CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2019 to March 31, 2020

BREEDING CRISPHEAD AND LEAFY LETTUCE

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SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, and *Verticillium* and *Fusarium* wilts into crisphead and the four leaf types suitable for California. Resistance for downy mildew is being introduced from 46 new sources and combined with resistance to *Verticillium* wilt race 1 and corky root. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes. New sources of resistance in our breeding program are more effective than the known resistance genes. Deployment of multiple new sources in combination will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. We continue to screen for high levels of resistance to *Verticillium* race 2 and have initiated the introduction of one QTL for resistance to *Verticillium* race 2.

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PROJECT TITLE: **BREEDING CRISPHEAD AND LEAFY LETTUCE**

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CONTINUING OBJECTIVES:

1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced crisphead and leafy breeding lines to maximize the likelihood of durable resistance.

2) To monitor variation in pathogen populations, particularly downy mildew, to facilitate the deployment of effective resistance genes.

3) To utilize the genetics of agriculturally important traits, particularly disease resistance.

4) To release advanced crisphead and leafy breeding lines which have resistance to multiple diseases, superior appearance and quality, high yielding ability, uniform maturity, and are slow bolting.

PROCEDURES AND RESULTS:

Development of Disease Resistant Lines

Breeding Strategy

The overall strategy used in the UC Lettuce Breeding Program continues to be to initiate crosses and grow early generations at Davis; later generations are trialed and selected at several different lettuce-growing areas in collaboration with USDA, Cooperative Extension in Salinas, and California growers. Backcross and/or single-seed descent strategies are employed for most of the early generations. We select for type, color, slow bolting, and yield as well as disease resistance in the crisphead and the four leaf lettuce plant types. As far as possible we use different sources of resistance for each plant type. This will diversify the selection pressure on the pathogen. The use of multiple sources of resistance will tend to increase the longevity of each resistance gene and decrease the chances that a single change in the pathogen will render multiple lettuce types susceptible.

When resistant advanced lines of the desired plant type have been generated for individual resistances, they are intercrossed to create lines with multiple disease resistances for lettuce downy mildew (LDM), corky root (CR), anthracnose (ANT), lettuce mosaic virus (LMV), and *Verticillium* race1 (*Ve1*). Additionally, we have screened germplasm to identify genetic resistances for *Verticillium* race 2 and *Fusarium* wilts, which are being incorporated into the breeding program.

Cultivars representing each type were selected to be the recurrent parents in backcross programs to introgress resistance genes based on their horticultural type and performance in California, their status in the public domain, and the presence of additional disease resistance genes. We are currently using Salinas, Green Towers, Tropicana, Red Fox, Red Tide, Bibb and Buttercrunch for the crisphead, romaine, green leaf, red leaf, and butterhead programs, respectively (Table 1). We welcome suggestions and further input on the field performance of these and other potential recurrent parents. The recurrent parents used in the final generations of backcrossing will be adjusted to reflect the industry standards as they advance.

Introgression of Resistance to Downy Mildew

We are continuing to develop crisphead and leafy lettuce lines with resistance genes from diverse sources to provide protection against downy mildew in California. We are focused on generating advanced breeding lines with new resistance genes identified through germplasm screens in previous years (Table 1). Mapping and characterization of the new sources are in progress to provide a pipeline of new genes for resistance. Backcrossing programs to introgress the next generation of genes for resistance to DM into crisphead and leafy types is a continual ongoing process.

Table 1: Status of introgression of new sources of DM resistance into crisphead and leafy types.

Accession	*Lactuca*	Type	Status	Category
PI491000	*L. saligna*	Green Leaf	BC_7S_1 testing for Hm	Advan. Donor
05G1411	*L.serriola*	Green Leaf	BC_7S_1 testing for Hm	Advan. Donor
ISR-380	*L. serriola*	Romaine	BC_7	Advan. Donor
KYRGY-237	*L. serriola*	Romaine	BC_7	Advan. Donor
GEOR-289	*L. serriola*	Romaine	BC_7	Advan. Donor
GEOR-297	*L. serriola*	Red Leaf	BC_7	Advan. Donor
CHEC-023	*L. serriola*	Iceberg	BC_7	Advan. Donor
CHEC-063	*L. serriola*	Iceberg	BC_7	Advan. Donor
CHEC-132	*L. saligna*	Green Leaf	BC_6	Advan. Donor
RUSS-635	*L. serriola*	Red Leaf	BC_6	Advan. Donor
CHEC-022	*L. serriola*	Iceberg	BC_6	Advan. Donor
CHEC-075	*L. serriola*	Iceberg	BC_6	Advan. Donor
CHEC-082	*L. serriola*	Iceberg	BC_6	Advan. Donor
CHEC-088	*L. serriola*	Iceberg	BC_6	Advan. Donor
CHEC-089	*L. serriola*	Iceberg	BC_6	Advan. Donor
CHEC-147	*L. saligna*	Romaine	BC_5	Advan. Donor
CHEC-083	*L. serriola*	Iceberg	BC_5	Advan. Donor
CGN5309	*L. serriola*	Green Leaf	BC_5	Advan. Donor
CHEC-136	*L. saligna*	Red Leaf	BC_4	Intermediate
GEOR-301	*L. serriola*	Romaine	BC_4	Intermediate
GEOR-292	*L. serriola*	Green Leaf	BC_4	Intermediate
GEOR-299	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-076	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-101	*L. saligna*	Romaine	BC_3	Intermediate
05G1421	*L.serriola*	Butterhead	BC_3	Intermediate
CHEC-108	*L. saligna*	Green Leaf	BC_3	Intermediate
AZER-805	*L.serriola*	Red Leaf	BC_3	Intermediate
AZER-811	*L.serriola*	Romaine	BC_3	Intermediate
AZER-822	*L.serriola*	Red Leaf	BC_3	Intermediate
AZER-840	*L.serriola*	Romaine	BC_3	Intermediate
AZER-843	*L.serriola*	crisphead	BC_3	Intermediate
AZER-853	*L.serriola*	Green Leaf	BC_3	Intermediate
AZER-854	*L.serriola*	Green Leaf	BC_3	Intermediate
KYRGY-247	*L. serriola*	Butterhead	BC_2	Early donor
CHEC-158	*L. saligna*	Green Leaf	BC_2	Early donor
CGN13330	*L. saligna*	Romaine	BC_1	Early donor
RUSS-653	*L.serriola*	Butterhead	BC_1	Early donor
GEOR-282	*L. serriola*	Butterhead	BC_1	Early donor
GEOR-284	*L. serriola*	Red Leaf	BC_1	Early donor
GEOR-288	*L. serriola*	Iceberg	BC_1	Early donor
CGN5157	*L. saligna*	Green Leaf	BC_1	Early donor
ARM09-158	*L. serriola*	crisphead	BC_1	Early donor
ARM09-169	*L. serriola*	crisphead	BC_1	Early donor
ARM09-172	*L. serriola*	crisphead	BC_1	Early donor
CGN5301	*L. saligna*	romaine	F_1	Early donor
AZER-871	*L.serriola*	Butterhead	F_1	Early donor

Each line is tested in each generation against a current, highly virulent isolate and the resistant progeny used as the resistant parent for the next generation. The isolate used for selection changes during the backcross process to reflect variation in *B. lactucae* in the field. A subset of advanced lines were tested for resistance against a set of six different isolates (See Table 2). Selfed generations of advanced lines (BC_6S_1) are then tested for resistance to a diverse panel of contemporary virulent Californian isolates as well as planted in trap crop nurseries (see below). Lines showing susceptibility are discarded. We are genetically characterizing the chromosomal position of all of our resistance genes. Once these genomic regions are identified, molecular markers will be used for MAS for selection of lines with multiple resistance genes that confer resistance to all Californian isolates. Release of lines with pyramids of several resistance genes should increase the durability of the individual genes and provide longer lasting resistance.

Table 2: Subset of 10 advanced BC_6S_1 lines tested for resistance against six different isolates of LDM (1825, 1485, 1909, 1691, 2000, and 1769). Selecting isolate = isolate previously used to select for resistance. Green indicates resistance and red susceptibility.

B. Line	selecting isolate	1825	1485	1909	1691	2000	1769
CHEC-132	1825		-				
RUSS-635	1909		-				
ISR-380	1825/1909		-				
	1825/1909		-				
	1825/1909		-				
	1825/1909		-				
	1825/1909		-				
	1825/1909		-				
KYRGY-237	1909		-				
	1909		-				
	1909		-				
	1909						
	2000						
	2000						
GEOR-289	1691						
	1691						
	1691						
	2000						
GEOR-297	1691						
	2000						
	2000						
	2000						
	2000						
CHEC-023	1825						
	1825						
	1825						
CHEC-063	1909						
	1909						
CHEC-076	1909						
	1909						
	1909						
	2000						
	2000						
	2000						
CGN5309	1825						
	1825						
	1825						
	1825						
	1825						
	1909						
	2000						
	2000						

Isolate 1691 was able to overcome the resistances of selections previously made with isolates 1825 and 1909. We are discontinuing the use of isolates 1825 and 1909 and prioritizing the use of isolates 1691 and 2000 for selection.

Downy Mildew Surveys

In order to ensure that we are breeding for resistance against virulent phenotypes of the pathogen *Bremia lactucae* currently present in California, we have continued to sample *B. lactucae* in collaboration with agricultural and seed companies, growers, and extension personnel. On average ~50 isolates have been characterized per year with a total of more than 2,243 isolates characterized since 1982. In most years, the sampling has been opportunistic; this provided a qualitative rather than a quantitative understanding of the phenotypes of isolates in the field. From September 2013 to June 2016, we were funded by the California Department of Food and Agriculture to conduct a detailed characterization of variation of *B. lactucae* in California. During this period the number of isolates characterized averaged ~180 isolates per year. This provided a comprehensive understanding of variation in California. Most sampling was again opportunistic in 2017 and 2018. Last year we received additional funding from the International Lettuce Genomics Consortium and characterized 173 isolates sampled from throughout the state. We also gathered isolates from lettuce lines specifically planted to trap naturally occurring *B. lacucae* in trap crop nurseries (see below). These isolates will be analyzed this year.

We maintain the *Bremia* database to display the virulence of California isolates characterized by us and others (http://bremia.ucdavis.edu/bremia_database.php). This database has information on the origin, virulence phenotype, mating type and fungicide sensitivity of isolates dating back to 2001. Isolates are entered as their phenotypes are characterized so that collaborators, pest control advisors, and others can access the data as soon as it is available; however, this is still several (4 to 8) weeks after an isolate is collected due to the need to increase each isolate prior to inoculation and scoring on the differential series of resistance cultivars. We have developed PCR-based assays that allows for more rapid characterization and analysis of large numbers of lesions (see below). We have data on 87 field isolates from 2019 and 2020. This year we will implement protocols to triage isolates into groups of similar isolates and to focus our phenotyping efforts on potentially novel and problematic isolates.

In 2014 the American *Bremia* Evaluation Board (ABEB) was initiated by interested breeding companies and UC Davis. This is a breeding company coordinated group that nominates official pathotypes (now races) for *B. lactucae* in the western US using a similar protocol to that used in Europe. In May 2015, it was decided to coordinate the activities in Europe and the Western US under a single IBEB-G(lobal) umbrella with two sub-groups IBEB-EU and IBEB–US responsible for activities in Europe and the US, respectively. Both groups now use the same core differential set of resistant cultivars so that data can be compared. Nomenclature has been standardized so that denominated isolates are designated Bl:#EU or US. The US pathotypes I to VIII are now designated as races Bl:1US to Bl:8US. When isolates of the same virulence phenotype are observed in multiple years and locations in California that overcome important *Dm* genes, they will be nominated by IBEB-US for designation as a new race. Nominated isolates will be distributed to the companies to confirm their phenotype and stability; if confirmed, they will be designated as an official race and used in cultivar resistance descriptions. There are currently nine official races in California; however, Races Bl: 1US to Bl: 6US have not been observed for many years; therefore, they are not of agricultural relevance and are not

available for distribution and screening. Reference isolates for Bl: 7 and 8 were ring tested by the companies and found to be unstable on some lettuce lines. Isolates representing these races are being filtered on lettuce lines with R-genes to increase stability; these will then be ring tested again. Reference isolate Bl: 9US is available for screening; this isolate was derived by passaging a common but unstable isolate (referred to as Bl: 9US-progenitor) on FrSAL-1. Individuals interested in participating in IBEB-US should contact Nicki Phillips (nicki.phillips@enzausa.com).

Over the past year, 173 isolates of *B. lactucae* representing a combination of samples provided by collaborators from several regions in California and targeted sampling, were characterized for virulence phenotype, mating type, and metalaxyl sensitivity. In 2019, none of the isolates analyzed were Race Bl: 5US, 6US, or 7US; 4% were race Bl:8US; 1% were race Bl:9US; 8% were the virulence pattern of Bl:9US before it was stabilized through filtering, Bl:9US progenitor. The majority of isolates (87%) had novel virulence phenotypes that differed from the designated races (Fig. 1).

Figure 1: Frequency of downy mildew Pathotypes detected in CA 2012-2019

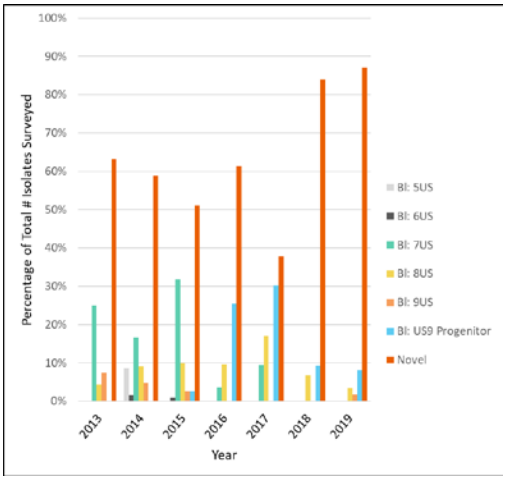
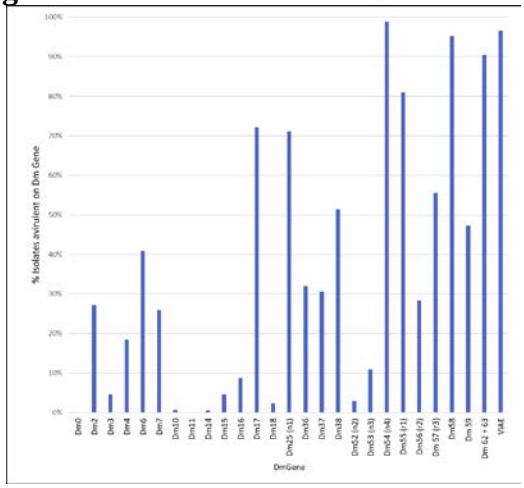


Figure 2: Frequency (%) of avirulence genes observed in California in 2019.



In 2017, we identified a group of novel isolates with similar virulence phenotypes. This virulence pathotype (VP-300) has been detected in multiple locations and over multiple years, and overcomes important *Dm* genes. It is characterized as being avirulent on *Dm6, Dm17, Dm25 (n1), Dm54 (n4), Dm55 (r1)* and Kibrille *(Dm53 + Dm11),* and virulent on *Dm38*. We identified 25 of these isolates (15% of typed isolates) in 2019. A candidate type isolate for this pathotype was distributed to seed companies for ring testing; however, its virulence phenotype was not stable. Therefore, we are filtering representative isolates on differential cultivars to produce a stable isolate and will then redistribute the isolate for ring testing.

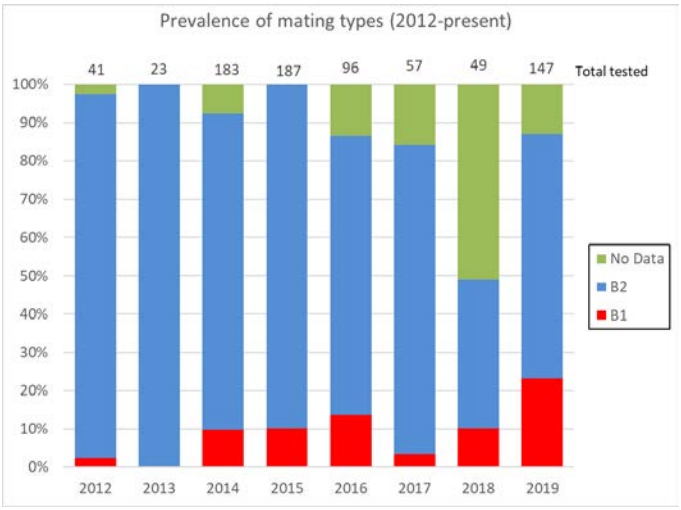
We have recently identified another group of isolates, usually collected from organic fields, with a common virulence pathotype (VP-301). This group of isolates is of the B_1 mating type and is characterized by being avirulent on *Dm2, Dm37, Dm38, Dm54 (n4),* and *Dm55 (r1),* and is virulent on NunDm17 (*Dm17),* RYZ2164 *(Dm25)* and Kibrille

(*Dm57 + Dm11*), all highly resistant lines. The avirulence phenotype has been found in 7 different locations and in 3 different years; it was identified 39 times in 2019 (23% of typed isolates). A candidate type isolate is being identified for ring testing this year.

The effectiveness of the resistance genes did not change significantly in 2019. The most effective resistance genes are in the newer lines Bartoli (*Dm54)* and RYZ20007 (*Dm58)* with 99% and 95% of all isolates typed being avirulent. *Dm17* remains moderately effective with 72% of isolates being avirulent. *Dm25* and *Dm55* are also moderately effective, with the frequency of *Avr25* decreasing to 71% and *Avr55* increasing to 81%. *Dm6, Dm36, Dm37, Dm38, Dm57* and *Dm59* continued to somewhat effect with 41%, 32%, 31%, 51%, 56%, and 47% of the isolates avirulent, respectively (Fig. 2).

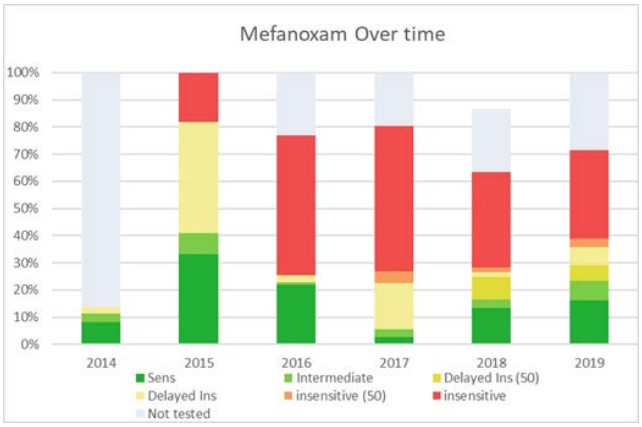
Of 128 isolates characterized for mating type in 2019, 60 (64%) and 14 (23%) were B_2 and B_1 respectively with the results for the 13% as inconclusive. The frequency of isolates of B_1 mating type varied during the years (Fig. 3) with the highest frequencies in years 2016, 2018 and 2019. Since 2018, B_1 mating type has been common in organic fields. Some of the recent increase in B_1 mating type reflects increased sampling of these fields.

Figure 3: Frequency of isolates with B_1 or B_2 mating types between 2012 – 2019.



Of the 56 isolates analyzed for metalaxyl sensitivity in 2019, 39%, 13%, 7%, and16% were insensitive, delayed insensitive, intermediate, and sensitive respectively (Fig. 4). Insensitivity is considered as the ability to sporulate by 15 dpi at 50 ppm or above. An intermediate reaction is considered as sporulation by 15 dpi at 5 or 10 ppm but not at 50 ppm. Sensitivity is considered as no sporulation by 15 dpi at 5 ppm or above. Isolates with sensitivity to metalaxyl are mostly found in organic fields.

Figure 4: Frequency of levels of (in)sensitivity from 2014-2019.



Trap Crop Nurseries

Trap crop nurseries were planted to assess the diversity of *B. lactucae* and to screen for isolates capable of breaking new resistance genes in lettuce lines under development. The nurseries were composed of small plots of resistant lines interspersed with highly susceptible wild and cultivated lines (Fig. 5). The resistant lettuce lines were resistance donors for UC breeding lines; resistant varieties from the EU-C differential series; and lines of interest for companies. The susceptible lines were a mixture of wild and cultivated lines. The trap crop nurseries were planted in Imperial Valley and Davis, California in November 2019 and February 2020 respectively. Due to the very dry weather, there was little disease pressure at either site. Isolates were collected from both fields in April 2020; however, *B. lactucae* was only observed on susceptible lines in Imperial Valley. Analysis of isolates from these trap nurseries is underway.

Figure 5. Part of the trap crop nursery growing at the Desert Research & Extension Center. Our thanks to Jairo Diaz-Ramirez and Gilberto Magallon who grew and scored this plot and collected isolates under difficult circumstances.

Development of molecular markers to quickly characterize isolates of *B. lactucae*

We are developing molecular markers to quickly characterize field isolates. Current phenotyping is too labor intensive and slow for real-time monitoring of *B. lactucae* populations. Genotyping isolates before phenotyping will allow increased sampling and a better understanding of population dynamics.

In 2019, simple sequence repeat (SSR) markers were developed and tested. The SF5 reference genome assembly and whole genome sequencing data from a diverse set of isolates was used to identify polymorphic regions in the genome of *B. lactucae*. Forty, PCR-based, SSR markers distributed over 14 scaffolds (90% of the genome) were identified. These markers were able to uniquely identify isolates from a diversity panel of 20 US isolates and a global collection of 26 other isolates. Using this data, twenty SSR markers were used to type 87 recently collected field isolates. In the next year, we will incorporate genotyping into our isolate typing protocol. We are distributing the SSR markers and preliminary data to interested collaborators.

Resistance to *Verticillium* wilt

Resistance to *Verticillium* wilt continues to be a high priority for our program. We previously developed an efficient, reliable, and contained method for screening for the reaction of lettuce to *V. dahliae* in the greenhouse. We utilize microplots within the greenhouse with restricted access to minimize the opportunity for spread of the pathogen (see previous reports). We continue to screen for resistance to *V. dahliae* strain VdLs17 (race 2) provided by Dr. Krishna Subbarao. We include cv. Salinas as the susceptible control genotype with cv. La Brillante representing a genotype that has resistance to race 1.

The majority of accessions screened for resistance over the past six years have been highly susceptible to race 2, although differences in symptoms have been observed. A few accessions of *L. serriola* showed delayed development of symptoms or were asymptomatic. In particular, seven lines from Armenia showed no symptoms when initially screened against VdLs17 (race 2) and exhibited no seed transmission. We developed a RIL population from a cross between *L. sativa* PI251246 that is highly susceptible to *Verticillium* race 2, and *L. serriola* acc. Arm09-170-1-5 that was asymptomatic. These lines have similar vegetative development patterns and flowering times. A QTL for resistance was identified in linkage group 6 that was in significant trials conducted in Davis and Salinas. The same QTL was significant in other populations (collaboration with German Sandoya; now at University of Florida). We are introgressing this QTL for resistance to *Verticillium* race 2 into cultivated lettuce.

In 2016, we received 67 new accessions of *L. serriola* from Azerbaijan; these were seed increased in 2017 and screened for resistance in the greenhouse in 2018. The experiment had random design without repetition. Up to six derived siblings were screened for each accession with six plants representing each sibling. Resistance was scored as absence of brown discoloration on the stem-root transition region. In addition, stems with no discoloration were plated on NP-10 medium to rule out the presence of *Verticillium.* For no accession were all the siblings tested was resistant. For five accessions, one or two siblings were resistant; these accessions may be segregating for resistance. We have crossed resistant individuals with susceptible lines (cvs. Salinas, La Brillante and Green Towers) and Armenian *L. serriola* that showed resistance previously to race 2. We have

now available F_2 seed from these crosses to confirm and study the genetic basis of this resistance.

Supply of Isolates

We have continued to supply current California isolates of downy mildew to breeding companies and other research groups. In particular, we have supplied isolates of the nominated races as well as candidate isolates for ring tests. We have trained personnel from the seed industry and others to handle lettuce downy mildew.