed hybrids. The main objects were 1) to
112 determine the time of onset of CLS epidemics on different hybrids; 2) to measure the local
113 dispersal pattern for both primary and secondary inoculum of CLS; 3) to assess the genetic
114 diversity of *C. beticola* populations during epidemics across different spatial and temporal
115 scales, including: on the same leaves; on neighboring plants; and across hybrids within and
116 between fields; 4) to determine the degree of clonality on different host populations and
117 identify the most likely sources of primary and secondary inoculum in natural field
118 infections.
119
120 **Methods**
121 **Field trials in Switzerland**
122 Two trial sites located in Switzerland (Rudolfingen, Trüllikon 47°38'18.6"N, 8°40'46.1"E and
123 Hendschiken, Othmarsingen 47°23'40.4"N, 8°13'41.1"E) were planted with four sugar beet
124 hybrids in March 2020. The fields had not been planted with sugar beet in the previous
125 three years. The four hybrids differed in their resistance to CLS, including two highly
126 resistant hybrids with the *BvCR4* gene – Hybrid_R2 and Hybrid_R3, and two susceptible
127 hybrids lacking *BvCR4*– Hybrid_S2 and Hybrid_S3 (Table 1). In each field we selected an

experimental area ~100m long, within the field, that contained plots (12-18 rows) of each of the four hybrids (Figure S1). In Hendschiken, each hybrid was planted in blocks of 12 rows for a total area of 1950 m^2 . In Rudolfingen, each hybrid was planted in blocks of 18 rows for a total area of 3600 m^2. Rows were separated by approximately ~45cm and the final distance between plants within rows was 0.18m. At both locations, there was no separation between rows planted to the four cultivars, but the experimental area was surrounded by a border of sugar beets to minimize edge effects. In some cases, the border rows included one or more of the sugar beet hybrids used in the main experiment. Sugar beets were harvested in early November. No fungicides were applied and the CLS infections were natural.

Temporal sampling strategy

Our sampling was designed to identify the naturally infected plants in each plot and determine how natural infection spreads among plants. We systematically searched each plot for infected plants once per week from July 21st to September 24th, 2020. Individual sugar beet plants served as sampling units and one leaf with CLS lesions was collected from a plant. The sampling method is shown in Figure S1a. All sampled plants were marked with flags to prevent resampling. In the early stages of the epidemic, we found single newly infected plants surrounded by healthy plants, which we referred to as solitary infections. As the epidemic progressed, the disease often spread from a single infected plant to neighboring plants to form a cluster of infected plants, hereafter called a hotspot. For each solitary infection, we recorded the GPS location (Garmin) and gave it a two-letter code (e.g. AA) and the number 1 to indicate it was the first infected plant at that GPS position (e.g. AA1). If the infected plant was immediately adjacent to a previously identified and flag-labeled plant, it was considered part of the same hotspot. Infected plants in the same hotspots were given the same alphabetic code and a number to indicate the sampled plants were the 2nd, 3rd, 4th or 5th plant found in the hotspot (e.g. AA4 was the fourth plant sampled in the AA hotspot). As mentioned, one leaf with CLS lesions was taken from each plant, with a preference for young and otherwise healthy leaves with multiple clear CLS lesions. The excised leaves were kept flat and cool and transported back to the laboratory for *C. beticola* isolation (see below for details). We sampled a maximum of five plants from each hotspot, which typically covered an area of ~1m^2 when sampling ceased. We stopped sampling a hybrid plot when it was no longer possible to find new solitary infected plants

because hotspots had coalesced. Our sampling design allowed us to track CLS epidemic progression and track hotspot formation within and between plots planted to resistant and susceptible hybrids.

Leaf storage

To store leaves flat, each leaf was placed on a labeled piece of paper and covered with paper towel. Multiple leaves were stacked between stiff cardboard sheets and secured with strong rubber bands. The stacked leaves were then stored in a cool box with ice blocks to maintain a low temperature during transport. Upon arrival at the laboratory, the leaves were air dried at room temperature in a drying cupboard. Dried leaves were stacked and placed in plastic bags with anhydrous silica gel and stored at 4°C until used for pathogen isolation.

Pathogen isolation and DNA extraction

To obtain pure strains of *C. beticola*, spores were collected from the dried leaves. Three or four pieces of leaf tissue with visible lesions were cut from each dried leaf. The tissue samples were surface sterilized by immersing them in a 2% sodium hypochlorite solution for 3 minutes, followed by two rinses in sterile de-ionized water. The leaf pieces were placed on dry paper towels to remove excess water, then were put into glass Petri dishes on an elevated sterile net placed on a piece of moist filter paper. The Petri dishes were sealed with parafilm and incubated at 24°C for 48-72 hours to induce sporulation. The induced lesions were checked for the typical black pseudostromata and hyaline conidia under a dissecting microscope (Olympus Schweiz AG). The spores were picked from sporulating lesions using hand-made fine glass needles and transferred to fresh potato dextrose agar (PDA, Difco). The plates were cultured under constant darkness at 24°C for 7-10 days. After a characteristic *C. beticola* colony had formed, a 5-mm agar plug was excised from the edge of each colony and transferred to a new PDA plate for 14 days to form a pure culture. Any contaminated plates were discarded. For long-term storage of each pure culture, 4-6 agar plugs were cut from the edges of a 2-week-old pure culture and put into cryotubes in duplicate - with and without anhydrous silica gel, and stored at 4°C. For most leaves, between 1-3 colonies were isolated from different lesions, but for some leaves, more than 3 strains were isolated to investigate pathogen diversity within the same leaf. In total, 1251 *C.*

192 *beticola* isolations were made. For practical reasons, DNA was not extracted from all
193 isolates. Instead, we aimed for a single isolation per leaf and preference was given to
194 isolates from hotpots, which were expected to be more virulent and capable of dispersing to
195 nearby healthy plants.
196
197 To obtain fresh mycelia for DNA extraction, we transferred hyphae from plates to liquid
198 culture by scraping small pieces of hyphae from the edge of each pure culture and placing
199 them into 150 mL flasks containing 50 mL potato dextrose broth (PDB, Difco). The liquid
200 cultures were incubated in the dark at 24°C on a shaker at 120 rpm for 10 days. The fungal
201 tissue was harvested, washed twice with sterilized water, and the supernatant was
202 discarded after centrifuging (Allegra X-12R, Beckman Coulter) at 3750 rpm for 10 mins. The
203 pellet was then transferred to 2 mL microcentrifuge tubes and lyophilized overnight. The
204 lyophilized pellets were ground into a fine powder using ~15 glass beads in a grinding
205 machine (Qiagen MM300 Retsch TissueLyser). A total of 560 DNA samples of isolates coming
206 from separate leaves were extracted using an automated DNA extraction robot, the
207 Kingfisher Flex (Thermo Fisher Scientific), using the recommended reagents and a protocol
208 for fungal samples (LGC Genomics GmbH). The resulting DNA samples were quantified using
209 a Nanodrop spectrophotometer (Thermo Fisher Scientific). We performed a polymerase
210 chain reaction (PCR) using species-specific primers (Knight and Pethybridge, 2020) to
211 confirm the samples were *C. beticola*. Only samples with a clear band at around 200 bp
212 (~85%) were retained.
213
214 **Simple sequence repeats analysis**
215 The six SSRs were amplified in 2 multiplexes (multiplex1: SSRCb1, SSRCb3, SSRCb21;
216 multiplex2: SSRCb22, SSRCb25, SSRCb27) (Groenewald et al., 2007; Vaghefi et al., 2017b).
217 The SSR primers were blasted against the reference genome to ensure they were not
218 physically linked (Table S1). The PCR amplifications were performed using Qiagen Type-it
219 kits (Qiagen) in a total volume of 11 µL. The reaction mixture contained 20 ng of template
220 DNA, 0.4 µM of each forward and reverse primer, 4 µL Type-it mix (with buffer and $MgCl_2$), 1
221 µL Q-solution (1 U Taq polymerase), and 2 µl deionized water. The initial denaturation was
222 carried out at 95°C for 5 mins, followed by 30 cycles of denaturation at 95°C for 30 s,
223 annealing at 58°C or 56°C for 90 s, elongation at 72°C for 30 s and final extension at 60°C for

224 30 mins. The PCR amplicons were analyzed on an ABI 3730xl DNA Analyzer (Applied
225 Biosystems) at the Genetic Diversity Centre (GDC) of ETH Zürich. Multiple samples were
226 repeated at different stages to create technical replicates to estimate SSR genotyping error
227 rates and ensure consistent allele calling.
228
229 SSR allele size was scored using the R package *Fragman* (Covarrubias-Pazaran et al., 2016).
230 To reduce the genotyping error, allele fragment sizes of each SSR marker were visualized
231 and binned using the R package MsatAllele v 1.05 (Alberto, 2009). Fragment stutter patterns
232 varied between the SSRs. Visual inspection of the allele calling and consistency between
233 repeats was used to assess the accuracy of the MsatAllele automatic binning. Automatic
234 binning was accurate for three SSRs (SSRCb27, SSRCb3, SSRCb21) but manual adjustment of
235 the bin sizes was required for the rest. The consistency of allele calling was evaluated using
236 isolates that were replicated (n=51). In total we obtained SSR genotypes for 475 isolates.
237 Genotype calling was repeated for 20% of isolates chosen at random. Samples with multiple
238 allele calls were assumed to be a mixture of strains and were excluded from the dataset. All
239 individuals with missing SSR data were also removed, leaving 340 fully genotyped isolates
240 (158 from Hendschiken and 182 from Rudolfingen) for downstream analyses.
241
242 **Statistical analysis**
243 Statistical analysis was performed in R version 4.3.1 (R Core Team, 2023) and R Studio
244 version 2023.6.0.421 (Posit team, 2023). To investigate the localized spread between plants
245 to form a hotspot, we used a generalized linear model with a binomial distribution. To
246 compare across hybrids, we needed to control for the fact that CLS continued to spread
247 after we stopped sampling in some hybrid plots, meaning that any new infection cases that
248 were identified at the end of the sampling period were not followed to determine if they
249 spread to neighboring plants. Our solution was to right-censor the data by excluding the
250 new infection cases observed 1 and 2 weeks before the final sampling date in each hybrid
251 plot. This is a common practice when sampling ends before an event has been observed. As
252 the sampling period varied for each hybrid, the right-censor date also varied (Table 2). The
253 rate at which a hotspot developed was analyzed using a linear model. The pairwise
254 distances between geotagged infection sites were calculated using the R package *geosphere*
255 v1.5-18 (Hijmans, R. J., 2022). Whether the distributions of geotagged plants differed

256 between hybrids or between the beginning and the end of the season were assessed using
257 Kolmogorov-Smirnov (K-S) tests in the R package R4PDE (Del Ponte, 2023). Multi-locus
258 genotypes (MLGs) were identified and analyzed with the R package *poppr* v.2.9.3 (Kamvar et
259 al., 2014; Kamvar et al., 2015).
260
261 We performed clone-correction for each site separately as our earlier analyses indicated it
262 was unlikely to observe the same MLG between two sites (whole genome sequence data of
263 256 isolates found only one case of the same clone present at the two sites (Chen et al.,
264 2024)). The number of alleles, number of unique MLGs, the expected number of MLGs
265 controlling for differences in sample size (eMLG) and Nei's gene diversity, Simpson's
266 diversity and evenness were calculated using the R package *poppr* v.2.9.3 for each hybrid
267 and site. Bootstrapped confidence intervals were calculated for genetic diversity statistics
268 using function diversity_ci in *poppr*. Clonal fraction was calculated as (N–number of
269 MLGs)/N, where N equals the number of isolates.
270
271 **Results**
272 **Onset and distribution of CLS disease**
273 The first symptoms of *C. beticola* were observed in the susceptible hybrids: for Hybrid_S2 on
274 July 21st and one week later in Hybrid_S3. The first infections on the resistant hybrids
275 appeared 21-30 days after the first infections appeared on the susceptible hybrids (Table 2).
276 The appearance of the first *C. beticola* infections on resistant hybrids with *BvCR4* varied
277 between fields. In Hendschiken, the disease appeared on Hybrid_R2 before Hybrid_R3,
278 while in Rudolfingen, the first symptoms were observed later in Hybrid_R2. GPS coordinates
279 were used to map the locations of infected plants. The spatial distribution of all infection
280 cases is shown in Figure 1.
281
282 The accumulation of geotagged infection cases is shown in Figure 2. The sampling period
283 varied for each hybrid because the dates of disease onset differed (Table 2) and because we
284 stopped sampling when the hotspots coalesced and solitary infections could no longer be
285 clearly distinguished. Thus, we stopped sampling first in Hybrid_S2, then Hybrid_S3 and
286 then Hybrid_R2. Sampling continued until our final visit (September 24) in Hybrid_R3 as
287 hotspots had not coalesced. Infections continued to increase in all hybrids after we stopped

288 sampling. It should be noted that due to some initial sampling problems, we missed the
289 start of the epidemic in Hybrid_S3. Hence caution must be taken when comparing the
290 temporal spread in Hybrid_S3 with other hybrids. By the end of the season, 454 plants were
291 sampled (one leaf per plant) from 196 geotagged infections in Hendschiken, and 503 plants
292 were sampled from 209 geotagged infections in Rudolfingen.
293
294 **Localized spread of CLS in each hybrid**
295 Across all hybrids, about 80% of infection cases spread to at least one neighboring plant,
296 resulting in the formation of hotspots (Table 3). There was no significant difference between
297 fields or hybrids in the probability that CLS spread from one solitary infected plant to
298 neighboring plants to form a hotspot of two or more infected plants, or to form a large
299 hotspot with at least five infected plants (hotspot: Field $F_{(1,209)}$ =1.23, p=0.26; Hybrid $F_{(3,206)}$
300 =4.84, p=0.1, Large hotspot Field $F_{(1,209)}$ =2.69, p=0.10; Hybrid $F_{(3,206)}$ =4.90, p=0.17). NB: the
301 numbers in Table 3 differ slightly compared to the total cases and hotspots (Figure 2) due to
302 the right-censoring of datasets.
303
304 To assess the rate of hotspot development in each hybrid, we calculated the rate of spread
305 as the number of infected plants within a hotspot divided by the number of days between
306 sampling periods for plants sampled in each hotspot. There was no difference in the rate of
307 spread between sites (Field $F_{(1,115)}$ =2.87, p=0.09), there was a significant effect of hybrid
308 (Hybrid $F_{(3,115)}$ =6.5, p<0.001) and no interaction (Field*Hybrid $F_{(3,115)}$ =2.17, p=0.09). In
309 Hendschiken, the rate of spread was slower in Hybrid_R3 and faster in Hybrid_R2 compared
310 to Hybrid_S2 (Hybrid_R2: t=3.95, p=0.002, Hybrid_R3: t=-2.73, p=0.008). In Rudolfingen,
311 there was no difference in the rate of spread, although Hybrid_R3 tended to be slower (t=-
312 1.82, p=0.07).
313
314 **Spatial distribution of infections**
315 We compared the distribution of geotagged infections at the beginning and the end of the
316 epidemic and across hybrids within each site. The first two sampling dates in each hybrid
317 plot were designated as the beginning of the epidemic and were used to investigate the
318 spatial distribution of the first CLS infections. The initial distributions of hotspots and
319 infection sites for most hybrids were randomly distributed (Table S2). During the epidemic,

320 the minimum distance between infection sites decreased or remained constant, while the
321 maximum distance increased throughout each epidemic (Table 4). The pairwise distances
322 between hotspots ranged from ~1 to 116 m over the course of the epidemics, with average
323 values of 24 m and 36 m in Hendschiken and Rudolfingen, respectively. In some cases, the
324 GPS was not recorded for an infection site, resulting in smaller numbers in Table 4
325 compared to Figure 2. The final distributions of hotspots and infection sites for most hybrids
326 were aggregated (non-random) (Table S2) with two exceptions: Hybrid_R2 in Hendschiken
327 and Hybrid_S2 in Rudolfingen, where the infection sites were randomly distributed.
328 Although the number of infection cases increased over the season and were more likely to
329 be aggregated, in pairwise comparisons of the spatial distribution of geotagged infections at
330 the beginning and at the end of the season few significant differences were found (Table S3,
331 Figure S2).
332
333 We compared the spatial distributions of geotagged infection sites in each hybrid and found
334 that in Hendschiken Hybrid_R3 more often exhibited a different distribution of diseased
335 plants compared to other hybrids (Table S4). In Rudolfingen, there were more differences:
336 Hybrid_R2 was different from both susceptible hybrids, Hybrid_R3 was also different from
337 Hybrid_S3, but was similar to Hybrid_S2, while Hybrid_S2 and Hybrid_S3 had different
338 spatial distributions.
339
340 **Genetic and genotypic diversity at different hierarchical levels**
341 The six SSR loci used in our study were polymorphic, resulting in 2 to 9 alleles per locus.
342 Allelic diversity after clone-correction ranged from 0.23 to 0.83 (Table S5) and there was
343 some variation in the number of alleles for each SSR between hybrids (Table S6). There were
344 some alleles unique to each field; for example, the 183 bp allele at SSRCb22 was found only
345 in Hendschiken and the 373 bp allele at SSRCb27 was only detected in Rudolfingen.
346
347 We investigated genetic diversity at different hierarchical levels, including within and
348 between hybrids and fields, and within leaves and hotspots. In Hendschiken, 158 isolates
349 were distributed among 82 unique MLGs. In Rudolfingen, 182 isolates resulted in 77 unique
350 MLGs (Table 5a). The genetic diversity ranged from 0.40 to 0.57 across different hybrid plots,
351 and the genotypic diversity ranged from 0.73 to 0.95 (Table 5a). There was little difference in

352 Nei's gene diversity between sites or hybrids. The clonal fraction differed significantly among
353 the hybrids in Rudolfingen (χ^2 = 11.15, df=3, p=0.01), while it was similar among hybrids at
354 Hendschiken (χ^2= 3.38, df=3, p=0.33). In Rudolfingen, the clonal fraction was lower in
355 Hybrid_S3 and higher in Hybrid_R2. When resistant and susceptible hybrids are combined
356 we saw a significantly higher clone fraction on resistant hybrids in Rudolfingen but not in
357 Hendshicken (Table 5b). The frequency of MLGs differed in each site (Figure 3). In
358 Rudolfingen, there was greater skew in MLG frequencies; R_MLG77 appeared in 46 isolates
359 (20 in Hybrid_R2 and 26 in Hybrid_R3) and was restricted to the resistant hybrids with
360 *BvCR4*. In Hendschiken, differences between the hybrids were less pronounced, with the
361 most frequent MLG (H_MLG75) appearing 13 times in the resistant hybrids with *BvCR4*
362 (Hybrid_R2 and Hybrid_R3) (3 and 10 times respectively) (Figure 3). The evenness was
363 significantly higher in susceptible hybrids in Rudolfingen, but no difference was found in
364 Hendschiken (Figure 4).
365
366 **Genotypic diversity within a leaf and hotspot**
367 To investigate genotypic diversity within individual leaves, multiple isolations were made
368 from the same leaf for a total of 48 leaves (Table 6a). The leaves included in this analysis
369 came from all four hybrids, were sampled over the time course of the epidemic, and had
370 typical levels of infection, with at least 10-15 discrete CLS lesions distributed across the leaf.
371 It was common to find more than one genotype per leaf, with 23 out of the 48 (0.48) tested
372 leaves having more than one genotype on the same leaf. The maximum number of MLGs
373 from one leaf was two, irrespective of the number of isolations (2-4). Although there was
374 not enough data to make statistical comparisons between hybrids or sites, we commonly
375 observed the coexistence of multiple genotypes in resistant hybrids. Specifically, in
376 Hendschiken 6 out of 14 (0.39) tested leaves from resistant hybrids had multiple strains and
377 10 out of 16 (0.63) resistant leaves had multiple strains in Rudolfingen. We also considered
378 the genotypic diversity within a hotspot (n= 48) (Table 6b). Just over half of the hotspots had
379 more than one MLG. In resistant hybrids, 8 out of 13 (0.61) tested hotspots had multiple
380 strains in Hendschiken and 11 out of 19 (0.57) hotspots had multiple strains in Rudolfingen.
381
382 **Genotypic diversity at the start of each epidemic**

383 Genotypic diversity and the spatial distribution of isolates from the first two sampling dates
384 in each plot were used to infer the most likely sources of primary inoculum in each
385 epidemic. In Hybrid_S2 (July 21st and 27th) in Rudolfingen we genotyped 13 isolates coming
386 from seven hotspots and identified nine MLGs (clonal fraction (CF) = 0.31). In Hendschiken,
387 we genotyped 14 isolates from six hotspots and identified seven MLGs (CF = 0.50), with five
388 isolates from one of the hotspots having the same MLG. At the beginning of the epidemic,
389 no two hotspots had the same MLG, indicating that different pathogen strains established
390 each hotspot. Only four out of the seven initial hotspots in Rudolfingen were geotagged,
391 with pairwise distances ranging from 18.4 to 64.7 meters. Three out of the six initial
392 hotspots in Hendschiken were geotagged, with pairwise distances ranging from 25.0 to 68.7
393 meters. For the first two sampling dates in Hybrid_S3, only one isolate was genotyped from
394 each site, and thus the distribution of MLGs cannot be compared.
395
396 For the resistant Hybrid_R2: In Rudolfingen, on the first sampling date (August 25th) 15
397 isolates from 11 hotspots were genotyped. These contained six MLGs (CF = 0.6) with
398 pairwise distances ranging from 1.3 to 49.2 m. At the beginning of this epidemic the
399 R_MLG77 genotype was found 10 times in eight hotspots that were separated by pairwise
400 distances ranging from 1.3 to 19.6 m. In Hendschiken, during the first sampling dates in
401 Hybrid_R2 (August 11th and 20th), five isolates were sampled from four hotspots composed
402 of four MLGs (CF = 0.2), with the pairwise distances ranging from 4.2 to 61.4 m. No two
403 hotspots shared a MLG.
404
405 For the resistant Hybrid_R3: In Rudolfingen, on the first two sampling dates (August 20th and
406 25th), four isolates from four hotspots were genotyped and each had a unique MLG (CF = 0),
407 with pairwise distances ranging from 5.7 to 46.0 m. The frequent MLG R_MLG77 appeared
408 in one of these hotspots. In Hendschiken, 15 isolates sampled from eight hotspots belonged
409 to nine MLGs (CF = 0.4) (four isolates from the same hotspot had the same MLG), with
410 pairwise distances between hotspots ranging from 6.2 to 58.3 m. One MLG was shared
411 between two hotspots that were 7.8 m apart.
412
413 **Distribution of isolates with the same MLGs over time**

414 The primary CLS infections in each plot gave rise to asexual conidia that provided secondary
415 inoculum. The spatial distribution of the resulting clones with identical MLGs provides
416 insights into the dispersal of secondary inoculum during a growing season. Common MLGs,
417 observed ≥ 5 times in each field (eight in Rudolfingen and five in Hendschiken), were used to
418 calculate the dispersal distance of the same genotype (Table 7). The minimum pairwise
419 distance of 0 m was not included in the table. It was common to find the same MLG
420 separated by distances exceeding 10 meters. The maximum distance between isolates
421 sharing the same MLG was 108.5 m in Rudolfingen and 68.7 m in Hendschiken.
422
423 **Discussion**
424 The major objective of this study was to investigate how host resistance influenced CLS
425 epidemics on sugar beet. We combined detailed epidemiological surveys with geo-
426 referenced sampling and SSR genetic data to understand the fine scale temporal and spatial
427 distribution of *C. beticola* genotypes on different resistant hybrids grown under the same
428 environmental conditions during a single season. The dynamics of different pathogen
429 populations were assessed on their respective hybrids during each epidemic. Resistant
430 hybrids delayed the onset of epidemics, however, the host genotype did not affect the
431 likelihood of spread to neighboring plants. No differences in genetic diversity were found
432 between pathogen populations on different hosts, but we found high frequencies of some
433 MLGs on resistant hosts. The distribution of CLS hotspots tended to be random at the
434 beginning and aggregated at the end of each epidemic in each hybrid, with a few
435 exceptions. We found that a single pathogen genotype could disperse over a hundred
436 meters during an epidemic, illustrating the possibility of long-distance dispersal of individual
437 clones within fields during a single growing season.
438
439 **Onset of the disease varied according to host genotype**
440 CLS emerged at least 3 weeks later in the hybrids with *BvCR4* (Hybrid_R2 and Hybrid_R3)
441 compared to the hybrids without *BvCR4* (Hybrid_S2 and Hybrid_S3). The delayed
442 appearance of CLS in resistant hybrids at both sites suggests that the resistant hybrids
443 suppressed the establishment of CLS early in the season, agreeing with observations from
444 earlier field trials conducted by KWS (personal communication). Similar delays (2 to 4
445 weeks) in the onset of CLS on other resistant sugar beet hybrids were also reported in

446 previous studies (Karaoglanidis et al., 2000; Weltmeier et al., 2011; Wolf and Verreet, 2005).
447 It is possible that we did not always detect the start of an epidemic due to the size of the
448 plots and our sampling frequency. For example, for Hybrid_R2 in Rudolfingen and Hybrid_S2
449 in Hendschiken, we collected leaves from 43 and 22 plants respectively on the first day of
450 sampling (Table 2), suggesting that the epidemic had already started in these plots in the
451 days between our sampling visits. For the six remaining hybrid plots, the number of plants
452 collected on the first sampling date was <10, suggesting that we caught the beginning of
453 those epidemics. If we consider only the six plots with less than 10 infected plants at the
454 onset of the epidemic, the average distance between infected plants was 23.0 m in
455 Hendschiken and 30.8 m in Rudolfingen. If we add the other two plots, the average distance
456 between infected plants was 23.9 m in Hendschiken and 25.7 m in Rudolfingen. Given that
457 sugar beets had not been grown at either field site for at least three years, our
458 interpretation in both cases is that the primary inoculum was airborne. This is consistent
459 with previous work demonstrating experimentally that the inoculum is dispersed mainly by
460 wind (Khan et al., 2008) and originates external to the field of interest (Knight et al., 2018).
461 Given the high genetic diversity found in the pathogen populations on these first infected
462 plants and the random distributions of primary infections across the fields in both locations
463 (Table S2), we hypothesize that the primary inoculum was mainly airborne ascospores.
464 Though the sexual stage of *C. beticola* has not yet been described, most population genetics
465 studies conducted thus far have indicated that sexual recombination is likely occurring
466 regularly in this pathogen (Birla et al., 2012; Groenewald et al., 2007b, 2006) and its closest
467 relatives include Mycosphaerella teleomorphs that are known to produce airborne
468 ascospores (Goodwin et al. 2001).
469
470 **Localized spread was not strongly affected by host genotype**
471 Host genotype did not affect the probability that a single infected plant would develop into
472 a hotspot, with about 80% of infection cases spreading to adjacent plants (Table 3), but the
473 speed of localized spread (involving 2-5 plants) varied between hybrids and fields. In
474 Rudolfingen, there was no difference among hybrids in how quickly CLS spread to
475 neighboring plants, but in Hendschiken, the spread was slower in Hybrid_R3 and faster in
476 Hybrid_R2 compared to the susceptible Hybrid_S2. This field-dependent pattern may be
477 attributed to differences in the distribution of the hybrid plots, in particular the

478 susceptibility of the hybrid planted in a neighboring plot. Previous studies suggested that
479 the level of host resistance did not influence the latent period, however, susceptible hybrids
480 produced more conidia per unit of necrotic leaf area (Oerke et al., 2019). Thus, plots planted
481 next to susceptible hybrids may experience higher local disease pressure. In Hendschiken,
482 Hybrid_R2 was planted next to the susceptible Hybrid_S3, while in Rudolfingen, Hybrid_R2
483 was planted next to the resistant Hybrid_R3, which might have acted as a buffer zone that
484 reduced the influx of conidia onto Hybrid_R2 (Figure S1). In addition to differences in local
485 spore production, differences in local microclimates may also have affected epidemic
486 development at each field site (Imbusch et al., 2021; Rossi et al., 2000; Vereijssen et al.,
487 2006). It also is possible that our weekly sampling interval was not frequent enough to
488 accurately monitor localized spread, as some hotspots spread from one to five plants
489 between two successive time points.
490
491 To summarize, our findings suggest that the host genotype strongly influenced the
492 emergence of CLS but had little impact on the probability that CLS would spread to adjacent
493 plants and form hotspots of infection. This is in line with previous work that demonstrated
494 that the latency of spore production was not influenced by host resistance (Rossi et al.,
495 2000). If *C. beticola* strains succeed in overcoming the resistance of a host and became
496 established on a resistant plant, their ability to spread to neighboring plants does not differ
497 from the speed of dispersal found on susceptible plants.
498
499 **Infection sites were not randomly distributed in fields**
500 At the end of the season, CLS infection sites were mostly aggregated (not randomly
501 distributed) with one exception (Table S2). The distributions of infections changed little
502 between the onset and the end of the epidemic, except for Hybrid_S2 and Hybrid_R2 in
503 Rudolfingen (Table S3). The minimum distance between infection cases was approximately
504 one meter, while the maximum distance reached 116 meters (Table 4). In line with earlier
505 findings, we observed qualitatively higher levels of CLS in Hybrid_S2, Hybrid_S3 and
506 Hybrid_R2 compared to Hybrid_R3 at the end of the season in both sites. Lesions coalesced
507 and caused wilting and death of entire leaves, leading to new growth from the roots in
508 hybrids S2, S3 and R2. Hybrid_R3 did not exhibit obvious lesion coalescence, leaf death, or
509 regrowth despite having many plants with CLS. This may reflect the shorter time interval

510 between disease onset and final sampling in Hybrid_R3. Studies have shown that later
511 infection results in lower yield losses for resistant varieties (Kaiser et al., 2010), and that the
512 delayed CLS onset observed in resistant hybrids was positively correlated with sugar yield
513 (Wolf and Verreet, 2009).
514
515 **Genotypic variation and spatial distribution of genotypes**
516 Earlier studies illustrated how pathogen populations with higher genetic diversity can have a
517 higher evolutionary potential to overcome R genes (McDonald and Linde, 2002; Stam and
518 McDonald, 2018). In our study, the average Nei's gene diversity based on six SSR markers
519 ranged from 0.40 to 0.57 (Table 5a). This is similar to what has been found in sugar beet
520 fields around the world (Knight et al., 2019). It is important to note that our estimates of
521 genotypic diversity were limited because only six SSR loci were used to identify putative
522 clones. To estimate how accurately the six SSRs identified clones, we compared the clones
523 identified using the SSR results with clones identified using whole genome sequences for
524 113 isolates where we obtained both types of data (Chen et al., 2024). The six SSR loci
525 correctly identified clones or unique genotypes in 84% of cases (47 out of 58 in Rudolfingen
526 and 48 out of 55 in Hendschiken). The incorrect assignments were a mix of wrongly
527 identifying two isolates as sharing the same genotype (underestimating genotypic diversity)
528 and failing to identify two isolates as being clones (overestimating genotypic diversity) at
529 roughly equal proportions (9.7% and 6.2% respectively). While this illustrates the potential
530 errors of our SSR genotypes, we expect that this error rate will be randomly distributed
531 across hybrids, so comparisons of relative genotypic diversity between hybrids remain valid.
532
533 The high genotypic diversity found in each population supports the hypothesis that *C.*
534 *beticola* populations undergo regular cycles of sexual recombination, as proposed in earlier
535 studies (Bolton et al., 2012; Groenewald et al., 2006; Groenewald et al., 2008; Vaghefi et al.,
536 2017a). We detected high genotypic diversity at small spatial scales, with 23 out of 48 tested
537 leaves infected by more than one MLG and 27 out of 48 tested hotspots containing more
538 than one MLG. Other studies also observed multiple SSR MLGs on a single leaf in table beet
539 and Swiss chard (Vaghefi et al., 2017c) and using other genetic markers (TAPD and DAMD)
540 on sugar beet (Moretti et al., 2006). Our data suggests that co-infection is common on both

541 resistant and susceptible hosts and that multiple strains may contribute to the spread of
542 disease within a CLS hotspot.
543
544 We hypothesized that virulent isolates would be more prevalent on *BvCR4* hybrids due to
545 their ability to overcome this resistance gene. If only a small fraction of isolates in the
546 primary inoculum are virulent and able to produce asexual secondary inoculum on the
547 resistant hybrids, we would expect these virulent clones to increase in frequency on
548 resistant hybrids during the epidemic, resulting in lower genotypic diversity and higher
549 clonal fractions on resistant hybrids compared to susceptible hybrids. Consistent with this
550 expectation, we detected higher clone fraction in resistant (BvCR+) hybrids in Rudolfingen
551 (Table 5b), but no difference at Hendschiken. Suggesting that the influence of this R gene on
552 pathogen genotypic diversity can vary between locations. Consistent with the clone fraction
553 variation, we observed skewed genotype frequencies on the resistant hybrids (Figure 3 and
554 4) most notably in Rudolfingen: one MLG (R_MLG77) appeared in 46 isolates exclusively in
555 resistant Hybrid_R2 and Hybrid_R3. In Hendschiken, the differences in frequency were less
556 striking, but one MLG (H_MLG75) appeared in 13 isolates solely found on the *BvCR4* hybrids.
557 A subset of the isolates collected from resistant hybrids were tested for virulence against
558 hybrids with and without *BvCR4* under field conditions in a later study. Under
559 experimentally elevated disease pressure, that study confirmed that the highest frequency
560 MLGs were virulent strains that could overcome *BvCR4* (Chen et al., 2024). It also found that
561 only half of the isolates collected from hybrids with *BvCR4* were virulent against this gene.
562 The lack of evidence for evolutionary bottlenecks in our study may be attributed to the
563 frequent coexistence of virulent and avirulent strains within the same hybrid. Most of our
564 temporal sampling occurred during the onset and development of the epidemic, thus we did
565 not capture the end-of-season dynamics which might show stronger bottlenecks following
566 clone competition. Transect or random sampling of the pathogen populations at the end of
567 the season may provide a better test of the effectiveness of resistant hybrids as selection
568 sieves.
569
570 **Source of inoculum and spatial distribution of MLGs**
571 One of our aims in this study was to identify the potential sources of primary inoculum for
572 CLS epidemics. We hypothesized that a CLS epidemic can be initiated by wind-dispersed

573 ascospores which are capable of long-distance dispersal over hundreds of meters or by
574 conidia on infected leaves from earlier crops that are moved by wind or watersplash. If the
575 primary inoculum in a plot originates from windborne ascospores coming from outside the
576 field, we would expect to find genetically different isolates sparsely and randomly
577 distributed across individual plants at the onset of an epidemic. Alternatively, if the primary
578 inoculum arises from locally produced asexual spores transmitted through watersplash from
579 crop debris or human activity (e.g. infested seeds or machinery), we would expect to find
580 the same genotype occurring simultaneously in different hotspots that might be aggregated
581 in the field (for the case of infested machinery). In plots planted with Hybrid_S2, at both
582 sites we observed unique genotypes in each hotspot, suggesting that unique genotypes led
583 to the establishment of each hotspot, and that epidemics were initiated by genetically
584 diverse primary inoculum coming from outside the fields. Similar patterns were observed in
585 Hybrid_R3 at both sites and in Hybrid_R2 in Hendschiken, with no or few isolates shared
586 between hotspots. But a different pattern was found for the resistant Hybrid_R2 in
587 Rudolfingen. In this epidemic, the genotypic diversity of the first infections was lower, and
588 the dominant genotype R_MLG77 appeared simultaneously 10 times in 8 hotspots
589 distributed widely across the field. The most parsimonious explanation is that asexual
590 spores originating from a neighboring unsampled border planted with the resistant hybrid
591 may have disproportionally affected the genotypic diversity found in the sampled Hybrid_R2
592 plot. We postulate that the R_MLG77 strain emerged from standing genetic variation and
593 attained a relatively high frequency in the border plots before being transmitted into the
594 sampled Hybrid_R2 plot (Figure S1). Except for Hybrid_R2 in Rudolfingen, we found that
595 genetically distinct primary inoculum was distributed widely across most plots (Table S2), as
596 expected for wind-dispersed ascospores originating from outside of the field. This finding is
597 in line with a previous study that found no sharing of MLGs between years, suggesting that
598 the dominant source of inoculum for an epidemic is external to the field of interest (Knight
599 et al., 2018).
600
601 Our experiment also aimed to better understand the dispersal pattern of secondary
602 inoculum within a season. In Rudolfingen, high frequency MLGs (≥5) were separated by
603 between 49 and 108 meters and in Hendschiken by between 9 and 68 m (Table 7). This is
604 the first study in the CLS pathosystem to measure the dispersal of individual genotypes

within a season. The dispersal distances we observe are unlikely to be due solely to rainsplash, as rainsplash can only disperse *C. beticola* spores short distances (several meters) (Carlson, 1967). Wind dispersal is a more likely mechanism, and although wind was already recognized as an important mechanism, it was not clear before how far wind could disperse *C. beticola* (Khan et al., 2008). Other possible dispersal mechanisms cannot be ruled out. It is possible that insects could also disperse the spores, along with human activity, including machine operations or spores moving on contaminated clothing during our weekly sampling visits.

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Data Availability Statement

The data that support the findings of this study are provided in the Supplementary information files.

Conflict of Interest

The work was co-authored and funded by the seed company KWS SAAT SE & CO. KGaA. PhD student Chen Chen was funded by a research grant from KWS SAAT SE & CO. KGaA to the McDonald lab at ETH Zurich. H. Keunecke, E. Nue, F. J. Kopisch-Obuch are employees of KWS SAAT SE & CO. KGaA.

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817 **Table 1**. Sugar beet hybrids and their resistance to Cercospora leaf spot.

Hybrids	**Cercospora leaf spot resistance level**
Hybrid_S2	Low quantitative resistance
Hybrid_S3	Moderate quantitative resistance
Hybrid_R2	*BvCR4* with low quantitative background resistance
Hybrid_R3	*BvCR4* with moderate quantitative background resistance

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820 **Table 2**. The first appearance of CLS, the number of plants sampled at the first sampling
821 date (No. plants), the number of days after the first appearance of symptoms on July 21st
822 (onset delay), the total length of the sampling period, and the last sampling date (the right
823 censored date is shown in parenthesis, see Methods) for each hybrid at each field site.
824

Location	Hybrid	First infection date	Onset delay	No. plants	Sampling period	Last sampling date (right censored date)
Hendschiken	Hybrid_S2	07-21-20	0	22	30	08-20-20 (08-11-20)
	Hybrid_S3	07-21-20	6	1	24	08-20-20 (08-11-20)
	Hybrid_R2	08-11-20	21	6	33	09-08-20 (09-03-20)
	Hybrid_R3	08-20-20	30	3	35	09-24-20 (09-03-20)
Rudolfingen	Hybrid_S2	07-21-20	0	7	30	08-20-20 (08-11-20)
	Hybrid_S3	07-27-20	6	2	24	08-20-20 (08-11-20)
	Hybrid_R2	08-25-20	35	43	14	09-08-20 (09-03-20)
	Hybrid_R3	08-20-20	30	4	35	09-24-20 (09-03-20)

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827 **Table 3.** The number of infection cases (IC) and the proportion of these that developed into
828 hotspots (≥2 infected plants) and the proportion that developed into large hotspots (≥5
829 infected plants) for each hybrid. The data has been right censored (see Methods).

Location	Hybrid	No. IC	Number of hotspots (proportion)	Number of large hotspots (proportion)
Hendschiken	Hybrid _S2	37	26 (0.70)	5 (0.14)
	Hybrid _S3	20	17 (0.85)	4 (0.20)
	Hybrid _R2	22	18 (0.82)	7 (0.32)
	Hybrid _R3	30	24 (0.80)	4 (0.13)
Rudolfingen	Hybrid _S2	23	19 (0.83)	9 (0.39)
	Hybrid _S3	18	17 (0.94)	6 (0.33)
	Hybrid _R2	29	26 (0.90)	9 (0.31)
	Hybrid _R3	31	23 (0.74)	5 (0.16)

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831

832 **Table 4.** The number of geotagged infection sites and the minimum, mean and maximum
833 pairwise distance (m) between infection sites at the beginning and the end of the sampling
834 period in each hybrid.
835

Location	Hybrid	Pairwise distance at the start of epidemics				Pairwise distance at the end of epidemics			
		Count	Min	Mean	Max	Count	Min	Mean	Max
Rudolfingen	Hybrid _S2	14	2.2	28.2	73.7	41	1.3	36.7	110.7
	Hybrid _S3	4	3.8	54.7	98.6	31	1.3	47.8	116.3
	Hybrid _R2	15	1.3	19.7	62.0	37	1.3	29.2	108.3
	Hybrid _R3	8	4.2	33.9	94.5	88	0.2	37.2	110.0
Hendschiken	Hybrid _S2	14	1.3	24.7	72.8	37	0.8	26.0	72.8
	Hybrid _S3	8	1.3	20.0	51.1	24	1.3	23.2	64.6
	Hybrid _R2	6	4.2	29.7	57.3	41	1.3	27.2	76.0
	Hybrid _R3	9	3.1	22.5	58.3	66	0.2	21.6	68.5

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838 **Table 5.** Indices of gene and genotype diversity based on six SSR loci for *Cercospora beticola*
839 populations collected from a) four sugar beet hybrids in two fields in Switzerland. Table
840 includes the number of strains (N), the number of multilocus genotypes (MLGs), the
841 expected number of MLGs after rarefaction (eMLGs), the clonal fraction (CF=1-(MLGs/N), the
842 Simpson's complement index (λ) and Nei's index of gene diversity before clone correction
843 (He_0) and after clone correction (He_{cc}). b) Genotype diversity compared between
844 Susceptible (BvCR-) and Resistant (BvCR+) hybrids combibned.

845 a)

Location	Hybrid	N	MLGs	eMLGs	CF	λ	He_0	He_{cc}
Hendschiken	Hybrid_S2	30	20	15.6	0.33	0.91	0.56	0.60
	Hybrid _S3	22	16	16	0.27	0.92	0.51	0.54
	Hybrid _R2	43	28	17.3	0.35	0.95	0.52	0.54
	Hybrid _R3	63	37	16.2	0.41	0.94	0.56	0.58
	Total/Mean	**158**	**82**	**-**	**0.48**	**0.97**	**0.56**	**0.56**
Rudolfingen	Hybrid _S2	49	27	16.7	0.45	0.95	0.51	0.54
	Hybrid _S3	23	19	19	0.17	0.94	0.57	0.60
	Hybrid _R2	40	16	10.7	0.60	0.73	0.40	0.56
	Hybrid _R3	70	35	14.1	0.50	0.85	0.52	0.57
	Total/Mean	**182**	**77**	**-**	**0.58**	**0.92**	**0.55**	**0.57**

846

847 b)

Location	Hybrid	N	MLGs	CF	Chisq	*p*-value
Hendschiken	Susceptible	52	36	0.31		
	Resistant	106	65	0.39	0.63	0.42
Rudolfingen	Susceptible	72	46	0.36		
	Resistant	110	51	0.54	**4.68**	**0.03**

848

849 **Table 6.** Genotypic diversity within (a) a leaf and (b) a hotspot (HS). The first column
850 indicates a frequency class relating to the number of isolations per leaf/hotspot. There are
851 three classes: 2, 3 or 4 isolations were made per leaf leaf/hotspot. The table includes the
852 number of leaves/hotspots that had only one MLG (all clones) and with more than one MLG.
853 The proportions are provided in parenthesis.
854
855 (a)

Number of isolations per leaf	Number of leaves	Single MLGs Number (proportion)	Multiple MLGs Number (proportion)
2	41	21 (0.51)	20 (0.49)
3	6	3 (0.50)	3 (0.50)
4	1	1 (1.00)	0
Total	**48**	**25 (0.52)**	**23 (0.48)**

856
857 (b)

Number of isolations per HS	Number of HS	Single MLGs Number (proportion)	Multiple MLGs Number (proportion)
2	34	17 (0.50)	17 (0.50)
3	11	3 (0.27)	8 (0.73)
4	3	1 (0.33)	2 (0.67)
Total	**48**	**21 (0.43)**	**27 (0.57)**

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859

860 **Table 7.** The median, mean and the maximum pairwise distance (m) between the most

861 frequent MLGs that were found at least five times in a field site.

	MLG	Count	Median	Mean	Maximum
Rudolfingen	R_MLG77	46	16.6	22.8	108.5
	R_MLG56	7	43.6	38.9	100.5
	R_MLG13	6	7.5	16.3	49.8
	R_MLG42	6	58.4	48.0	107.8
	R_MLG15	5	51.9	43.5	105.0
	R_MLG20	5	14.9	28.5	74.0
	R_MLG46	5	31.9	23.2	64.4
	R_MLG55	5	19.2	30.0	77.1
Hendschiken	H_MLG75	13	4.2	7.8	20.5
	H_MLG51	11	15.7	18.9	46.7
	H_MLG9	9	3.7	4.2	9.4
	H_MLG77	8	15.0	13.5	36.7
	H_MLG82	8	29.3	27.1	68.7

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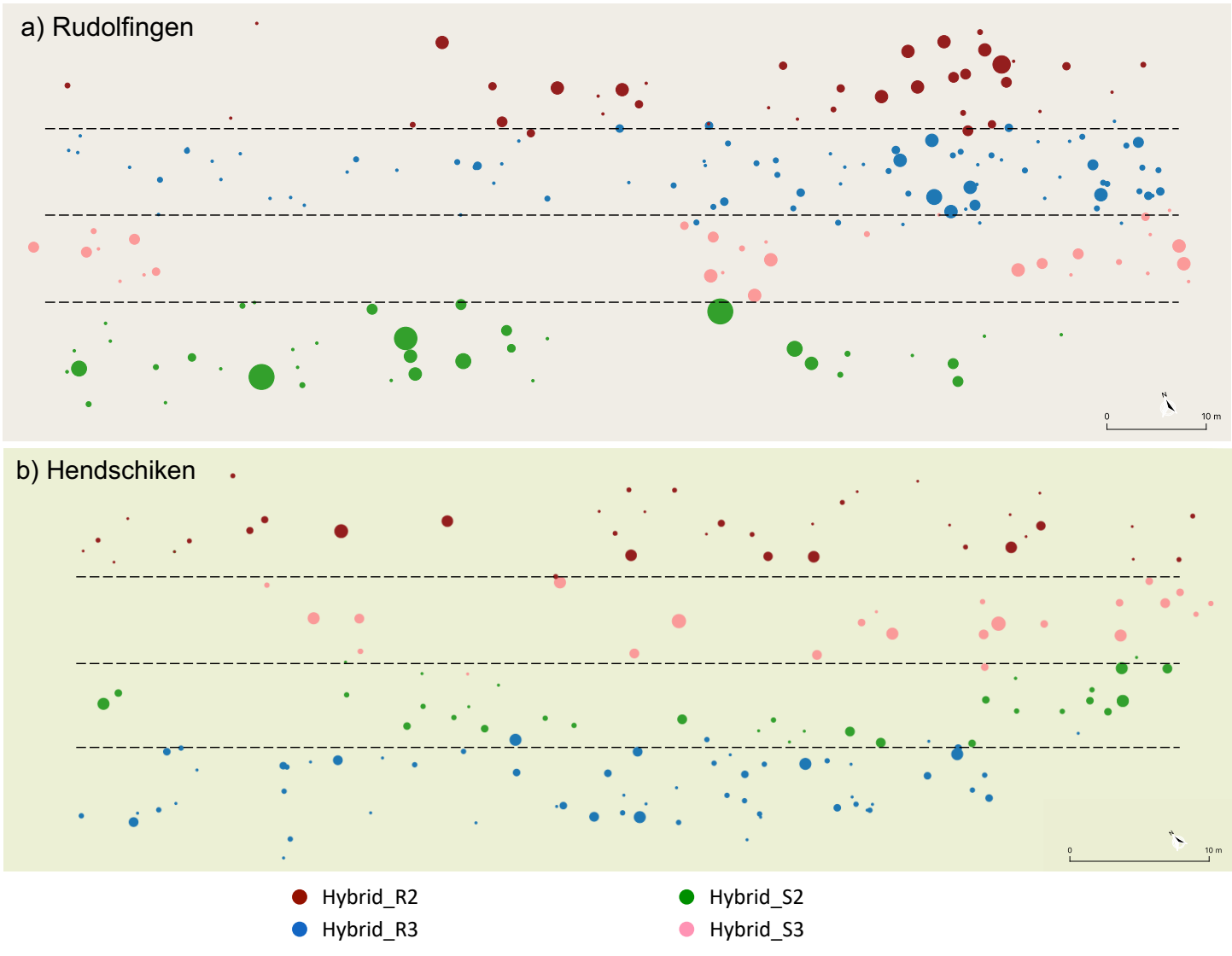
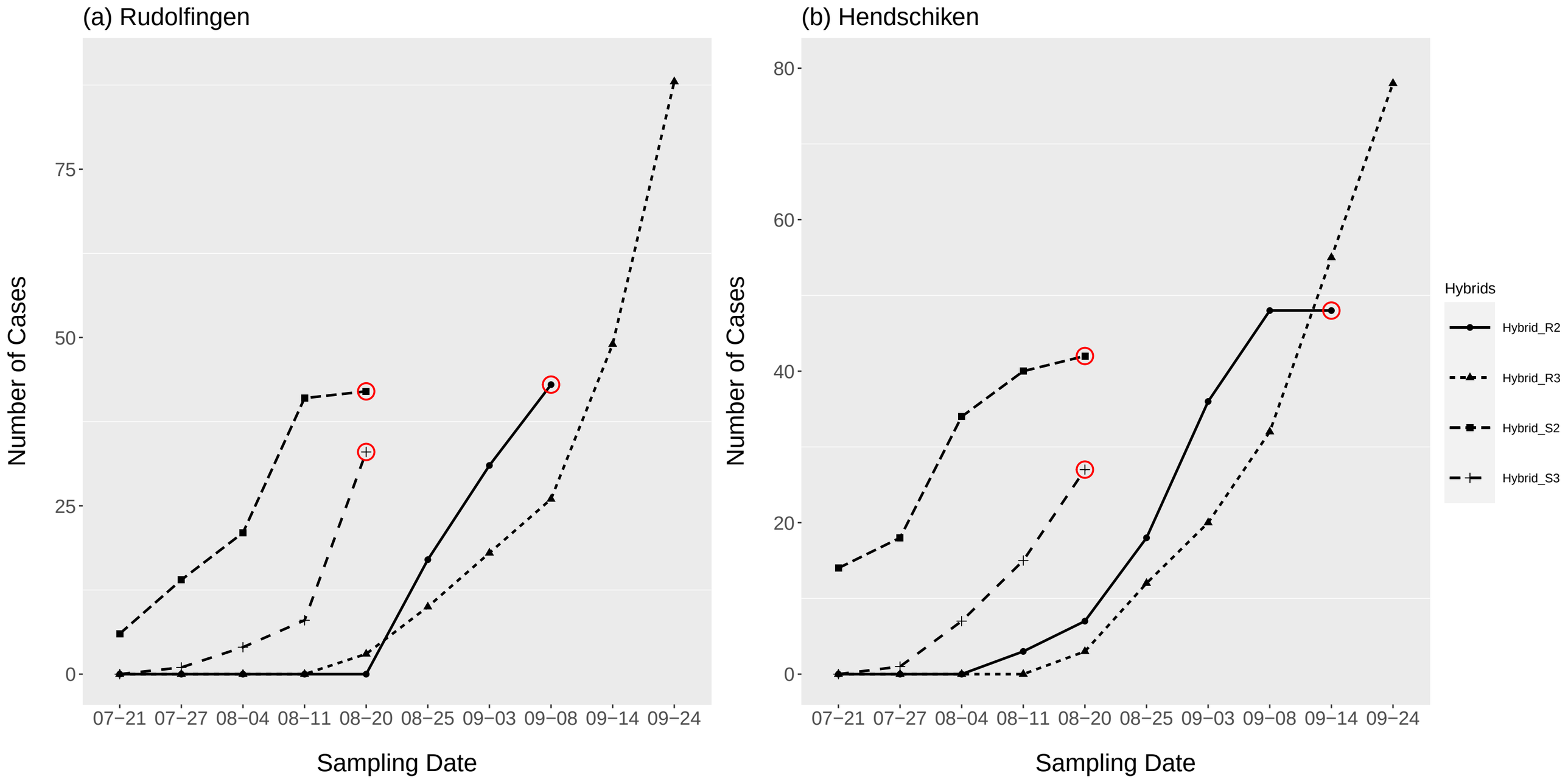
Figure 1. Spatial distribution of CLS in Rudolfingen (a) and Hendschiken (b). Each point represents a geotagged infection case. The size of the point indicates the number of sampled plants within the hotspot. The point color indicates the plant hybrid. Plant hybrid was determined by counting the planted rows from a reference point and using the different hybrid morphology. The dashed lines approximate the separation of the different hybrids, but there was no physical separation between hybrids. Thus, it can appear that one plot contains more than one hybrid (points with different colors), but this is an artifact of plotting and scaling the figure.

Figure 2. Accumulation of geotagged infection cases over time in Rudolfingen (a) and Hendschiken (b) in each hybrid. Sampling was terminated on different dates in each hybrid (indicated by red circles) when the disease had progressed to a point where new isolated infections could not be differentiated from already sampled hotspots (see methods and Table 2).

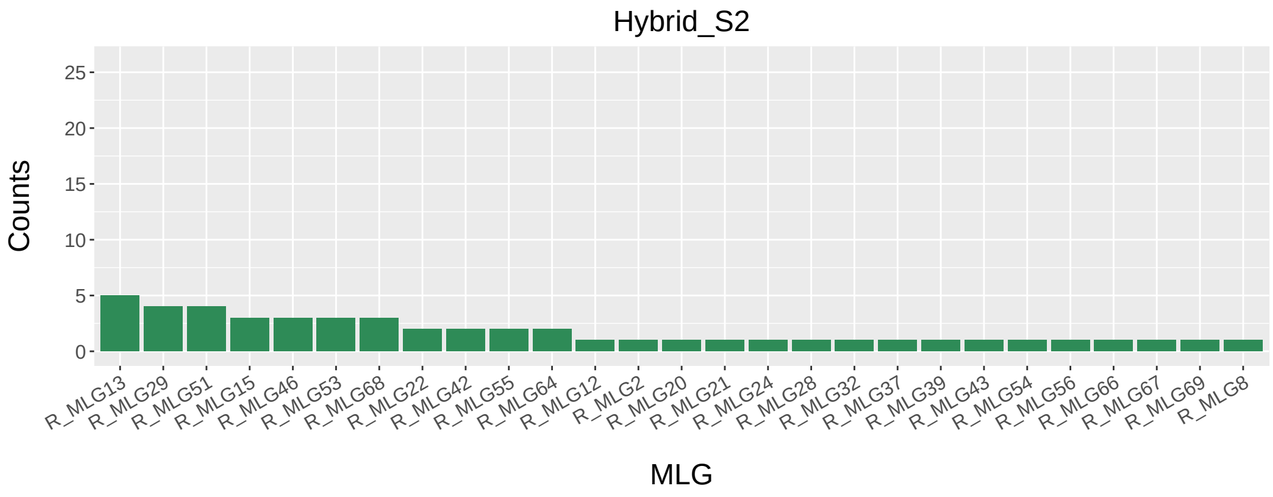
Figure 3. Frequency of each multilocus genotypes (MLGs) in each hybrid in Rudolfingen (a) and Hendschiken (b).

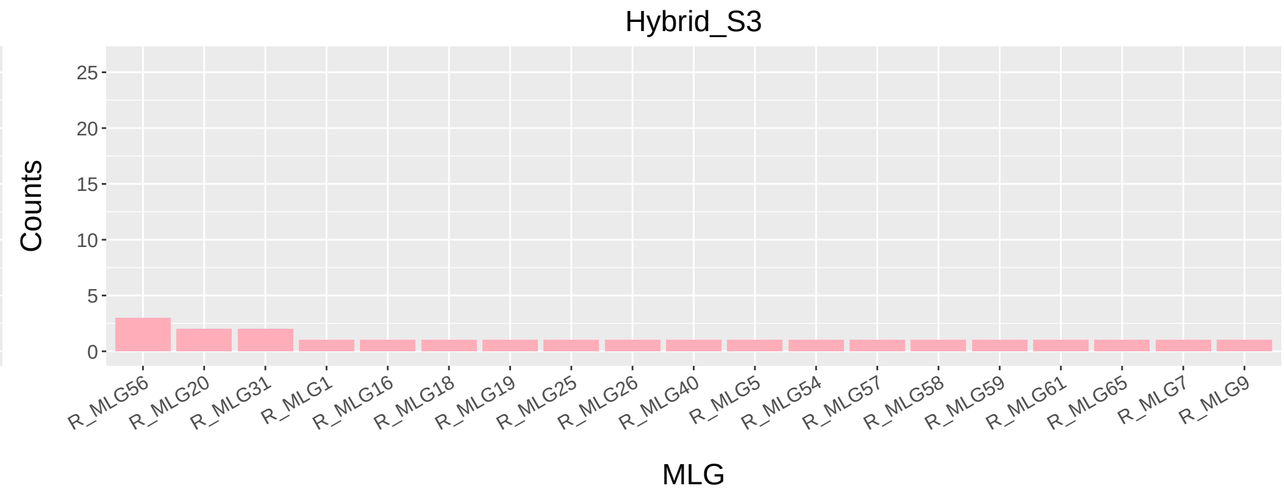
Figure 4. Evenness of multilocus genotypes (MLGs) in each hybrid in Rudolfingen (a) and Hendschiken (b). Points are the values of evenness and the error bars indicate the bootstrapped 95% confidence intervals.

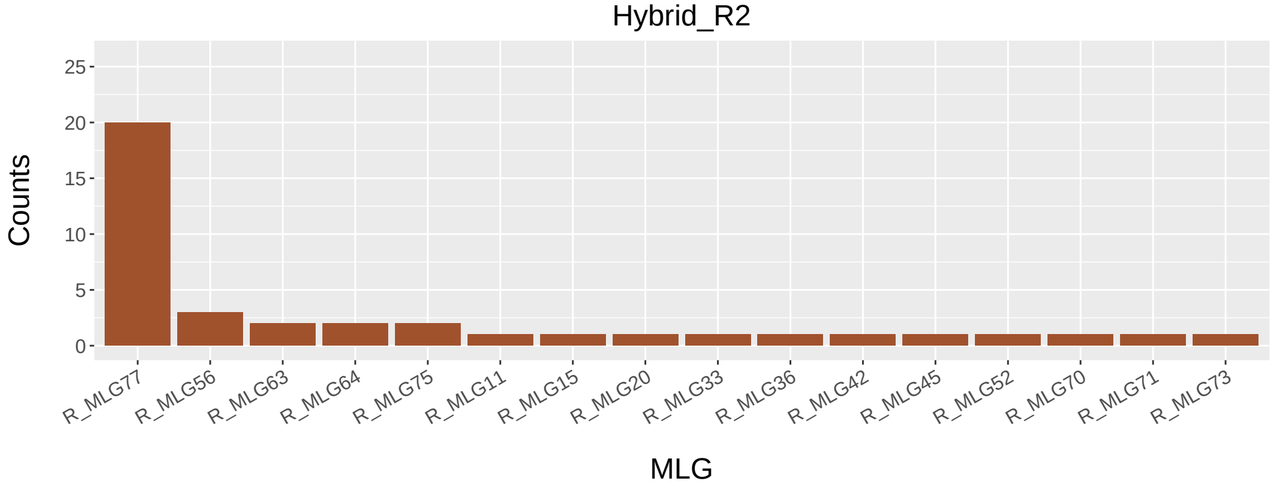

a) Rudolfingen
0
10 m
b) Hendschiken
0
10 m
Hybrid_R2
Hybrid_R3
Hybrid_S2
Hybrid_S3

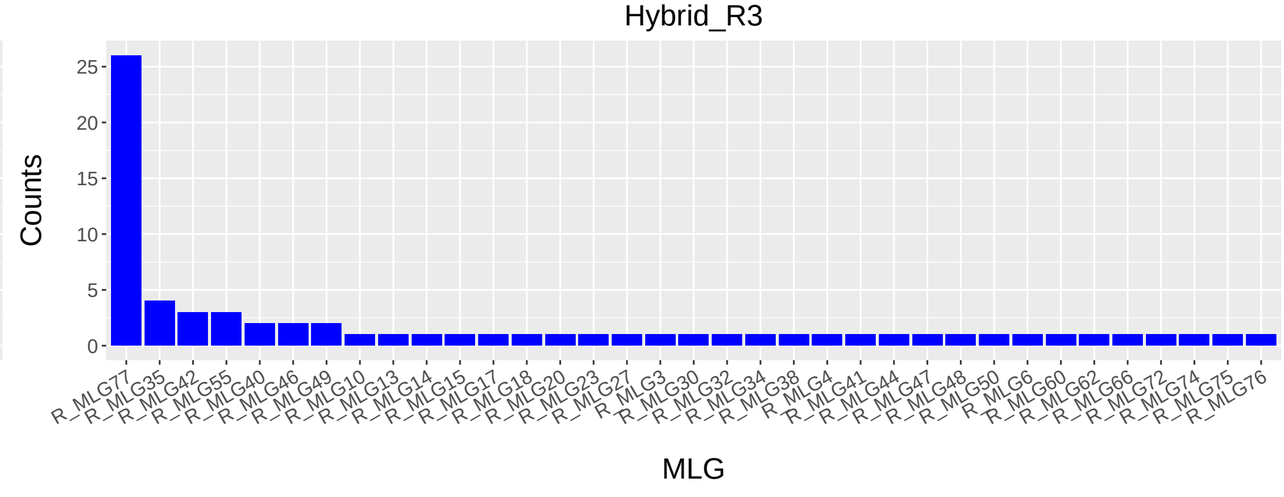

(a) Rudolfingen
(b) Hendschiken
Number of Cases
Sampling Date
0
25
50
75
20
40
60
80
07−21
07−27
08−04
08−11
08−20
08−25
09−03
09−08
09−14
09−24
Hybrids
Hybrid_R2
Hybrid_R3
Hybrid_S2
Hybrid_S3

(a) Rudolfingen

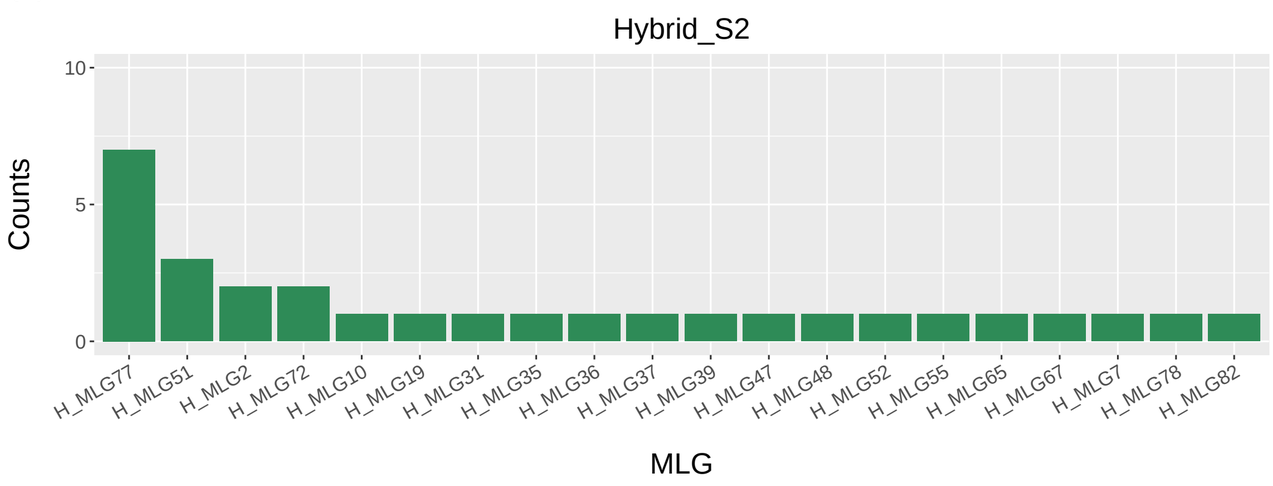


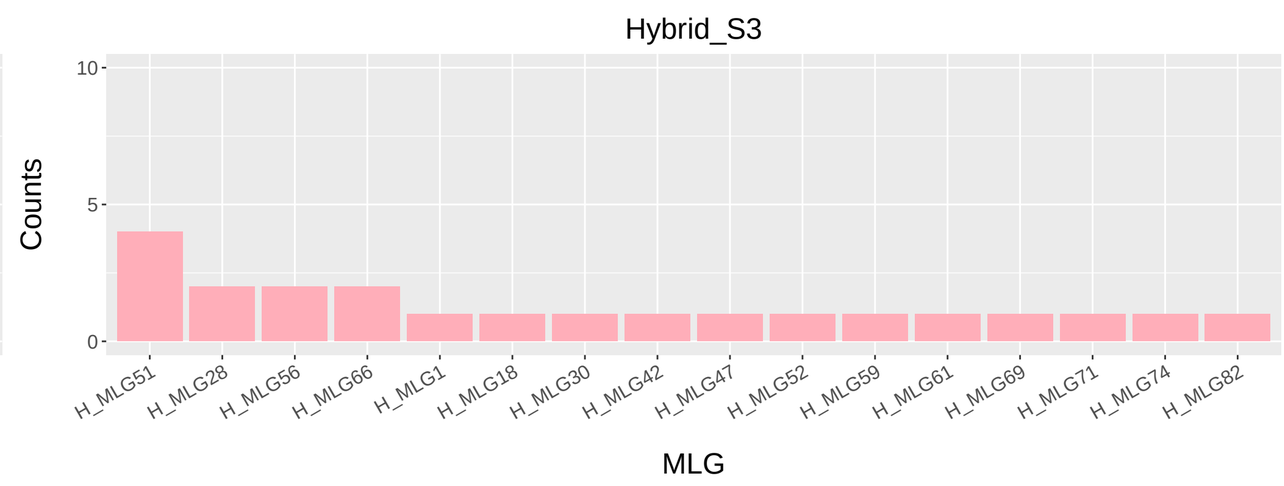


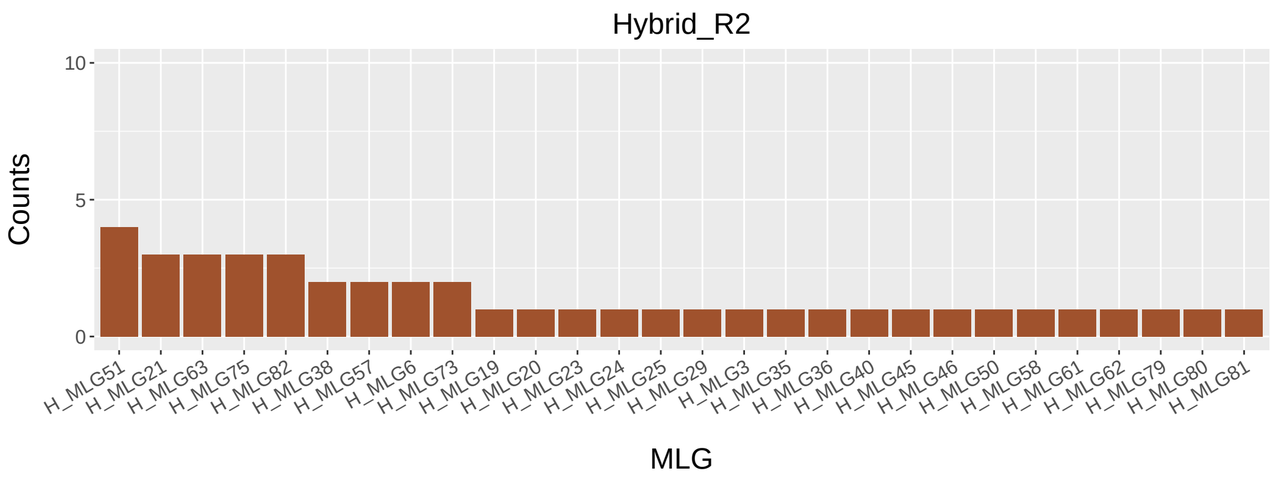


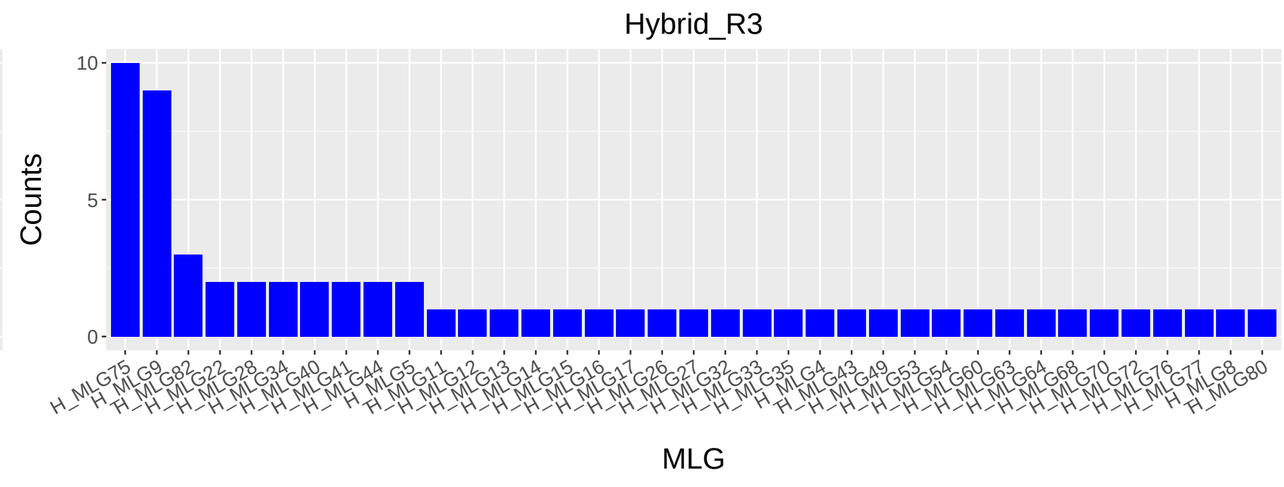


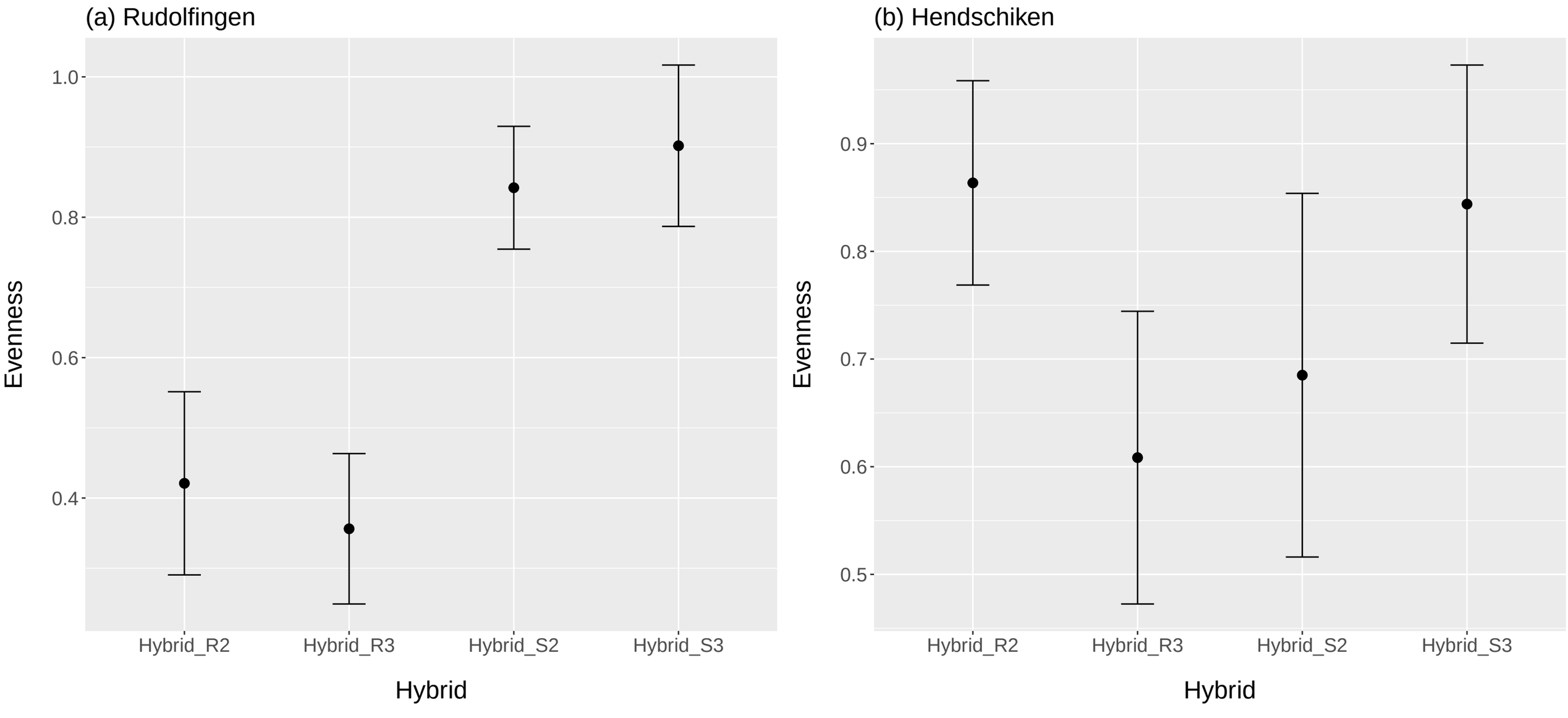
(b) Hendschiken










(a) Rudolfingen
Evenness
1.0
0.8
0.6
0.4
Hybrid_R2
Hybrid_R3
Hybrid_S2
Hybrid_S3
Hybrid
(b) Hendschiken
Evenness
0.9
0.8
0.7
0.6
0.5
Hybrid_R2
Hybrid_R3
Hybrid_S2
Hybrid_S3
Hybrid