FINREP98

COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

PROJECT TITLE: **Breeding improved cotton varieties.**

PROJECT CODE: CSP70C

ORGANISATION: **CSIRO Cotton Research Unit**

Locked bag 59

Narrabri 2390

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Plain English summary

The CSIRO cotton breeding program based at Narrabri has been very successful in developing varieties adapted to all cotton production regions in Australia. At least 90% of the cotton grown in Australia are varieties developed at Narrabri.

The breeding program includes many objective including: high yield, high fibre quality, disease resistance and tolerance to insect damage. These targets are applied to cotton production areas that range from hot locations such as central Queensland to cool districts such as the upper Namoi valley and southern NSW. Such diverse locations require different characteristics for optimum production.

Breeding requires large numbers of experiments and breeding lines. This program now has in excess of 15,000 yield plots at 15 different locations; there are also about 30,000 single plants harvested at the early generation stage of the breeding process.

New varieties released to the Australian cotton industry in recent years include conventional types Siokra S-101, Sicot 189, Siokra V-16 and Sicala 40 and transgenic varieties Siokra L-23i, Sicot 189i, Sicala V-2i, Siokra V-15i and Sicot 50i. There are new breeding lines close to commercial release.

Background:

The CSIRO field breeding program is a mature project in that the breeding lines and procedures have now been established for a number of years. The following table shows the evolution of the program from the 1970's. Of particular note is the continued emphasis on yield and adaptation to Australian conditions and the change in emphasis of characters such as fibre quality, verticillium wilt tolerance and Bt cotton from being 'special purpose' to overall objective - all varieties are aimed to have those characteristics.

	1972-74	1975-91	1992-94	1994+
OVERALL OBJECTIVES	Yield Adaptation	Yield Adaptation Quality Blight res	Yield Adaptation Quality Blight res	Yield Adaptation Quality Blight res Verticillium tol Alternaria tol Bt genes
TB1 Full season project objectives	Host Plant Resistance (frego bract, glabrous leaf)	HPR (okra leaf, frego, glabrous, nectariless)	HPR (okra, frego, glabrous, nectariless, allelochemical)	HPR (okra, frego, glabrous, nectariless, allelochemical)
TB2 Objectives of special purpose projects	Earliness, quality	Earliness Verticillium tol Dryland	Earliness Verticillium tol Dryland Bt genes Nutrition Cold tolerance	Earliness Cold tolerance Verticillium tol+ Fusarium tol Dryland (WUE) *G barbadense* Herbicide tol Coloured cotton

Until this breeding program released varieties, Australian growers were completely dependent on foreign-bred cultivars with their shortcomings. This program is dedicated to producing cottons that will increasingly provide resistance to local diseases and pests besides progressively increasing yield and improving quality. By this means the stability and competitiveness of the Australian industry will be strengthened and fostered, assuring its long term future. In 1998, CSIRO varieties had about 90% of the Australian cotton planting seed market. International sales are now occurring through AgrEvo/CSI.

The research program involves crossing (about 100 per year), single plant selection plots (about 30000), progeny rows and replicated trials on the Australian Cotton Research Institute (about 8000 plots) and replicated trials on district sites (14 sites with about 8000 plots). As indicated above, there are a number of objectives in the breeding program with the final outcome being commercial varieties. Some of those specific objectives in addition to yield include:

Bt cotton. The crossing program for cotton plants expressing the Monsanto gene from *Bacillus thuringiensis* is now well advanced. All commercial varieties and promising breeding lines have one Bt gene or two different Bt genes. This material is now at the stage where large numbers of lines are being evaluated to ensure that the very best characteristics (including yield, quality, disease resistance, etc) that were present in the original variety, are present in the Bt transgenic lines. It must be emphasised that the breeding program has increased in size and complexity as a result of this aspect of the project. We are at the stage where the original non-transgenic program is now being duplicated to accommodate Bt lines. Transgenic material is provided by the Canberra group who also play an important role in screening transgenic lines. Many new genes will enter the breeding program over the next few years.

Herbicide tolerance. At least four different genes for herbicide tolerance are being incorporated with CSIRO varieties. Limited field plots were sown in 1995/96, with more detailed experiments since.

Fusarium wilt. This disease has now been confirmed as being present at Brookstead and Boggabilla. In 1994/95, we had preliminary experiments at Brookstead to evaluate possible differences between local and overseas lines that can be exploited by conventional breeding. The results of that experiment determined our next breeding steps, including a comprehensive crossing and screening program starting in 1996.

Adaptation to specific growing circumstances. Of particular note are early maturity for short season varieties and at the other end of the spectrum are types adapted to the hotter areas such as Central Qld (and possibly the Ord). We have advanced lines in the program with exceptional performance for different locations. The cold tolerance lines from Ross Downes' program (CSP64C) are being used for crossing and evaluated as part of this objective.

Host Plant Resistance. Conventional plant characters such as okra, sub-okra, super okra, frego bract, glabrous leaf, nectariless and allelochemicals (tannin, gossypol) have been incorporated into a high yielding background. This material is at various stages of the breeding program. Of particular interest is the search for positive interactions between some of these HPR characters and Bt cotton. This aspect is being examined for CSIRO varieties.

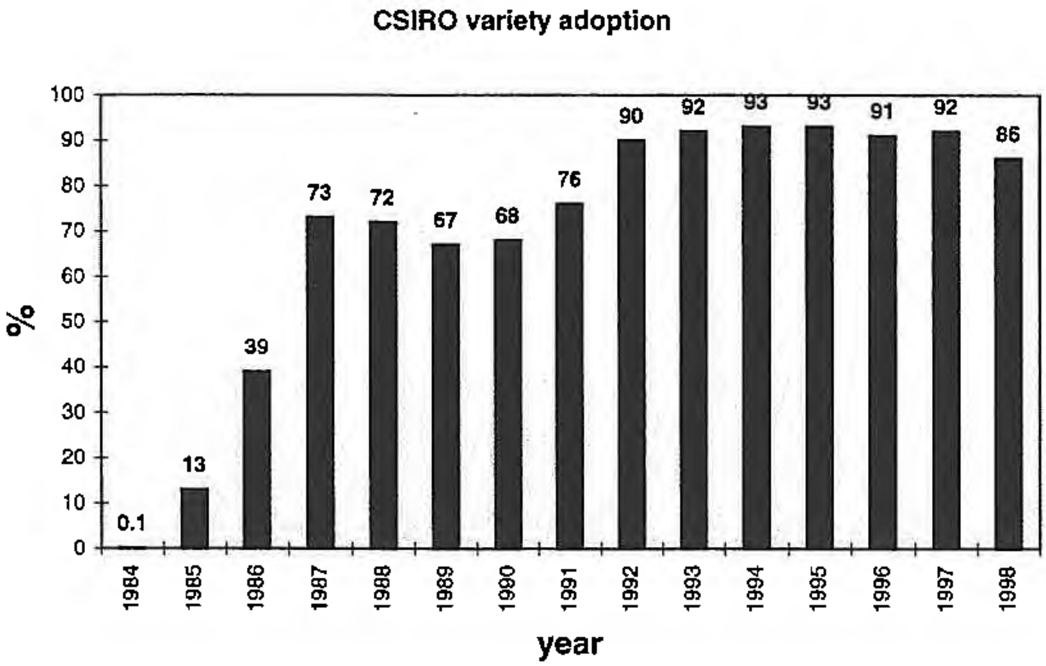
Fibre Quality. We are continually aiming to improve fibre properties in all new varieties. Although there are high prices at present it is important to maintain as high a quality as possible for years when supply is high. *Coloured cotton*: A small project has been initiated which aims to produce lines of Siokra V-16 with green or brown lint. The effort is small at this stage, but this market may grow in the next ten years and we should be ready to supply high yield and quality types if they are needed. *Pima cotton:* A separate CRC project aims to produce a bacterial blight resistant locally adapted Pima line (SIPIMA 2000). That project is fully integrated with the main breeding scheme.

Narrow rows. A number of lines are being developed with plant architecture which may be suitable for ultra-narrow row production. Some producers are testing narrow rows. This production technique may be suitable for southern NSW.

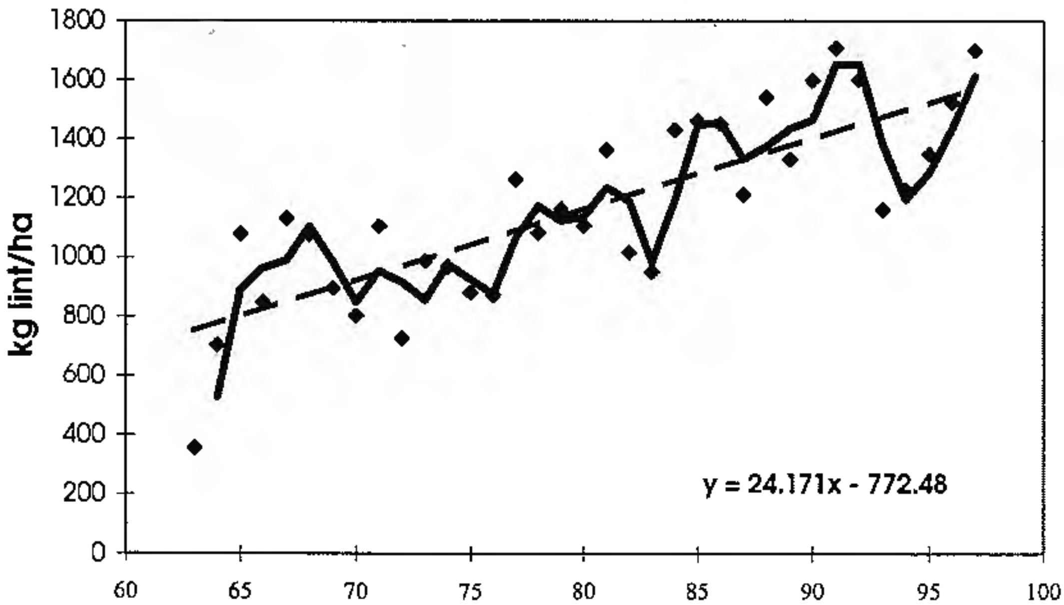
Water use efficiency. A separate CRC/CRDC project (CSP55C) is researching possible differences in WUE that can be exploited by breeding. That project is fully integrated with the main breeding scheme.

Objectives and achievements

The ongoing aim of developing improved cotton varieties adapted to Australian growing conditions and markets has been achieved to a large extent: CSIRO varieties have been adopted on approximately 90% of the crop area in Australia over the past seven seasons (figure 1).



Yield records over the past 30 years show a steady increase (figure 2) and our estimates are that 30% of that increase (8 kg/ha/year) have been due to the higher yield of CSIRO varieties.



Results

Collaboration/CRC linkages The CSIRO field breeding program is fully coordinated with the Biotechnology sub-program (headed by Dr. D. Llewellyn) based in Canberra. Their material is sent to Narrabri for evaluation as soon as it becomes available. All Narrabri and Canberra CSIRO staff are full time in kind contributions to the CRC for Sustainable Cotton Production. Dr Bruce Lyon at the University of Sydney is also collaborating with the breeding program in a number of projects. Mr Gavin Mann of DPI Queensland undertakes sowing and harvesting of Qld sites in Central Qld and Darling Downs.

Experiments. The research program involves crossing (about 100 per year), single plant selection plots (about 40,000), progeny rows and replicated trials on the Australian Cotton Research Institute (about 8,000 plots) and replicated trials on district sites (14 sites with about 8,000 plots). As indicated above under 'Background', there are a number of objectives in the breeding program with the final outcome being commercial varieties.

The last stage of the breeding program is the multisite (13 irrigated; 3 raingrown) Cotton Cultivar Trial. That data provides the most detailed data set on control varieties and new breeding lines. In the three years of this stage of CRDC funding, the following varieties were released to Australian cotton growers:

Conventional	*Transgenic*
Siokra S-101	Sicala V-2i
Sicot 189	Siokra V-15i
Siokra V-16	Sicot 50i
Sicala 40	Siokra L-23i
	Sicot S-8i
	Sicot 189i

Performance data on those varieties is shown in the attached tables.

Discussion

The comprehensive tables attached show the yield of 48 entries at all 13 sites in Australia. This procedure of multiple sites and seasons allows detailed analysis of interactions between genotype and environment. As such we have been able to identify new varieties with specific adaptation for particular regions, particularly hot areas in central Queensland and cooler areas in eastern Darling Downs, eastern Namoi Valley and southern NSW.

Assessment of impact on the cotton industry

Approximately 90% of seed sales in Australia are of varieties bred at ACRI. Adoption of new varieties by the Australian cotton industry is very quick and dynamic. The release and adoption of Sicala 40 for the 1998 sowing is a good example: about 16% of the seed sales (for the record sowing) was of this variety in its first year of release.

Recommendations

Plant breeding is a long term process and this project is on-going. Priorities for the program for the short term are:

- Continue heavy selection for high yield while maintaining or improving quality.
- Two gene insect transgenics to guard against insect resistance to the first Bt gene.
- Improved resistauce to diseases, particularly Fusarium wilt.
- Increased attention to varieties for hot areas (Central Qld, Bourke, NW Australia).

Publications

Constable, G.A., Reid, P.E. and Thomson, N.J. (1996). CSIRO cotton varieties - performance 1995/96 In 'CSD Variety Trial Results 1996', pp.44.

Constable, G.A., Reid, P.E. and Ferguson, G. (1996). Performance of new CSIRO INGARD varieties In 'Proc. 8th Aust. Cotton Conf.' , pp.561-564

Reid, P.E., Constable, G.A., Thomson, N.J., Mann, G., Heal, L.J. and Patrick, C.M. (1996). CSIRO small scale variety trials for 1994/95 and 1995/96 In 'Proc. 8th Aust. Cotton Conf.' , pp.573-575

Patent and Licensing Agreements - Plant Breeders Rights for Sicala V-2i, Siokra V-15i, Siokra L-23i, Sicot S-8i and Sicot 50i.

9410YMEANS

	1994/95 ACCT LINT YIELD													
		WA	BR	MV	MN	BK	CO	MO	BB	SG	DD	TH	BI	EM	MEAN
1	DPL 16	1065	864	1041	1431	1226	1536	1278	1849	1412	966	1329	1656	1550	1323
2	DPL 90	1141	927	1275	1447	1483	1650	1351	2022	1552	1318	1554	1803	1975	1500
3	NAMCALA	1396	1033	1307	1473	1317	1545	1361	1857	1406	1422	1371	1549	1395	1418
4	SIOKRA 1-1	1008	1096	1092	1635	1244	1894	1645	2116	1454	1062	1358	2042	1653	1485
5	SIOKRA L22	1349	960	1134	1635	1377	1743	1446	1989	1446	1279	1443	1916	1765	1499
6	SIOKRA L23	1222	1050	1391	1672	1489	1745	1511	2033	1436	1336	1652	2081	1924	1580
7	SIOKRA S324	1391	1359	1312	1759	1358	1971	1590	2193	1600	1355	1398	2062	1525	1606
8	SIOKRA 1-4/649	1361	1112	1164	1652	1327	1930	1589	2288	1605	1094	1618	2070	1803	1586
9	SIOKRA V-15	1652	1378	1936	1912	1477	1894	1814	2351	1735	2054	1557	2063	1772	1815
10	SICALA 34	1267	1139	1124	1566	1338	1923	1373	1942	1373	1143	1493	2057	1612	1488
11	SICALA V-2	1709	1225	2011	1859	1639	1947	1840	2235	1858	2128	1620	1921	1691	1822
12	CS 8S	1548	1429	1957	1743	1470	1901	1880	2195	1663	1935	1493	2210	1681	1777
13	CS 50	1271	1198	1314	1801	1423	1944	1417	2192	1601	1285	1609	2068	1908	1618
14	CS 189+	1548	1124	1722	1824	1606	1703	1701	2107	1632	1904	1639	1929	1831	1713
15	83055-33-613	1749	1238	1981	1868	1603	1928	1815	2281	1848	2127	1598	1986	1698	1825
16	86001-130-542	1560	1115	1943	1824	1630	1700	1797	2199	1731	2002	1792	2024	1790	1777
17	86001-130-1220	1600	1168	1762	1652	1675	1822	1661	2158	1696	1978	1742	2072	1836	1756
18	87029-176-58	1892	1481	2097	2262	1625	2083	1981	2454	1805	1913	1678	2139	1756	1936
19	87029-176-353	1779	1472	2089	2154	1638	2082	1990	2531	1832	2184	1572	2256	1804	1953
20	87029-176-405	1732	1476	2022	1849	1571	2022	1898	2402	1779	2101	1667	2148	1852	1886
21	87029-176-500	1616	1485	2118	1876	1561	1913	1958	2435	1715	2048	1539	1992	1776	1849
22	87029-176-506	1709	1480	2035	1948	1577	2016	1977	2424	1841	2081	1611	2157	1865	1902
23	87029-176-562	1704	1379	2048	2026	1586	1954	1984	2388	1710	2068	1788	2170	1807	1893
24	87029-176-834	1754	1521	1908	1924	1640	2070	1899	2497	1781	2181	1543	2131	1820	1898
25	87029-176-1031	1685	1463	2018	1959	1611	1998	1908	2415	1748	2066	1679	2076	1817	1880
26	87029-176-1100	1756	1520	1895	1930	1557	2060	2101	2405	1794	2033	1804	2213	1848	1917
27	87262-168	1339	821	1488	1700	1586	1671	1708	2043	1536	1443	1768	2033	2004	1626
28	88201-343	1575	1239	1694	1787	1546	1983	1702	2162	1602	1890	1643	2093	1736	1742
29	88203-97	1605	1180	1731	1702	1623	1961	1520	2224	1526	1863	1774	2060	1870	1741
30	88208-214	1492	1324	1705	1696	1537	1810	1451	1998	1655	1853	1555	1900	1625	1662
31	88210-115	1488	746	1723	1717	1423	1727	1485	2025	1523	1476	1659	1821	1509	1563
32	88210-442	1439	810	1821	1639	1514	1707	1327	1951	1578	1598	1621	1929	1571	1577
33	89007-33	1550	1440	1749	1901	1439	1929	1873	2330	1744	2077	1627	2032	1532	1786
34	89007-239	1584	1479	1935	1825	1462	1924	1592	2109	1569	1892	1372	1973	1460	1706
35	89009-45	1518	1185	1862	1825	1589	1891	1741	2055	1673	1982	1683	2136	1790	1764
36	89013-114	1735	1581	1913	1850	1579	2085	1897	2430	1718	2257	1424	2056	1447	1844
37	89026-309	1702	1215	1778	1775	1644	1855	1851	2219	1776	2090	1486	1919	1543	1758
38	90001-113	1565	1334	1952	1785	1517	1855	1784	2300	1789	1993	1595	2116	1595	1783
39	90001-781	1821	1236	2049	1886	1681	1896	1914	2327	1847	2199	1761	2132	1749	1884
40	90001-894	1572	1306	1942	1899	1538	1889	1848	2292	1802	1953	1438	1982	1668	1779
41	90003-118	1447	1303	1817	1764	1704	1809	1647	2151	1861	1975	1774	2252	1687	1784
42	90003-217	1646	1116	1779	1833	1797	1863	1733	2223	1766	1822	1800	2044	1807	1787
43	90003-332	1685	1274	1783	1824	1653	1816	1594	2106	1859	1918	1634	2069	1645	1758
44	90005-456	1528	1302	1687	1712	1576	1868	1662	2330	1671	1834	1682	2268	1760	1760
45	90005-674	1752	1366	1683	1819	1530	1920	1774	2255	1711	1978	1720	2008	1704	1786
46	90005-757	1850	1560	1930	1914	1612	2156	1942	2480	1822	2082	1555	2039	1585	1887
47	90012-26	1558	1321	1817	1804	1459	2005	1798	2268	1686	2044	1634	2109	1785	1791
48	90012-512	1639	1026	1912	1880	1613	1856	1755	2347	1507	1812	1939	2084	1933	1793

	9510 LINT YIELD															
		WA	BR	MV	MN	BK	CO	MO	BB	SG	DD	TH	BI	EM	MEAN	CV
1	DPL 16	1352	831	1272	1105	1370	1546	1536	1491	1286	1335	1770	1747	1709	1412	18.8
2	DPL 5690	1688	1024	1579	1271	1654	1743	1823	1582	1521	1609	1934	2015	2034	1652	17.2
3	NAMCALA	1483	1022	1355	1261	1501	1428	1551	1229	1326	1419	1613	1346	1573	1393	11.7
4	SIOKRA 1-1	1599	1037	1611	1152	1493	1774	1610	1717	1281	1687	1923	1958	2022	1605	18.8
5	SIOKRA L22	1503	1016	1559	1290	1601	1816	1710	1595	1336	1834	1863	1940	1936	1615	17.2
6	SIOKRA L23	1561	980	1620	1294	1664	1805	1828	1637	1430	1855	1952	2086	1977	1668	18.3
7	SIOKRA S324	1643	1260	1708	1232	1674	2005	1837	1882	1544	1968	2007	1868	2067	1746	15.6
8	SIOKRA 1-4	1635	1067	1759	1345	1658	2108	1825	1968	1566	2029	2007	2051	2158	1783	18.1
9	SIOKRA V-15	1949	1420	1814	1461	1948	2031	2139	1844	1805	2014	2081	2022	2089	1894	12.0
10	SICALA 34	1625	1060	1505	1243	1574	1794	1579	1621	1162	1858	1854	1938	1946	1597	18.3
11	SICALA V-2	1891	1337	1727	1513	2022	1967	2229	1634	1889	1817	2045	1976	1970	1847	13.1
12	CS 8S	1728	1334	1610	1305	1855	1837	2071	1893	1567	1790	2156	1946	1999	1776	14.8
13	CS 50	1627	1049	1625	1342	1719	2044	1820	1795	1429	1930	2047	2000	2115	1734	18.2
14	CS 189+	1783	1152	1640	1393	1779	1667	1933	1611	1546	1732	2023	1809	1969	1695	14.1
15	SICOT 189	1669	1193	1522	1494	1894	1842	1926	1777	1774	1861	2147	2135	2005	1788	15.0
16	87029-176-353	2025	1571	1880	1549	2069	2100	2187	1815	1937	2056	2203	2233	2195	1986	11.5
17	87029-176-506	1950	1506	1730	1437	1961	2168	2264	1842	1881	2062	2243	2086	2119	1942	13.4
18	87029-176-643	2010	1597	2082	1576	2076	2087	2195	1909	2011	2112	2269	2051	2182	2012	10.4
19	87029-176-977	1930	1473	1773	1475	1970	2043	2108	1763	1860	2027	2229	2041	2039	1902	12.1
20	87029-176-1100	2008	1486	1849	1502	2039	2011	2193	1795	1895	2122	2309	2129	2177	1963	12.9
21	87262-168	1642	961	1509	1400	1614	1664	1964	1600	1270	1627	1905	2043	1934	1626	18.6
22	88201-343	1640	1330	1630	1330	1862	2091	1939	1918	1718	2038	2089	2035	2089	1824	15.0
23	89007-33	1827	1567	1879	1487	2014	2061	2116	1905	1982	1944	2042	1946	2047	1909	9.9
24	89007-58-506	1823	1631	1805	1487	2171	2043	2108	1874	2001	2123	2195	2008	2163	1956	11.3
25	89007-110-10	1930	1539	1930	1581	2143	2084	2116	1853	2045	2137	2244	1932	2170	1977	11.0
26	89007-110-52	1969	1694	1893	1618	2121	2359	2135	1980	2081	2210	2130	2057	2217	2036	10.2
27	89007-110-222	2087	1719	1902	1608	2173	2178	2146	1876	1949	2273	2210	2050	2151	2025	10.0
28	89007-110-437	1939	1467	1729	1538	1907	2014	2018	1748	1876	1882	2077	1869	2001	1851	10.0
29	89009-78-209	1910	1519	1858	1517	2062	1891	2214	1682	1823	2009	2143	2150	2043	1909	12.0
30	89009-78-408	1887	1346	1793	1608	2181	2138	2106	1739	1789	1924	2124	2117	2097	1911	13.1
31	89009-179-57	1884	1510	1765	1516	2030	2110	2190	1944	1934	2166	2219	2161	2215	1973	12.6
32	89009-179-164	1910	1481	1754	1485	2023	2098	2210	1862	1973	2079	2158	2063	2158	1943	12.4
33	89009-179-491	1785	1468	1777	1488	1925	2157	2212	1899	1968	2192	2219	2206	2147	1957	13.6
34	SIOKRA S-101	1775	1625	1802	1495	2263	2214	2091	1959	1921	2162	2248	1936	2118	1970	12.4
35	89205-134	1657	1112	1604	1437	1752	1875	1866	1670	1487	1738	2113	1978	2049	1718	15.9
36	89205-401	1570	1137	1647	1349	1688	1933	1918	1698	1392	1896	1925	2135	2037	1717	17.2
37	90001-781	1993	1383	1853	1554	2129	2011	2177	1601	1939	1935	2269	2002	2142	1922	13.7
38	90003-118	1845	1287	1709	1425	1961	1763	1961	1586	1698	1930	2220	2086	2005	1806	14.7
39	90003-217	1865	1238	1769	1501	1897	1875	2087	1722	1756	1915	2147	2004	2054	1833	13.6
40	90005-757	1828	1637	1927	1514	2126	2183	2299	1962	2055	2229	2303	2112	2174	2027	12.1
41	90012-512	1756	1249	1770	1433	1832	1804	2072	1620	1698	1871	2147	2058	2098	1801	14.7
42	90031-169	1864	1542	1900	1568	2099	2184	2173	2022	2032	2125	2233	2058	2173	1998	11.2
43	91014-211	1957	1565	1851	1359	2146	2001	2076	1929	1868	2105	2097	1977	1959	1915	11.7
44	91203-60	1619	1228	1500	1403	1847	2003	1843	1757	1609	1870	2185	2073	2055	1769	16.1
45	91203-228	1698	1503	1687	1407	1905	2204	1903	1934	1849	2148	2156	2194	2135	1902	14.0
46	91203-566	1555	1289	1553	1323	1843	1966	1731	1734	1680	1897	2083	2040	2089	1753	15.3
47	91203-667	1547	1201	1636	1418	1834	1940	1837	1814	1711	1915	2203	2116	2135	1793	16.2
48	90219-218	1881	1422	1809	1468	2007	1949	2049	1850	1730	2004	2063	2131	2001	1874	11.8

	1996/97 ACCT ALL SITES														
		wa	br	mv	mn	bk	co	mo	bb	sg	dd	th	bi	em	mean
1	DPL 16	1681	910	1315	1560	1212	1410	1179	2009	1657	1358	1806	1614	1728	1495
2	DELTA PEARL	2039	1235	1701	1821	1674	1805	1475	2165	2121	1785	2356	2052	1924	1858
3	NAMCALA	1705	846	1191	1466	1264	1433	1215	1849	1613	1202	1481	1319	1479	1389
4	SIOKRA 1-1	1786	1344	1526	1867	1371	1655	900	2195	1775	1543	1842	1456	1642	1608
5	SIOKRA 1-4	1748	1323	1475	1859	1500	1581	807	2173	1841	1574	2069	1831	1932	1670
6	SIOKRA L23	1896	1240	1646	1835	1398	1802	1472	2365	1982	1703	2389	2026	1798	1812
7	SIOKRA S-101	2036	1547	1832	1915	1705	1756	1158	2272	2008	2197	2004	1470	1900	1831
8	SIOKRA V-15	2200	1397	1919	1910	1758	1936	1610	2311	2074	2038	2112	1620	1868	1904
9	SIOKRA V-16	2235	1387	2102	2004	1792	2150	1676	2430	2336	2064	2338	1848	1957	2025
10	SICALA V-2	2199	1327	1908	1878	1602	1993	1650	2264	2159	1964	1969	1743	1900	1889
11	SICOT 189	1959	1303	1754	1727	1391	1890	1534	2363	2098	1810	2388	2222	2060	1885
12	CS 50	1938	1301	1713	1832	1556	1841	1388	2284	1984	1708	2463	1935	1865	1833
13	CS 8S	2089	1374	2043	1896	1782	1780	1662	2273	2119	1959	2034	1984	1832	1910
14	89007-110-10	2149	1522	1915	1856	1872	1880	1678	2319	2103	1963	1891	1682	1873	1900
15	89007-110-222	2230	1516	1864	1872	1915	1822	1937	2389	2103	2129	2128	1984	2000	1991
16	89007-110-470	2198	1535	1934	2022	1746	1866	1697	2392	2145	2029	2040	1734	1813	1935
17	89007-110-52	2288	1570	1894	1943	1848	1927	1725	2493	2156	2055	2047	1742	1811	1961
18	89007-33	2052	1504	1891	1779	1675	1777	1571	2276	2065	1978	2033	1787	1830	1863
19	89007-58-506	2095	1455	1842	1915	1781	1884	1635	2292	2030	2037	2058	1484	1878	1876
20	89009-179-491	1998	1417	1787	1866	1584	1858	1518	2313	2215	2123	2073	1698	1977	1879
21	89009-179-57	2074	1307	1850	1805	1481	1942	1632	2347	2216	2067	2150	1701	2029	1892
22	89201-805	1881	1432	1814	1862	1718	1765	1440	2529	2079	1791	2546	2007	1921	1907
23	89230-341-1024	1958	1460	1813	1715	1403	1688	1478	2234	1993	1912	1828	1670	1741	1761
24	89230-341-1074	1993	1396	1775	1763	1428	2030	1490	2266	2126	1883	1971	1779	1673	1813
25	89230-341-826	2077	1415	1739	1902	1615	1787	1461	2220	2118	1944	1711	1564	1679	1787
26	89230-341-847	2076	1448	1724	1825	1560	1814	1305	2381	1997	2047	1921	1525	1718	1795
27	90001-781	2141	1376	2038	1886	1579	2111	1621	2458	2090	2120	2244	2036	1947	1973
28	90005-757	2239	1553	1961	1998	1844	2058	1528	2455	2167	2244	2034	1676	1746	1962
29	90031-169	2103	1548	1980	2044	1615	1854	1463	2292	1980	2127	1962	1617	1849	1880
30	90050-221	2201	1483	1842	1946	1738	2045	1427	2351	2154	1993	2030	1692	1879	1906
31	91011-163	2300	1482	2000	1918	1877	1974	1719	2461	2172	2064	2167	1723	1845	1977
32	91011-223	2282	1540	2100	1967	1915	2025	1445	2471	2276	2182	2045	1590	1949	1984
33	91011-380	2243	1491	1906	1961	1787	2070	1396	2446	2187	2116	2061	1759	1938	1951
34	91011-472	2155	1501	1857	1916	1826	1979	1568	2260	2109	1997	1895	1616	1744	1879
35	91011-888	2148	1506	1914	1943	1738	2053	1437	2426	2165	1971	1901	1574	1830	1893
36	91011-940	2212	1468	2076	1976	1761	2036	1716	2350	2254	1974	2090	1561	1817	1945
37	91203-163	2134	1534	1819	1882	1761	1840	1436	2346	2039	2138	1982	1417	1805	1856
38	91203-228	2028	1463	1728	1820	1554	1807	1532	2337	2126	1865	2097	1914	1975	1865
39	91203-335	2128	1431	1909	1749	1628	1882	1499	2306	2141	1994	1909	1715	1839	1856
40	91203-667	2000	1156	1822	1743	1447	1732	1483	2193	2137	1736	2398	1875	2008	1825
41	91223-28	2081	1431	1945	1764	1621	1775	1687	2187	2104	1873	2143	1721	1863	1861
42	SIOKRA L-23i	1989	1434	1839	2047	1535	1744	1575	2435	2011	1959	2390	2031	1711	1900
43	SIOKRA V-15i	2054	1607	1976	1923	1855	1833	1375	2322	2078	1949	1924	1565	1701	1859
44	SICALA V-2i	2234	1596	2078	2081	1868	1808	1600	2348	2188	2128	2067	1844	1757	1969
45	SICOT S-8i	2067	1360	2029	1931	1809	1843	1771	2319	2065	2125	2333	1972	1840	1959
46	SICOT 50i	2111	1422	1789	1975	1571	1792	1578	2374	2049	1874	2382	1955	1944	1909
47	SIOKRA V-15 1076	1996	1443	1876	1873	1676	1820	1524	2188	2011	1932	2065	1862	1884	1858
48	NUCOTN 37	1896	1231	1769	1653	1623	1781	1644	2376	1906	1738	2293	2195	1894	1846

COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

PROJECT TITLE: **DYNAMICS OF Bt PROTEIN IN INGARD COTTON:**
MECHANISMS OF VARIABLE EFFICACY AGAINST *HELICOVERPA*

PROJECT CODE: **CRC3C**

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DYNAMICS OF Bt PROTEIN IN INGARD COTTON:
MECHANISMS OF VARIABLE EFFICACY AGAINST *HELICOVERPA*

There were at least ten commercial fields of Ingard in the 1996/97 season with low efficacy pre flowering and requiring up to three insecticide sprays. Most other commercial Ingard fields required none or one insecticide when adjacent conventional cotton has been sprayed for *Helicoverpa* five times. Variability was also evident in 1997/98 although with lower pest pressure in some districts that year, performance of Ingard was relatively better

This project set up pilot studies to investigate physiological causes of variation in Ingard efficacy. The information will assist with crop management to minimise efficacy problems and plant breeders may utilise the findings to breed for improved stability of efficacy.

It was found that shade and low temperature could affect Bt levels and efficacy against *Helicoverpa*. Those preliminary studies require further study to confirm the result and to clarify the mechanism.

CRC linkages

This project is the initiative of the CRC in response to the problems experienced in 1996/97 with Ingard cotton. The fact that a coordinated project can be put together is a positive consequence of the CRC concept. Research into physiology issues is a sub-program of the Cropping Systems Program of the CRC.

Research proposal summary

Pilot studies were done in 1997. A significant proportion of the research was done in growth cabinet, glasshouse and phytotron where particular environmental conditions could be controlled. *Targeted outcomes were hypotheses of the cause of low efficacy in Ingard cotton which would contribute to field management of future Ingard crops as well as specific plant breeding strategies.*

Background

Ingard cotton was approved for limited commercial use (30,000 ha) in the 1996/97 season. There are various proposals and ideas on increasing areas for future seasons, eg 90,000 to 150,000 ha in 1997/98. Most regulatory and research concerns with the technology are to ensure a management strategy is in place to minimise the likelihood of *Helicoverpa* developing resistance to the Bt protein.

Previous (but limited) trials with Ingard have shown very good efficacy against *Helicoverpa* except after crop cutout when larvae survival has indicated a reduction in Bt protein levels and so in some instances at least one insecticide spray was required late season. Early season efficacy has even been good in Kununurra with extreme *Helicoverpa* pressure, but one occasion in January 1996 at Narrabri, showed a reduction in efficacy which was attributed to weather conditions such as cool temperature, cloud and/or waterlogging.

There are up to ten commercial fields of Ingard in the 1996/97 season with low efficacy pre flowering and requiring up to three insecticide sprays. Most other commercial Ingard fields have required none or one insecticide when adjacent conventional cotton has been sprayed for *Helicoverpa* five times. There is generally no question that all Ingard fields will have a good yield.

This situation of varying efficacy has raised a number of concerns, not the least being the dissatisfaction by cotton growers of the value of Ingard - requiring insecticide applications when they have been charged $245/ha for the licence. Of additional concerns are:

- Bad publicity with insecticide being applied to this technology, when it has been promoted as drastically reducing pesticide use. Overall the technology may not achieve that objective if the low efficacy is common.

- The Bt resistance management strategy relies on a high dose. With such high survival of *Helicoverpa* neonate larvae occurring this season, clearly the dose does not always qualify as 'high'.

There has been no clear indication of the primary cause of the low efficacy. Following are brief comments on some of the hypotheses:

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Low temperatures. Nitrogen uptake and protein synthesis are tied to photosynthesis, so factors reducing crop growth, including temperature extremes and cloudy weather, could potentially reduce protein production, including Bt protein. Chilling injury, even to seedlings, has been shown to interfere with subsequent protein synthesis. Whether Bt protein is different in these reactions to plant protein is yet to be determined.

Waterlogging. Previous research has shown that waterlogging of cotton will reduce nitrogen uptake. The reduced nitrogen status could affect protein levels.

Growth dilution. A number of the problem fields are in the western side of the cotton producing belt (Bourke, St George, Mungindi). In most cases these fields are in very fertile rotations and have less cool weather than eastern locations. The reduction in efficacy with some of these fields coincided with rapid vegetative growth in November-December. Rapid expansion of leaves and squares may dilute the Bt protein to levels which allow survival of *Helicoverpa* larvae. A combination of cool weather, followed by good conditions for rapid growth, may exaggerate the single effects of each factor.

Variety. Bioassays have shown that there are differences between varieties in efficacy. Field observations are consistent with that data, although the major problem Ingard fields include most varieties. Some experimental varieties contain different promoters; the full difference between these promoters derived from different viruses has not been fully measured. Maybe they have different reactions to climate and plant growth stage or plant part.

Objectives and achievements

Study the effect of different environmental and plant stresses on plant protein dynamics.

Methodology

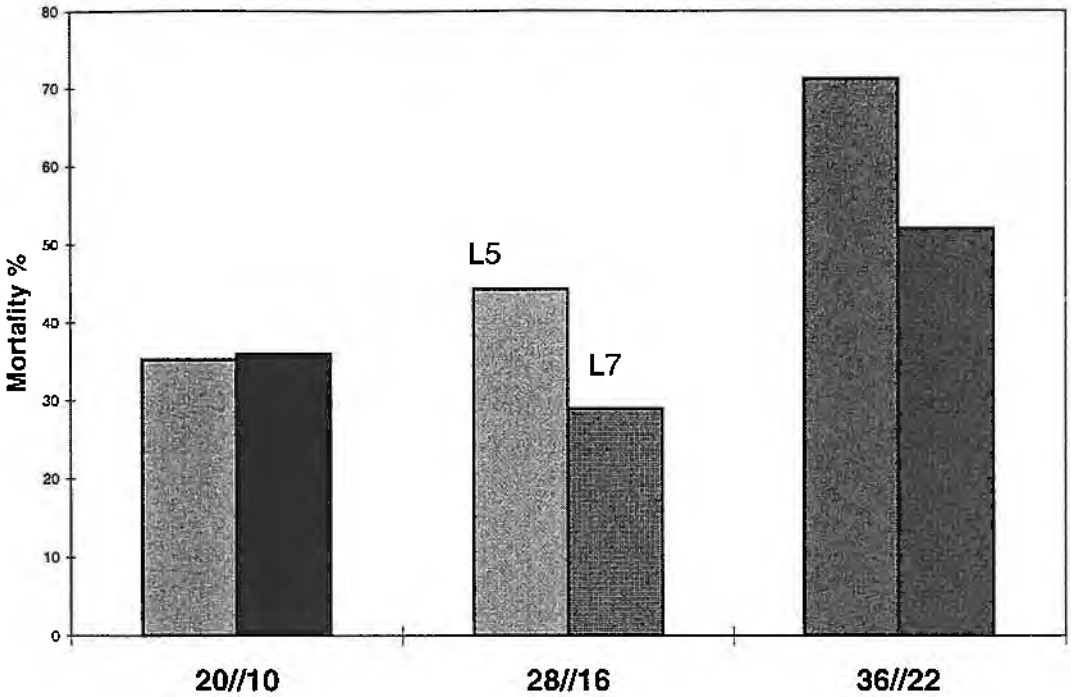
Field and controlled environment studies are required to separate the various environmental and plant interactions and their effect on Bt level/efficacy. A range of people/skills are to be integrated in the preliminary stage of this project.

- Field experiments on soil-plant nutrient status;
- Field or glasshouse waterlogging treatments;
- Cabinet studies on various combinations of temperature during germination;
- Phytotron studies on combinations of high/low temperature and light.

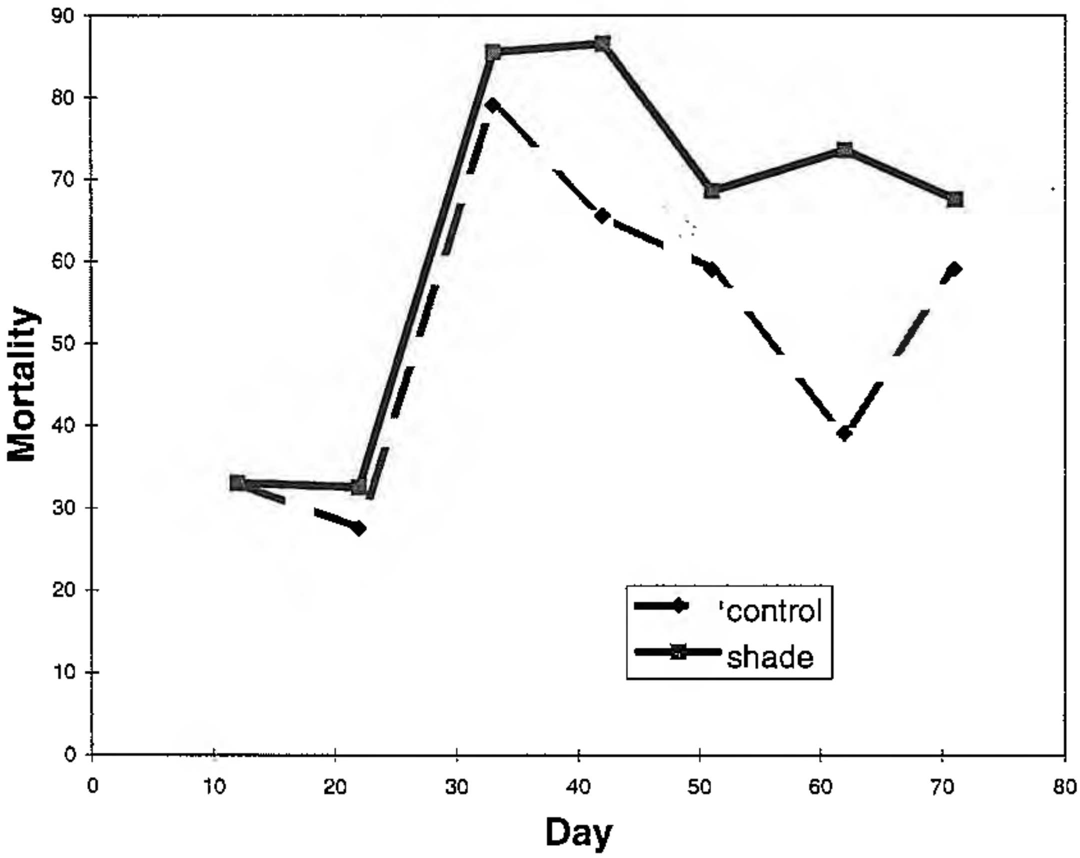
When critical combinations of environmental effects are identified, they should be repeated on the various combinations of genotype, promoter, gene and construct. Two gene Ingard should be studied as well, because we should be moving in that direction anyway. The two gene Ingard should therefore be seed increased (even if not fully developed yet for yield) to be included in extensive field trials for Ingard efficacy.

Results and discussion

Two pilot experiments were established in the phytotron in Canberra to establish known differences in light and temperature history on Ingard (Siokra V-15*i* plants). The following figures summarise the results.



Mortality of *Helicoverpa* larvae feeding on two different leaves (leaf 5 or leaf 7) for cotton plants grown at three diurnal temperatures for one week. All plants were grown at 28/16 for the rest of the experiment



Mortality of *Helicoverpa* larvae feeding on cotton leaves grown at full sun or in shade for one week up to day 10.

There was clear evidence for shade and higher temperatures to increase the subsequent efficacy of Bt in cotton leaves. These results may not be intuitive and the reduced efficacy for young leaves is not common, but the differences created by shade or temperature were clear. Explanations for the results need to consider a number of factors such as direct effects of treatment on Bt production, indirect effects such as growth dilution for rapidly growing leaves, and possible effects of treatment on activity of promoters associated with the Bt gene.

Recommendations

These data showed there was potential for single climatic events to affect Ingard efficacy. The preliminary experiments actually raised more questions than were answered and more detailed experiments are required, particularly on waterlogging effects. New experiments should be undertaken which would allow the direct effects of climatic factors to be separated from factors associated with the transgene construct-events.

The greatest limitation to progress in this research area is a coordinated approach. CRDC have funded a number of separate projects in this general area without addressing a balanced approach to research disciplines of field pest management (especially Ingard thresholds), plant physiology and molecular biology.